

Volatile Phenol Determination in Wine

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Twelve volatile phenols were identified in a red wine manufactured and stored without any contact with wood and determined by using exchange chromatography, gas chromatography, and combined capillary GC-MS.

Many analytical, semiquantitative, or quantitative results on volatile compounds of wines or other fermented alcoholic beverages are now available, but only few of them are dealing with minor constituents such as volatile phenols. However, Williams (1974) and Tressl (1978) stressed the importance of volatile phenols in cider and beer aroma and suggested that phenols could contribute to beer off-flavors at too high levels. Dubois et al. (1971) identified volatile phenols in red wines and suggested that they should contribute to the overall aroma. Tanner (1972) studied an off-flavored wine contaminated by phenol and cresols. Schreier and Drawert (1977) studied acidic constituents in grape and wine aroma, and Dzhakhua et al. (1978) determined the volatile phenol composition of white wines prepared with different proportions of the stem in the fermenting medium. Singleton and Esau (1969), Singleton and Noble (1976), and Rapp et al. (1977) surveyed knowledge of volatile phenols in wine, and Maga (1978) compiled references about simple phenols and phenolic compounds in food flavors. These papers indicate that phenols arising from the alcoholysis of lignin could be important in the flavor of wood-aged alcoholic beverages but did not allow judgement to be made of the strict contribution of phenols resulting from microbiological pathways because no distinction could be made between phenols extracted from wood by alcohol and phenols produced from the juice during fermentation. We tried here to determine volatile phenolic compounds resulting from microbiological pathways in a wine manufactured and stored in inert tanks so that it has not been in contact with wood at any stage.

EXPERIMENTAL SECTION

Materials. A red wine of "Corbières" produced in 1976 by our institute at the experimental station of Pech Rouge with the following method was used. Grapes were stored for 8 days in an inert tank filled with CO₂ (>98%). After this period of anaerobic metabolism, grapes were crushed and the juice was allowed to ferment in inert tanks with the solid parts of the grape. Such a method gives a red wine in which the influences of wooden tanks are eliminated.

Most reference samples of organic compounds were obtained from reliable sources. Others were synthesized by established methods: 4-vinylphenol and 2-methoxy-4-vinylphenol by decarboxylation of the corresponding cinnamic acids (Sovish, 1959) and propiovanillone by a Fries reaction on phenylpropionate (Coulthard et al., 1930). All compounds were odorant pure as judged by the absence of any odor other than that from major compound when chromatographed with a sniffing technique.

Aroma Recovery. Wine (1.75 L), adjusted to 8% NaCl in order to avoid emulsion formation, was extracted for

48 h by 100 mL of Freon 11 in a continuous liquid-liquid extractor (Quickfit).

Isolation of the Acidic Fraction. Acidic materials were removed from the Freon extract by fivefold extraction using 40 mL of 5% sodium hydroxide solution. The aqueous basic solution was washed twice with 20 mL of dichloromethane and then carefully brought down to pH 1 with 20% HCl. The solution was then extracted 5 times with 40 mL of dichloromethane and the organic phase was dried over sodium sulfate. The acidic fraction thus isolated was slowly concentrated to 1 mL through a Vigreux column.

Isolation and Concentration of a Volatile Phenolic Fraction. Isolation of the volatile phenols from the acidic concentrate was performed by a DEAE-cellulose chromatography according to Zinkel and Rowe (1964). Lactones and phenols were eluted on a 1 × 10 cm column packed with 1.8 g of DEAE-cellulose (Serva; 0.62 mequiv/g) with 100 mL of an ether-methanol-water mixture (89:10:1). After dilution to 500 mL with water, phenols were extracted with 5 × 40 mL of CH₂Cl₂. The organic solution was concentrated to 1 mL through a Vigreux column. Quantitative analysis was carried out with addition of *n*-pentadecane as the internal standard.

