Determination of Fixed Acids in Commercial Wines by Gas–Liquid Chromatography

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The polybasic acids in wines (succinic, fumaric, malic, tartaric, and citric) were separated from other solid material by precipitating the acids as the lead salts of the respective acids. Trimethylsilyl derivatives were formed directly from the precipitates and were identified and quantitated by vapor phase chromatography. It is interesting to note that blackberry, peach, pineapple, elderberry, loganberry, honey, and cherry wines did not contain tartaric acid in detectable amounts (10 mg/100 cm^3 of sample). The citric acid concentration in commercial wines in some cases accounted for approximately 90% of the total fixed acid content. Grape wine in all cases contained malic and tartaric acids and in most cases contained citric acid.

Customarily the separations of carboxylic acids by glc are preceded by the conversion of the acids to alkyl (usually methyl) esters. Since the esters are more volatile, they can be separated at lower temperatures. These esters are less strongly adsorbed than acids and give less tailing; therefore, they can be more accurately determined.

Phenols (Ismail, 1963; Langer *et al.*, 1958) were the first compounds to be separated by gas chromatography as their silyl derivatives. Silyl ester derivatives have been used for identification of the anomers of pentoses and hexoses by gas chromatography (Bentley *et al.*, 1963; Sweeley *et al.*, 1963; Martin and Eib, 1968). Flavenoids (Furuya, 1964) and inositols (Lee and Ballou, 1965) have also been converted to silyl derivatives for gas chromatography. The silyl ether derivatives of large complex molecules such as morphine (Brochmann-Hanssen and Svendsen, 1963; Martin and Swinehart, 1966) and steroids (Hammond and Leach, 1965) and mono- and diglycerides (Tallent *et al.*, 1966; Wood *et al.*, 1963) have been chromatographed. The use of silyl esters is a logical extension of this work to gas partition chromatography and the gas chromatography of trimethylsilyl esters of fatty acids (Birkofer and Donike, 1967; Dalgliesh *et al.*, 1967), amino acids (Mason and Smith, 1966; Ruhlmann and Giesecke, 1961), metabolic acids (Horii *et al.*, 1965), acids in wine (Brunelle *et al.*, 1967), acids in whiskey (Martin *et al.*, 1965), phenolic acids (Burkhard, 1957; Blakley, 1966), resin acids (Zinkel *et al.*, 1968), and fruit acids (Fernandez-Flores *et al.*, 1970).

The various reagents that are used to synthesize the alkyl esters do not react with alcohol groups such as those on hydroxyl acids. If, however, hydroxycarboxylic acids are combined with trimethylchlorosilane, both the acid and alcohol hydroxy groups react to give the trimethyl siloxy group. Therefore, the trimethylsilyl derivatives of hydroxycarboxylic acids are more volatile and less easily adsorbed than alkyl esters of hydroxycarboxylic acids. The silyl ether esters are superior to the methyl esters for gas chromatographic separations of hydroxycarboxylic acids (Martin and Swinehart, 1968).

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METHOD

Apparatus and Reagents. GAS CHROMATOGRAPH. A Hewlett-Packard Model 402 gas chromatograph equipped with a flame ionization detector was used for the analysis. This instrument was placed in tandem with a Hewlett-Packard Integrator Model 3370A. The glass column for the instrument was cleaned with acetone and ethanol, and then allowed to air dry. A 250-ml solution of a 5% dimethyldichlorosilane (DMCS) in benzene was passed through the column, after which it was allowed to air dry.

The glass column for the analysis was 6-ft long, 1/4-in. o.d., and was packed with a 3% OV-3 on Chromosorb W HP 80–100 mesh. The glass wool plugs were likewise treated with 5% DMCS in benzene.

The column was operated isothermally for 10 min at 118° C and then programmed at the rate of 3°/min to a temperature of 220° C. The detector block and injection port were operated at 250° C. Helium was used as the carrier gas at the rate of 40 ml/min.

Lead acetate buffer solution 8 g $Pb(OAc)_2 \cdot 3\ H_2O$ plus 1 ml of acetic acid, and the final solution *qs* to 100 ml with water.

Celite. Celite 545 (Fisher Chemical).

Sulfuric acid. 0.1 *N* sulfuric acid.

Glutaric acid. A 1-mg/ml solution of glutaric acid (Applied Science Laboratories, Inc., 99.9% purity) in a 50/50 ethanol water solution.

Standard acid solution. A 5×10^{-2} *M* solution each of succinic acid (Fisher Certified), fumaric acid (Eastman Organic Chemicals) 98% + purity, l malic acid (Eastman Organic Chemicals) 98% + purity, tartaric acid (Eastman Organic Chemicals) 98% + purity, and citric acid (Fisher Scientific) anhydrous certified grade were placed in a 50/50 ethanol water solution.

