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New sensitive method for the examination of the volatile flavor fraction of cabernet sauvignon wines[a]

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ABSTRACT

A new design of solvent extractor and a new low-temperature, high vacuum, two stage concentrator apparatus were constructed that enabled the quantitative and qualitative examination of trace level concentrations of the volatile flavor components of *Vitis vinifera* grape musts and wines to be conducted reproducibly. The sensitivity of the new technique, in the ppb range, was demonstrated by the detection and identification of 2-methoxy-3-isobutylpyrazine, an organoleptically significant flavor compound in Cabernet Sauvignon wine for the first time. 2-Hydroxybenzothiazole and ethyl 4-acetyloxybutanoate were also identified for the first time as components in this wine.

INTRODUCTION

It is estimated that the concentrations of volatile flavor compounds in grape musts and wines range between 1 g/l and 1 ng/l [1]. Since the concentrations at which volatile compounds influence the flavor of a given food system are extremely low, sometimes at concentrations well below the detection limits of some of the most sophisticated analytical instruments, it is imperative that the volatile fraction of the grape must or wine be isolated from the bulk of the food sample and concentrated. The extremely low odor threshold values of some of the more significant flavor compounds demand that isolation techniques be aimed at isolating these trace level compounds from other less organoleptically significant components which may be present at concentrations several orders of magnitude higher. Several techniques have been used to achieve this: solvent extraction [2–4]; simple, fractional, steam, atmospheric and vacuum distillation [5,6]; adsorption onto charcoal, Tenax, silica gel, Porapak Q and Chromosorb [7]; and headspace techniques [8]. The isolated extract is usually enriched

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by rotary evaporation, a gentle stream of inert gas, or vacuum techniques to concentrate individual components to within the range of the detectors to be used. Gas chromatographic techniques are employed to separate and identify the components in the volatile extract. Most of these methods can detect only major and minor components but not trace-level components. Very few reports appear in the literature on the simultaneous detection and identification of multiple trace volatile flavor components in wine in the part per billion (ppb) and part per trillion (ppt)[a] concentration range [9,10].

In 1986, Boison *et al.* [11] reported the detection and identification of the organoleptically significant volatile flavor compound, 2-methoxy-3-isobutylpyrazine, as a trace component in Cabernet Sauvignon wine for the first time. Even though this compound, believed to impart a herbaceous aroma to Cabernet Sauvignon grapes and wines, was first identified in the grape in 1966 [12] and confirmed in 1975 [13], its presence in the wine could not be confirmed. Slingsby *et al.* [14] were also unable to establish the presence of this compound in wines they had made from the grapes of the *Vitis vinifera* cultivar Cabernet Sauvignon. Quite recently, however, Harris *et al.* [10] have also reported the identification and quantification of several alkoxypyrazines, including 2-methoxy-3-isobutylpyrazine, in wines and grape juices. This paper decribes in detail the new technique that enabled the detection and identification of 2-methoxy-3-isobutylpyrazine as a trace level component in Cabernet Sauvignon wines.

EXPERIMENTAL

Reagents

All flavor compounds used in this investigation were purchased from one of the following chemical companies: BDH (U.K.), Eastman-Kodak (U.S.A.), Fisher (Canada), Sigma (U.S.A.), Aldrich (U.S.A.), Pyrazine Specialities (U.S.A.) and McArthur (Canada). All compounds were checked for chromatographic purity before use. Freon 11 (Canadian Liquid Air) was purified by vacuum distillation before use. The 1972 red wine bottled by Chateau Pichon Lalande de Pauillac was from the private collection of one of the authors (R.H.T.). The composition of this red wine was approximately 4:1:1 Cabernet Sauvignon, Malbec, and Petit Verdot.

Apparatus

The all-glass solvent extraction apparatus that was used in this analysis is illustrated in Fig. 1. It was operated in the batch mode as follows: The solvent pot, T (onto which an extruding bulb U, with an internal volume of 700 μl and an outer dimension of 2.5 cm × 0.8 cm O.D., had been blown), initially contained 250.0 ml of freon 11 (b.p. 24.1°C) and a 7 mm × 12 mm egg-shaped PTFE-coated stirring magnet. The pot, T, sat in a warm water bath held at 35°C by a heater-stirrer which also maintained vigorous stirring in T. The resulting freon vapor condensed into the reflux syphon, D, which provided reflux solvent to the fractionating column, S, (50 cm × 2.5 cm O.D., packed with 3 mm glass helices) and to the intermittent syphon, F, which provided a critical volume of freon (1.15 ml) sufficient to form a single bead in the

[a] Throughout this article, the American billion (10^9) and trillion (10^{12}) are meant.