Gas Chromatography and Mass Spectrometry. Gas chromatographic analyses of the volatile phenolic fractions were performed using a 40-m capillary column (0.3-mm i.d.) coated with FFAP. Temperature was programmed from 60 to 200 °C/min at 3 °C/min. The gas chromatograph (Girdel 3000) was equipped with a flame ionization detector. The quantitative results were obtained by using a LTT 9210 integrator. GC-MS identifications were performed by using a Varian M.A.T. Model CH5 mass spectrometer linked with a glass capillary column (0.8-mm i.d.) coated with FFAP or C 20 M via a helium Watson-Biemann glass separator: carrier gas flow rate (helium), 16 mL/min; injection port temperature, 250 °C; column temperature, from 60 to 200 °C at 3 °C/min; electron energy, 70 eV; electron current, 150 μA; ion source and separator temperature, 250 °C. Identification of components was made by comparison of retention time and mass spectral patterns of the unknowns with those of authentic compounds.

Quantitative Analysis. The determination of each phenol concentration is possible by using the equation

$$a_i = \frac{A_i}{A_m} \frac{m}{K_{extr_i} K_{resp_i}} \frac{1000}{V} \quad (1)$$

a_i = concentration in ppm of the phenol i in wine. A_i = area tabulated by the integrator for the chromatographic response of phenol i . A_m = area tabulated for the internal standard. m = mass in milligrams of the internal standard added before GC analysis. K_{extr_i} = extraction coefficient (including Freon 11 extraction, NaOH extraction, DEAE-cellulose chromatography, CH₂Cl₂ extraction, concentra-

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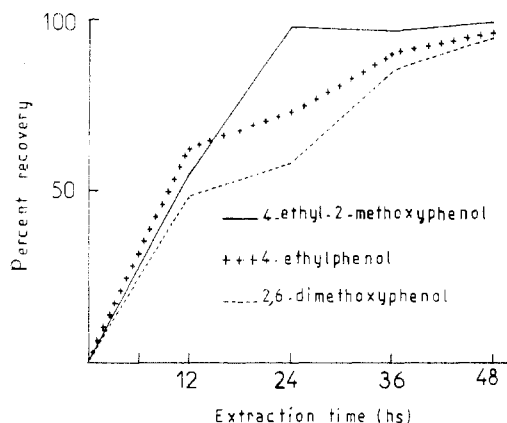


Figure 1. Extraction of 3 ppm range differently substituted phenols from water with Freon 11 in a continuous liquid-liquid extractor.

Table I. Identification and Recovery of Volatile Phenols in Corbières

GC peak	phenol	MS identification	retention time	Kextr, %
5	2-methoxyphenol	positive	+	106
8	phenol	positive	+	37
8	<i>o</i> -cresol	positive	+	102
9	4-ethyl-2-methoxyphenol	positive	+	87
10	2-ethylphenol	positive	+	109
11	<i>p</i> -cresol	positive	+	88
12	<i>m</i> -cresol	positive	+	88
13	4-ethylphenol	positive	+	103
14	2-methoxy-4-vinylphenol	positive	+	13
16	4-vinylphenol	positive	+	48
18	acetovanillone	positive	+	35
19	propiovanillone	positive	+	67

tion, and GC separation) for phenol i . K_{resp_i} = response coefficient for the phenol i of the flame ionization detector. V = volume of wine used (1.750 mL). This equation can only be solved if the value of $K_{resp_i}K_{extr_i}$ for each phenol is known. $K_{extr_i}K_{resp_i}$ was estimated from eq 1 after GC analysis of volatile phenolic fractions obtained with the above-described extraction method on model aqueous solutions containing 12% ethanol, 8% NaCl, and the studied phenols at known concentration ($a_i < 1$ ppm) and brought to pH 3.5 with citric acid.

Phenol and *o*-cresol were not separated on our capillary column. The phenol to *o*-cresol ratio was calculated by GC mass fragmentometry with the 94 fragment ion of phenol and the 108 fragment ion of *o*-cresol in the wine extract and in a synthetic mixture.

