COMPUTER-AIDED TECHNIQUE FOR BLENDING WINE: APPLICATION OF SIMPLEX OPTIMIZATION TO HEADSPACE GAS CHROMATOGRAPHIC PROFILES OF WINE

By

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ABSTRACT

An objective method to blend wines for standardizing flavor quality was developed. Aroma volatiles of varietal and white stock wines were analyzed at 6°C and 37°C by headspace gas chromatography with cryofocussing. Pattern similarity constants of the chromatographic profiles were entered into the simplex optimization program which determined the best blending ratios of wines to simulate the target wine. Thirteen and 23 vertices were required to give the optimum response for trials 1 and 2, respectively. For both trials the computer optimized blends could not be differentiated from the target wines by a sensory taste panel consisting of both untrained and expert judges.

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INTRODUCTION

Maintaining product quality constant is of primary concern to food manufacturers. Blending is a common and necessary practice for standardizing product quality in the food industry for many foods including wine (Vine, 1981; Peynaud, 1984; Jackish, 1985; Rankine, 1988), distilled beverages (Lang, 1983), tea (Theobald, 1977), citrus juices (Charley, 1969; Cook, 1983; Anon., 1987), coffee, processed cheese and processed meat products. A large proportion of the world's wines, both ordinary and fine, are blended (Jackish, 1985). Wines may be blended for color, taste, alcohol and body, and aroma (Peynaud, 1984). Blending for all of these quality parameters except aroma is guided by physical or chemical analysis. Blending for aroma, on the other hand, is dependent on sensory analysis alone; it is a delicate operation that requires a great deal of experience and skill.

If the winemaker is attempting to duplicate the aroma of a wine blend from a previous year, the strategy will be to first to identify the strengths and weaknesses of the available stocks. Normally, one wine is selected as the primary stock while the others are identified as secondary blending stocks. Trial blends are made by the winemaker on the basis of sensory evaluation. These blends are then tested by experienced wine tasters prior to the final blending. Many trials may be necessary to match the flavor of the target product, especially if it is a complex blend.

Traditional methods for blending for the purpose of maintaining uniformity of wine flavor are difficult, time consuming and subjective. Clearly, a more rapid and objective quality test method is desired. Aishima et al., (1987) developed an objective system for finding the best blending ratios of strawberry essences with the concentrated strawberry juice to simulate the aroma of fresh juice. The idea was to maximize the similarity coefficient calculated between gas chromatographic patterns of the fresh juice and a blend of the concentrate with the essences using two different simplex optimization programs, computerized and experimental. Both programs were successful in finding optimum blending ratios. This method, however, can be improved to make it more feasible for quality control purposes by employing a less tedious and complicated method of volatile analysis and eliminating the experimental simplex optimization that requires many trial blends to be analyzed.

The objectives of the present research were:

(1) To develop a headspace gas chromatographic method to analyze wine.
(2) To use computerized simplex optimization to determine blending ratios of varietal and white stock wines to simulate the aroma of the previous year's wines.
(3) To compare blends of the computer-aided technique and traditional method with the target product on the basis of sensory tests.

LITERATURE REVIEW

A. COMMERCIAL WINEMAKING OPERATION

Harvesting

The making of wine really begins at the time of harvesting; the grapes must be picked at the proper stage of maturity and they must be of sound quality (Amerine and Singleton, 1977). To follow the progress of ripening, grape berries should be sampled regularily for determining the average concentration of sugar and acidity of the crop so that the harvest date can be set. Harvesting should be done as to avoid damaging and bruising berries. Oxidation and maceration of the grapes before they reach the winery can be detrimental to the final quality of the wine (Peynaud, 1984).

Stemming and Crushing

Processing begins as soon as the fruit arrives at the winery. The first step is to remove stems as they contribute to the tannic acid astringency (Vine, 1981). Crushing breaks open each of the berries to allow release of pulp and juice during pressing. For making red wine, this operation facilitates contact and fermentaion by yeast (Vine, 1981; Amerine and Singleton, 1977). Both stemming and crushing operations are normally accomplished together in a crusher-stemmer. This machine consists of large horizontal cylinder that is perforated with holes large enough to allow berries to

pass through but not the stems and a rotating axle fitted with a series of paddles. When this axle rotates at high speeds, the crushed berries, now called must, are released through the holes into a collecting basin (Amerine and Singleton, 1977).

Sulfiting

While the practice of employing sulfur dioxide as an antiseptic agent for wines is of ancient origin (Ough and Amerine, 1988), sulfiting the must is a relatively recent practice (Peynaud, 1984). Compressed sulfur dioxide is commonly employed by large wineries while smaller operations add potassium or sodium metabisulfite, sodium sulfite or sodium bisulfite. Sulfur dioxide is added to musts or wines to control undesirable microorganisms, to inhibit browning enzymes and to serve as an antioxidant (Amerine and Singleton, 1977).

Pressing

Different winemaking procedures are carried out once the sulfited must is ready for further processing. If white grapes are being vinified, the crushed grapes are pressed to separate the juice from the solids (skins, seeds, some pulp) called pomace, before fermentation. Red grapes, on the other hand, are pressed after fermentation. Rack and cloth presses, basket presses and continuous presses are used by wineries (Amerine and Singleton, 1977).

Fermentation

White grapes are not fermented on skins as reds because the leucoanthocyanin pigment contributes undesirable colors and flavors to white wine (Vine, 1981). The must, fresh juice or crushed grapes, is inoculated with a pure starter culture of *Saccharomyces cereviseae*. Fermentation is conducted at low temperatures of 18° to 20°C (Peynaud, 1984) but even lower temperatures (10° to 15.5°C) are recommended (Amerine and Singleton, 1977). Higher temperatures of 26° to 30°C are suited for making red wine to allow for thorough maceration of the grapes and rapid fermentaion (Peynaud, 1984).

Clarification, Aging and Filteration

After fermentation is complete, the wine is allowed to stand to collect yeast cells and other fine suspended material called lees at the bottom of the container. The wine is racked by pumping it out of the fermentation container without disturbing the lees. Racking may be carried out several times prior to the aging period. Wine is clarified further by fining. Bentonite is a montmorilonite clay that has been used for wine clarification and stabilization, especially for clearing cloudiness caused by precipitating proteins. It removes proteins, metallic hazes and adsorbs nutrients necessary for microbial growth and enzymes (Vine, 1981).

Now the wine is ready to be aged in stainless steel tanks or in wooden barrels. A final filteration is carried out just before bottling.

B. THE FLAVOR OF WINE

A recent review by Nykanen and Soumalainen (1983) indicated that some 1,300 volatile compounds have been identified in alcoholic beverages. Over 550 flavor components have been reported in wines (Williams, 1982). Flavor substances occur in wines in a wide range of concentrations from nanograms to grams per liter (Schreier, 1979). A certain proportion of wine volatiles are believed to originate from the grape itself and are thought to remain unchanged during the winemaking process. Some of these original components, however, act as precursors and are changed during the fermentation step (Williams, 1982; Schreier, 1979). A considerable number of new aroma products result from the activity of yeasts on the sugar substrate. Aging too contributes to the final flavor of the wine.

Although several factors may be considered important in imparting flavor components to wine, it is generally assumed that most of the aroma constituents arise through the action of yeasts during fermention (Webb and Muller, 1972; Soumalainen, 1971) and that they are responsible for the body of wine aroma (Nykanen and Soumalainen 1983; Nykanen, 1986; Montedoro and Bertuccioli, 1986; Schreier, 1979). Also, unlike some foods, it is not possible to refer to any 'character impact' compound that is responsible for the typical aroma of wine (Nykanen and Soumalainen, 1983); the flavor character of wine results from a complex mixture of aroma components (Webb and Muller, 1972).

Higher Alcohols (Fusel Oils)

Higher alcohols produced during fermentation, contribute to the complex flavor of wine (Ough and Amerine, 1988). Quantitatively, fusel alcohols are the largest group of flavor compounds in wines (Nykanen, 1986). This group includes aliphatic alcohols such as 1-propanol, 2-methyl-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol and an aromatic alcohol, phenethyl alcohol (Nykanen and Soumalainen, 1983; Nykanen, 1986). Originally it was thought that fusel alcohols resulted from the catabolic conversion of the amino acids to the alcohols or the Ehrlich mechanism. Later investigations, however, showed that fusel alcohols can be formed by an anabolic process from sugars and that the Ehrlich pathway could account for only a small portion of the fusel oils formed.

Fatty Acids

A large number of free fatty acids have been identified in wines but relatively few are sufficiently volatile to contribute to odor. Acetic, propionic and butanoic acids are found in high enough concentration to contribute to the aroma of wine. All of the aliphatic acids are odorless at the levels present in wine (Montedoro and Bertuccioli, 1986). Fatty acid synthesis by the yeast cell requires acetyl-coA.

Fatty Acid Esters

Like the fatty acids, fatty acid esters are formed during fermentation and also require acyl-coA for synthesis (Montedoro and Bertuccioli, 1986). They are the largest group of flavor compounds and are considered of much importance to the odor of alcoholic beverages.

Esters

Esters contribute extensively to the aroma of wines, particularly to wines with strong fruity aromas (Williams, 1982). Lower fermentation temperatures seem not only to raise the total content of esters but also the content of those that increase the fruity sensory response (Ough and Amerine, 1988). Isoamyl-acetate, hexyl acetate (Simpson, 1979a) and especially 2,6-6-trimethyl-2 vinyl-4-acetoxytetrahydro-pyrane (Williams, 1982) strongly contribute to wines with intensive fruity aroma. Esters important to the flavor of wines may also originate from the grape itself or may be formed during the aging period. Methylanthranilate, a 'character impact' compound of native American grape varieties (*Vitis labrusca*) and related hybrids, is not found in European *Vitis vinefera* grape varieties (Schreier, 1979; Shewfelt, 1986). It can be isolated from grapes and wines with Freon 11 (Ough and Amerine, 1988). With storage, the volatile ester content increases making wines more flavorsome.

Carbonyls

Although many aldehydes and ketones have been found in wines, most of them especially ketones, have little sensory importance compared to esters. Diacetyl and acetaldehyde are more important contributors to the aroma of sherries than other wines. Acetaldehyde, an intermediatary product of yeast metabolism from pyruvate, has a dry choking aroma that is responsible for the oxidized note of table wine (Montedoro and Bertuccioli, 1986). Its concentration is an indicator of the oxidation state of a wine (Schreier, 1979).

Other aldehydes, hexenal, trans-hex-2-enal and cis-hex-3-enal have a grassy odor that may be typical of wines made from unripe grapes (Montedoro and Bertuccioli, 1986). Diacetyl and 3-hydroxybutan-2-one have sweet sugary aromas and occur in wines in concentrations up to 3 and 30 mg/L, respectively. Many of the aldehydes found in wines may be products of lipoxygenase activity. Linoleic and linolenic acids are found in the bloom of the skin of grapes and are considered to be possible precursors of these components (Schreier, 1979).

Only a few aldehydes have been detected among the wine aroma constituents. Studies have shown that aldehydes can be reduced to their respective alcohols during fermentation. Also, some aldehydes are converted to bisulfite addition products when they react with sulfur dioxide and are not extracted in isolation procedures because of their high water solubility (Schreier, 1979).

Ketones in wines normally have little sensory impact

(Schreier, 1979).

Terpenes

The delicate aroma of many classical wines has been attributed to terpenes and their various oxidation products, including linalool, geraniol and rose oxide, and linalool oxide. Most terpenes originate in grapes occurring as glucosides (Williams, 1982) while some may be metabolized by microorganisms (Schreier, 1979).

Hydrocarbons

Many C12 and C18 alkanes, styrene and terpene hydrocarbons have been identified in wines but one that is of importance to aged wine is 3,8-8-trimethyldihydronaphthalene. It develops upon storage and at concentrations of 20 to 100 ppb and imparts a kerosene or bottle-aged character to the wine. (Williams, 1982).

Volatile Phenols

The importance of phenolic substances to the taste and odor of wines has been reviewed by Singleton and Noble (1976). Polyphenols (tannins) are responsible for the astringency of wine. They precipitate proteins of the saliva and the mucous surfaces causing a contracting dry mouthfeel. Volatile phenols in wine range from phenol, cresols with medicinal odors to more pleasant odorants such as vanillin and methyl salicyclate. All odorous phenolic compounds with the exception of aceto-

vanillone, are not found in grapes; it is likely that they are metabolic products of microorganisms or cleavage products of higher phenols that originate in the grapes (Shreier, 1979). Etievant (1981) demonstrated that volatile phenols originate from microbiological pathways.

C. VOLATILE ANALYSIS OF WINE

Flavor compounds constitute only a very small part of foods and beverages, as they are present at parts per million to parts per billion levels (Flath and Sugisawa, 1981). Isolation of these aroma substances is made more difficult for wines and other alcoholic beverages because they contain large quantities of water and ethanol. Rapp (1981) emphasized that the method selected for analyzing volatiles should result in an ethanol-free aroma concentrate and that although methods such as freezing out, salting or distilling achieve enrichment, they also concentrate ethanol and therefore are not appropriate enrichment techniques for trace components in alcoholic beverages. Many isolation and concentration techniques described in the literature for wines can be grouped either as liquid-liquid extraction or gas extraction.

Liquid-Liquid Exraction

One of the commonly employed methods for isolating flavor compounds is by extraction with an organic solvent. Most

researchers have relied on the selectivity of solvents or mixtures of solvents such as pentane (Williams and Tucknott, 1973; Chaudhary et al., 1968), pentane-dicholromethane (Lamikanra, 1987; Schaefer et al., 1983), Freon 11 (trichloromonofluoromethane) (Hardy, 1969; Williams and Tucknott, 1973; Stevens et al., 1969; Cobb and Bursey, 1978; and Nelson et al., 1978), Freon-methylene chloride (Guntert et al., 1986), and Freon 113 (Nelson and Acree, 1978) to obtain an ethanol-free extract. Methylene chloride (Brander, 1974; Brander et al., 1980; Kwan and Kowlaski, 1980; van Wyk et al., 1967a,1967b; Slingsby et al., 1980) was used in studies which involved separating organic and neutral fractions from wine.

Hardy (1969) investigated the suitability of Freon 11 as a solvent for analysing alcoholic beverages. Recoveries of alcohols, ketones and esters from 10% aqueous ethanol in a model system using continuous extraction were high for alcohols of C5 and above and low for C4 and below. Extraction efficiency of the other volatiles was high. This demonstrated that Freon 11 is a well suited solvent for alcoholic beverages. Furthermore, Hardy (1969) found that by adding propylene glycol to Freon 11, higher alcohols which were the major components of the Freon 11 extracts were removed. This allowed esters and other minor components to be concentrated a further five to ten times.

Pentane seems to behave much like the extractant Freon-propylene glycol. In a comparative study, Williams and Tucknott (1973) examined ether, pentane and Freon 11.

Separatory funnel extractions of ethanol solutions of esters and alcohols showed preferential removal of esters by pentane. Freon 11 and ether were less selective or not selective at all. When a continuous liquid-liquid extractor was used, pentane was no longer selective. Since ether is not selective against ethanol, the authors concluded that for exhaustive extraction of all components other than ethanol, pentane or Freon 11 should be used in a continuous extractor and that for selective extraction of esters in the presence of alcohols pentane should be employed with little fractionation of the solvent.

In a later comparative study, Cobb an Bursey (1978) supported the finding of Hardy (1969) and Williams and Tucknott (1973) in that Freon is a suitable solvent for isolating volatiles from wines. Commonly used extracting solvent for grape juices and wines including diethyl ether, dichloromethane, 2-methylbutane and Freon 11 were compared. A model system containing nine flavor compounds found in Concord wine in a 12% ethanol-water mixture were extracted with these four solvents using a separatory funnel. Substantial losses occurred during the isolation procedure, in particular for ether and isopentane. Overall, Freon 11 extracted more of the compounds at a higher efficeincy than the other solvents. Dichloromethane was a close second having similar results while ether and isopentane faired much poorer.

One innovation in flavor extraction in foods is the use of supercritical carbon dioxide. Supercritical carbon dioxide extraction has become very popular recently in food flavor

analysis although no applications for wine have been reported. One explanation for this may be that this extractant may behave like liquid carbon dioxide in that it will selectively extract alcohols. Schultz and Randall (1970) have reported that ethanol is fully miscible with liquid carbon dioxide.

Headspace Extraction Techniques

Headspace techniques offer several advantages over extraction techniques in particular, they produce results more representative of the volatiles as they would be smelled. In general, solvent extraction methods isolate and concentrate all of the volatile components that contribute to the flavor of a food while headspace methods usually extract the lower boiling point components that are present in high concentration. In addition solvent methods require long sample preparation times. For example, extraction of volatiles from Pinot noir wines with pentane-dichloromethane required 8 hrs. (Schreier et al. 1980) while the method of Hardy (1969) took 17 hrs. to complete. Finally, total volatile methods have the additional problem of potentially enriching trace impurities that may be present in the solvent.

It is important at this point to make a distinction between the two types of gas extraction techniques that have been used in wine aroma analysis namely, equilibrium or static headspace and purge and trap or dynamic headspace. In purge and trap analysis, the aqueous sample is bubbled with an inert gas to remove volatile components which are then adsorbed onto a

polymer trap. Normally, the trap is developed by heat and the aroma substances are then cooled prior to being injected in the gas chromatograph. With static headspace techniques, on the other hand, a sample of gas immediately above the food that has been equilibrated at a particular temperature and is contained in a closed vessel is removed and analyzed by gas chromatography.

Purge and Trap Headspace Analysis

Researchers employing solvent extraction techniques had to depend on the selectivity of organic solvents to eliminate removal of ethanol from alcoholic beverages. Workers using headspace concentration methods have used polymer traps to eliminate ethanol from the isolated volatiles. Porous resins such as Porapak Q, Tenax GC and Chromosorb 105 have low affinity for dominant compounds in wine namely, water and ethanol (Heide, 1985; Simpson 1979b). They provide a means for concentrating all but the low molecular aroma compononents (Simpson, 1979b).

Using Porapak Q, Jennings et al. (1972) developed a method to follow changes in the volatile composition of beer. Purified nitrogen was purged through the sample and through the polymer trap for adsorption of the released aroma components. To rid the trap of ethanol and water, nitrogen was passed through the column with one end of the column open to atmosphere. Aroma volatiles were collected in a glass trap cooled with dry ice by heating and backflushing the Porapak Q

trap. Cordner et al. (1978) employed the same procedure to examine the effect of crop level on chemical composition and headspace volatiles of Zinfandel grapes and wines but used a Tenax GC trap instead.

Murray (1977) introduced an new headspace technique using a Chromosorb 105 column that fit into a sophisticated introducer system of a laboratory constructed gas chromatograph. Diluted brandy was among the samples that were analyzed. Williams and Strauss (1977) adapted this technique for examining wines and other alcoholic beverages but used the introducer with a conventional commercial gas chromatograph. Two Chromosorb 105 traps were used in series. A 30 L volume of sweep gas was passed through the sample and the trap. Excess ethanol and water were removed by passing nitrogen through both traps. Chromatograms of the table wine revealed that the first trap adsorbed only a few of the higher concentration alcohols while the second trap adsorbed many volatiles including the ones adsorbed by the first but in lower proportion. The authors cautioned that the desorption conditions should be adequate to avoid selective retention of some volatiles on the trap.

Two German researchers Rapp and Knipser (1980), introduced a new technique of headspace analysis of wine that was essentially a combination of headspace and solvent extraction methods. Instead of using a porous polymer trap to collect volatiles stripped from the sample by a purging gas, the aroma components were collected into 10 % aqueous ethanol.

As this collection was being carried out, the ethanol solution was continuously extracted with Freon 11. Several advantages of this technique were cited in this original publication. In comparison to the polymer trap methods in which the sample volatiles can be analyzed only once, the headspace sample obtained by this procedure can be examined several times using different detection systems (flame ionization detector or GC-mass spectrometer). Also, no special injector system is needed as with the method of Murray (1977). No impurities are contained in the extract and artefact formation is unlikely to occur since the operation is carried out at 28°C.

Owing to the superiority and success of this procedure, the method has gained popularity. Drucret (1984) employed the techique described by Rapp and Knipser (1980) for comparing headspace volatiles of carbonic maceration and traditional wine. More recently, Craig (1988) used this technique to compare headspace volatiles of kiwifruit wine and grape wine.

Static Headspace Analysis

It was mentioned earlier that headspace analysis represents the composition of aroma compounds as they would be sensed by the nose. According to Dravnieks and O'Donnell (1971), however, this is only true for static headspace methods and not purge and trap methods. Extraction procedures involving bubbling of inert gas in the sample and subsequent trapping of volatiles onto porous polymer columns, or cooling traps do not lead to the 'natural' composition of the headspace

of the food (Bertuccioli and Montedoro, 1974).

Firstly, in accordance with Henry's law, the headspace concentrations of volatiles are not determined by the vapor pressures of their respective volatiles and their analytical concentrations in the food alone; they also depend on their activity coefficients. The activity coefficient is affected by the content of water, lipids, proteins, carbohydrates, polyphenols and other materials in the food and will vary during sampling as in the case of purging methods. Henry's law is only valid when the headspace gas above the food is allowed to equilibrate with it (Dravnieks and O'Donnell, 1971).

In addition, when a continuous loss of volatiles occurs, diffusion rates of volatiles from the bulk of the food to its interface with air become further rate limiting factors that can vary the ratios of volatiles in the headspace (Dravnieks and O'Donnell, 1971).