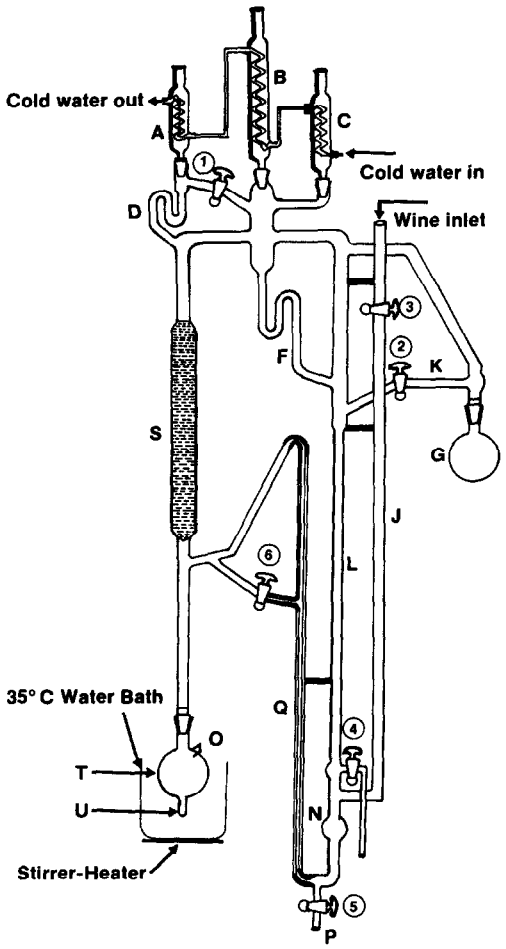

Fig. 1. Solvent extraction apparatus. A, B, C = Condensers; D = reflux syphon; F = intermittent syphon; G = wine/must receiving bulb for continuous extraction; J = inlet tube; K = overflow transfer tube; L = extracting tube; N = collection bulb; O = glass post mounted on the side of the solvent pot for harnessing the horseshoe magnet retriever; P = wine/must outlet tube; Q = capillary tube; S = fractional distillation column; T = solvent pot; U = extruding bulb for handling small sample volumes; 1–6 = taps.

extracting tube, L. L was filled with 95.0 ml of wine through the entry tube, J. The wine was held stationary in L with pressurized, inert gas in the entry tube and with stopcock 3 closed. The freon bead fell through the length of L into the collection bulb, N, at a rate of 4 beads/min. The contents of N could be withdrawn through stopcock 5. As the consecutive freon beads fell, the freon and extracted volatile flavor components flowed into T through the capillary tube, Q. The extracted volatile flavor components collected in T and the warmed, stirred, freon solution in T underwent fractional distillation in S. This enabled purified freon to recycle and reextract the sample in L. After 2.5 h, the 35°C water bath was removed, and T was removed from the extractor and connected, through the transfer tube, K, to the low-temperature, high-vacuum, concentrator (Fig. 2) held under a vacuum of 13–130 mPa. The transfer tube, K, was