Validity of Quantitative Results. Experiments on wine and on the model solution were both repeated 5 times. The Student's t test as described by Steel and Torrie (1960) was used to calculate 95% confidence limits of $K_{extr_i}K_{resp_i}$ for the experiments on the model solution and of $A_i/A_m \times m(10^3/V)$ for the experiments on wine. Minimum and maximum a_i values were then calculated by using eq 1.

Sensory Evaluation. The duo-trio (Larmond, 1969) was adopted to assess simultaneously by smelling and tasting the difference between pure wine and the wine in which phenols identified were added at the average calculated concentrations. The test was performed once by each of 16 untrained judges. Each sample (50 mL) was allowed to reach room temperature in a wine glass covered with a watch glass before the test started. The panelists

Table II. Quantitative Results for Volatile Phenols in Corbières Wine. Estimation of the Precision of the Method

	95% confidence limits of $K_{extr_i}K_{resp_i}$		SE of mean	95% confidence limits of $(A_i/A_m) \times (10^3 m/V)$		a_i , ppm, = IV/I	a_i min, ppm, = V/III	a_i max, ppm, = VI/II
	min (II)	max (III)		min (V)	max (VI)			
2-methoxyphenol	0.55	0.75	6.34×10^{-4}	4.4×10^{-3}	5×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-2}
phenol	0.215	0.30	2.24×10^{-3}	1.3×10^{-2}	3×10^{-2}	6×10^{-4}	1×10^{-4}	1×10^{-1}
<i>o</i> -cresol	0.72	0.88	4.54×10^{-3}	2.6×10^{-2}	2×10^{-2}	4×10^{-4}	4×10^{-4}	5×10^{-2}
4-ethyl-2-methoxyphenol	0.545	0.66	1.55×10^{-3}	1.2×10^{-2}	1×10^{-2}	5×10^{-3}	5×10^{-3}	3×10^{-2}
2-ethylphenol	0.75	0.95	1.91×10^{-4}	1.4×10^{-3}	1×10^{-3}	4×10^{-4}	4×10^{-4}	3×10^{-3}
<i>p</i> -cresol	0.545	0.74	7.83×10^{-5}	9.7×10^{-4}	1×10^{-3}	7×10^{-4}	7×10^{-4}	3×10^{-3}
<i>m</i> -cresol	0.54	0.74	3.53×10^{-4}	3.7×10^{-3}	4×10^{-3}	2×10^{-3}	2×10^{-3}	1×10^{-2}
4-ethylphenol	0.705	0.87	3.33×10^{-4}	3.8×10^{-3}	5×10^{-3}	4×10^{-3}	4×10^{-3}	1×10^{-2}
2-methoxy-4-vinylphenol	0.075	0.13	2.43×10^{-4}	1.8×10^{-3}	1×10^{-2}	3×10^{-3}	3×10^{-3}	9×10^{-2}
4-vinylphenol	0.32	0.50	4.96×10^{-5}	4.1×10^{-4}	8×10^{-4}	3×10^{-4}	3×10^{-4}	3×10^{-3}
acetovanillone	0.25	0.35	6.53×10^{-3}	7.3×10^{-2}	2×10^{-1}	1×10^{-1}	1×10^{-1}	5×10^{-1}
propiovanillone	0.355	0.56	2.79×10^{-3}	2.9×10^{-2}	6×10^{-2}	2×10^{-2}	2×10^{-2}	2×10^{-1}