In view of the above, Bertuccioli and Montedoro (1974) developed and optimized a method to analyze the 'natural' headspace composition of wine. Samples of wine (100 mL) were placed in a large syringe (2 L size) and equilibrated at 20°C for 15 min. The headspace volume of the syringe was passed through a Porapak Q trap which was connected to the syringe. The motor driven syringe was set to release the headspace gas at a flow rate of 100 to 150 mL/min. Volatiles were desorbed by heat. Many new components in wine not previously reported by headspace methods were detected by Bertucciolli and Viani (1976) when they replaced the Porapak Q trap with Tenax GC.

Noble (1978) and Noble et al. (1979) employed the same principle as Bertuccioli and Montedoro (1974) but used a different apparatus to investigate the reproducibility of the headspace method. The headspace volume over the wine sample was displaced by fluid rather than a motor driven syringe. Noble et al. (1980) and Noble (1981) employed principal component analysis of wine headspace volatiles collected by displacement to classify wines by variety.

To study changes caused by *Botrytis cinerea* in finished wine, Flath et al., (1972) used a precolumn cooling trap in place of a polymer trap to analyze headspace volatiles. A 10 mL volume of sample vapor was removed with a gas tight syringe and injected into the precolumn, a stainless steel tubing immersed in a cold bath. The condensed volatiles were transfered into the gas chromatograph oven by removing the refrigerant and heating the tubing with a hot air gun.

In a more recent study, Gelsomini (1985) reported a direct headspace analysis with capillary columns using an automatic headspace chromatographic system. Wine samples saturated with anhydrous sodium sulfate were conditioned in the thermostatic bath for 1 hr. at 50°C. Differences in red and white wine were apparent from the headspace chromatograms that contained a handful of peaks. Use of this system was recommended for quality control of wines in the industry.

A comment about capillary columns is warranted here. In the discussion of extraction methods for volatile analysis, no mention was made of capillary columns. Enrichment of aroma

compounds, whether by solvent extraction or headspace methods, results in the concentration of volatiles that are composed of hundreds of individual components which contain many different classes of constituents, and a wide range of boiling points. Separation of such complex mixtures that vary in concentration by several magnitude, demands extremely high efficiency of the column which could have only been possible using capillary column gas chromatography (Rapp, 1981).

D. SIMPLEX OPTIMIZATION

Optimization has been described as finding the best possible method of carrying out some operation (Bayne and Rubin, 1986). Even today a popular optimization procedure is the classical one-factor-at-a-time method (Bayne and Rubin, 1986). This sequential approach requires that all factors except one be held constant while the factor being tested is evaluated at varying levels.

Using a two factor example, Massert et al., (1988) described in a very simple and understandable manner the sequential single factor-at-a-time strategy. If there is no interaction between the two factors, then the optimum can be found; the ridge on the response surface of this example would lie parallel to the factor axes (Fig. 1). The no interaction case is, however, an exception; in general, factors do not operate independently on the response. When interactions occur, a plot of the response surface reveals that the ridge

(optimum) does not lie parallel to the axes; it is instead oblique with respect to the other factor axes (Fig.2), thus making the task of finding the optimum difficult. A one-factor-at-a-time approach can improve the response but not optimize it. The optimum may be found if the factors are changed together in the direction of the axis of the ridge.

EVOP

Noting the inadequacy of the one-factor-at-a-time method, Box (1957) suggested an alternative optimization technique that was capable of finding the optimum even when interactions between factors existed. Evolutionary operation (EVOP) strategy was based on factorial designs in which each of the variables would be altered slightly at the same time (Saguey, 1986). EVOP is valuable for industrial process optimizations since factors are not varied extensively but it has limited application for research purposes where extensive shifts in experimental conditions may be required. Another drawback of this method is the large number of experiments in each factorial design needed to complete the optimization (Nakai and Arteaga, 1989; Berridge, 1985).

Simplex EVOP

In 1962 Spendley et al. introduced the sequential simplex method called simplex EVOP which overcame major limitations of the factorial EVOP method. This fixed-size sequential simplex consisted simply of reflection rules (Massert et al., 1988).

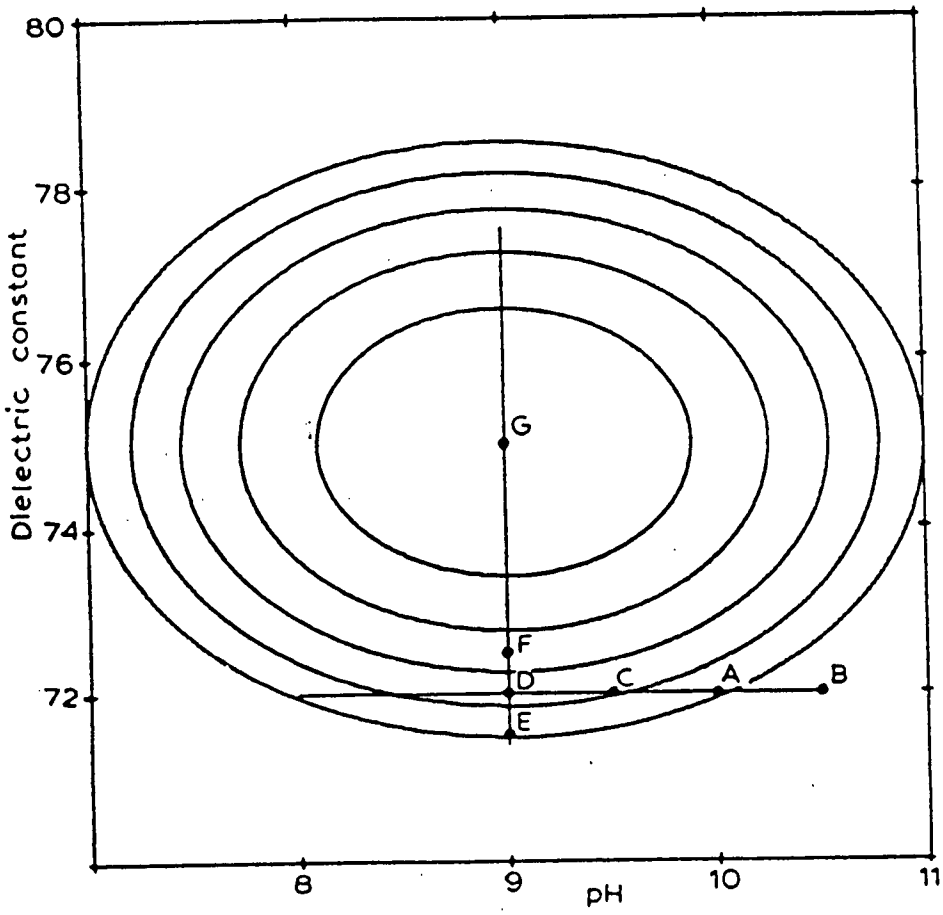

Fig. 1. Single-factor-at-a-time strategy on a well behaved response surface (Massert et al., 1988).

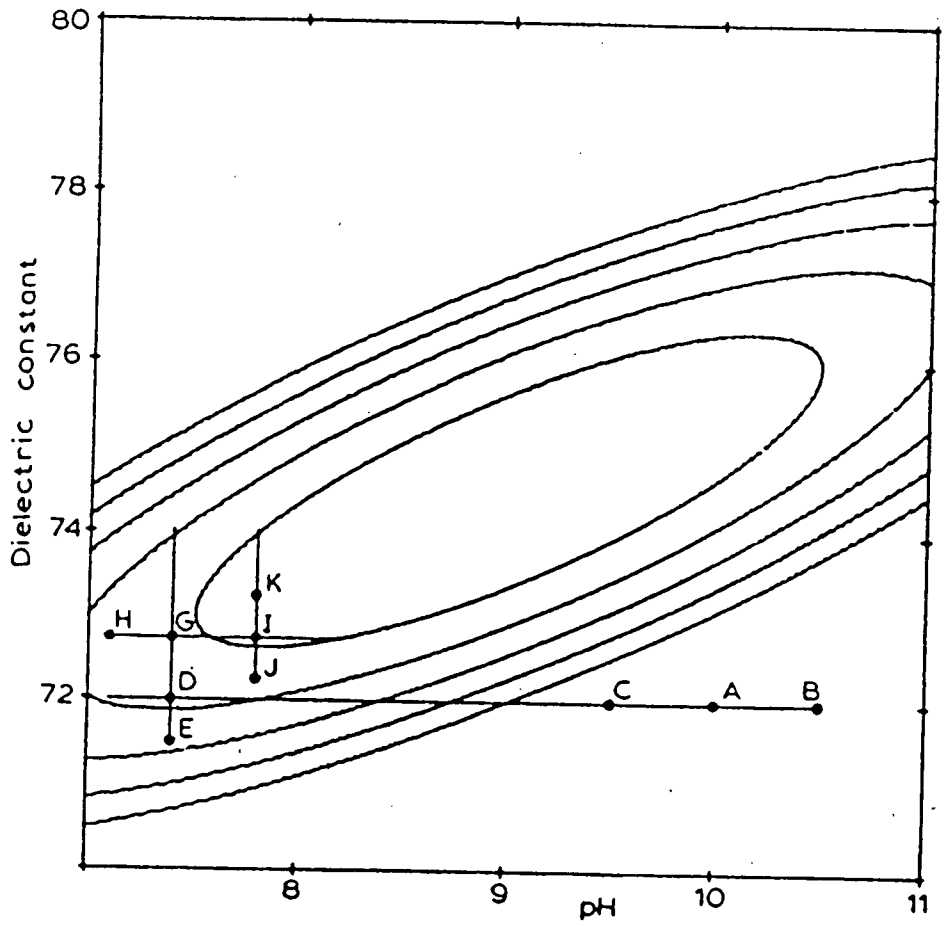

Fig. 2. Single-factor-at-a-time strategy on a response surface exhibitaing a diagonal ridge (Massert et al., 1988).

Nedler and Mead (1965) improved the original simplex by giving the simplex the ability to accelerate in favorable directions and to decelerate in unfavorable directions (Morgan and Deming, 1974).

Simplex Method

A simplex is a geometric figure that has n+1 vertices where n is the number of factors. For two factor optimization, the simplex will be a triangle and for a four factor case, the simplex will be a tetrahedron. Once the factor ranges are determined, the simplex is established by carrying out n+1 observations. Each move of the simplex in the search for the optimum requires one observation of the response (Jurs, 1986). Because the search is without perspective of the response surface, it has been called a "search in the dark" (Aishima and Nakai, 1986).

The movement of the simplex is determined by a set of rules for reflection (move away from the worst response), contraction (if a step is made in a wrong direction) and expansion (if a move is made in a desirable direction). These rules have been illustrated with simple examples by numerous authors (Morgan and Deming, 1974; Berridge, 1985; Massert et al., 1988 and Jurs, 1986).

Improvements in the Method

Modifications of the original simplex have been made by

several researchers (Routh et al., 1977; Ryan et al., 1980; Nakai, 1982; Nakai et al., 1984 and Nakai and Kaneko, 1985). Improved optimization schemes have been developed for two reasons: (1) because the simplex optimization is an iterative search, the speed of the search is high at the beginning but slow near the end (2) the possibility of the simplex optimization finding the local optimum rather than the global one. Nakai et al., (1984) introduced mapping and simulataneous shift in order to improve the efficiency of the optimization with a new mapping super-simplex optimization program. Response values are plotted against each factor and the data points are grouped. From the maps of all the factors, target values are predicted. If the direction of search is evident, simultaneous shift is executed. A shift in all factor levels from the best response vertice is made toward the target value. This procedure significantly expedited the optimization at the later stages (Nakai and Kaneko, 1985).

Nakai (1982) advocated the use of simplex optimization for application to food product and process development. Optimization methods including fractional factorial, one-at-a-time search, pattern search method, response surface and simplex optimization methods of Morgan and Deming (1974), Routh et al., (1977), Ryan et al., (1980) and Nakai (1982) were compared for efficiency using two mathematical models. Many food applications of simplex optimization and other optimization techniques can be found in a current publication

by Nakai and Arteaga (1989).

MATERIALS AND METHODS

A. SAMPLES

Orange Juice

Fresh orange juice made by an in-store juicing machine was purchased from a local supermarket and stored at 8°C. Samples were analyzed within three days of the purchase.

Grape Juice Concentrates

Seven different grape juice concentrates received from Sun-Rype Productions Ltd. (Kelowna, B.C.) and stored frozen at 0°C.

Apple Juices

Eight varietal apple juices from apples grown and processed at the Summerland Research Station were obtained from Agriculture Canada and stored at 0°C.

Wines

All wine samples were obtained from Brights Wines (Oliver, B.C.). Wine samples were obtained directly from the winery's large stainless steel holding vats by discarding the first 400 to 600 mL of wine and filling sterile dark green glass bottles until there was little headspace. Bottles were then screwcapped and sealed with parafilm before storing at 5°C.

Two commercial white table wines of Brights Wines were optimized for blending using headspace gas chromatography and the simplex optimization program. Trial 1 involved blending eigth possible wines to formulate the housebrand wine called Leibesheim. Four of these wines were varietal (Chenin blanc, vats 7, 14, 20 and 25) and the rest were white stock wines from vats 61, 70, 69 and 12. For the second blending trial, to formulate Cuvee White, five varietal wines (Verdelet, vats 74, 47, 51, 71,and 58), two white stock wines (vats 70 and 12), and one premium white stock wine (vat 22) were available for the blending problem.

B. SAMPLE PREPARATION

Samples of 15 mL size were pipetted into 20 mL glass vials, capped with butyl rubber teflon faced septa (Hewlett Packard) and crimped with aluminum caps with a pressure release safety feature. The vial containing the sample was then placed in the heated carousel for the specified time. Wine samples analyzed at 6°C were refrigerated for more than 4 hrs. prior to sampling.

All glassware used in this work was soaked in detergent (Extran) overnight or longer, rinsed five times with tap water and distilled water, and oven dried.

C. INSTRUMENTAL ANALYSIS

Wine and Apple Juice

i. Gas Chromatography

Chromatography was performed on a Varian Vista 6000 gas chromatograph equipped with a flame ionization detector and a Series 651 Data System. The detector settings were range 12 attenuation 1, and temperature 325 °C. The injection port was operated at 200°C. Chromatograms were plotted on a Hewlett Packard Thinkjet Plotter at a chart speed of 1 cm/min.

All gases connected to the gas chromatograph were pre-purified grade (Linde, Vancouver) and operated at the following conditions: helium (carrier) 3 mL/min., hydrogen 36 mL/min., nitrogen (make-up) 18 mL/min., and air 300 mL/min. Linear gas velocities were measured with a bubble flow meter at 40°C oven temperature. Moisture traps (Chemical Research Service Inc.) were installed in all gas lines between the gas cylinder and the chromatograph. An additional hydrocarbon trap (Chromatographic Specialties) was installed for the carrier gas.

A one ramp oven temperature programming sequence was used: an initial temperature of -20°C was held for 2 min. and then increased to 300°C at a rate of 10°C/min. and held at 300°C for 10 min. The final high temperature programming was a cleaning step between runs that eliminated any high boiling components remaining in the column.

ii. Headspace Sampling

Aroma constituents above the samples contained in glass vials were withdrawn and injected into the gas chromatograph by a heated transfer line that connected the headspace sampler (Dani HSS 3950 Sampling Unit and Dani HSS 3950 Programmer) to the gas chromatograph.

Pressurization and vent times were set at 3 sec. and the injection time for 44 sec. The carousel bath temperature was 37°C while the manifold temperature was set to maximum at 150°C. The pressures for the gases were: carrier 1.4 bar, air 3.4 bar, and auxiallary air 0.6 bar.

Orange and Grape Juice Concentrates

i. Gas Chromatography

A Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector and a HP 3396A integrator was used. The detector temperature, range and attenuation were set at 250 °C, 0 and 0, respectively. The injection port was operated at 225°C. For orange juice samples, a three step column temperature programming sequence was as follows: initial column temperature (-20°C) was held for 2 min. and then advanced at 20°C/min. to 40°C for 3 min. holding time. A second temperature program was initialized at 10°C/min. to 90°C for 4 min. holding time and followed by a third programming sequence at 4°C/min. to a final temperature of 170°C for a final holding time of 4 min. The same

temperature programming procedure was used for grape juice concentrates except that the initial column was lowered to -40°C.

Each gas line was equipped with oxygen traps while the carrier gas line was fitted with an additional moisture trap. Gas flows were: helium (carrier) 2 mL/min., helium (make-up) 30 mL/min., hydrogen 30 mL/min. and air 400 mL/min.

ii. Headspace Sampling

Two 1 mL headspace samples from two different vials containing the same sample, were injected into the gas chromatograph by a multiple headspace injection technique as described by Wylie (1986). This was achieved by programming the Hewlett Packard 19395A headspace sampler.

D. INTERNAL STANDARD

Several alcohols were examined as possible standards including 3-pentanol, 2-methyl-1-propanol, 2-methyl-2-butanol and 2-methyl-2-propanol. 3-pentanol has been identified in grapes (Stevens et al., 1969) but not in wines. Cordner et al. (1978) used 3-pentanol as an internal standard for purge and trap analysis of wine. Since this component was not available in high purity and it was not separated well from other peaks in a wine chromatogram, it could not be used as an internal standard. Both 2-methyl-1-propanol and 2-methyl-1-

butanol were available in chromatography grade but were rejected as possible standards because they have been identified by purge and trap and static headspace analysis of wines (Bertuccioli and Viani, 1976; Noble, 1981; Cordner et al., 1978; Craig 1988).

The best choice was 2-methyl-2-propanol as it was available in high purity (Polyscience Corp.), it was resolved from other peaks in the chromatogram and no headspace method (static or purge and trap) had reported its presence in wines. Stevens et al. (1969) identified this substance in Grenache rose wine in trace amounts by extracting the wine with Freon 11.

One uL of 2-methyl-2-propanol was added to 15 mL of wine in a 50 mL test tube and throughly mixed by vortexing. The sample was then immediately transferred to a glass vial which was then capped and crimped.

E. OPTIMIZATION

The main objective of this work was to determine what is the best blending ratio of the varietal wines and white stock wines to match the target wine. In blending optimization the premise is that only the volatile components contribute to the aroma of the product, therefore, gas chromatographic data together with simplex optimzation could be used for finding the best blending ratios (Nakai and Arteaga, 1989). The first step was to analyze the wine samples to be used for blending

by headspace gas chromatography to determine how similar the flavor profiles of these wines were to the target wine. This was accomplished by calculating a pattern similarity coeffient shown below:

$$\text{(Equation 1)} \qquad S(AB) = \Sigma X_i X_i' / \sqrt{\Sigma X_i^2 X_i'^2} \qquad 0<S(AB)<1.0$$

This equation represents the similarity of two chromatograms, $A = (X_1, X_2 \ldots X_n)$ and $B = (X_1', X_2' \ldots X_n')$ where X_i and X_i' are areas of peaks i in the chromatograms of samples A and B, respectively. As in regression analysis, the pattern similarity coefficient varys from 0 to 1. If two chromatograms have identical profiles, then the similarity coefficient would be calculated as 1 while two completely dissimlar profiles would have a coefficient of 0 (Nakai and Arteaga, 1989).

Peak selection of the pattern similarity coefficient was based on two rules. The first criterion was that the peak in question had to be present in the target wine as well as in at least one of the blending wines. The second important condition was that the standard deviation of a peak area had to be high in order for it to be selected. If the peak areas of the target wine and the blending wines are very close, then this data is not of value in calculation of the similariy coeffient because the idea is to detect differences in the patterns.

The pattern similarity coefficient formula is a

subprogram subroutine of the simplex program. When the data of the reference and blending wines is entered into the simplex program, it searches for the optimal blending ratios that give the highest similarity coefficient between the gas chromatographic pattern of the blend and the target wine. This optimization procedure is called an automated sequential simplex technique (Nakai and Arteaga, 1989).

F. TITRATABLE ACIDITY AND TOTAL SOLUBLE SOLIDS

To decide if corrections of acidity and sweetness were necessary for the blends of trial one and trial 2, total soluble solids and titratable acidity were determined at the winery using their procedures.

Titratable Acidity

A 5 mL sample of wine was pipetted into a 250 mL beaker and diluted with 100 mL of distilled water. The diluted sample was stirred while being titrated with 0.1 N NaOH to an end point of pH 8.2. Titratable acidity was calculated as mg tartaric acids per 100 mL wine.

Total Soluble Solids

Samples of 200 mL were adjusted for temperature to 20°C. Total soluble solids were measured as °Balling with a hydrometer.

G. SENSORY EVALUATION

To determine if there were detectable differences between the blends and the target wine, two sets of triangle tests were carried out, one for the subjective blend and the other for the objective blend. For trial 1, the panel consisted of 12 technical staff form the Agriculture Research Station at Summerland. Three of the panelists were experienced judges as they were previously trained in evaluation of wines and tested wines regularly. Fifteen members evaluated the wines blends of the second trial. Twelve were from the Research Station and the other three were wine experts form the winery. In total, seven judges were experienced in tasting wines for this trial.

Samples of 40 mL size were presented at or near room temperature in wine glasses under red light to eliminate any possible bias due to color.

RESULTS AND DISCUSSION

A. Method Development

Headspace gas chromatography (HSGC) was selected as the method of volatile analysis mainly because the procedure of Aishima et al. (1987) was not suited for quality control purposes. In this method volatiles from strawberry essences and concentrates were isolated using a modified Likens Nickerson apparatus to carry out simultaneous distillation and extraction with methylene chloride for two hours. The resulting extracts were concentrated with a Kuderna Danish concentrator and further enriched under a stream of nitrogen. Separation of the total volatiles by GC required 90 min. to complete. It is therefore apparent that this sample preparation procedure is not only complicated but far too time consuming, making it impractical for routine application in the industry. In contrast, static headspace sampling permits direct analysis of the sample vapor without prior isolation and concentration treatments (Jennings and Rapp, 1983).