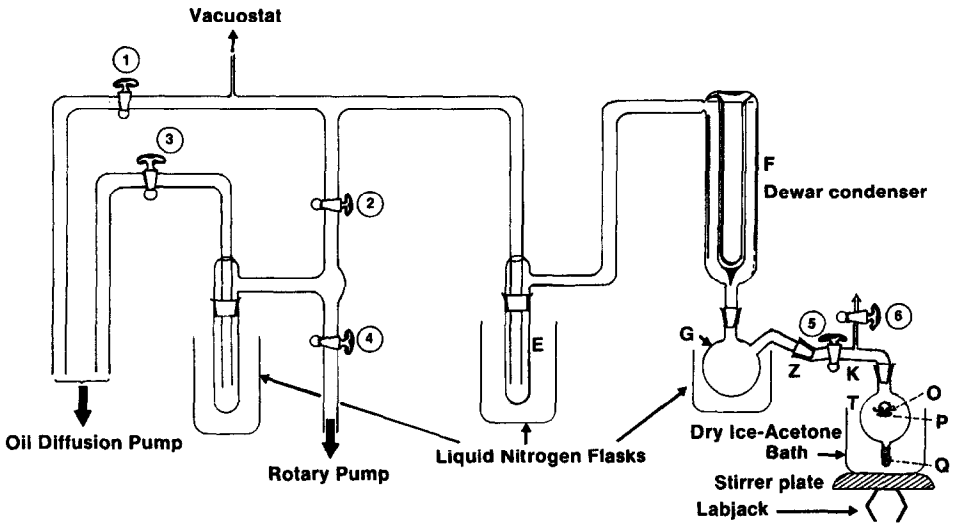

Fig. 2. Low-temperature, high-vacuum, concentrator apparatus used in the first stage of the enrichment procedure. E = Cold trap; F = cold finger of a Dewar condenser; G = distillation bulb; K = transfer tube; O = glass post supporting horseshoe magnet retriever; P = retrieved bar magnet; Q = micro bar magnet for stirring solutions in extruding bulb; T = solvent pot; 1–6 = taps.

designed to provide the desired configuration for performing the necessary distillation procedures. T was held at an initial angle of 45°C to the horizontal. The contents of T were initially frozen in liquid nitrogen and then maintained at −78.1°C by immersion into a dry ice–acetone slush bath. When the contents of T had melted, stopcock 5 was opened with stopcock 6 closed, and liquid nitrogen was poured onto the cold finger, F, and the dewar surrounding the distillation bulb, G, while vigorous stirring was maintained in T. Distillation was continued until visual inspection indicated that the volume of solution left in T was approximately 2 ml, at which time the apparatus was slowly rotated about joint, Z, downward into a vertical position while the adjustable laboratory jack was lowered so that the bulk of the solution in T was slowly transferred into U. The magnetic bar, P, was retrieved with a horseshoe magnet and harnessed in position by O. Vigorous stirring was still maintained in U with the micro magnetic bar, Q, and distillation was continued until the desired volume in U (100 μl) was attained.

The 100 μl concentrated extract in U was transferred into the reservoir bulb, R, (Fig. 3) with a cooled 500-μl syringe. R was cooled in liquid nitrogen in a dewar and connected to the main vacuum line through the adaptor, K, with stopcocks 5, 6 and 7 closed. R was connected to the air-jacketed tube, V (20 cm × 4 mm I.D.) which was maintained at a temperature of 55°C during the distillation, and the sample receiver, Y. With the contents of R still frozen, stopcocks 5, 7, 9 and 10 were opened while stopcocks 6, 11 and 12 were still closed and the system was evacuated. When a suitable vacuum (13–130 mPa) was attained in this system, stopcock 7 was closed and the contents of R were allowed to melt to dislodge any dissolved air. Stopcock 5 was then closed. Then Y was cooled in a liquid nitrogen dewar while the contents of R were allowed to warm up to 40°C (40°C water bath) and stirred vigorously with the

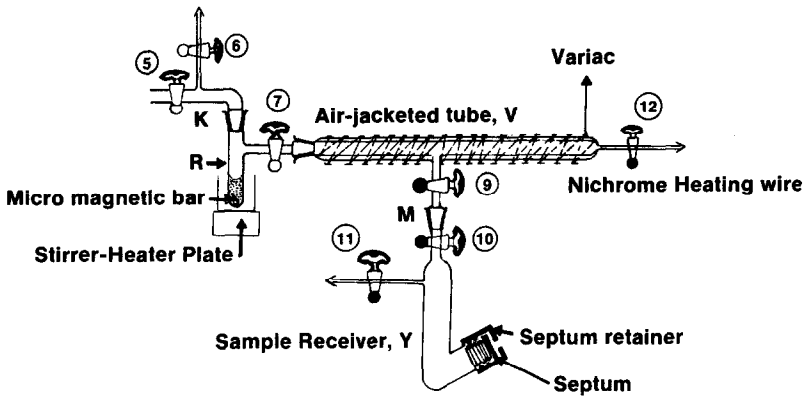

Fig. 3. Low-temperature, high-vacuum, concentrator apparatus used in the final stage of the enrichment procedure. K = Transfer tube; R = reservoir bulb; Y = sample receiver; 5–7, 9–12 = taps.

micromagnetic bar. Forty minutes after the commencement of this process, stopcock 10 was closed, all warming and stirring in R were discontinued. The water bath around R was replaced with liquid nitrogen in a dewar and any uncondensed vapors in V were thus condensed back into R. Stopcock 7 was closed and Y was disconnected from the system by opening stopcock 11. The contents of the reservoir bulb, R (25 μl) and the receiving trap, Y (75 μl) were then ready for analysis by capillary gas chromatography–mass spectrometry (GC–MS).