Table I. Total Acid Values by Titrimetric Method, Individual Acids by the Glc Procedure

Name	Gas Liquid Chromatography					Titrimetric		
	Milliequivalent				Mg/100 cc.	Mg/100 cc.		
	Succinic Acid	Malic Acid	Tartaric Acid	Citric Acid	Total Fixed Acid*	Fixed Acid*	Volatile Acid*	Total Acids*
Apple Wine	1.253	6.205		0.484	596.0	543.0	47.0	590.0
Blackberry Wine	0.627	1.193		4.950	508.0	579.0	81.0	660.0
Blackberry Wine	0.881	2.685		3.826	555.0	642.0	128.0	770.0
Chianti Wine	1.626	1.462	2.692	0.437	467.0	554.0	76.0	630.0
Concord Grape Wine	1.135	0.746	2.999		366.0	480.0	130.0	610.0
Danish Blackberry Wine	0.406	0.985	0.133	2.077	270.0	295.0	232.0	527.0
Danish Cherry Wine	0.745	6.862	1.346	0.250	691.0	673.0	66.0	739.0
Dry Red Wine I (Before Treatment)	0.932	0.149	4.544	0.281	518.0	533.0	108.0	641.0
Dry Red Wine II (Before Treatment)	0.982	0.149	4.598	0.281	451.0	545.0	96.0	641.0
Dry Red Wine III (Before Treatment)	1.745	Trace	3.505	0.531	434.0	580.0	134.0	714.0
Dry Red Wine IV (After Treatment)	1.423	Trace	3.691	0.562	426.0	557.2	166.8	724.0
Elderberry Wine	0.999	0.567		5.075	498.0	576.0	104.0	680.0
Grape Base Wine	0.999	0.627	2.559	Trace	314.0	341.0	71.0	412.0
Loganberry Wine	0.627	1.119		9.072	812.0	612.0	98.0	710.0
Muscatel Wine	0.881	1.298	1.626	1.499	398.0	296.0	74.0	370.0
Peach Wine		0.269		2.171	183.0	193.0	186.0	379.0
Polish Cherry Wine	1.135	1.566		5.434	610.0	658.0	150.0	808.0
Port Wine	0.406	1.477	2.132	0.312	325.0	332.0	108.0	440.0
Pure Cherry	0.745	3.058		2.030	438.0	508.0	92.0	600.0
Natural Grape Wine Base	0.999	0.507	3.252	0.484	393.0	377.0	110.0	487.0
Red Dry Wine	1.507	2.506	5.735		732.0	672.0	98.0	770.0
Straight Sherry Wine	1.762	0.627	2.132	0.968	412.0	304.0	86.0	390.0

* Expressed as Tartaric Acid

Pyridine. Pyridine (Eastman Organic Chemicals) spectrograde was dried for 48 hr over KOH granules.

Silylating reagent. Trimethylchlorosilane (TMS) and hexamethyldisilazane (HMS) were obtained from Applied Science Laboratories, Inc.

Ethanol water solution. 80% anhydrous U.S.P. and 20% distilled water.

Sample Preparation. A 2-ml aliquot of wine was placed in a 15-ml centrifuge tube, and 1 ml of lead acetate buffer solution, 10 mg of celite 545, and 1 ml of sulfuric acid were added in that sequence. The precipitate was shaken with 10 ml of ethanol water solution, after which it was centrifuged for 10 min. The tube was removed and the supernatant decanted. The mat was broken with a stirring rod, and a second 10-ml

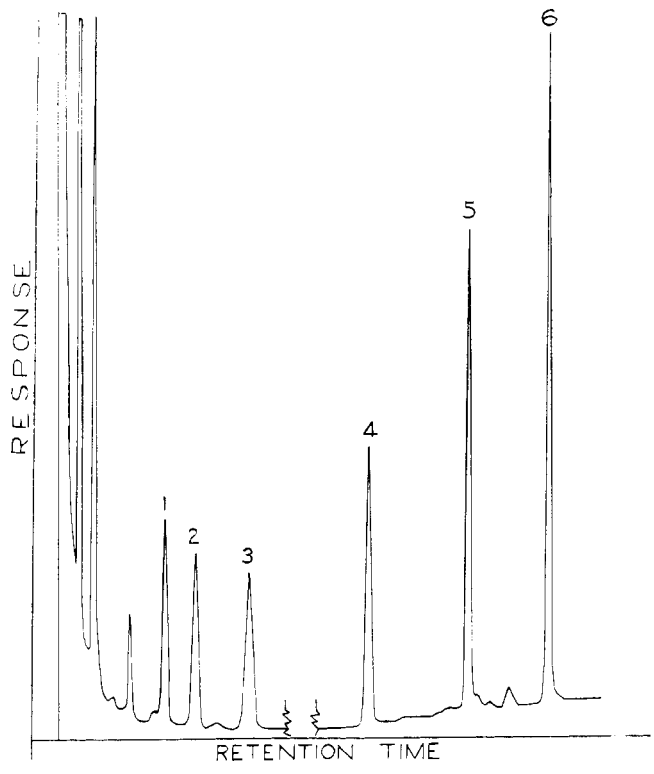

Figure 1. Chromatogram of a standard solution of acids: 1, succinic; 2, fumaric; 3, glutaric; 4, malic; 5, tartaric; 6, citric