The first goal of the current study was to develop a rapid and simple method of aroma extraction using an automated headspace sampling system. A number of different samples including orange juice, grape juice concentrate, apple juice and wine were analyzed because a reliable source of samples from the industry was not available until contact with the winery (Brights Wines, Oliver, B.C.) was made.

Preliminary Work

An examination of applications of equilibrium headspace methods for food in the literature revealed that the method lacked sensitivity (Heath and Reineccius, 1986; McNally and Grob 1985; Issenberg and Hornstein, 1970; Reineccius and Anandaraman, 1984; Shibamoto; 1984; Hachenberg and Schmidt, 1977 and Ioffe and Vitenberg, 1984). Headspace analysis of 15 mL of fresh orange juice confirmed the limitation of this method. The chromatogram of the orange juice in Fig. 3 shows that only about 10 major peaks eluted. In light of this, preliminary work was carried out to improve the lower detection limit of the concentration headspace components. Two general texts on static headspace analysis (Hachenberg and Schmidt, 1977 and Ioffe and Vitenberg, 1984) have addressed this problem. Hachenberg and Schmidt (1977) discuss two methods of increasing sensitivity: raising the incubation temperature and addition of electrolytes for aqueous samples.

Increasing the thermostating temperature improved results. Orange juice samples were analyzed at 40, 50, 60 and 70°C and wine samples were analyzed at 6, 37 and 55°C. As expected, new peaks appeared and the peak areas of components that were detected at lower temperatures increased. The effect of raising temperature while keeping all other parameters constant on total peak area is shown in Fig. 4 and 5. The overall effect is an increase in total peak area with temperature. The result of raising temperature on enhancing sensitivity (E_{HS}) is related by the following equation:

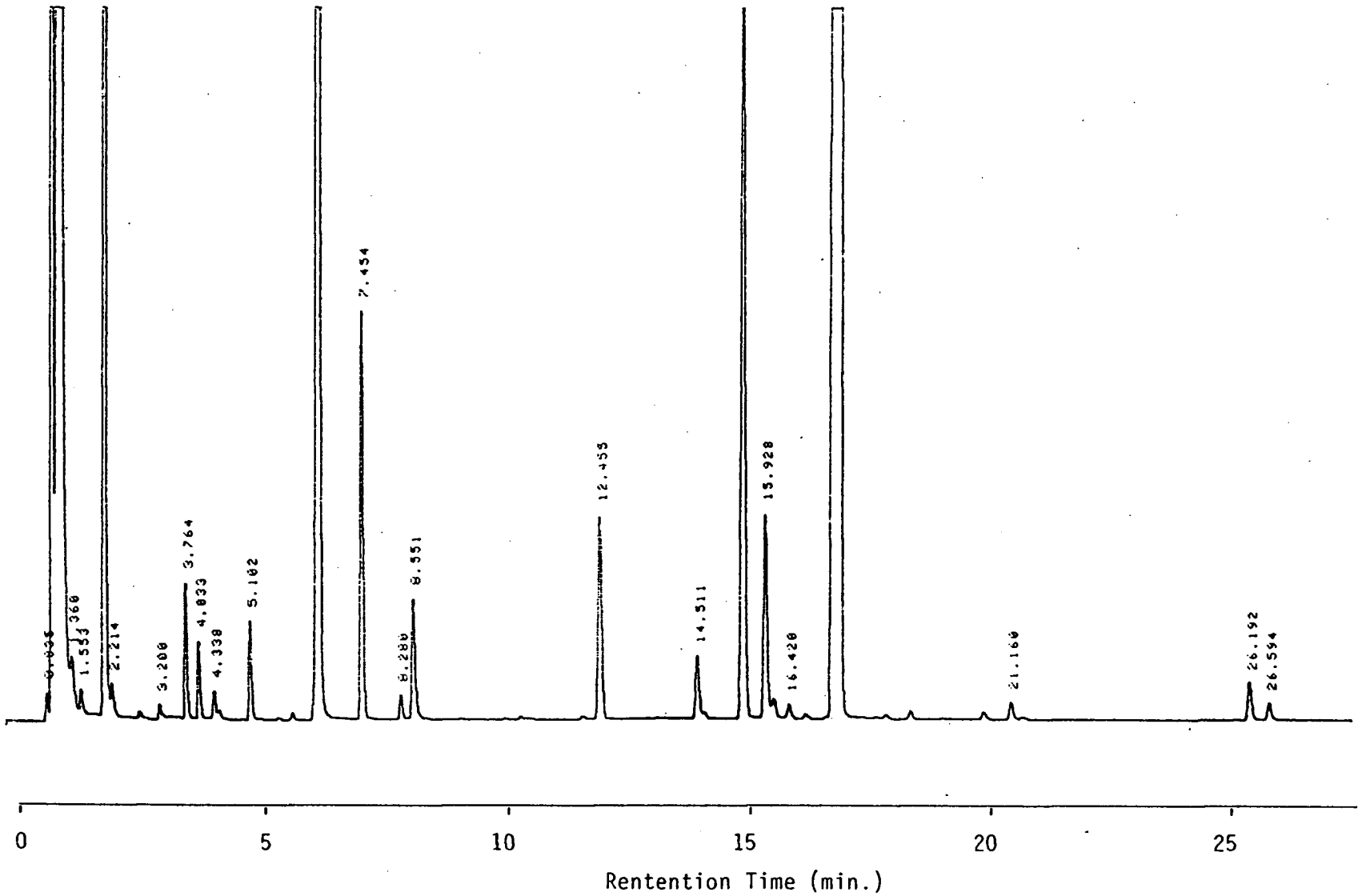

Fig. 3. Chromatogram of headspace volatiles from fresh orange juice analyzed at 70·C.

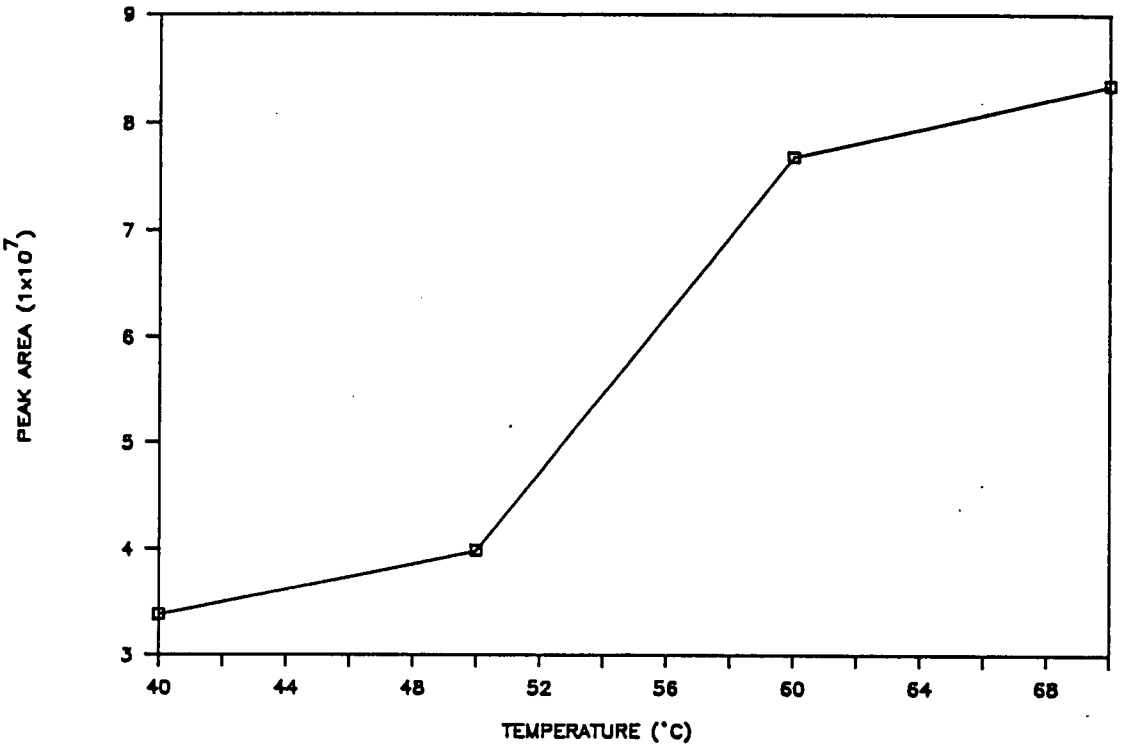

Fig. 4. The effect of temperature of equilibration on peak area for orange juice.

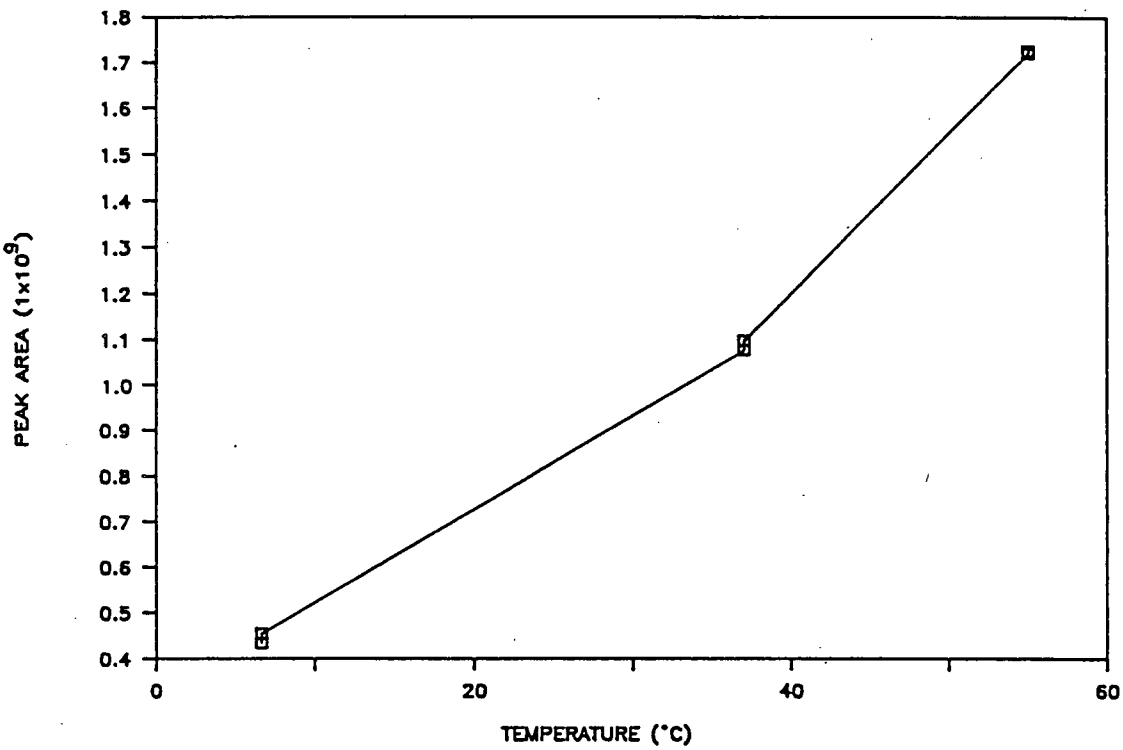

Fig. 5. The effect of temperature of equilibration on peak area for wine.

$$E_{HS} = c_i \rho_{oi} \gamma_i \qquad \text{(Equation 2)}$$

where c is the concentration of component i, ρ_{oi} is the saturated vapor pressure of the pure substance and γ_i is its activity coefficient (Hachenberg and Schmidt, 1977). The saturated vapor pressure of the components in the headspace of the food increases with temperature.

Fig. 4 and 5 show interesting trends not related to sensitivity. The curve for orange juice is sigmoidal; between 40 to 50°C and 60 to 70°C there is a small change in total peak area but there is a sharp increase between 50 and 60°C. Wine, in contrast, showed a very different trend; the relationship between the detector response and equilibrating temperature is almost linear. Some comments about differences in the two figures can be made even though the samples were not analyzed at identical temperatures. It is possible that in orange juice there may be an abundance of volatile flavor components with boiling points in the 50 to 60°C range. Wine, however, may contain aroma substances that are more or less equally spread out in number over the boiling point range tested.

Enhancing sensitivity by increasing temperature has limited application because thermally induced chemical changes such as oxidation, hydrolysis and non-enzymatic browning reactions can occur. Analysis of flavor components at elevated temperatures may be appropriate when the interest

is to isolate cooked volatiles. High temperatures of 60°C or more can induce non-enzymatic Maillard browning reactions (Heath and Reineccius, 1986).

Sensitivity of headspace analysis may also be improved by the addition of salts. Salts cause the value of the activity coefficient to increase in equation 2. The solubility of mainly polar substances is lowered so that they are forced into the headspace (Ioffe and Vitenberg, 1984). Ammonium sulfate and sodium chloride were added to orange juice at saturated levels but the chromatograms showed little improvement.

Cold Trapping

The use of an enrichment technique called cold trapping or cryofocussing appeared to the most successful procedure for upgrading sensitivity of headspace sampling. Fig. 3 and 6 illustrate the concentration effect. The first chromatogram shows headspace analysis of fresh orange juice without any cooling while the second chromatogram resulted from cryofocusing at -20°C. The number of major peaks doubled from 10 to 20 demonstrating the striking increase in sensitivity due to enrichment. In addition, it should be mentioned that the combination of cryofocussing and headspace analysis still represents a true equilibrium analysis (Kolb et al., 1986), unlike enrichment by addition of salts in which not all of the volatiles are affected equally; the degree of enrichment is different for esters, aldehydes and alcohols

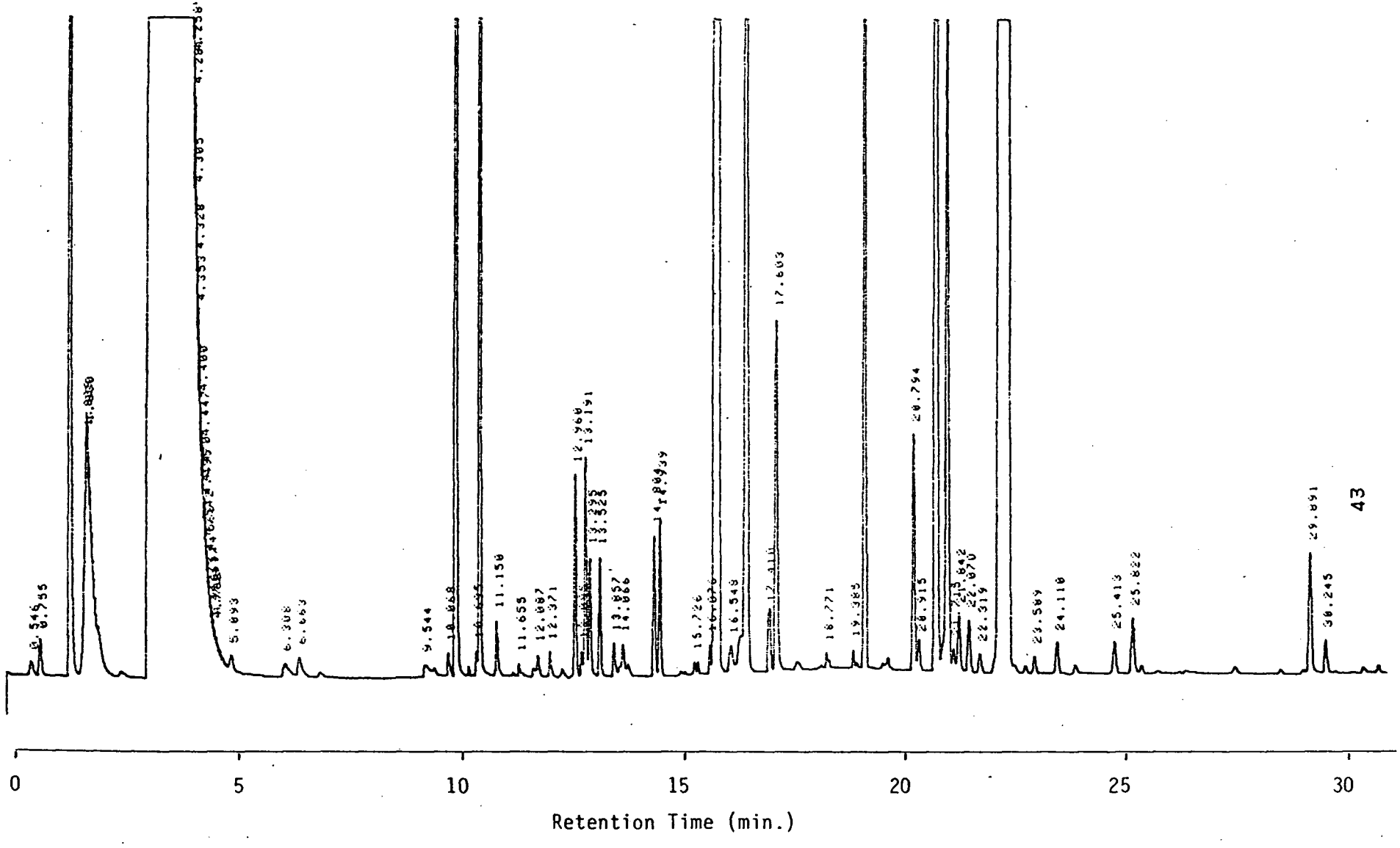

Fig. 6. Chromatogram of headspace volatiles from fresh orange juice analyzed at 70·C using cryofocussing.

(Poll and Flink, 1984).

Cryogenic focussing is a relatively recent development for static headspace analysis in which a band focussing effect of the aroma substances occurs as a result of cooling the first part of a capillary column or by cooling the entire column (Kolb, 1985). A coolant, either liquid carbon dioxide or liquid nitrogen is employed to achieve subambient temperatures. The headspace sample is introduced into a cold column causing the sample components to condense in a narrow band at the head of the column. Volatiles do not actually freeze by cryogenic trapping; their migration rates through the column slow down (Kolb et al., 1986).

For orange juice samples, cold trapping was used in combination with delivering a larger headspace volume which is another technique to improve the detection limit of the headspace method. Injecting larger gaseous samples into the GC is not possible to be carried in the absence of cooling because resolution deteriorates (Jennings and Rapp, 1983). Peaks that should be sharp and well separated appear as wide bands and are poorly resolved. Increasing sample volume without cryofocussing does little or nothing to better sensitivity. The method of Wylie (1986) was applied for orange juice (Fig. 6) and grape juice concentrates (Fig. 7 and 8). Multiple Headspace Injection, MHI, not to be confused with multiple headspace extraction which is used for quantitation when the matrix effect is of concern, allows the headspace sampler to make several rapid injections from each

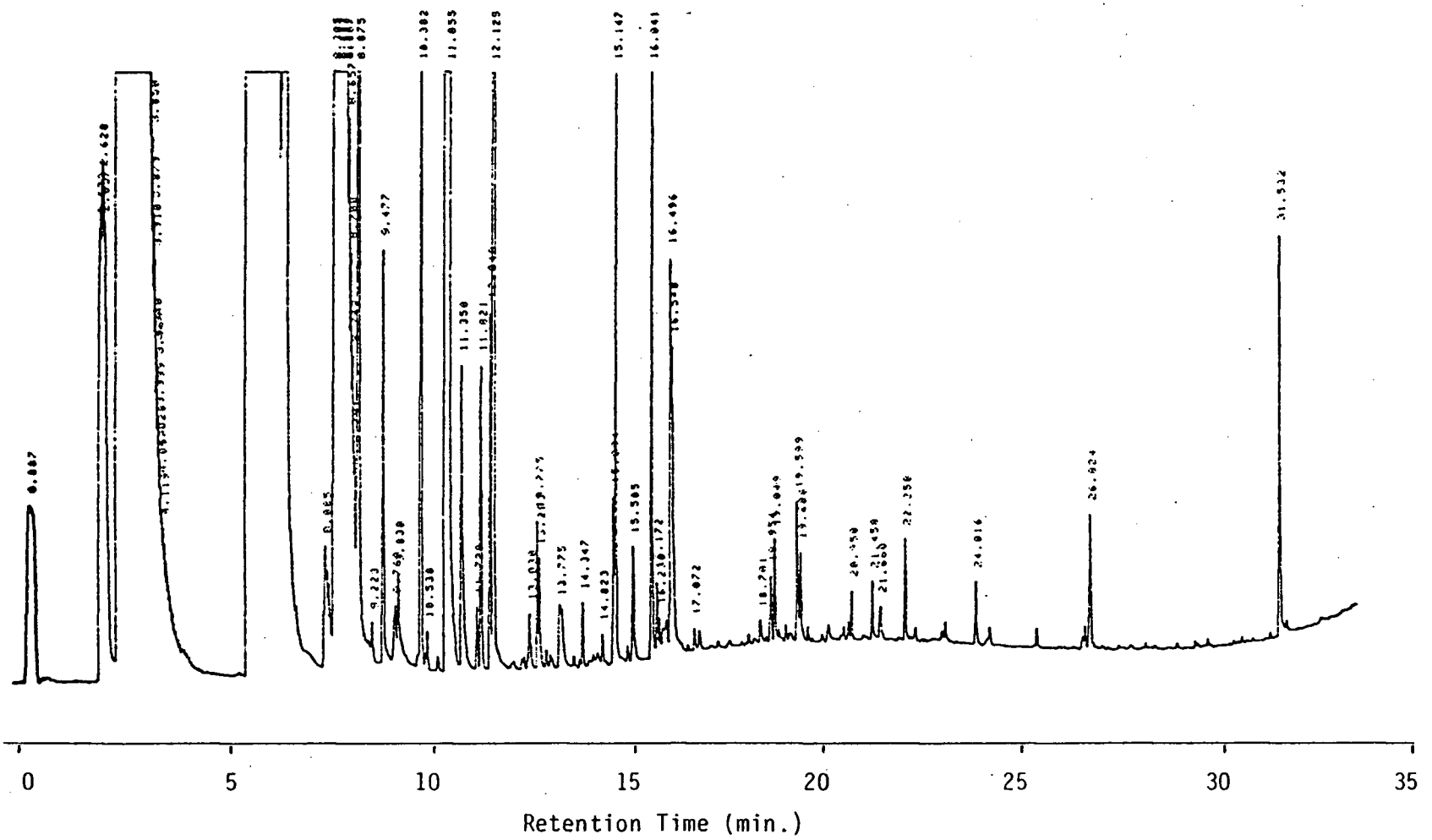

Fig. 7. Headspace volatiles from grape juice concentrate at 55·C (sample 1).