Wine analysis

A 95.0-ml amount of a 1972 red wine bottled by Chateau Pichon Lalande de Pauillac, France, were extracted in a batch mode for three hours with 250.0 ml of freon 11. The freon extract was enriched 2500-fold to 1000 μl using the low-temperature, high-vacuum concentrator (Fig. 2). The concentrated extract was treated with 100 μl 5% sodium bicarbonate and then 100 μl propylene glycol [14]. The separated freon fraction was concentrated further before analysis by GC–MS (Fig. 3).

Capillary GC analysis

For capillary GC analysis, the isolated and concentrated volatile flavor extract was spiked with a homologous series of *n*-alkanes (concentrations ranging from 1 to 10 ng/μl) and 20 ng/μl of acetophenone as internal standard. Sample sizes of 0.5 to 1.0 μl were injected into a 250°C split/splitless injector of a Varian 3700 GC system which housed either a 50 m × 0.25 mm I.D. fused-silica non-bonded Carbowax 20M (J & W Scientific), or a 30 m × 0.25 mm I.D. fused-silica non-bonded SE-54 capillary column (J & W Scientific), with the split valve closed. After 25 s, the split valve was opened to provide the desired flow of prepurified helium (30 cm/s, linear velocity) through the column while the GC oven was held at 50°C. The GC oven temperature was then programmed to 210°C at 4°C/min for the Carbowax column, 50–280°C for the SE-54 column, and held at the final temperature for 20 min. A microsample splitter, fitted at the end of the capillary column, provided a 1:1 split ratio of the chromatographic effluent to the flame ionization detection (FID) system and thermionic sensitive detection (TSD) system, also known as a nitrogen-specific

detection system, both held at 250°C. When the mass spectrometer (a double-focussing, high-resolution, magnetic sector instrument, MM70-70F, VG Analytical Altrincham, U.K.) served as the detector for the GC effluent, the following operating conditions were used: ion source temperature, 200°C; ionization voltage 70 eV; accelerating voltage, 4 kV; trap current, 100 μA; electron multiplier amplifier gain, 1; sensitivity, 10^{-7} A; response time 0.1 ms. The mass range was scanned repetitively in an exponential downscan mode at 1 s/decade at resolving powers of $\geqslant 1000$ (10% valley) to obtain electron impact (EI) mass spectra. To obtain chemical ionization (CI) mass spectra, the ion source temperature was held at 150°C, the source energy at 500 μA, and the electron energy at 50 eV. Isobutane was used as the reactant gas.

The concentration $C(X)$ of a component X in the aqueous alcoholic or wine solution was estimated from eqn. 1:

$$C(X) = A(X)/A(\mathrm{IS}) \cdot C(\mathrm{IS}) \cdot M(X)/M(\mathrm{IS}) \cdot \frac{1}{RWR} \cdot V(f)/V(i) \quad (1)$$

where $C(X)$ = concentration of component X in ng/μl of sample
$C(\mathrm{IS})$ = concentration of internal standard in ng/μl (*i.e.* 20 ng/μl)
$M(X)$, $M(\mathrm{IS})$ = molecular weights of component and internal standard
RWR = relative weight response factor
$V(i)$ = initial volume of sample taken for extraction
$V(f)$ = final volume of extract after enrichment
$A(X)$ = area under the selected ion retrieval (SIR) chromatogram for the identifying m/z value for component X
$A(\mathrm{IS})$ = area under the selected ion retrieval (SIR) chromatogram for the identifying m/z value for the internal standard.

RESULTS AND DISCUSSION

The solvent extraction and concentration apparatuses and their constituent

TABLE I

EXTRACTION EFFICIENCY OF THE SOLVENT EXTRACTOR FOR VOLATILE FLAVOR COMPONENTS IN 12% ETHANOL SOLUTION

Compound	Recovery ± S.D. (n=4) (%)		
	2 ppm	0.02 ppm	0.002 ppm
Ethyl hexanoate	99 ± 3	97 ± 3	96 ± 4
Ethyl 3-hydroxybutyrate	98 ± 7	97 ± 5	98 ± 6
Methyl furoate	98 ± 5	99 ± 4	95 ± 5
Diethyl succinate	99 ± 5	99 ± 7	97 ± 5
Hexanoic acid	97 ± 7	96 ± 4	96 ± 4
2-Phenethyl alcohol	100 ± 4	100 ± 6	98 ± 4
Octanoic acid	98 ± 3	99 ± 3	96 ± 4
Diethyl malate	100 ± 4	98 ± 4	98 ± 6
Methyl anthranilate	100 ± 3	99 ± 7	97 ± 4
Decanoic acid	96 ± 7	97 ± 4	97 ± 7