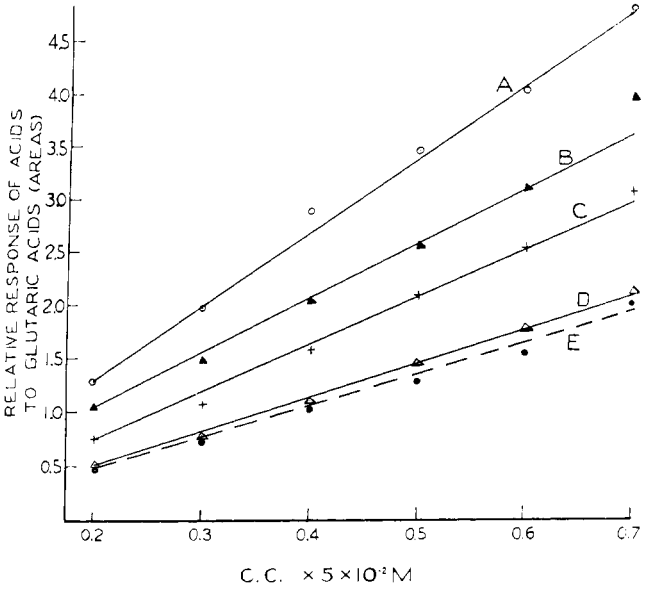

Figure 2. This gives the relative response of A, citric, B, malic, C, tartaric, D, succinic, and E, fumaric, to glutaric acids *vs.* cm³ molar concentration

ethanol–water solution was employed to wash the precipitate. The mixture was centrifuged for another 10 min, and again the supernatant was decanted. Boiling chips were added, along with 1.5 ml of glutaric acid solution, to the precipitate, and the mixture was placed in an oven for 12 hr at 105° C. Pyridine, 1.5 ml, was added to the mixture, which immediately was capped with a rubber septum and then placed in a sand bath for 24 hr at 137° C. The sample was removed from sand bath, and 0.5 ml of TMS and 0.5 ml of HMS were added. The resulting solution was allowed to stand at room temperature for 4 hr. A 2.5 μl sample was used for the chromatographic analysis.

Standard Solution. 0.2, 0.3, 0.4, 0.5, 0.6, and/or 0.7 ml of a 5×10^{-2} *M* solution of succinic, fumaric, malic, tartaric, and citric acids were placed in a 15-ml centrifuged tube, respectively. The standard was treated in the same manner as the sample solution.

Calculation. The amount of organic acids was calculated in the following manner:

$$\frac{Ao/Gs}{Astd/Gstd} \times Mg \times 50 = mg/100\ cm^3\ of\ sample$$

where Ao = area of organic acid, Gs is the area of glutaric acid in sample, Astd is the area of organic acid in standard solution, and Gstd is the area of glutaric acid in standard solution. A blank sample (before introduction of internal standard) was chromatographed to ascertain possible interference from the presence of glutaric acid or other compounds with similar retention times. The recovery of fixed acids by the glc procedure was compared with recovery by titrimetric methods. The amount of fixed acids found by titrimetric methods was determined as the difference between total acids and volatile acids.

The procedural modifications employed were as follows. The end point was determined by a Fisher Automatic Titrimeter at pH 8.4 instead of the visual point of phenolphthalein.

DISCUSSION

The di- and tricarboxylic acids used in the determination were succinic, fumaric, glutaric, malic, tartaric, and citric. The retention times of the various acids for the standard solution were noted and compared with those of the sample solution.

In prior work, an alkanoic acid (C_{11}) was used as an internal standard, but because of its high degree of sublimation and its monocarboxylic character, it necessitated the use of dicarboxylic or tricarboxylic acid not present in wines. Since glutaric acid was not found to occur naturally in any of the samples analyzed by glc it was selected as the internal standard of choice. The various acids were determined in wines, but further studies would be highly desirable as to varieties within a particular type of wine, *i.e.*, catawba grapes wine. Imported and domestic commercial samples were selected in order to provide a fair degree of diversification.

RESULTS

Figure 1 shows the various acids, namely succinic, fumaric, glutaric, malic, tartaric, and citric, as sharp symmetrical peaks 1, 2, 3, 4, 5, and 6, respectively.

Figure 2 gives linearity of response of molar concentration of acid to glutaric acid by peak areas. The acids fall into a sequence which is proportional to their molecular weights.

Table I gives data for blackberry, cherry, elderberry, apple, loganberry, peach, and various classes of grape wines. In many cases, the citric acid content accounts for 90% of the fixed acid content for the commercial wines. As was expected, the malic acid is found in apples, pineapple, blackberry, loganberry, cherry, grape, elderberry, and peach wines. Tartaric acid is found in wines of grape origin, but it is not found in apple, blackberry, elderberry, loganberry, cherry, or peach wines at detectable levels (10 mg/100 ml).

In most cases, the fixed acid values found by glc procedures are lower than titrimetric values.

Samples of Dry Red Wine I and II, as well as III and IV, show before and after cation exchange treatment. For this particular treatment, there appears to be no significant change in the malic, succinic, citric, or tartaric concentration.

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