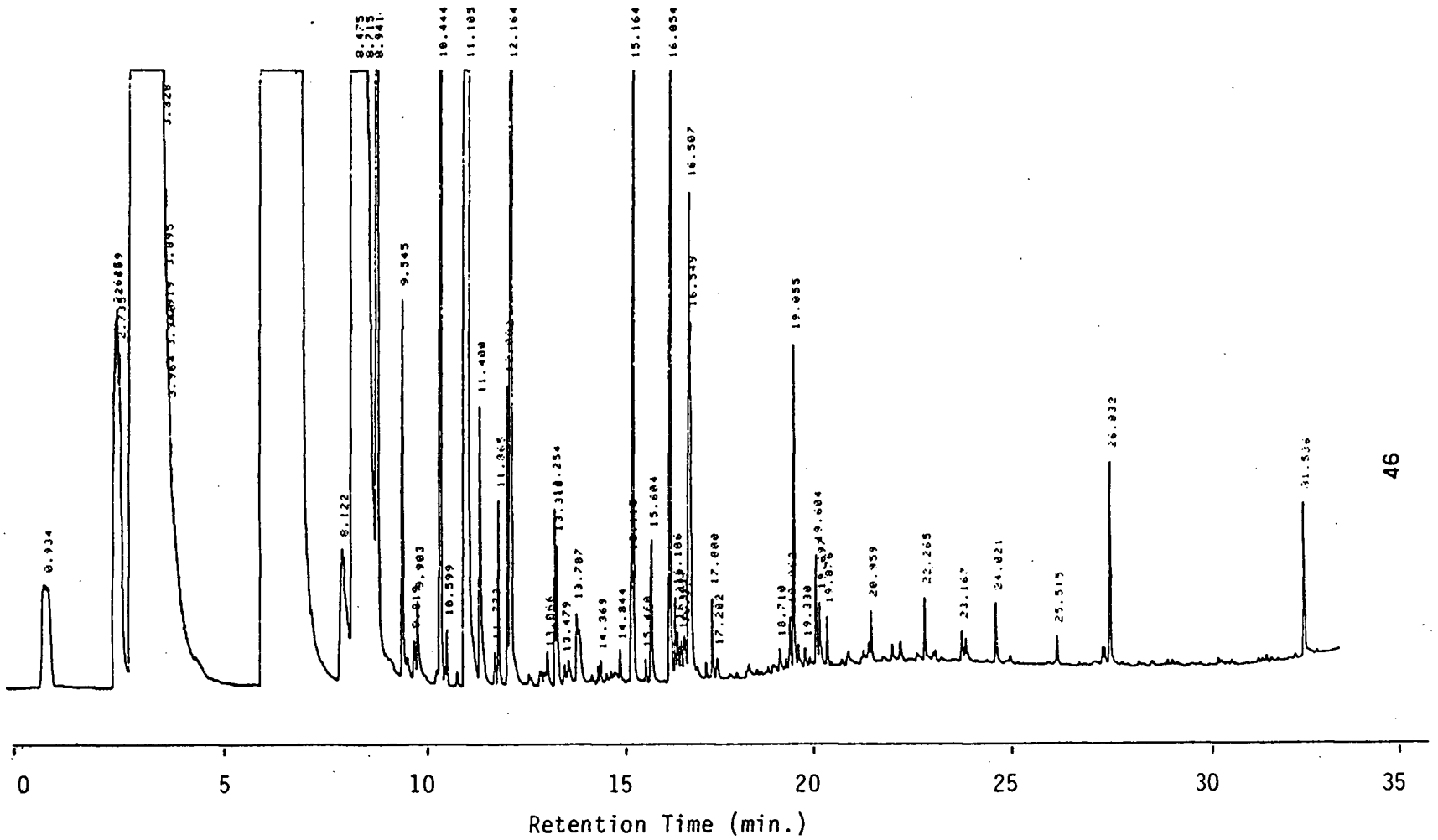

Fig. 8. Headspace volatiles from grape juice concentrate at 55·C (sample 2).

of one or more vials or to make one injection from each of two or more vials by programming the HP 19395A. In the case of orange juice and grape juice concentrates, two fast injections were made from two vials containing the same sample while the column temperature was -20°C.

Apple juice and wine samples were analyzed with only one headspace injection using a different but similar automated sampling system. In this system the headspace samples were introduced directly into the capillary column. The needle of the heated transfer line that connects the headspace sampler to the GC fit snuggly around the capillary column like a sleeve. Sample introduction for the headspace system used for orange juice and grape juice concentrates was of different design as the needle of the heated transfer line was connected to the injection port, not to the column directly. The transfer of the headspace sample from the injection port to the column results in dilution of the sample since the carrier gas is entering the injection port as well (Takeoka and Jennings, 1984). Because no dilution was occurring with the new sampling system, one injection of the headspace sample was sufficient to increase the needed sensitivity; MHI was not required. Chromatograms of apple juice varieties and a wine sample are illustrated in Fig. 9 and 10 and 11.

Although it is generally accepted that purge and trap headspace analysis is far more sensitive in comparison to static headspace methods, the latter can be made equally sensitive if not better provided that the headspace injections

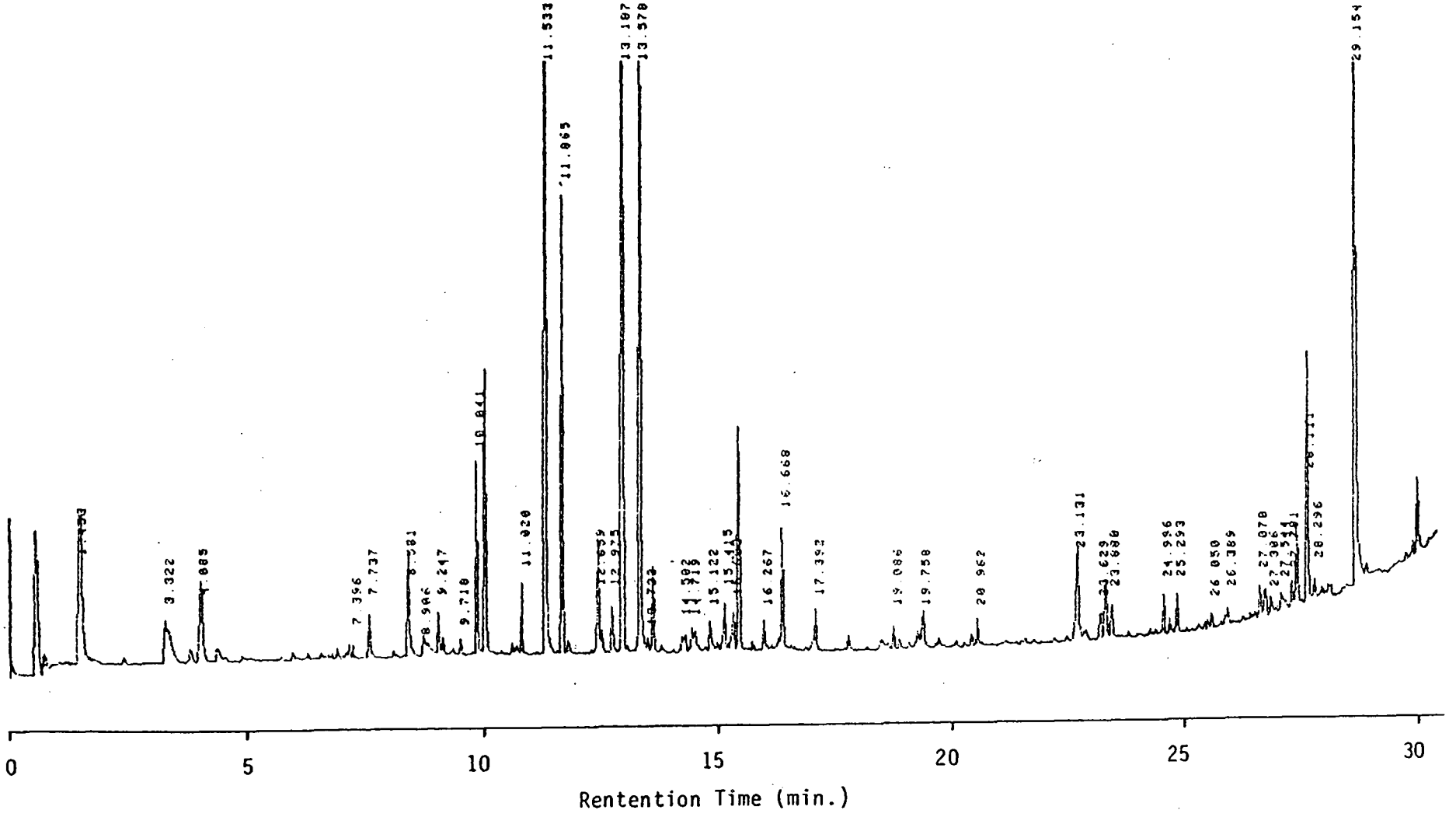

Fig. 9. Analysis of Winesap variety apple juice at 55·C.

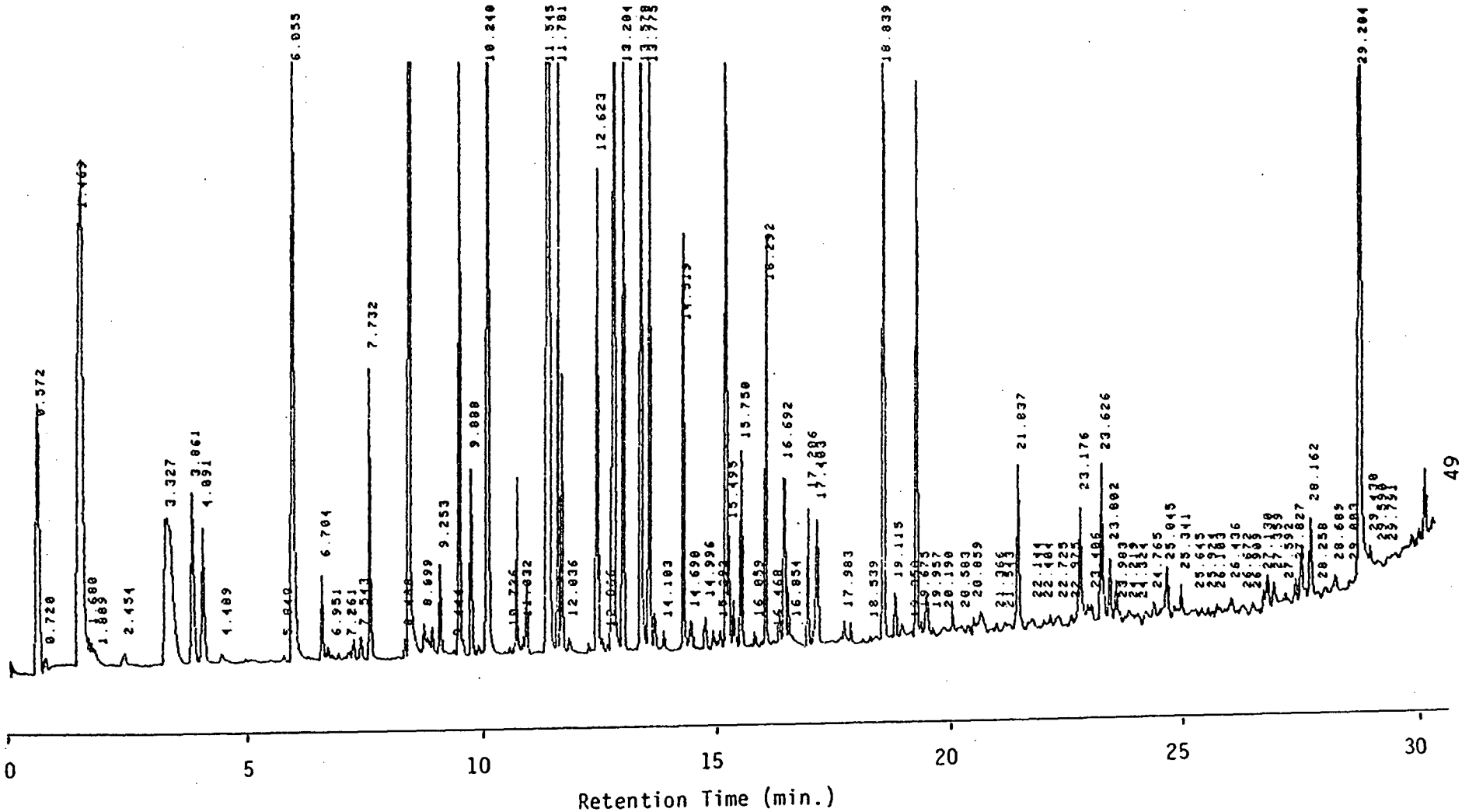

Fig. 10. Analysis of Sinta variety apple juice at 55·C.

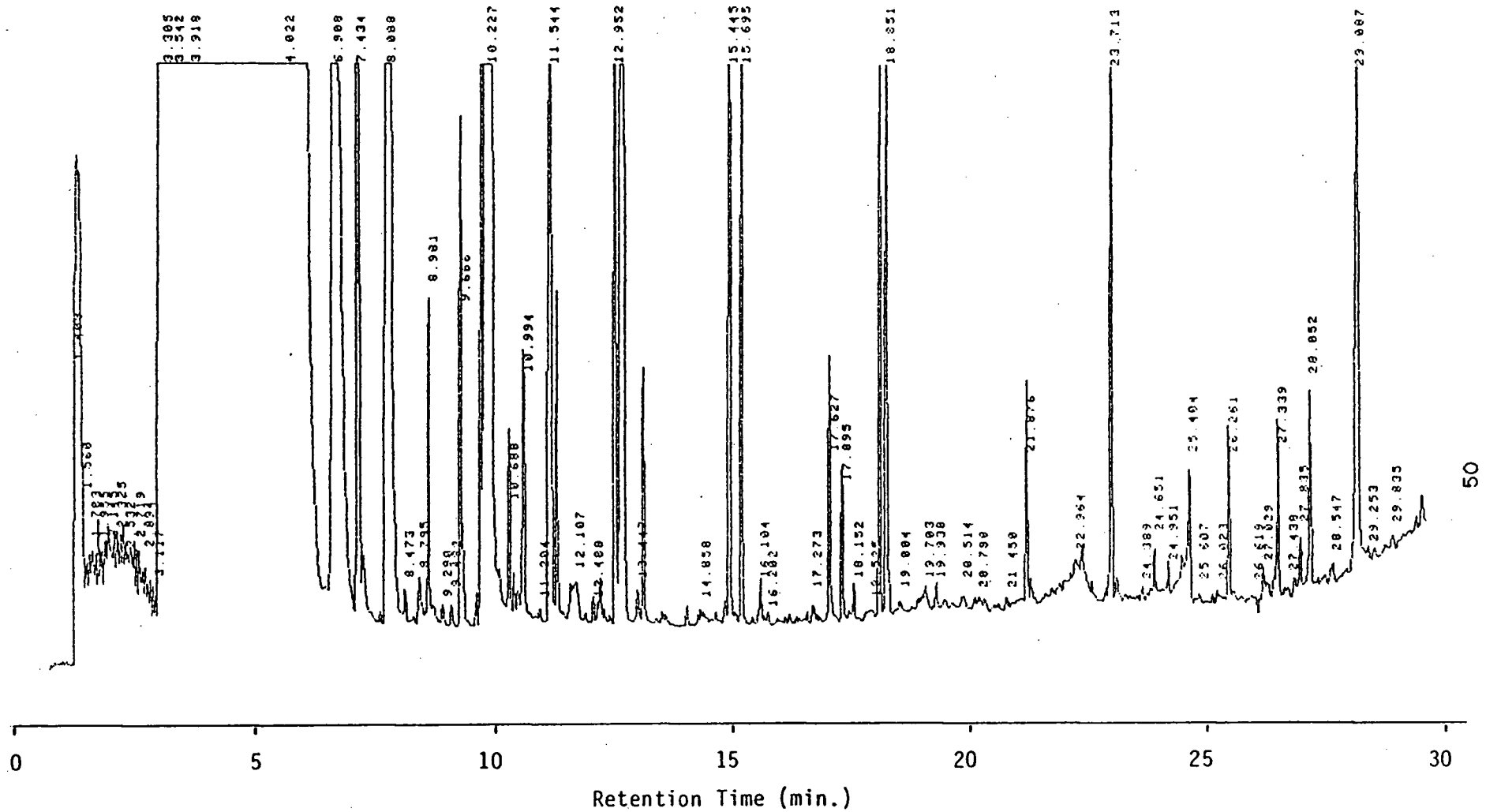

Fig. 11. Headspace analysis of wine (Leibesheim) at 55·C.

are properly executed. According to Takeoka and Jennings (1984), if a headspace sample is injected directly into the interior of a small bore capillary column and it is cryofocussed, then for some samples, results of static headspace can rival and be superior to those obtained by conventional dynamic headspace analysis.

Capillary Column

All samples were analyzed with one column for the entire duration of this study. A non-polar fused silica capillary column crosslinked with 5% phenylmethyl silicone liquid stationary phase was used. Many of the headspace studies of wines, however, have been conducted with columns coated with polyethylene glycol (PEG), also called Carbowax 20M, liquid stationary phase (Bertuccioli and Montedoro, 1974; Gelsomini, 1985; Williams and Strauss, 1977; Simpson, 1979 and Murray, 1977) or related stationary phases such as Carbowax 400 (Bertuccioli and Viani, 1976).

Carbowax 20M columns are popular among researchers working with headspace studies because the retention of low molecular weight polar components is significantly higher (Takeoka and Jennings, 1984). On fused silica, however, this stationary phase has several limitations (Takeoka and Jennings, 1984; Jennings, 1987). PEG phases are susceptible to damage by water. This is of concern for headspace methods because headspace samples usually contain appreciable amounts of water unless some provision has been made to remove it.

Another disadvantage of Carbowax columns is that they posses relatively low high temperature and high low temperature limits. At temperatures of 50 to 60°C the stationary phase solidifies resulting in a drastic loss of resolving power of the column. This drawback would make it impossible to use cold trapping procedures with PEG columns.

Other drawbacks of this columnn are its high affinity for oxygen which causes the columns to deteriorate faster than other types of columns. Even traces of oxygen can have adverse effects at high temperatures. Although new PEG columns (DB-WAX) which are resistant to water and remain liquid at subambient temperatures of down to 0°C are available commercially (Takeoka and Jennings, 1984), they may not be entirely applicable for cryogenic methods which often require temperatures of -100 to -20°C. Craig (1988) investigated headspace volatiles of wine with a DB-WAX column but no cold trapping of volatiles in the column was used.

Careful consideration was given to the choice of the column in this study. A relatively non-polar column was selected essentially because it was necessary to perform cryofocussing to increase the lower detection limit of the headspace method. Other advantages offered by this column were its tolerance for water and its selectivity. Water was present at high amounts in the headspace of the samples analyzed unlike most of the headspace studies on wine, with the exception of Gelsomini (1985), in which some type of trap with low affinity for water was used. In addition, this

column offers increased selectivity towards different classes of solutes compared to other non-polar columns due to the presence of the phenyl group in the stationary phase (Jennings, 1987). A 1 um thick film was chosen to increase the sample capacity of the column.

B. WINE HEADSPACE ANALYSIS

Volatile analysis of alcoholic beverages is more complicated than it is for other food products because of the presence of high concentrations of ethanol. Researchers analyzing such products have, therefore, developed methods which are selective against ethanol. Ethanol-free concentrates of volatiles have been obtained by employing selective solvents such as pentane and Freon 11 or in the case of headspace methods, by the use of polymers such as Tenax GC, Chromosorb 105 or Porapak Q. In this study, however, no effort was made to rid the heaspace sample of ethyl alcohol. Fig. 11 shows the large ethanol peak eluting early in the chromatogram which obliterates a large portion of the chromatogram, masking an unknown number of aroma components of the wine that elute in this area. Because the focus of this study was directed towards developing a method for quality control for the industry, a quick method with minimum sample handling was desired. Developing a headspace procedure that included steps to eliminate ethyl alcohol would have made the technique complicated, tedious, long and basically impractical

for routine use in the industry. Although it is possible that the masked peaks may have been important in calculation of the pattern similarity constant of the wines, it was hoped that the objectives of this work could be attained without this extra information.

Besides ethanol, water is also a dominant component of wine that can cause problems in aroma analysis techniques. Large volumes of headspace volatiles are often preconcentrated in chilled traps, a technique similar in principle to cryofocussing, but the major volatile recovered is water (Jennings et al., 1972). When 1 mL of wine headspace sample is preconcentrated by cold trapping and analyzed by GC, the same situation probably results especially at higher temperatures such as 55°C. Even though water may be the principle component in the headspace sample, the flame ionization detector (FID) is insensitive to water. Aqueous samples can be analyzed by the FID without a large solvent peak obscuring the first part of the chromatogram (Rowland, 1974). Flame ionization detection is particularly suited for headspace analysis not only because of its lack of response to water, but also because of its high sensitivity to organic compounds and its large linear range (Nawar, 1966). Thermal conductivity detectors, in contrast, have universal response but poor sensitivity (Rowland, 1974).