TABLE II

EFFICIENCY OF THE FRACTIONAL DISTILLATION COLUMN, S, IN PURIFYING FREON SOLVENT FOR RE-EXTRACTION

Compound	% (w/w) in distillate[a]
Ethyl hexanoate	0.2
Ethyl 3-hydroxybutyrate	nd[b]
Methyl furoate	nd
Diethyl succinate	0.5
Hexanoic acid	nd
2-Phenethyl alcohol	nd
Octanoic acid	nd
Diethyl malate	nd
Methyl anthranilate	0.8
Decanoic acid	nd

[a] Each component had an approximate concentration of 2 ppm in the solution.
[b] nd = Not detected.

TABLE III

EFFICIENCY OF RECOVERY AND REPRODUCIBILITY OF THE FIRST STAGE OF THE ENRICHMENT PROCEDURE

Compound	Recovery ± S.D. ($n=4$) (%)		
	Approx. concn. of each component		
	2 ppm	0.02 ppm	0.002 ppm
Ethyl hexanoate	98 ± 8	96 ± 7	97 ± 8
Ethyl 3-hydroxybutyrate	99 ± 8	93 ± 7	92 ± 4
Methyl furoate	98 ± 2	98 ± 3	97 ± 5
Diethyl succinate	98 ± 3	96 ± 4	98 ± 6
Hexanoic acid	99 ± 9	98 ± 3	98 ± 4
2-Phenethyl alcohol	100 ± 2	103 ± 5	101 ± 4
Octanoic acid	98 ± 8	96 ± 4	95 ± 5
Diethyl malate	100 ± 3	98 ± 4	97 ± 5
Methyl anthranilate	99 ± 8	99 ± 5	100 ± 6
Decanoic acid	97 ± 8	99 ± 6	93 ± 6

TABLE IV

EFFICIENCY OF THE SODIUM BICARBONATE TREATMENT IN REMOVING ORGANIC ACIDS FROM THE PARTIALLY CONCENTRATED FREON EXTRACT

Compound	Recovery obtained on the freon extract enriched 2500-fold (%)[a]
Ethyl hexanoate	98
Ethyl 3-hydroxybutyrate	96
Methyl furoate	99
Diethyl succinate	97
Hexanoic acid	nd[b]
2-Phenethyl alcohol	96
Octanoic acid	nd
Diethyl malate	97
Methyl anthranilate	98
Decanoic acid	nd

[a] Only 1 analysis was performed.
[b] nd = Not detected.

TABLE V

EFFICIENCY OF THE PROPYLENE GLYCOL TREATMENT IN REMOVING FUSEL ALCOHOLS FROM THE PARTIALLY CONCENTRATED FREON EXTRACT

Compound	Recovery (%)[a]
Ethyl hexanoate	98
1-Pentanol	1
1-Hexanol	1
Isoamyl hexanoate	97
1-Octanol	2
γ-Butyrolactone	95
Diethyl succinate	98
2-Phenethyl alcohol	1
Octanoic acid	96
Methyl anthranilate	98
Decanoic acid	98

[a] Only 1 analysis was performed.

TABLE VI

RECOVERIES AND DISTRIBUTION OF VOLATILE FLAVOR COMPONENTS DISTILLED INTO SAMPLE RECEIVER, Y, IN THE FINAL STAGE OF THE ENRICHMENT PROCEDURE

Compound (Mol. wt.)	B.p. (°C)	Recovery[a] ± S.D. ($n=4$) (%)
Ethyl hexanoate (144)	168	95 ± 4
1-Pentanol (88)	137	97 ± 3
Ethyl 2-hydroxyisopropionate (132)	150	96 ± 4
Amyl butyrate (158)	186	97 ± 6
Ethyl lactate (118)	160	95 ± 3
1-Hexanol (102)	158	93 ± 4
cis-3-Hexen-1-ol (100)	156	97 ± 5
cis-2-Hexen-1-ol (100)	158	93 ± 4
Acetic acid (60)	118	97 ± 6
Ethyl octanoate (172)	208	2.0 ± 0.1
Isoamyl hexanoate (186)	225	3.0 ± 0.4
2-Methylbutanoic acid (102)	176	95 ± 7
Hexanoic acid (130)	223	96 ± 3
Octanoic acid (144)	265	95 ± 4
Decanoic acid (172)	270	95 ± 6

[a] These values have been corrected for the degree of enrichment.