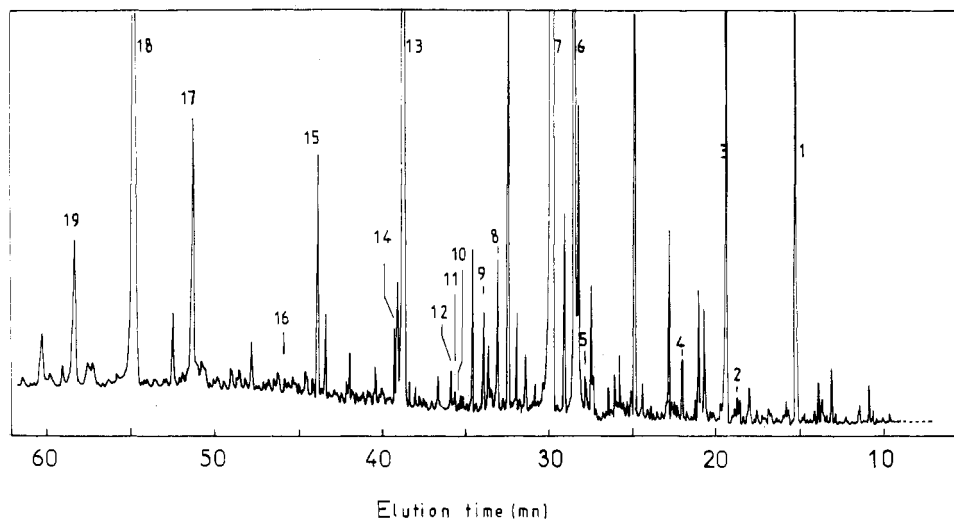


Figure 2. Analysis of a volatile phenolic fraction of Corbières by using a FFAP (30 m \times 0.3 mm i.d.) glass capillary column, temperature programmed from 60 to 200 °C at 3 °C/min. Peak 1, *n*-pentadecane; peak 2, γ -lactone; peak 3, γ -butyrolactone; peak 4, γ -lactone; peak 5, 2-methoxyphenol; peak 6, benzyl alcohol; peak 7, 2-phenylethanol; peak 8, phenol and *o*-cresol; peak 9, 4-ethyl-2-methoxyphenol; peak 10, 2-ethylphenol; peak 11, *p*-cresol; peak 12, *m*-cresol; peak 13, 4-ethylphenol; peak 14, 2-methoxy-4-vinylphenol; peak 15, phthalide; peak 16, 4-vinylphenol; peak 17, diethylphthalate; peak 18, acetovanillone; peak 19, propiovanillone.

were isolated during the test.

RESULTS AND DISCUSSION

Freon 11 was chosen for the continuous extraction of volatiles in order to avoid thermal degradation. In spite of its weak polarity, this solvent proved to have a good ability to recover volatile phenols differently substituted through continuous extraction (Figure 1).

Most authors usually split the aromatic extracts into acidic and phenolic fractions with NaOH and NaHCO₃ aqueous solutions. This method was not used here, as it gives a phenolic fraction highly contaminated with long-chain organic acids (C8; C10; C12), a consequence of the great difference in concentration between organic acids and volatile phenols in wines. An anionic chromatography of the acidic fraction as used by Nishimura and Masuda (1971) seemed more attractive to make possible the isolation of a phenolic fraction mostly constituted of phenols and devoid of acids. A chromatogram of this phenolic extract is shown in Figure 2.

Most of phenols identified here (Table I) have previously been reported in wine (Singleton, 1976; Van Straten, 1977), but 2-ethylphenol has only been isolated in oak-aged wine (Webb, 1972) and whisky (Van Straten, 1977). Propiovanillone was found in rum (Dubois and Brule, 1972) but not in wine.

The extraction efficiency was rather good for all identified phenols (Table I), if we consider the numerous steps involved in the isolation of the phenolic extract, but the yields were lower with phenol itself and the more conjugated substances. This may be explained by the relatively long elution times observed for these compounds during the exchange chromatography. An estimation of the precision of the method can be seen in Table II: a minimum and a maximum concentration value was calculated for each identified phenol from the 95% confidence limits obtained by the use of the Student's *t* test. As threshold values of volatile phenols in wine are not known, we cannot decide if they contribute to the overall aroma of the wine when present, for example, at the minimum calculated

concentrations. All we can say is that using the duo-trio test 14 panelists out of 16 succeeded in distinguishing between the pure wine and the same wine in which natural phenolic concentrations were doubled. This result is significant ($p < 0.01$).

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