One other aspect of headspace analysis of wine needs to be discussed. Fig. 11 shows that prior to the elution of ethanol, there appears to be some peak distortion occurring.

Kolb et al. (1986) reported this phenomenon in the headspace analysis of cheese when a large volume of headspace gas was introduced into the column. Since peak distortion disappeared when the headspace sample volume was reduced, they attributed the malformed peaks to column overload. Split or malformed peaks have been called the "Christmas tree effect." According to Jennings (1987), this results from the exposure of fused silica column to non-uniform heating from the oven heater. Peak distortion occurs when the front of the chromatograting band is exposed to a higher temperature and the back of the band is decelerated by a lower temperature.

It is unlikely that the splitting peaks are occurring because of non-uniform heating in this study because apple juice samples were analyzed using the same instrument and under almost identical conditions but no such phenomenon was observed. If sample overloading is the cause, it is not clear why the problem was exclusive to wine; all of the other samples analyzed did not exhibit peak distortion. One explanation may be that unlike other samples, wine may contain high concentrations of some extremely volatile low boiling substances, like acetaldehyde, which are overloading the column and causing peak splitting.

Column overloading has also been blamed for this problem by Guntert et al. (1986). Another cause for the poor peak shape of some of the components eluting in the front part of the chromatogram may be related to the type of liquid stationary phase used. Guntert et al. (1986) reported a

similar problem in analysing for low volatility components in wine. They believe that many acidic wine compounds of relatively high concentration that are eluted early are only slightly soluble in the stationary phases such as DB-5 (the column used in this work) and DB-1701 are responsible for this problem.

C. PRECISION AND INTERNAL STANDARD

Precision

Repeatability of the HSGC profiles was determined with an apple juice sample. Thirteen peaks of varying areas were selected from the chromatogram. Table 1 shows the means, standard deviations and coefficents of variation of the 13 peaks for 3 injections. The mean coefficient of variation of the peaks ranged from 1.70 to 9.28% and the average coefficent of variation of all the 13 peaks was 5.26%. Reproducibility of the internal standard with 18 replicates of wine samples was 5.08% as shown in Table 2.

Precision of manual headspace extractions with gas-tight syringes is usually not adequate compared to automated headspace sampling units that use high precision pneumatic sampling (Closta et al., 1983). Rodriquez and Culbertson (1983) used gas tight syringes to quantitate selected compounds in the headspace of orange juice. Relative standard deviations ranged from 10 to 40%. Ettre et al. (1980) tested the repeatability of analyzing an n-alkane mixture with an

Table 1. Repeatablity of headspace method using apple juice samples (n=3).

Peak	Mean	St. Dev.	Cof. Var.
1	42848	2636	6.15
2	6746	626	9.28
3	8635	783	9.07
4	26953	459	1.70
5	276607	9878	3.57
6	18837	1166	6.19
7	9770	655	6.70
8	103416	2356	2.28
9	28583	1691	5.92
10	19251	858	4.46
11	35713	986	2.76
12	207358	12716	6.13
13	19141	786	4.11

Table 2. Repeatability of the internal standard in the wine samples analyzed.

	Average Peak Area*
	7632522
	7552853
	7409673
	7286683
	7434091
	7245762
	6435650
	7057862
	7780734
Mean	7315092
Standard Deviation	371546
Coefficient of Variation (%)	5.08

* average of two replicates

automated headspace sampler and found that the relative standard deviation for the 4 compounds was less than 1.0%. Using the same headspace sampling system, Geiger (1978) examined the headspace composition of beer. Reproducibility of 6 selected components varied from 2.1 to 7.6% with an overall mean coefficient of variation of 4.2%. Results of this study should be compared to the results of Geiger (1978) rather than Ettre et al. (1980) because an alcoholic beverage was tested instead of a high purity of one class (n-alkanes) components. In view of this, the repeatibility of the method is comparable to that of Geiger (1978).

Internal Standard

Even though an automated headspace sampling system was used, an internal standard was added because of possible errors arising from the sample preparation steps, or from adsorption of volatiles by the glassware or other parts of the sampling units. Adsorption of volatiles has been reported on the walls of the glass syringes used for sampling (Buttery et al., 1965) but the problem can be overcome by coating the inside walls of the syringe with Teflon, silane or other inert materials (Franzen and Kinsella, 1975). To determine if any adsorption was occurring on the glass vials or septum, or the transfer line and valves and tubings of the sampling unit, the effect of concentration on the peak area of the internal standard was tested. Fig. 12 shows that the relationship is linear (R = 0.9998) indicating that no adsorption of aroma

components was occurring in the headspace sampling unit or the glassware. This is also supported by the fact that a blank run showed a clean chromatogram. Blank runs between samples also indicated that there was no carry-over occurring.

Before discussing wine blending optimization, it is important to mention why trial 1 and 2 blends were analyzed at different equilibrating conditions. Trial 1 wines were conditioned at 6°C while trial 2 wines were incubated at 37°C for headspace analysis. The chromatogram shown in Fig. 11 is the analysis of a wine sample at 55°C. Although there are many peaks present indicating good sensitivity, there may be adverse reactions taking place at this temperature. In addition, volatile analysis at 55°C does not reflect the headspace composition of wine as it would be when consumed. Since white wine is often served at chilled or refrigeration temperature, 6°C was selected for trial 1. A temperature of 37°C was chosen because when wine is taken into the mouth it is at body temperature.

Representative chromatograms of trial 1 and 2 wines including an example of the varietal wine, white stock and the target are shown in Fig. 13 to 15 and 16 to 18. As expected, sensitivity dropped when wines were analyzed at 37°C and especially at 6°C. A quick examination of the chromatograms reveals that at each temperature the patterns of the varietal, white stock and target wines are quite similar. A closer examination of the chromatographic report, however, indicates that there are significant differences in areas of identical

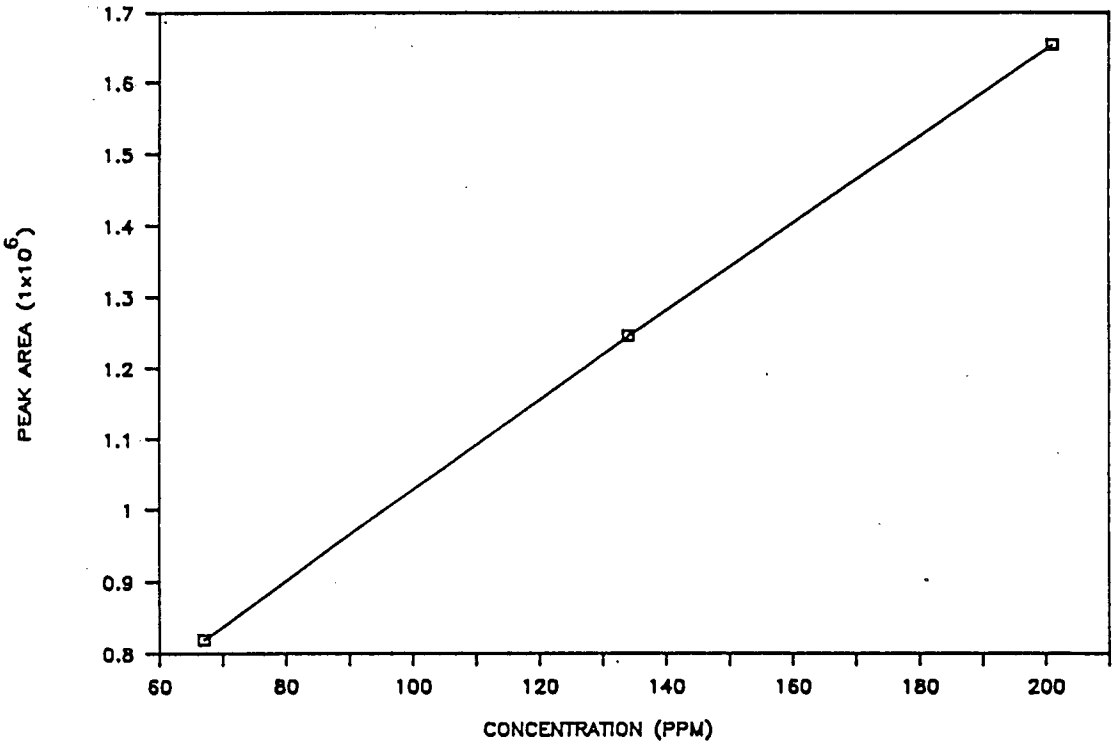

Fig. 12. The Effect of increasing concentration of internal standard on peak area.

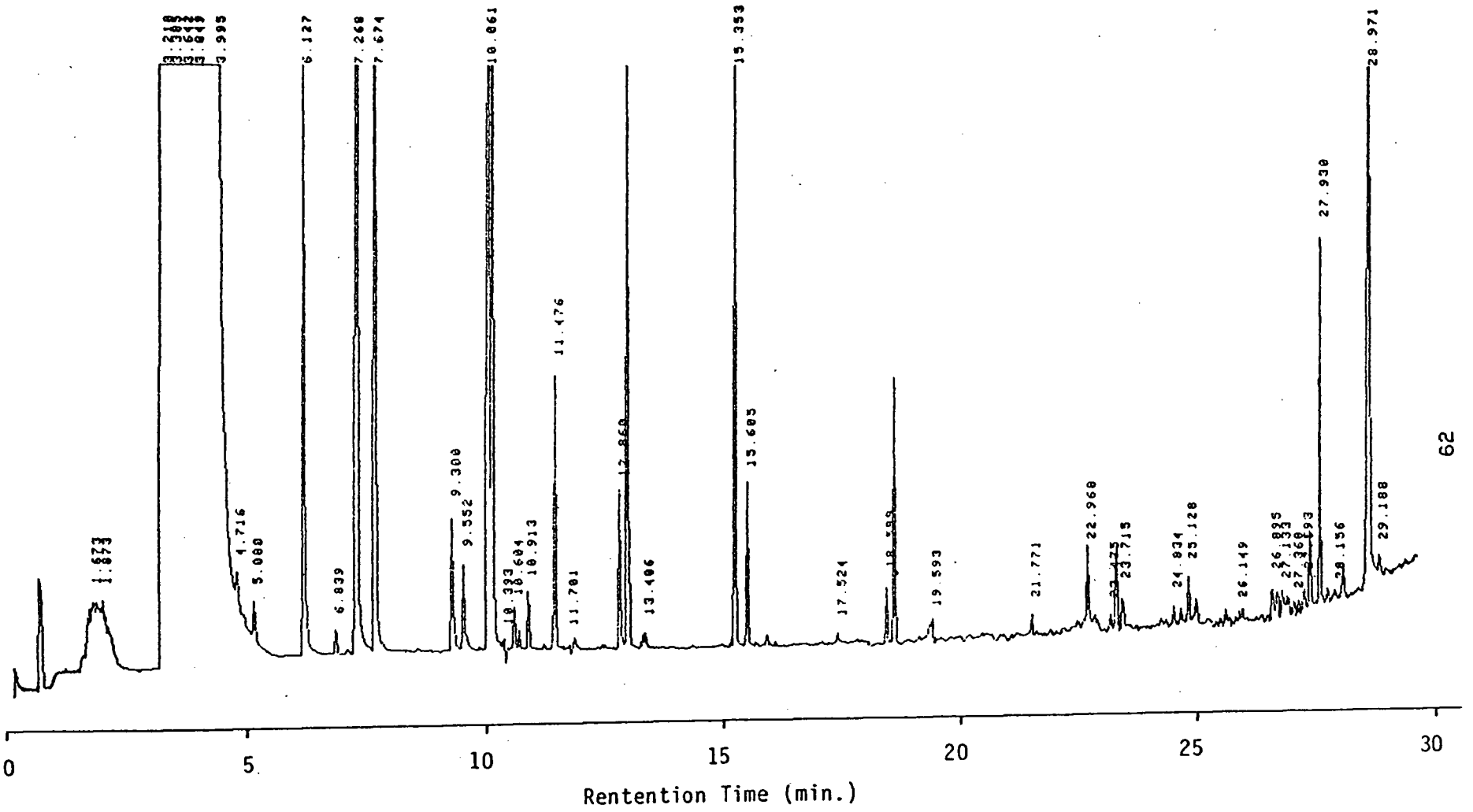

Fig. 13. Representative HSGC profile of a Chenin Blanc varietal wine of trial 1.

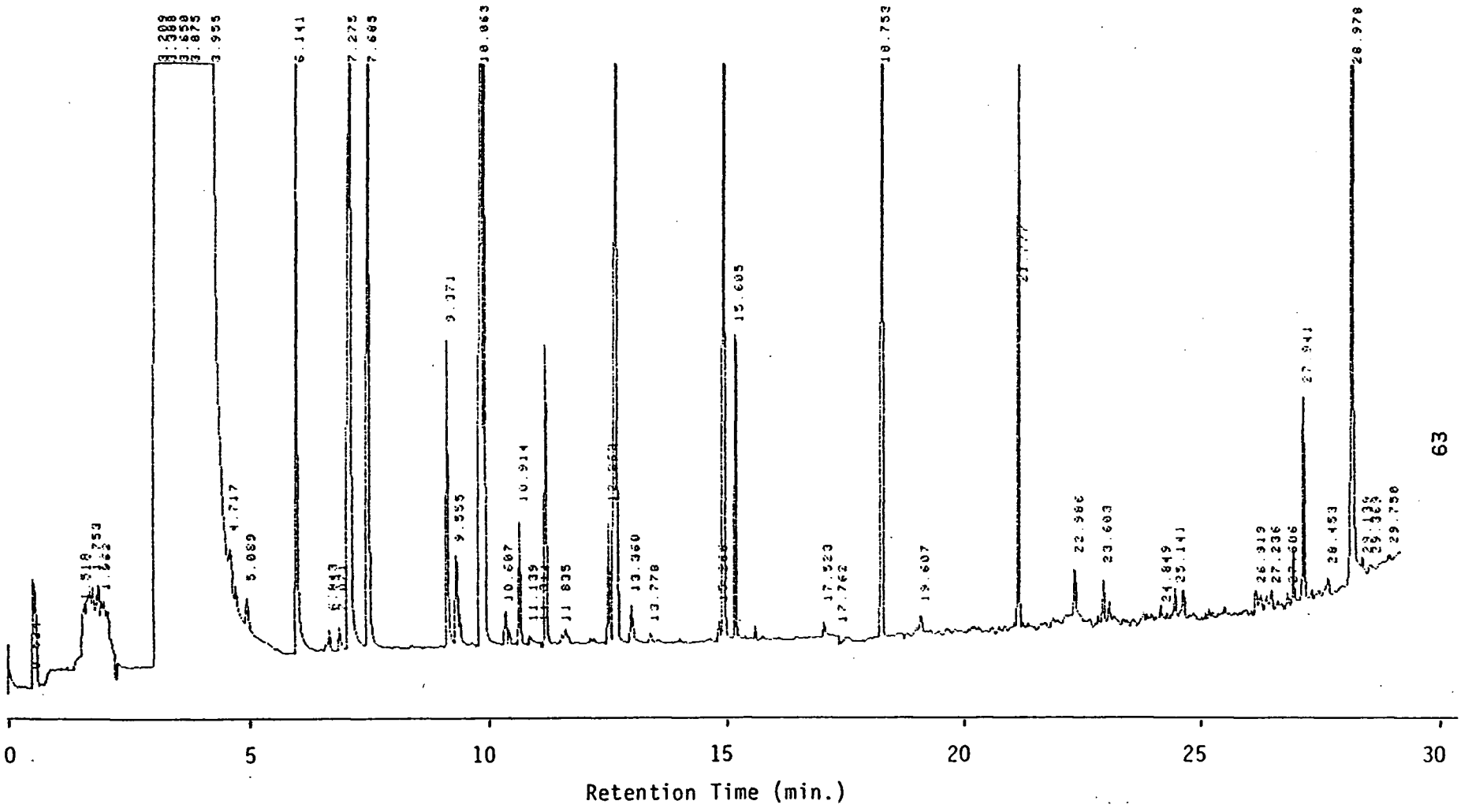

Fig. 14. Representative HSGC profile of a white stock wine of trial 1.

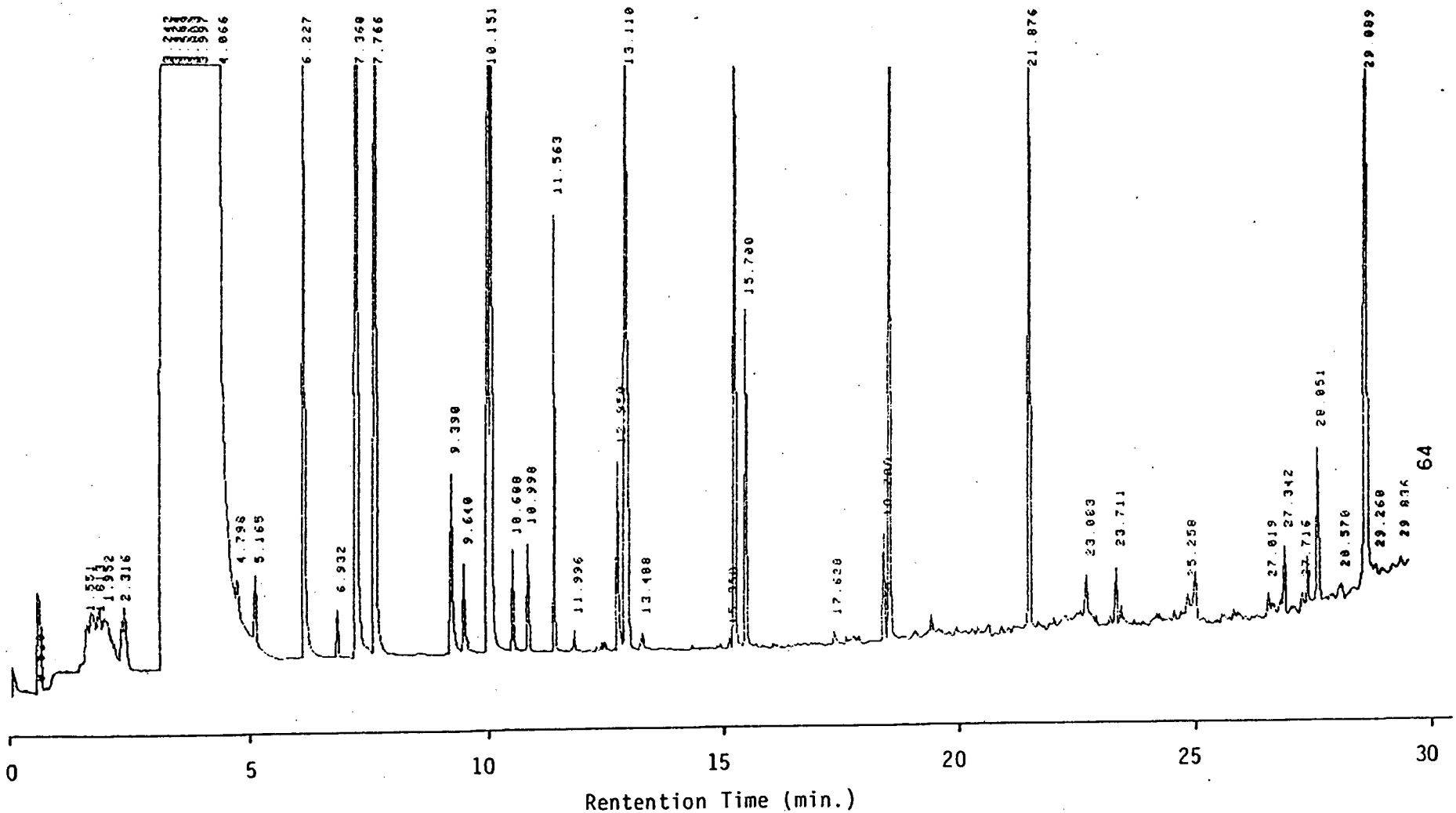

Fig. 15. HSGC profile of the target wine, Leibesheim, for trial 1.