TABLE VII

RECOVERIES AND DISTRIBUTION OF VOLATILE FLAVOR COMPONENTS LEFT BEHIND IN RESERVOIR BULB, R, IN THE FINAL STAGE OF THE ENRICHMENT PROCEDURE

Compound (Mol. wt.)	B.p. (°C)	Recovery ± S.D. (n=4) (%)
Ethyl octanoate (172)	208	96 ± 4
Isoamyl hexanoate (186)	225	96 ± 7
Benzaldehyde (106)	178	89 ± 4
Ethyl 2-hydroxyisopropionate (132)	185	91 ± 5
1-Butyl lactate (146)	200	95 ± 3
1-Octanol (130)	195	89 ± 2
Methyl furoate (126)	181	92 ± 4
Isophorone (138)	215	95 ± 6
γ-Butyrolactone (86)	206	93 ± 4
Ethyl decanoate (200)	241	97 ± 6
Neral (152)	230	91 ± 4
Isoamyl octanoate (214)	270	97 ± 5
Diethyl succinate (174)	217	90 ± 4
Geranial (152)	229	92 ± 7
2-Phenethyl acetate (164)	233	98 ± 1
Ethyl laurate (228)	273	98 ± 6
2-Phenethyl alcohol (122)	219	89 ± 7
trans-Cinnamaldehyde (132)	253	88 ± 3
Diethyl malate (190)	253	91 ± 3
Methyl anthranilate (151)	256	96 ± 4
Ethyl anthranilate (165)	268	97 ± 3
Phthalide (134)	290	98 ± 7

TABLE VIII

ESTIMATION OF THE ACCURACY AND PRECISION OF THE DEVELOPED ANALYTICAL PROCEDURE AT A CONCENTRATION LEVEL OF 2000 ppb

Compound	Concentration of component in model solution (ppb)	Concentration of component found by experiment (mean[a] ± S.D., n=4) (ppb)	Deviation (%)
Ethyl hexanoate	1835	1740 ± 140	5.2
Ethyl 3-hydroxybutyrate	2205	2030 ± 120	7.9
Methyl furoate	2601	2360 ± 220	9.3
Diethyl succinate	2314	2080 ± 220	10.1
Hexanoic acid	2049	1850 ± 60	9.7
2-Phenethyl alcohol	2000	nd[b]	nd
Octanoic acid	1849	1720 ± 160	7.0
Diethyl malate	2517	2260 ± 190	10.2
Methyl athranilate	2594	2340 ± 150	9.8
Decanoic acid	2174	2000 ± 140	8.0

[a] These values refer to the experimentally determined concentrations of volatile flavor components in standard 12% ethanolic solutions following solvent extraction, enrichment and GC–MS analysis after correcting for the degree of enrichment.

[b] nd = Not detected. Alcohols were extracted into propylene glycol but this fraction was not analyzed.

TABLE IX

ESTIMATION OF THE ACCURACY AND PRECISION OF THE DEVELOPED ANALYTICAL PROCEDURE AT A CONCENTRATION LEVEL OF 2 ppb

Compound	Concentration of component in model solution (ppb)	Concentration of component found by experiment (mean ± S.D., $n=4$) (ppb)	Deviation (%)
Ethyl hexanoate	1.8	1.7 ± 0.1	5.6
Ethyl 3-hydroxybutyrate	2.2	2.0 ± 0.1	9.1
Methyl furoate	2.6	2.4 ± 0.3	7.7
Diethyl succinate	2.3	2.1 ± 0.2	8.7
Hexanoic acid	2.1	1.9 ± 0.1	9.5
2-Phenethyl alcohol	2.0	nd	nd
Octanoic acid	1.8	1.7 ± 0.2	5.6
Diethyl malate	2.5	2.3 ± 0.2	8.0
Methyl anthranilate	2.6	2.3 ± 0.2	11.5
Decanoic acid	2.2	2.0 ± 0.1	9.1