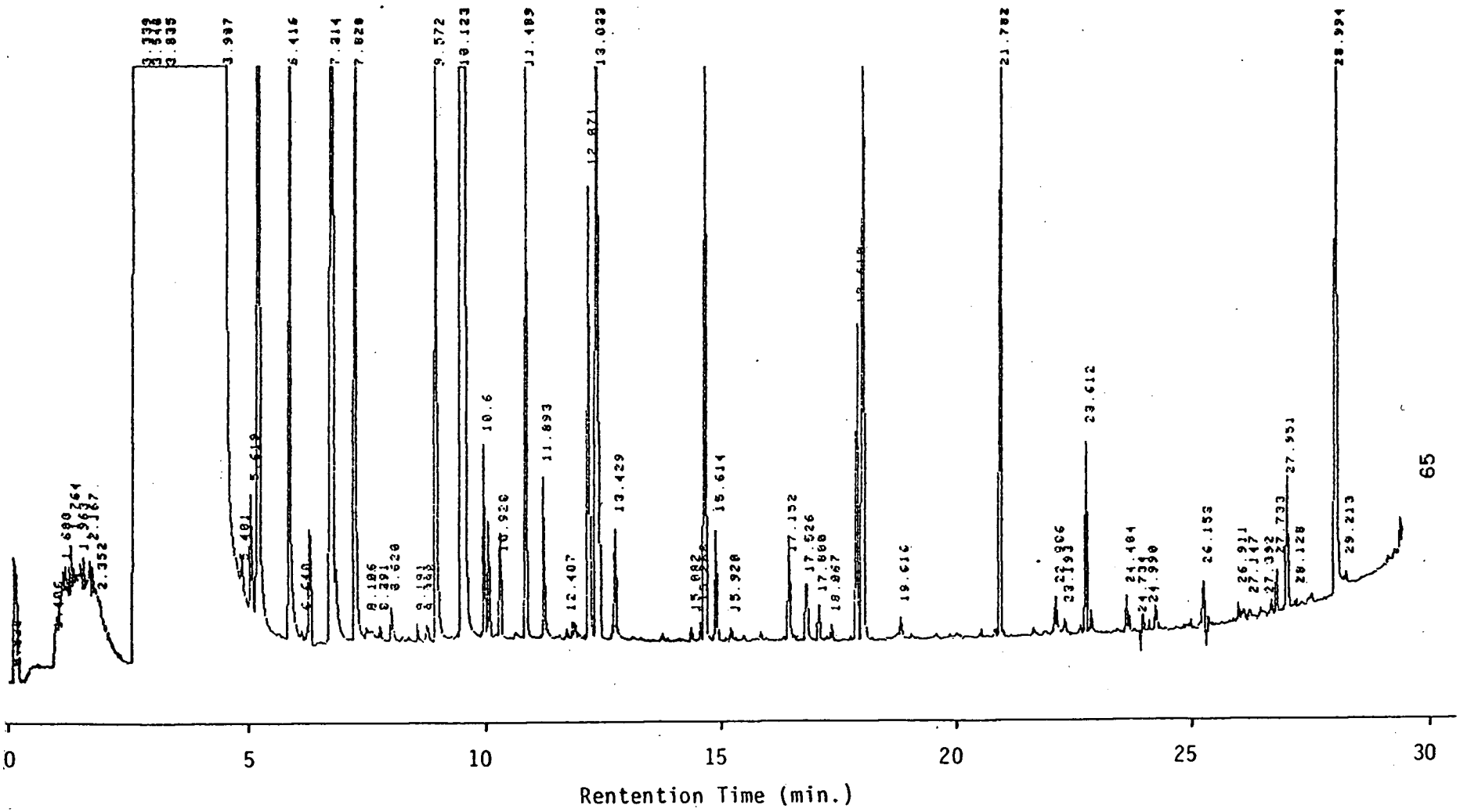

Fig. 16. Representative HSGC profile of a Verdelet varietal wine of trial 2.

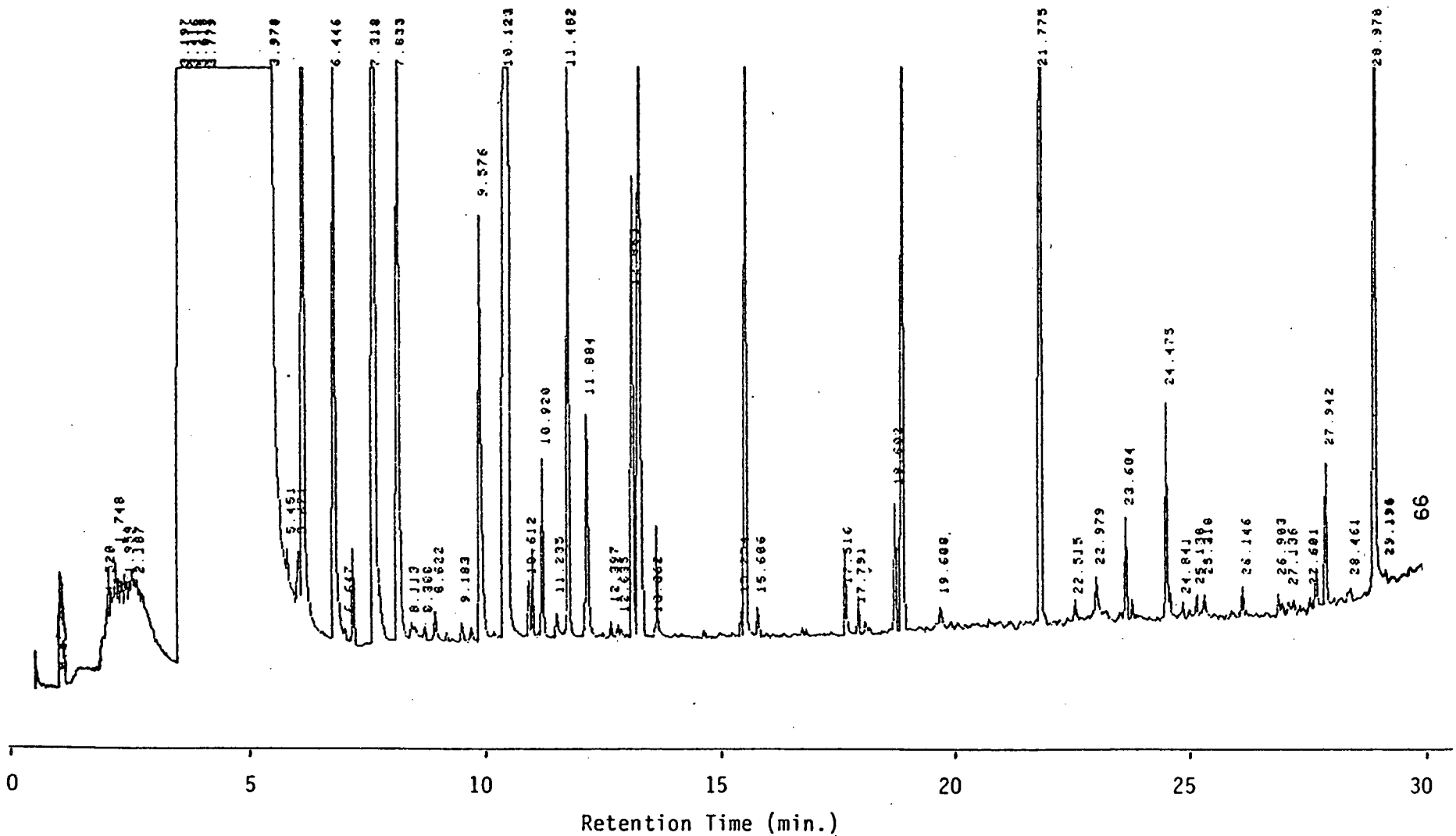

Fig. 17. Representative HSGC profile of a white stock wine of trial 2.

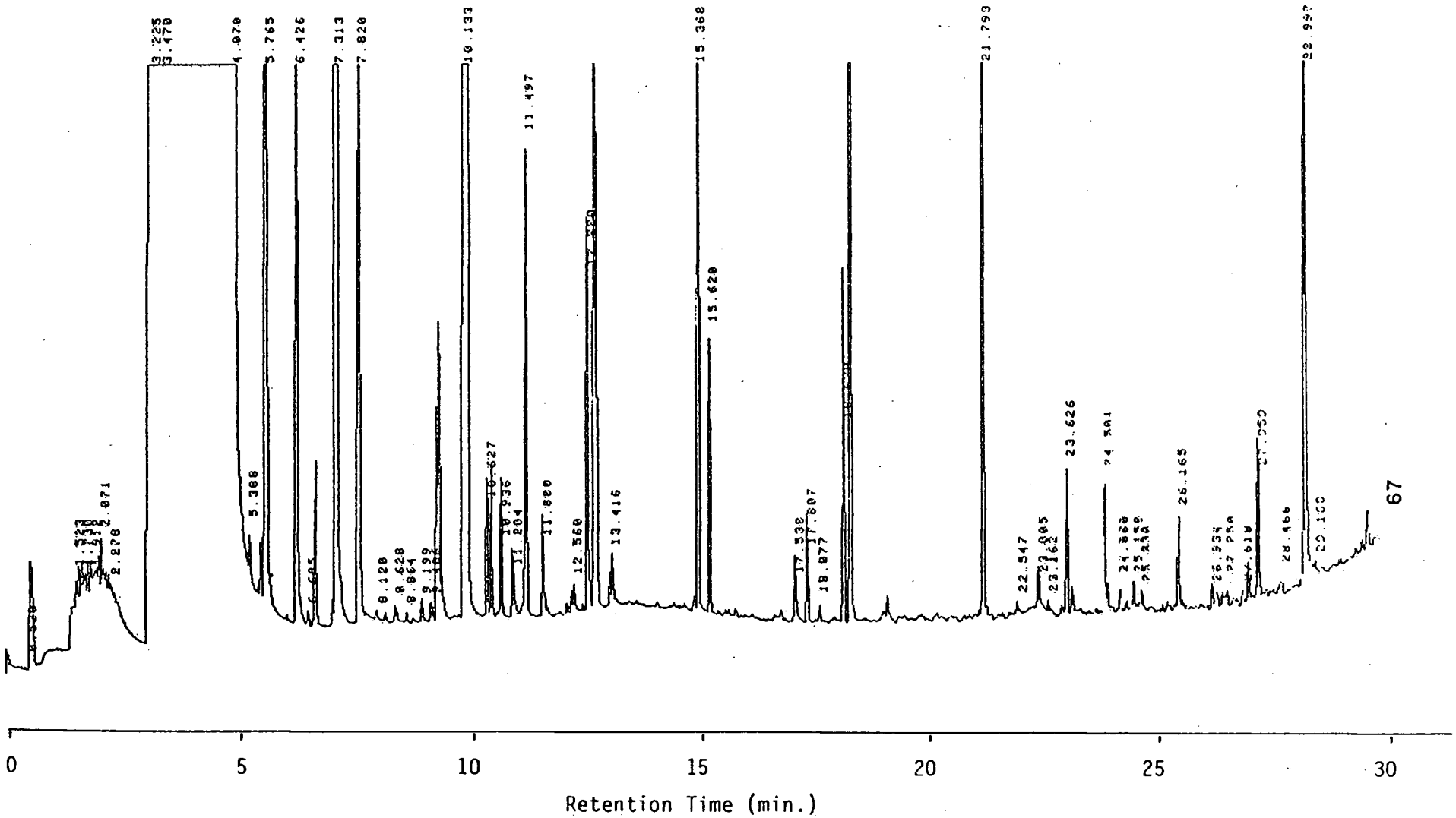

Fig. 18. HSGC profile of the target wine, Cuvee White, for trial 2.

retention time peaks. These results are in agreement with earlier findings. Brander (1974) suggested that essentially the same volatile components are present in all wine varieties and that aroma differences among varieties are due to these components being present in varying ratios. Differences are quantitative rather than qualitative.

Moreover, Nykanen (1986) suggested that the basic flavor components of alcoholic beverages including wines, brandy and whiskey are the same because most of them are formed during fermentation and that differences in taste and smell are due to differences occuring in the quantities of compounds. Also Craig, (1988) reported that the aromagrams of kiwifruit and grape wine appeared quite similar but that significant differences occurred in the quantities of many peaks. Stern et al. (1975), however, stressed the importance of trace quantities of substances to the aroma of wine. These volalites may be present at too low concentration to be detected but may be significant organoleptically.

D. SIMPLEX OPTIMIZATION

The first step of the optimization was to calculate the similarity constants of the varietal and white stock wines to the target wine. Table 3 shows all of the blending and target wines for both trials 1 and 2. Twenty peaks for trial 1 and 36 peaks for trial 2 with distinct variation were selected based on the criterion discussed in the materials and methods

section. The relative standard deviation of these peaks was greater than 20%. Peaks in the early portion of the chromatogram including ethanol were omitted from the selection procedure. Using the GC Data Entry program, the chosen peaks were entered into an IBM PC computer. Considering the large data entry required, any errors made in entry could be corrected with the next program called GC Data Correction. This program also normalized the peak areas using the area of the internal standard. Normalized data was recalled by the Similarity Constant program to calculate the similarity coefficients of the varietal and white stock wines.

Table 4 show the similarity constants of trial 1 and 2 wines. The values for trial 1 are close together and quite high around 0.969 to 0.991 with the exception of 0.817 and close together. In contrast, the similarity constants of trial 2 are more varied, ranging from 0.598 to 0.901.

Before explaining what these results mean, it is important to discuss why there is such a large variation in the similarity constants of trial 1 and 2. Because trial 1 wines were analyzed at a very low temperature, only a small number of very volatile peaks could be analyzed by the headspace method. Since fewer peaks, almost half the number, were available, the similarity constants for most of these wines were very high. Despite this situation, the results still showed some validity. For example, wine sample 7 was a preblended wine therefore, it had one of the highest match with the target. Sample 25 and 14 were white stock wines made

Table 3. Blending and target wines for trials 1 and 2.

Trial	Target	Varietal	White Stock	Premium Stock
1	Leibesheim	(Chenin blanc) 7,14,20,25	61,70,69,12	
2	Cuvee White	(Verdelet) 74,47,51,58,71	70,12	22

Table 4. Pattern similarity constants of trial 1 and 2 wines.

	Sample No.	Similarity Constant
Trial 1	7	0.991
	69	0.976
	14	0.987
	20	0.995
	25	0.817
	61	0.978
	70	0.969
	12	0.985
Trial 2	71	0.901
	74	0.805
	22	0.648
	47	0.856
	51	0.598
	58	0.610
	70	0.738
	12	0.778

from grapes originating from the same vineyard except that for sample 25, the grapes were picked early in the season and for sample 14, they were harvested late. Wine made from the less ripe grapes had a similarity constant value of 0.817 while the wine made from the more ripe grapes had a similarity constant value 0.985. Even though less data was available to calculate the pattern similarity values, the program could still discern differences in the wine samples. For this reason, it was decided to continue carrying out the blending optimization on trial 1.

According to commercial practice, the winemaker grades the varietal wines by sensory methods and selects the best varietal wine as the principal wine which will be used for blending with the white stock wines. This procedure is based on the blender's experience with varietal wines alone. Since in this study varietal and white stock wines were being compared to the target, which is a blended wine, it was not an appropriate comparison to make. It would have been better to grade varietal wines by comparing them to a known best varietal wine. Because this was a first-time study on blending optimization of wines, it was not possible to classify these wines in terms of flavor quality without having some GC data of varietal wines that have been graded for flavor quality by wine experts. Due to the lack of this information, the varietal wines judged as best by the winemaker who was cooperating with this study, were selected as the primary blending stock. Chenin blanc, sample 14, and

Verdelet, sample 47, were the principal blending wines for trials 1 and 2, respectively.

Blending Optimization

Because this was an initial study on wine blending the optimization problem was kept relatively simple. Practical wine blending takes into account regulations for commercial winemakers, for example the amount of foreign stock allowed to be blended with domestic stock wine, availability of blending stocks, consumer preferences and cost considerations (Jackish, 1985). However, this is not to imply that the optimization program is incapable of accomodating such factors. This program can easily be modified to handle any of the above mentioned constraints. In this study, however, wines were optimized for aroma with only one constraint since the objective of the work was to determine if a computer-aided approach to blending could be successful. More complex blending problems could be investigated later after the outcome of this study.

Once a principal varietal wine had been selected, factor ranges for the rest of the white stock wines were entered into the Blending Optimization program. Table 5 shows the ranges of the white stock wines for both trials. For trial 1 all the upper limits were set at 40% but for trial 2 since wine sample 22 was a premium white stock wine, more costly than regular white stock wines, the upper limit was set at 20%. The rest of the white stock wines of this trial were assigned

Table 5. Factors and their limits for the blending optimization of trial 1 and 2 wines.

	Factors		Lower Limit	Upper Limit
Trial 1	Sample	70	0.000	0.400
		12	0.000	0.400
		69	0.000	0.400
Trial 2	Sample	70	0.000	0.400
		12	0.000	0.400
		22	0.000	0.200

limits of 40%.

The optimization program used the pattern similarity subprogram and the previously entered GC data of the principal blending stock, the white stock, and the target wine to search for the optimal blending ratios of the varietal and white stock wines which gives the highest similarity constant values between the GC profiles of the blend and the target wine. Results of the theoretical optimization for trial 1 are shown in Table 6. After 13 vertices, a similarty coeffiecient of 0.993 was obtained. Vertices 11 to 13 were averaged to calculate the final blending ratio of 40.0, 49.0 and 22.9% of white stock wines 70, 12 and 69 with the varietal wine 14. For the actual blending, the total available volume of sample 14 would be taken as 100% and the the amounts of the other wines would be a percentage of the total varietal wine as determined by the optimization program. For example, if there was 10.0 L of the primary stock wine available for blending, and 40.0% was the dictated ratio of a white stock wine, then 4.0 L of this wine would be required for the formulation.

For the blending optimization of trial 2, the program iterated 23 vertices to reach a similarity constant of 0.861 (Table 7). A ratio of 26.9% of wine sample 70 was needed to blend with wine sample 47, the principal blending wine. As in the case for trial 1, the three final vertices were averaged to obtain the final blending ratios. Results of this optimization suggest that wine samples 12 and 22 do not contribute favorably to the formulation of the target wine.

Table 6. Blending optimization of trial 1 wine.

	Vertex	Sample Ratios			Response
		No. 70	No. 12	No. 69	
Initial Simplex	1	0.000	0.000	0.000	0.988
	2	0.377	0.094	0.094	0.993
	3	0.094	0.377	0.094	0.991
	4	0.094	0.094	0.377	0.992
Reflection	5	0.377	0.377	0.377	0.993
Expansion	6	0.566	0.566	0.566	0.993
Reflection	7	0.471	0.000	0.471	0.993
Expansion	8	0.660	-0.189	0.660	0.992
Reflection	9	0.723	0.220	0.251	0.993
Reflection	10	0.670	0.304	0.639	0.993
Contraction	11	0.400	0.147	0.230	0.993
Reflection	12	0.400	0.000	0.258	0.993
Expansion	13	0.400	0.000	0.199	0.993
Final Average Value		0.400	0.049	0.229	0.993

Table 7. Blending optimization of trial 2 wine.

	Vertex	Sample Ratios			Response
		No. 70	No. 12	No. 22	
Initial Simplex	1	0.000	0.000	0.000	0.856
	2	0.377	0.094	0.047	0.856
	3	0.094	0.377	0.377	0.850
	4	0.094	0.094	0.189	0.854
Reflection	5	0.220	-0.251	0.110	0.854
Contraction-R	6	0.189	-0.094	0.094	0.859
Reflection	7	0.283	-0.094	-0.094	0.861
Expansion	8	0.377	-0.189	-0.236	0.850
Reflection	9	0.566	-0.063	0.031	0.858
Reflection	10	0.314	-0.262	-0.026	0.857
Contraction-R	11	0.330	0.000	0.000	0.861
Reflection	12	0.000	0.000	0.000	0.856
Contraction-W	13	0.400	0.000	0.016	0.860
Reflection	14	0.400	0.031	0.000	0.859
Contraction-W	15	0.263	0.000	0.034	0.860
Reflection	16	0.184	0.000	0.000	0.860
Reflection	17	0.268	0.000	0.000	0.861
Reflectin	18	0.400	0.000	0.000	0.860
Contraction-W	19	0.239	0.000	0.000	0.861
Reflection	20	0.197	0.000	0.000	0.861
Contraction-W	21	0.297	0.000	0.000	0.861
Reflection	22	0.230	0.000	0.000	0.861
Contraction-W	23	0.280	0.000	0.000	0.861
Final Average Value		0.269	0.000	0.000	0.861

Blending ratios and the corresponding actual volumes of wines used for simulating the target are shown in Table 8. This table also shows the ratios of the commercial blend for this year.

E. ADJUSTMENTS FOR ACIDITY AND SWEETNESS

Once the wines were blended to match the aroma of the target wine, the aroma optimized wines had to be matched for the other component of flavor, taste. Making adjustment to a blend for acidity and sweetness is one of the final steps in practical wine blending in the industry. Procedures identical to the ones followed by the winery were used. First titratable acids of the blends were measured by titration with 0.1 N NaOH to pH 8.2. Titratable acidity of the target wine and the computer optimized blend of both trials are shown in Table 9. For trial 1, the acidity value of the blend was 0.622 g per 100 mL compared to the target wine with a value of 0.570 g per 100 mL. To reduce the acid or sourness character of the wine, this blend was diluted with water (2% of the total volume of wine). Canadian regulations permit a maximum of 10% dilution of wine with water. After dilution, the blend now had a titratable acid value of 0.555 which was close to the target. No adjustments for acidity were needed for the trial two blend as the values were judged to be close enough to the target.

Total soluble solids were determined by a Brix hydrometer

Table 8. Blending ratios of computer-aided blends and commericial blends for trials 1 and 2.

Trial 1				
Computer-aided blend			Commercial blend	
Wine Sample	Percent*	Volume (mL)	Wine Sample	Percent
14	59.5	1500	7	70
70	23.8	600	69	15
69	13.7	345	12	15
12	3.0	70		
Trial 2				
47	78.7	1200	58	60
70	21.3	324	12	40

* percent of total volume of wine

Table 9. Titratable acidity of the computer optimized blends, commercial blends and the target wines.

	Trial 1 (g/100 mL)	Trial 2
Target wine	0.570	0.525
Commercial blend	0.600	0.570
Computer optimized blend	0.555*	0.540

* ameliorated with distilled water

as a measure of the sweetness character of wine. For both trials the levels of sugars were too low compared to the target wine and had to be ameliorated with liquid invert sugar. The level of the total soluble solids before and after adjustment are shown in Table 10. This table also shows the acid and sugar levels of the commercial blends that were made to simulate the previous years wines, Leibesheim and Cuvee White. These wines were formulated using the same available stock wines as were available for this study for the computer-aided optimization.