parts were evaluated using model solutions of selected wine flavor components in 12% aqueous ethanol. The results of these studies, presented in Tables I–X, demonstrated that this apparatus was highly efficient and reproducible and yields almost 100% recovery of volatiles present in wine at the ppb level. Volatile flavor compounds with boiling points lower than 190°C and the fatty acids generally distilled into the sample receiver, Y, while the higher boiling volatile flavor components were left behind in the reservoir bulb, R (Tables VI and VII). This selective partitioning of flavor components in the final stage of the concentration procedure made it very convenient to carry out GC–MS analysis on essentially two fractions of volatile flavor compounds. Fig. 4 shows a partial total ion chromatogram (TIC) of a 1-μl sample of concentrated and simplified volatile flavor extract (fraction from receiver, R) of the 1972 red wine injected onto a 50 m × 0.25 mm I.D. fused-silica Carbowax 20M capillary column coupled directly to a VG70-70F mass spectrometer source. A total of 260 flavor components were detected in this wine in this analysis, including 16 compounds that still remain to be identified, 15 compounds that have been assigned tentative identities that had not been previously reported as flavor components in this wine [15], and 226 compounds previously reported as flavor components in this wine and confirmed in this analysis. Three new compounds were identified in this wine, namely, ethyl 4-acetyloxybutyrate, 2-hydroxybenzothiazole and 2-methoxy-3-isobutylpyrazine (Table X).

The mass spectrum and retention indices measured on both Carbowax 20M and SE-54 matched those of an authentic sample of this compound obtained from Pyrazine Specialities (U.S.A.). Both Boidron *et al.* [12] and Bayonove *et al.* [13] provided no quantitative data on the amounts present in the grapes. Bayonove *et al.* [13] however predicted that the concentration in the grapes would be about 5 ppb, which is ten times the concentration we found in this wine. The lower concentration may explain why its presence in the wine could not be confirmed by previous investigators.

Subsequent to the publication of a Ph.D. thesis on this work in 1985 by Boison [15], and the presentation of this finding at a scientific meeting in 1986, Harris *et al.* [10]

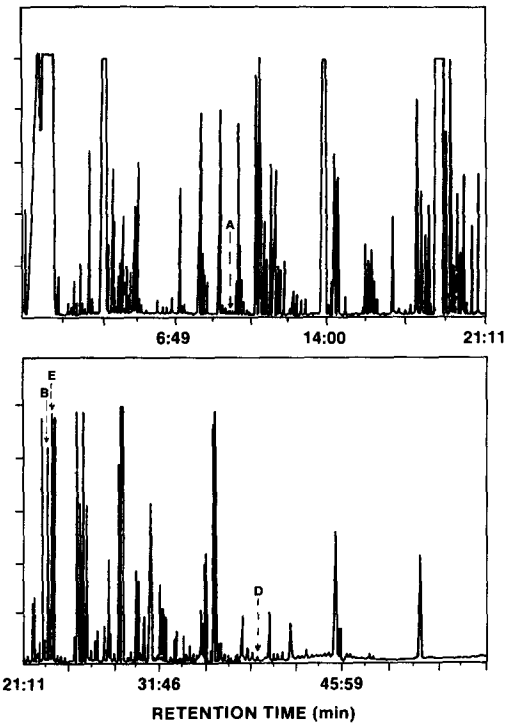

Fig. 4. Partial TIC chromatogram of the volatile flavor extract of Cabernet Sauvignon wine injected onto a 50 m × 0.25 mm I.D. Carbowax 20M fused-silica capillary column coupled directly to a VG70-70F mass spectrometer source. A = 2-Methoxy-3-isobutylpyrazine; B = ethyl 4-acetyloxybutyrate; D = 2-hydroxybenzothiazole; E = ethyl 2-acetyloxy-4-methylpentanoate.

TABLE X

QUANTITATIVE RESULTS AND RETENTION PARAMETERS OF NEW COMPOUNDS IDENTIFIED IN CABERNET SAUVIGNON WINE

	2-Methoxy-3-isobutyl pyrazine	Ethyl 4-acetyl-oxybutyrate	2-Hydroxy-benzothiazole
Mol. wt. confirmed by CI-MS	165	174	151
Odor threshold (ppb)	0.002[a]	—[b]	—[b]
Odor description	Herbaceous/ bell-pepper	Sweet/ mildly fragrant	Smokey/ nutty
Fragment ion monitored by SIR (*m/z*)	124	87	151
Experimental temperature-programmed retention index on Carbowax 20M	1473	2049	—
Experimental temperature-programmed retention index on SE-54	895	1591	1755
Concentration in wine (ppb) (mean ± S.D., $n=4$)	0.5 ± 0.07	476 ± 21	7.7 ± 0.6

[a] Ref. 6.
[b] Values not determined or listed in the literature.

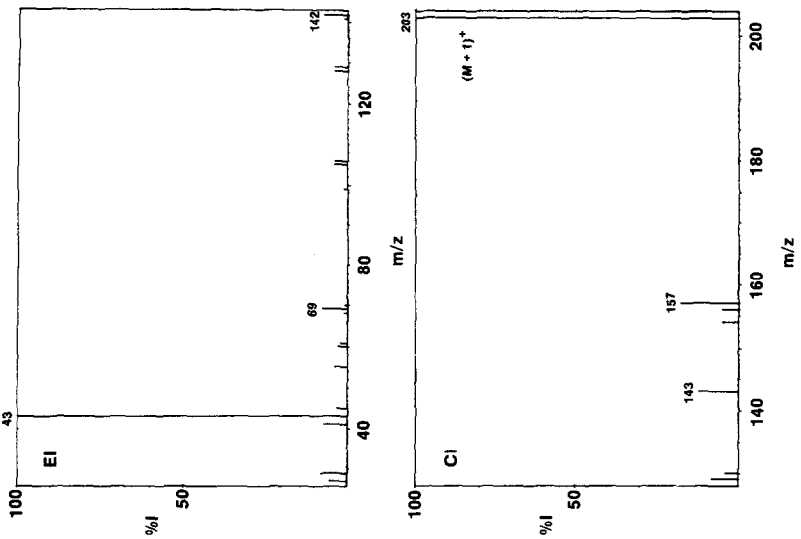

Fig. 5. EI and CI mass spectra of ethyl 4-acetyloxybutyrate. I = Ion intensity.

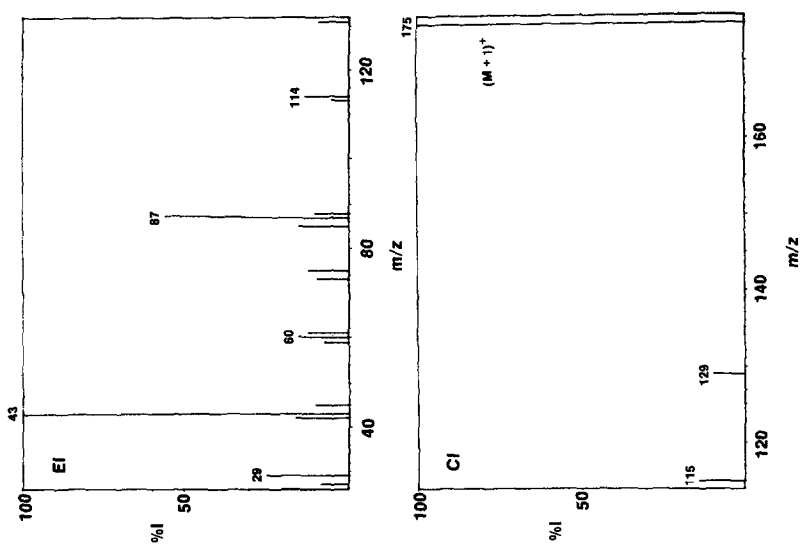

Fig. 6. EI and CI mass spectra of ethyl 2-acetyloxy-4-methylpentanoate.

have published some very interesting findings that have confirmed the finding that the organoleptically significant volatile flavor component, 2-methoxy-3-isobutylpyrazine, is present in Cabernet Sauvignon wines at trace level concentrations.

Fig. 5 shows the EI and CI mass spectra of ethyl 4-acetyloxybutyrate identified in Cabernet Sauvignon wine in this investigation. Retention and mass spectral parameters matched those of an authentic sample of this compound. The presence of another acetyloxy compound, ethyl 2-acetyloxy-4-methylpentanoate (Fig. 6), was established in this wine. There was no indication in the literature that it had been previously reported as a flavor constituent in Cabernet Sauvignon wine. These acetyloxy esters may be considered to be derivatives of the corresponding hydroxy esters arising through fermentation processes. The occurrence of ethyl 2-acetyloxy-4-methylpentanoate in Cabernet Sauvignon wine is of particular interest because it is widely believed that it exhibits a much stronger analgesic effect than acetylsalicylic acid [16]. The anaesthetic effect of the precursor of this ethyl ester, 4-hydroxybutanoic acid has already been discussed by Makoto and Hoshino [17].

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