F. VERIFICATION OF RESULTS

To confirm the results of the computer optimization of blending wine, sensory tests were conducted with untrained consumers and expert taste panels. In addition, the theoretically optimized blends were analyzed by HSGC to determine how close the actual similarity constants of these blends would be to the predicted value.

Sensory Evaluation

To determine if there was a detectable difference in the computer optimized blends and the target, triangle tests were conducted. Commercially blended wines were also tested against the target. Samples were served at near room temperature in coded wine glasses. Twelve judges evaluated trial 1 wines. Each judge received 3 coded samples: 6 judges

Table 10. Total soluble solids for the blends and targets of trials 1 and 2.

	Trial 1 (°Balling)	Trial 2
Target wine	+0.30	-1.05
Subjective blend	-0.60	-1.10
Computer optimized blend	-1.50	-1.70
Computer optimized blend after adjustment	+0.35	-1.15

tested 2 samples of the computer-optimized blend and one of the target blend, and the other six judges evaluated one computer-optimized blend and two target blends. The order of the 3 samples was randomized for every panelist. The same scheme was used for the 15 member panel that tested trial 2 blends.

Results of the triangle difference test are shown in Table 11. Six judges correctly identified the odd sample in comparing the computer-aided blend while 4 judges successfully picked out the odd sample for the commercially blended wine. Results of trial 2 blends and the commercial blend tests were similar with 8 out of 15 judges and 5 out of 15 judges correctly identifying the odd sample, respectively. Since 9 correct judgements out of 12 and 10 correct judgements out of 15 are necessary to establish a significant difference at the 99% level of confidence, it may be concluded that there is no detectable difference between the computer optimized blend and commercial blend, and the target wines. This implies that both the conventional and innovative blending schemes were successful in standardizing the flavor quality of Leibesheim and Cuvee White wines.

It is interesting to note that in their evaluations of these wines, nearly all of the judges, both untrained and trained, expressed difficulty in selecting the odd sample. Many of them commented that they had to rely on their sense of smell alone to detect differences.

Table 11. Results of the triangle test comparing the computer optimized blends and commercailly formulated blends with the target wine.

	Comparison	Correct response
Trial 1	Objective blend	6*
	Subjective blend	4*
Trial 2	Objective blend	8*
	Subjective blend	5*

* not significant

Similarity constants of the Blends

Similarity constants of trial 1 and 2 blends and the corresponding commercial blends were determined. All blends were analyzed by HSGC (Fig. 19 and 20) and the peak data was entered into the Similarity Constant program of the optimization program. The predicted similarity constants for both trials were 0.993 and 0.861, respectively while the actual values were 0.997 and 0.865, respectively. The closeness of the actual values to the predicted values supports the original assumption that experimental optimization was not necessary in blending optimization.

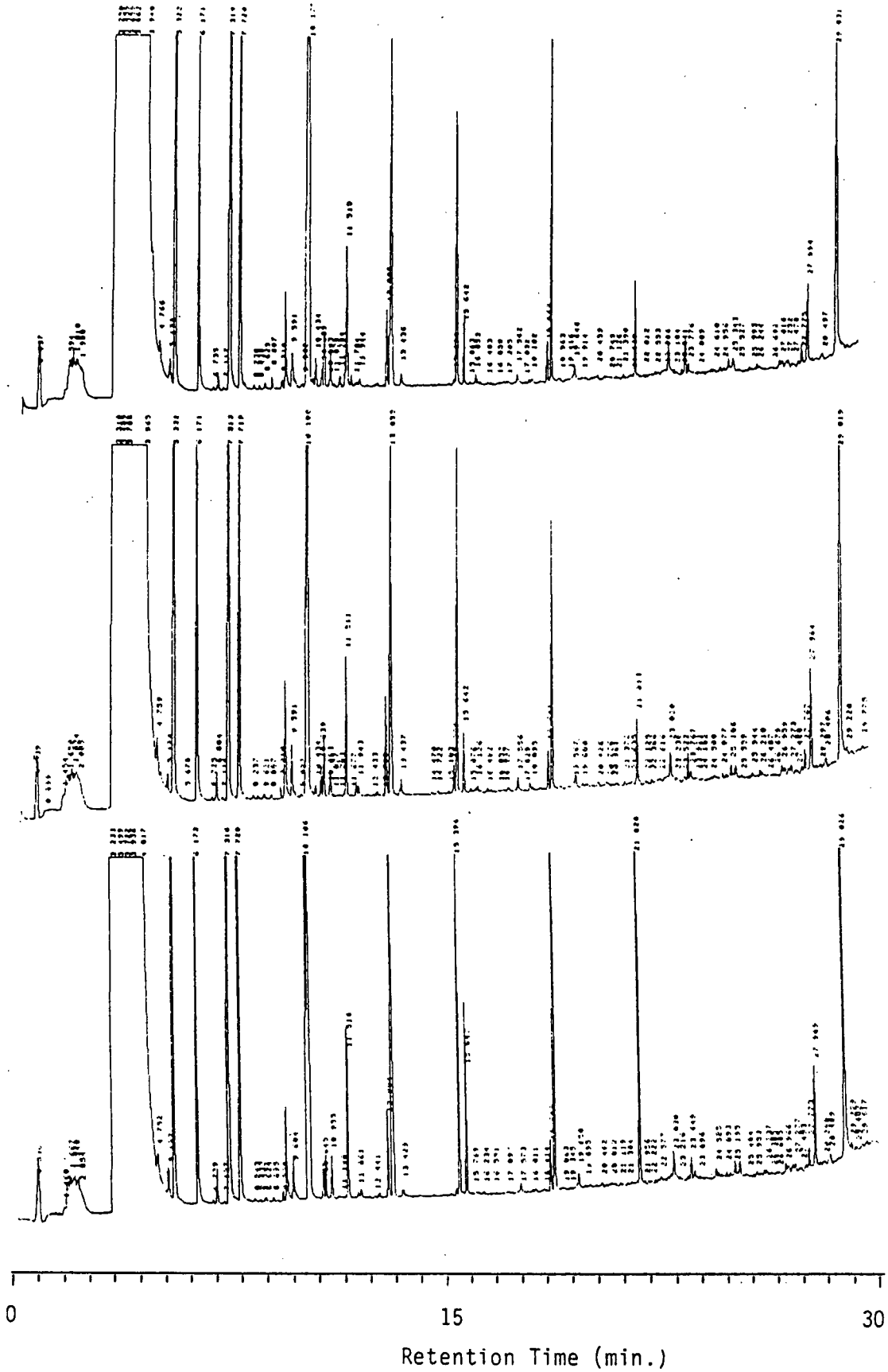

Fig. 19. Chromatograms of headspace volatiles from (a) the target, (b) commercial blend and (c) the computer optimized blend for trial 1.

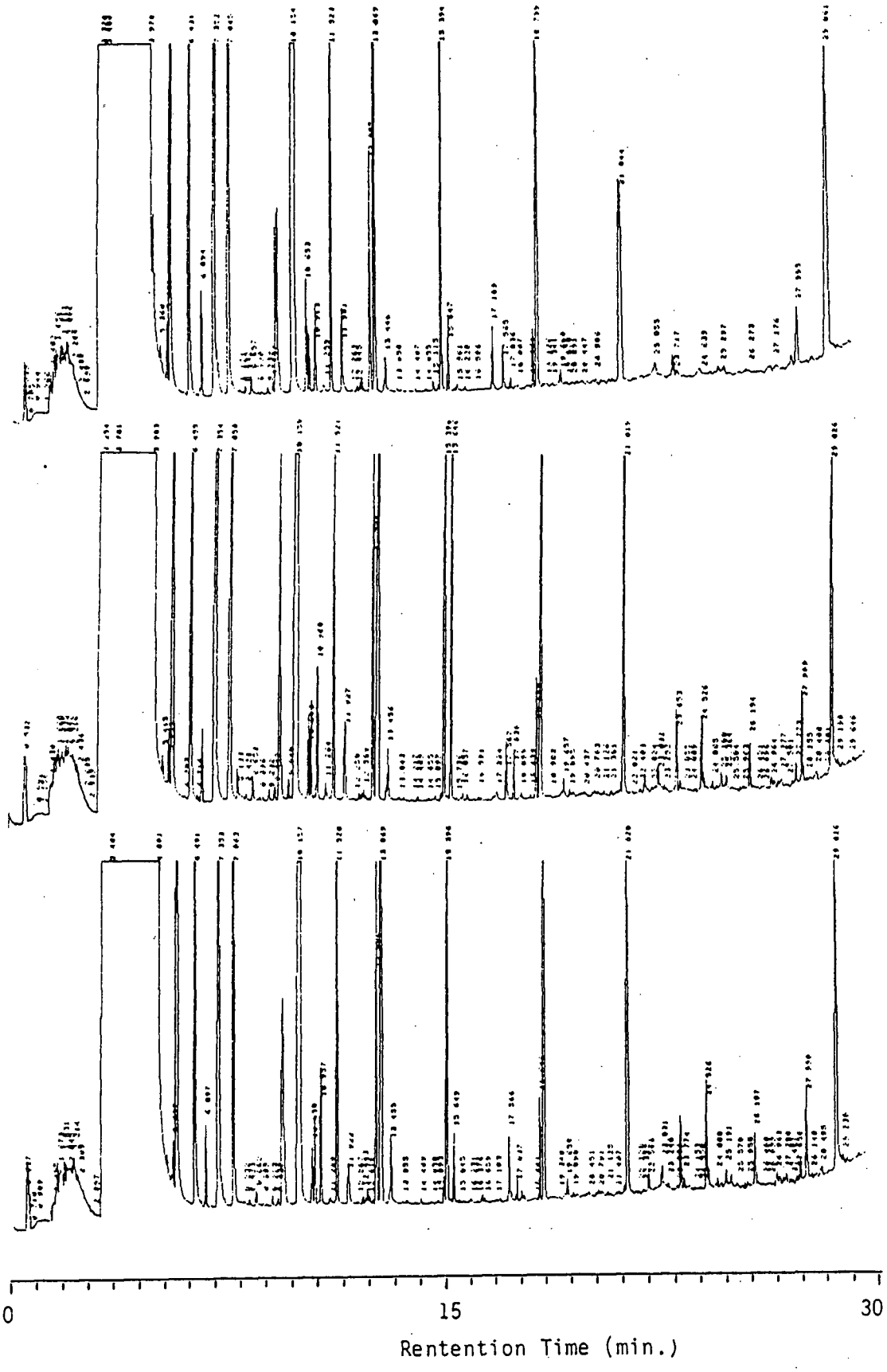

Fig. 20. Chromatograms of headspace volatiles from (a) the target, (b) commercial blend and (c) the computer optimized blend for trial 2.

CONCLUSION

Although sugars, acids and phenols are responsible for sweet, sour and bitter and astringent sensations of wine, respectively, odor is the most important sensation in the perception of wine quality (Acree and Cottrell, 1985). Volatile flavor components responsible for the aroma of wine have been separated and identified by GC and GC-mass spectrometric methods.

In this study, white wine aroma constituents were analyzed by HSGC using a cold trapping technique. Sensitivity was significantly improved by this method compared to other methods of enhancing the lower detection limit of headspace methods. The procedure being simple and rapid, could be easily employed by wineries.

Based on the aroma profiles of a number of blending component wines including varietal and white stock, blending to standardize two widely selling commercial wines, Leibesheim and Cuvee White, was carried out using simplex optimization. The optimization program was successful in determining the optimum blending ratios of the white stock wines and varietal wines for simulating the reference or target wine for both trials. Results of the sensory analysis indicated that no significant difference existed between the computer optimized blend and the previous year's commercial blend. In addition, the similarity coefficient values of the blends were very close to the predicted values by the program. These results

confirmed the initial assumption that computerized simplex optimization could be used for the blending problem instead of experimental simplex optimization.

Despite the fact that both trials 1 and 2 for blending wines analyzed at 6°C and 37°C, respectively were successful in simulating the reference wine, the higher temperature of analysis is recommeneded for future work. At refrigeration temperature the chromatogram contained fewer peaks thus when the similarity constant values were calculated by the optimization program, almost all of the wines had very high and similar values. Headspace analysis of wines at this temperature did not provide enough peak data to adequately differentiate trial 1 wines as did the analysis at 37°C for trial 2 wines.

Sensory evaluation data provided further support to this finding. Even though all of the triangle test trials were statistically not significant, more panelists had difficulty selecting the odd sample when presented with the commercially prepared blend and the target wine for both the Leibesheim and Cuvee White wines. Headspace anlaysis and subsequent results of the pattern similarity constants of trial 2 wines were consistent with these findings as the commercial blend had a slightly higher similarity values than the computer optimized blend. For trial 1, however, the sensory and similarity constant results did not agree. From the above findings it appears that headspace analysis of wines for blending optimization should be conducted at 37°C rather than at 6°C

for more accurate results.

With further work, application of this research on blending of wines for use as a quality control method in wineries that could replace conventional sensory based blending procedures appears promising. However, it should be stressed that the results of this study cannot be considered conclusive as the number of blending trials conducted were too few to make any definitive conclusions. The study does, nevertheless, provide an important basis for further research to define clearly the potential use of HSGC and computerized simplex optimization procedure for product formulation.

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Appendix 1. GC Data Entry computer program.

```
CLEAR:KEY OFF:CLS:DIM F(150),G(10,150)
INPUT "Number of peaks? ",N1
INPUT "Number of samples to be blended? ",NN:PRINT
DO K=0 TO NN-1
  PRINT "Entry No.";K+1
  INPUT "   Sample No.? (2 digits) ",P(K)
ENDDO
PRINT:PRINT "To keep entering, press ENTER;"
PRINT "To reenter for correcting misentry, press M":PRINT
PRINT "Target"
INPUT "   Int Stand Pk Area    ",A
DO B$=""
  B$=INKEY$
ENDDO
IF B$="m"
  B$="M"
ENDIF
IF B$="M"
  INPUT "   Int Stand Pk Area    ",A
ENDIF
INPUT "   Volume               ",V
DO C$=""
  C$=INKEY$
ENDDO
IF C$="m"
  C$="M"
ENDIF
IF C$="M"
  INPUT "   Volume               ",V
ENDIF
PRINT
DO I=1 TO N1
  PRINT "   Peak  ";I;
  INPUT "          Peak area? ",F(I)
  DO D$=""
    D$=INKEY$
  ENDDO
  IF D$="m"
    D$="M"
  ENDIF
  IF D$="M"
    INPUT "                   Peak area? ",F(I):D$=""
  ELSE
    D$=""
  ENDIF
ENDDO
DO K=0 TO NN-1
  PRINT
  PRINT "Sample";P(K)
  INPUT "   Int Stand Pk Area   ",A(K)
  DO E$=""
```

```
    E$=INKEY$
  ENDDO
  IF E$="m"
    E$="M"
  ENDIF
  IF E$="M"
    INPUT "   Int Stand Pk Area  ",A(K):E$=""
  ELSE
    E$=""
  ENDIF
  INPUT "   Volume                ",V(K)
  DO F$=""
    F$=INKEY$
  ENDDO
  IF F$="m"
    F$="M"
  ENDIF
  IF F$="M"
    INPUT "   Volume                ",V(K):F$=""
  ELSE
    F$=""
  ENDIF
  PRINT
  DO I=1 TO N1
    PRINT "   Peak   ";I;
    INPUT "        Peak area? ",G(K,I)
    DO G$=""
      G$=INKEY$
    ENDDO
    IF G$="m"
      G$="M"
    ENDIF
    IF G$="M"
      INPUT "                   Peak area? ",G(K,I):G$=""
    ELSE
      G$=""
    ENDIF
  ENDDO
ENDDO
LPRINT TAB(5) "Target";
LPRINT TAB(12) "No.";P(0);
LPRINT TAB(19) "No.";P(1);
LPRINT TAB(26) "No.";P(2);
LPRINT TAB(33) "No.";P(3);
LPRINT TAB(40) "No.";P(4);
LPRINT TAB(47) "No.";P(5);
LPRINT TAB(54) "No.";P(6);
LPRINT TAB(61) "No.";P(7);
LPRINT TAB(68) "No.";P(8):LPRINT
DO I=1 TO N1
  LPRINT USING "###";I;
  LPRINT USING "########";F(I);
  DO J=0 TO NN-2
    LPRINT USING "########";G(J,I);
```

```
  ENDDO
  LPRINT USING "########";G(NN-1,I)
ENDDO
KEY ON:PRINT
PRINT "Store data; diskette ready in Drive B?
       Press F5 for storing":STOP
OPEN "O", #1, "B:DATA"
PRINT #1, N1,NN,A,V
DO K=0 TO NN-1
  PRINT #1, A(K),V(K),P(K)
ENDDO
DO I=1 TO N1
  PRINT #1, F(I)
  DO J=0 TO NN-1
    PRINT #1, G(J,I)
  ENDDO
ENDDO
CLOSE #1
PRINT:PRINT "END":END
```

Appendix 2. GC Data Correction computer program.

```
CLEAR:CLS:DIM F(150),G(10,150)
PRINT "Recall data; diskette ready in Drive B?
       Press F5 for recalling":STOP
OPEN "I", #1, "B:DATA"
INPUT #1, N1,NN,A,V
DO K=0 TO NN-1
  INPUT #1, A(K),V(K),P(K)
ENDDO
DO I=1 TO N1
  INPUT #1, F(I)
  DO J=0 TO NN-1
    INPUT #1, G(J,I)
  ENDDO
ENDDO
CLOSE #1
DO
  CLS:PRINT TAB(29) "----- MENU -----":PRINT
  PRINT TAB(15) "1: Correction  2: Deletion
                 3: Insertion":PRINT:PRINT
  INPUT "Menu No.? ",Z
  IF Z=1
    PRINT:KEY OFF
    INPUT "Correct data for target?(Y/N) ",
          A$                'Data Correction
    IF A$="y"
      A$="Y"
    ENDIF
    IF A$="Y"
      INPUT "   How many data to correct? ",B
        DO I=1 TO B INPUT "   Data No.? ",C PRINT "   Stored
        data";F(C) INPUT "   Correct data? ",F(C)
      ENDDO
    ELSE
      CLS
    ENDIF
    INPUT "How many samples to correct? ",U
    DO K=1 TO U
      INPUT "Sample No.? ",W
      DO M=0 TO NN-1
        IF W=P(M)
          L=M
        ENDIF
      ENDDO
      PRINT "  Sample ";P(L)
      INPUT "    How many data to correct? ",E
      DO J=1 TO E
        INPUT "    Data No.? ",F
        PRINT "    Stored data ";G(L,F)
        INPUT "    Correct data? ",G(L,F)
```

```
    ENDDO
  ENDDO
  CLS
ELSEIF Z=2
  INPUT "Delete data for target?(Y/N) ",
        D$                'Data Deletion
  IF D$="y"
    D$="Y"
  ENDIF
  IF D$="Y"
    INPUT "   How many data to delete? ",B
    PRINT "   If more than one deletion,
              start from the bottom data"
    DO I=1 TO B
      INPUT "   Data No.? ",E
      DO I=E TO N1-1
        F(I)=F(I+1)
      ENDDO
    ENDDO
  ENDIF
  INPUT "How many samples to delete? ",U
  DO K=1 TO U
    INPUT "Sample No.? ",W
    DO M=0 TO NN-1
      IF W=P(M)
        L=M
      ENDIF
    ENDDO
    PRINT "  Sample No.";P(L)
    INPUT "    How many data to delete? ",B
    PRINT "    If more than one deletion,
               start from the bottom data"
    DO J=1 TO B
      INPUT "    Data No.? ",E
      DO I=E TO N1-1
        G(L,I)=G(L,I+1)
      ENDDO
    ENDDO
  ENDDO
  CLS
ELSE
  INPUT "Insert data for target?(Y/N) ",
        I$                'Data Insertion
  IF I$="y"
    I$="Y"
  ENDIF
  IF I$="Y"
    INPUT "   How many data to insert? ",B
    PRINT "   If more than one insertion,
              start from the bottom data"
    DO I=1 TO B
      INPUT "   Data No.? ",C
      DO I=N1 TO C+1 STEP-1
        F(I+1)=F(I)
```

```
        ENDDO
        INPUT "   Data to be inserted? ",F(C+1)
      ENDDO
    ENDIF
    INPUT "How many samples to insert?  ",U
    DO K=1 TO U
      INPUT "Sample No.? ",W
      DO M=0 TO NN-1
        IF W=P(M)
          L=M
        ENDIF
      ENDDO
      PRINT "  Sample No.";P(L)
      INPUT "    How many data to insert? ",E
      PRINT "    If more than one insertion,
                 start from the bottom data"
      DO J=1 TO E
        INPUT  "    Data No.? ",F
        DO I=N1 TO F+1 STEP -1
          G(L,I+1)=G(L,I)
        ENDDO
        INPUT "    Data to be inserted? ",G(L,F+1)
      ENDDO
    ENDDO
    CLS
  ENDIF
  INPUT "Correction completed?(Y/N) ",H$
  IF H$="y"
    H$="Y"
  ENDIF
  IF H$="Y"
    INPUT "New number of peaks, if changed? ",N1
  ENDIF
ENDDO H$="Y"
LPRINT "GC DATA"
GOSUB @TTL
DO I=1 TO N1
  LPRINT USING "###";I;
  LPRINT USING "########";F(I);
  DO J=0 TO NN-2
    LPRINT USING "########";G(J,I);
  ENDDO
  LPRINT USING "########";G(NN-1,I)
ENDDO
PRINT "Store corrected GC data; diskette ready in Drive B?
       Press F5":STOP
OPEN "O", #1, "B:DATA"
PRINT #1, N1,NN,A,V
DO K=0 TO NN-1
  PRINT #1, A(K),V(K),P(K)
ENDDO
DO I=1 TO N1
  PRINT #1, F(I)
  DO J=0 TO NN-1
```

```
    PRINT #1, G(J,I)
  ENDDO
ENDDO
CLOSE #1
DO I=1 TO N1
  F(I)=F(I)/(A*V)
  DO J=0 TO NN-1
    G(J,I)=G(J,I)/(A(J)*V(J))
  ENDDO
ENDDO
LPRINT:LPRINT:LPRINT "STANDARDIZED DATA"
GOSUB @TTL
DO J=1 TO N1
  LPRINT USING "###";J;
  LPRINT USING "####.##";F(J);
  DO I=0 TO NN-2
    LPRINT USING "####.##";G(I,J);
  ENDDO
  LPRINT USING "####.##";G(NN-1,J)
ENDDO
KEY ON:PRINT
PRINT "Store standardized data; diskette ready in Drive B?
      Press F5":STOP
OPEN "O", #2, "B:DATA1"
PRINT #2, N1,NN
DO K=0 TO NN-1
  PRINT #2, P(K)
ENDDO
DO I=1 TO N1
  PRINT #2, F(I)
  DO J=0 TO NN-1
    PRINT #2, G(J,I)
  ENDDO
ENDDO
CLOSE #2
PRINT:PRINT "END":END
@TTL
LPRINT TAB(5) "Target";
LPRINT TAB(12) "No.";P(0);
LPRINT TAB(19) "No.";P(1);
LPRINT TAB(26) "No.";P(2);
LPRINT TAB(33) "No.";P(3);
LPRINT TAB(40) "No.";P(4);
LPRINT TAB(47) "No.";P(5);
LPRINT TAB(54) "No.";P(6);
LPRINT TAB(61) "No.";P(7);
LPRINT TAB(68) "No.";P(8):LPRINT
RETURN

LPRINT TAB(47) "No.";P(5);
LPRINT T
```

Appendix 3. Similarity Constant computer program.

```
CLEAR:KEY OFF:CLS:DIM F(150),G(10,150)
PRINT "Recall data; diskette ready in Drive B?
       Press F5 for recalling":STOP
OPEN "I", #2, "B:DATA1"
INPUT #2, N1,NN
DO K=0 TO NN-1
  INPUT #2, P(K)
ENDDO
DO I=1 TO N1
  INPUT #2, F(I)
  DO J=0 TO NN-1
    INPUT #2, G(J,I)
  ENDDO
ENDDO
CLOSE #2
CLS:PRINT "SIMILARITY CONSTANT":PRINT
LPRINT "SIMILARITY CONSTANT":LPRINT
DO I=0 TO NN-1
  A=0:B=0:C=0
  DO J=1 TO N1
    A=A+F(J)*G(I,J)
    B=B+F(J)*F(J)
    C=C+G(I,J)*G(I,J)
  ENDDO
  R=A/SQR(B*C)
  PRINT "   Sample No. ";P(I);
  PRINT TAB(30) USING "###.###";R
  LPRINT "   Sample No. ";P(I);
  LPRINT TAB(30) USING "###.###";R
ENDDO
PRINT:PRINT "END":END
```

Appendix 4. Blending Optimization computer program.

```
CLEAR:CLS
DIM X(10,100),Y(100),F(150),G(10,150),H(150),D(10,150)
PRINT "Recall data; diskette ready in Drive B?
       Press F5 for recalling":STOP
OPEN "I", #2, "B:DATA1"
INPUT #2, N1,NN
DO K=0 TO NN-1
  INPUT #2, P(K)
ENDDO
DO I=1 TO N1
  INPUT #2, F(I)
  DO J=0 TO NN-1
    INPUT #2, G(J,I)
  ENDDO
ENDDO
CLOSE #2
DO I=1 TO N1
  DO J=0 TO NN-1
    D(J,I)=G(J,I)
  ENDDO
ENDDO
MM=NN
DO
  CLS:INPUT "Sample No. of the principal ingredient? ",T
  DO I=0 TO MM-1
    IF T=P(I)
      L=I
    ENDIF
  ENDDO
  DO J=1 TO N1
    G(0,J)=D(L,J)
  ENDDO
  INPUT "How many samples for blending with the
         principal ingredient? ";A
  DO I=1 TO A
    INPUT "   Enter sample No. ",A(I)
    DO J=0 TO MM-1
      IF A(I)=P(J)
        B(I)=J
      ENDIF
    ENDDO
  ENDDO
  DO I=1 TO N1
    DO J=1 TO A
      K=B(J)
      G(J,I)=D(K,I)
    ENDDO
  ENDDO
  NN=A:PRINT:MV=100
  INPUT "Terminating difference value? ",TERM
  INPUT "How many vertices without
```

```
       prohibit-trespassing? ",Z:PRINT
DO I=1 TO NN
  PRINT "Sample No. ";A(I)
  INPUT "   Enter lower then upper limits ",L(I),U(I)
ENDDO
LPRINT "Vertices without prohibit-trespassing ";Z
LPRINT "Terminating difference value";USING "###.####";
       TERM:LPRINT
LPRINT "Principal ingredient is sample ";T
LPRINT "Lower and upper limits":LPRINT "   LL:    ";
DO J=1 TO NN
  LPRINT USING "###.###";L(J);
ENDDO
LPRINT:LPRINT "   UL:    ";
DO K=1 TO NN
  LPRINT USING "###.###";U(K);
ENDDO
LPRINT:LPRINT
P=(1/(NN*SQR(2)))*(NN-1+SQR(NN+1))
Q=(1/(NN*SQR(2)))*(SQR(NN+1)-1)
DO J=1 TO NN                                    'Initial simplex
  M(1,J)=L(J)
ENDDO
DO I=2 TO NN+1
  DO J=1 TO NN
    IF I-1=J
      M(I,J)=L(J)+P*(U(J)-L(J))
    ELSE
      M(I,J)=L(J)+Q*(U(J)-L(J))
    ENDIF
  ENDDO
ENDDO
DO M=1 TO NN+1
  DO J=1 TO NN
    S(J)=M(M,J)
  ENDDO
  GOSUB @FTN
  B(M)=R
ENDDO
DO XX=1 TO NN+1
  DO I=1 TO NN
    X(I,XX)=M(XX,I)
  ENDDO
ENDDO
DO Y=1 TO NN+1
  Y(Y)=B(Y)
ENDDO
LPRINT TAB(12) "No.";A(1);
LPRINT TAB(19) "No.";A(2);
LPRINT TAB(26) "No.";A(3);
LPRINT TAB(33) "No.";A(4);
LPRINT TAB(40) "No.";A(5);
LPRINT TAB(47) "No.";A(6);
LPRINT TAB(54) "No.";A(7);
```

```
LPRINT TAB(61) "No.";A(8);
LPRINT TAB(70) "Response":XX=XX-1:Y=Y-1
LPRINT:LPRINT TAB(12) "(Initial simplex)"
DO J=1 TO XX
  LPRINT "Vertex ";USING "###";J;
  DO K=1 TO NN
    LPRINT USING "###.###";X(K,J);
  ENDDO
  LPRINT TAB(66) USING "    ###.###";Y(J)
ENDDO
LPRINT
SEARCH
  WORST=B(1):WL=1                    'Find WORST
  DO I=2 TO NN+1
    IF B(I)<WORST
      WORST=B(I):WL=I
    ENDIF
  ENDDO
  BEST=B(1):BL=1                     'Find BEST
  DO J=2 TO NN+1
    IF B(J)>BEST
      BEST=B(J):BL=J
    ENDIF
  ENDDO
  T=0                                'Compute NEXT to the worst
  DO I=1 TO NN+1
    T=T+B(I)
  ENDDO
  NXT=(T-WORST-BEST)/(NN-1)
  DO K=1 TO NN                                    'Centroid
    S=0
    DO L=1 TO NN+1
      S=S+M(L,K)
    ENDDO
    S=S-M(WL,K):N(K)=S/NN
  ENDDO
  C=1:C$="(Reflection)":GOSUB @SRC               'Reflection
  DO M=1 TO NN
    R(M)=S(M)
  ENDDO
  REFL=R
  IF REFL>BEST
    C=2:C$="(Expansion)":GOSUB @SRC               'Expansion
    IF R>REFL
      DO N=1 TO NN
        Q(N)=S(N)
      ENDDO
      GOSUB @WRPL
    ELSE
      DO I=1 TO NN
        Q(I)=R(I)
      ENDDO
      R=REFL
      GOSUB @WRPL
```

```
    ENDIF
  ELSEIF REFL>NXT
    DO J=1 TO NN
      Q(J)=R(J)
    ENDDO
    R=REFL
    GOSUB @WRPL
  ELSEIF REFL>WORST
    C=0.5:C$="(Contraction-R)":GOSUB @SRC      'Contraction-R
    IF R>REFL
      DO I=1 TO NN
        Q(I)=S(I)
      ENDDO
      GOSUB @WRPL
    ELSE
      C=0.25:C$="(Massive contrac-
      tion-R)":GOSUB @SRC   'Massive contrn.-R
      IF R<REFL
        DO K=1 TO NN
          Q(K)=R(K)
        ENDDO
        R=REFL
        GOSUB @WRPL
      ELSE
        DO L=1 TO NN
          Q(L)=S(L)
        ENDDO
        GOSUB @WRPL
      ENDIF
    ENDIF
  ELSE                                          'Contraction-W
    C=-0.5:C$="(Contraction-W)":GOSUB @SRC
    IF R>WORST
      DO J=1 TO NN
        Q(J)=S(J)
      ENDDO
      GOSUB @WRPL
    ELSE
      C=-0.25:C$="(Massive contrac-
      tion-W)":GOSUB @SRC  'Massive contrn.-W
      DO K=1 TO NN
        Q(K)=S(K)
      ENDDO
      GOSUB @WRPL
    ENDIF
  ENDIF
EXITIF XX>MV
ORELSE
  A=XX:B=XX-1:C=XX-2                'Termination
  IF ABS(Y(A)-Y(B))>TERM
    T$="N"
  ELSEIF ABS(Y(B)-Y(C))>TERM
    T$="N"
  ELSEIF ABS(Y(A)-Y(C))>TERM
```

```
      T$="N"
    ELSE
      T$="Y"
    ENDIF
  ENDLOOP T$="Y"
    DO I=1 TO NN                          'Average of last three
      AV(I)=(X(I,A)+X(I,B)+X(I,C))/3
    ENDDO
    BV=(Y(A)+Y(B)+Y(C))/3:LPRINT
    LPRINT "Final average values":LPRINT "          ";
    DO I=1 TO NN
      LPRINT USING "###.###";AV(I);
    ENDDO
    LPRINT TAB(66) USING "###.###";BV
  ENDSRCH
  PRINT:PRINT:PRINT
  INPUT "Another combination of ingredients
        for blending?(Y/N) ",H$
  IF H$="n"
    H$="N"
  ENDIF
ENDDO H$="N"
PRINT "END":END
@SRC                                  'New vertex
DO I=1 TO NN
  S(I)=N(I)+C*(N(I)-M(WL,I))
ENDDO
IF XX>Z-1
  DO J=1 TO NN              'Prohibit trespassing
    IF S(J)<L(J)
      S(J)=L(J)
    ELSEIF S(J)>U(J)
      S(J)=U(J)
    ENDIF
  ENDDO
ENDIF
GOSUB @FTN
Y=Y+1:XX=XX+1
Y(Y)=R
DO J=1 TO NN
  X(J,XX)=S(J)
ENDDO
LPRINT "Vertex ";USING "### ";XX;
LPRINT C$:LPRINT "          ";
DO I=1 TO NN
  LPRINT USING "###.###";X(I,XX);
ENDDO
LPRINT TAB(66) USING "    ###.###";Y(Y)
RETURN
@WRPL                                  'W replacing
B(WL)=R
DO I=1 TO NN
  M(WL,I)=Q(I)
ENDDO
```

```
RETURN
@FTN                                    'Function
DO L=1 TO N1
  H(L)=0
ENDDO
DO I=1 TO N1
  DO J=1 TO NN
    H(I)=H(I)+S(J)*G(J,I)
  ENDDO
  H(I)=H(I)+G(0,I)
ENDDO
A=0:B=0:C=0
DO K=1 TO N1
  A=A+F(K)*H(K)
  B=B+F(K)*F(K)
  C=C+H(K)*H(K)
ENDDO
R=A/SQR(B*C)
RETURN
H(I)+G(0,I)
ENDDO
A=0:B=0:C=0
DO K=1 TO N1
  A=A+F(K)*H(K)
  B=B+F(K)*F(K)
  C=C+H(K)*H(K)
ENDDO
R=A/SQR(B*C)
```

Appendix 5. Similarity Constant of Blend computer program.

```
CLEAR:KEY OFF:CLS:DIM F(150),G(10,150)
INPUT "Recall data (Fresh) from diskette?(Y/N) ",A$
IF A$="y"
  A$="Y"
ENDIF
IF A$="Y"
PRINT:KEY ON
  PRINT "Diskette ready in Drive B? Press F5 for
        recalling":STOP
  OPEN "I", #1, "B:DATA"
  INPUT #1, N1,NN,A,V
  DO K=0 TO NN
    INPUT #1, A(K),V(K),P(K)
  ENDDO
  DO I=1 TO N1
    INPUT #1, F(I)
    DO J=0 TO NN
      INPUT #1, G(J,I)
    ENDDO
  ENDDO
  CLOSE #1
ELSE
  CLS:INPUT "Number of peaks? ",N1
  PRINT "Fresh"
  INPUT "   Int St Pk Area    ",A
  DO B$=""
    B$=INKEY$
  ENDDO
  IF B$="m"
    B$="M"
  ENDIF
  IF B$="M"
    INPUT "   Int St Pk Area    ",A
  ENDIF
  INPUT "   Volume            ",V
  DO C$=""
    C$=INKEY$
  ENDDO
  IF C$="m"
    C$="M"
  ENDIF
  IF C$="M"
    INPUT "   Volume            ",V
  ELSE
    CLS
  ENDIF
  PRINT
  DO I=1 TO N1
    PRINT "Peak  ";I;
    INPUT "       Peak area? ",F(I)
    DO D$=""
```

```
      D$=INKEY$
    ENDDO
    IF D$="m"
      D$="M"
    ENDIF
    IF D$="M"
      INPUT "                Peak area? ",F(I):D$=""
    ELSE
      D$=""
    ENDIF
  ENDDO
ENDIF
KEY OFF: PRINT:INPUT "Number of GLC patterns? ",NN
DO K=1 TO NN
  INPUT "   Pattern title? ",P$(K)
  INPUT "   Int St Pk Area   ",A(K)
  DO E$=""
    E$=INKEY$
  ENDDO
  IF E$="m"
    E$="M"
  ENDIF
  IF E$="M"
    INPUT "   Int St Pk Area  ",A(K):E$=""
  ELSE
    E$=""
  ENDIF
  INPUT "   Volume           ",V(K)
  DO F$=""
    F$=INKEY$
  ENDDO
  IF F$="m"
    F$="M"
  ENDIF
  IF F$="M"
    INPUT "   Volume            ",V(K):F$=""
  ELSE
    F$=""
  ENDIF
  PRINT
  DO I=1 TO N1
    PRINT "Peak   ";I;
    INPUT "     Peak area? ",G(K,I)
    DO G$=""
      G$=INKEY$
    ENDDO
    IF G$="m"
      G$="M"
    ENDIF
    IF G$="M"
      INPUT "               Peak area? ",G(K,I):G$=""
    ELSE
      G$=""
    ENDIF
```

```
  ENDDO
ENDDO
LPRINT TAB(9) "Fresh";
LPRINT TAB(17) P$(1);
LPRINT TAB(27) P$(2);
LPRINT TAB(37) P$(3);
LPRINT TAB(47) P$(4);
LPRINT TAB(57) P$(5):LPRINT
DO I=1 TO N1
  LPRINT USING "###";I;
  LPRINT USING "###########";F(I);G(1,I);G(2,I);
               G(3,I);G(4,I);G(5,I)
ENDDO
PRINT:PRINT "Store data; diskette ready in Drive B?
           Press F5 for storing":STOP
OPEN "O", #2, "B:DATA2"
PRINT #2, N1,NN,A,V
DO K=1 TO NN
  PRINT #2, A(K),V(K),P$(K)
ENDDO
DO I=1 TO N1
  PRINT #2, F(I)
  DO J=1 TO NN
    PRINT #2, G(J,I)
  ENDDO
ENDDO
CLOSE #2
PRINT:PRINT "END":END
```

Appendix 6. Similarity Constant of Blend, Data Correction computer program.

```
CLEAR:CLS:DIM F(150),G(10,150)
PRINT "Recall data; diskette ready in Drive B?
       Press F5 for recalling":STOP
OPEN "I", #2, "B:DATA2"
INPUT #2, N1,NN,A,V
DO K=1 TO NN
  INPUT #2, A(K),V(K),P$(K)
ENDDO
DO I=1 TO N1
  INPUT #2, F(I)
  DO J=1 TO NN
    INPUT #2, G(J,I)
  ENDDO
ENDDO
CLOSE #2
PRINT:KEY OFF:INPUT "Correct data for fresh?(Y/N) ",
                    A$    'Data Correction
IF A$="y"
  A$="Y"
ENDIF
IF A$="Y"
  INPUT "How many data to correct? ",B
  DO I=1 TO B
    INPUT "Data No.? ",A
    INPUT "Correct data? ",F(A)
  ENDDO
ELSE
  CLS
ENDIF
INPUT "Correct data for GLC pattern?(Y/N) ",E$
IF E$="y"
  E$="Y"
ENDIF
IF E$="Y"
  DO L=1 TO NN
    PRINT "PATTERN:  ";P$(L)
    INPUT "How many data to correct? ",E
    DO K=1 TO E
      INPUT "Data No.? ",F
      INPUT "Correct data? ",G(L,F)
    ENDDO
  ENDDO
ELSE
  CLS
ENDIF
INPUT "Delete data for fresh?(Y/N) ",
       D$                    'Data Deletion
IF D$="y"
  D$="Y"
ENDIF
```

```
IF D$="Y"
  INPUT "How many data to delete? ",B
  PRINT "If more than one deletion, start from the bottom"
  DO I=1 TO B
    INPUT "Data No.? ",E
    DO I=E TO N1-1
      F(I)=F(I+1)
    ENDDO
  ENDDO
ELSE
  CLS
ENDIF
INPUT "Delete data for GLC pattern?(Y/N) ",D$
IF D$="y"
  D$="Y"
ENDIF
IF D$="Y"
  DO L=1 TO NN
    PRINT "PATTERN:    ";P$(L)
    INPUT "How many data to delete? ",B
    PRINT "If more than one deletion, start from the bottom"
    DO I=1 TO B
      INPUT "Data No.? ",E
      DO I=E TO N1-1
        G(J,I)=G(J,I+1)
      ENDDO
    ENDDO
  ENDDO
ELSE
  CLS
ENDIF
INPUT "Insert data for fresh?(Y/N) ",
       I$                    'Data Insertion
IF I$="y"
  I$="Y"
ENDIF
IF I$="Y"
  INPUT "How many data to insert? ",B
  PRINT "If more than one insertion, start from the bottom"
  DO I=1 TO B
    INPUT "Data No.? ",C
    DO I=N1 TO C+1 STEP-1
      F(I+1)=F(I)
    ENDDO
    INPUT "Data to be inserted? ",F(C+1)
  ENDDO
ELSE
  CLS
ENDIF
INPUT "Insert data for GLC pattern?(Y/N) ",I$
IF I$="y"
  I$="Y"
ENDIF
IF I$="Y"
```

```
  DO L=1 TO NN
    PRINT "PATTERN:    ";P$(L)
    INPUT "How many data to insert? ",E
    PRINT "If more than one insertion, start from the bottom"
    DO K=1 TO E
      INPUT  "Data No.? ",F
      DO I=N1 TO F+1 STEP -1
        G(L,I+1)=G(L,I)
      ENDDO
      INPUT "Data to be inserted? ",G(L,F+1)
    ENDDO
  ENDDO
ELSE
  CLS
ENDIF
INPUT "New number of peaks, if changed? ",N1
LPRINT "GLC DATA"
LPRINT TAB(9) "Fresh";
LPRINT TAB(19) P$(1);
LPRINT TAB(29) P$(2);
LPRINT TAB(39) P$(3);
LPRINT TAB(49) P$(4);
LPRINT TAB(59) P$(5):LPRINT
DO I=1 TO N1
  LPRINT USING "###";I;
  LPRINT USING "###########";F(I);G(1,I);G(2,I);G(3,I);
                            G(4,I);G(5,I)
ENDDO
DO I=1 TO N1
  F(I)=100*F(I)/(A*V)
  DO J=1 TO NN
    G(J,I)=100*G(J,I)/(A(J)*V(J))
  ENDDO
ENDDO
LPRINT:LPRINT:LPRINT "STANDARDIZED DATA"
DO J=1 TO N1
  LPRINT USING "###";J;
  LPRINT USING "######.###";F(J);G(1,J);G(2,J);G(3,J);
                            G(4,J);G(5,J)
ENDDO
LPRINT:LPRINT:LPRINT "SIMILARITY CONSTANT"
DO I=1 TO NN
  A=0:B=0:C=0
  DO K=1 TO N1
    A=A+F(K)*G(I,K)
    B=B+F(K)*F(K)
    C=C+G(I,K)*G(I,K)
  ENDDO
  R=A/SQR(B*C)
  LPRINT "    ";P$(I);
  LPRINT TAB(30) USING "###.###";R
ENDDO
PRINT:PRINT "END":END
```