Discussion

The separation of sodium, potassium, magnesium, and calcium on an Amberlite (H) column has been reported by Obara and Suzuki (*4*). The separation of iron and aluminum from each other, however, was not reported.

By employing essentially the same technique with Dowex 50W, X-12, 200- to 400-mesh, (H) form ion-exchange resins, the separation of the uni- and bivalent cations was found to be complete. The separation of iron and aluminum was largely complete. The unresolved portions, if any, were small and did not alter the absorbances by a significant amount.

The analytical scheme of Obara and Suzuki employs titration of the effluent acid from the column to determine sodium and potassium, complexometric titration with EDTA for magnesium and calcium, and two different colorimetric schemes for iron and aluminum.

To simplify the analyses, it was desirable to reduce the number of procedures involved. Sutton and Almy (*6*) used an indirect chloride titration to determine magnesium and calcium after removal of the uncombined chlorides by evaporation. This method was found to be applicable for the determination of sodium, potasium, magnesium, and calcium.

Molot and Kul'berg (*2*) reported the use of aluminon for the simultaneous determination of iron and aluminum. Iron was determined separately by the potassium thiocyanate method. It was found, however, that the suggested wave length of 530 mμ did not correspond to the absorbance maximum of either iron (550 mμ) or aluminum (525 mμ). By combining and modifying the procedures of Molot and Kul'berg (*2*), Obara and Suzuki (*4*), and Sutton and Almy (*6*), a simplified analytical scheme for all six cations was obtained.

Literature Cited

(1) Honig, P., *Proc. Tech. Sessions Bone Char* **1959** (Pub. 1961), 101.
(2) Molot, L. A., Kul'berg, L. M., *Uch. Zap. Saratovsk. Gos. Univ.* **42,** 79 (1955).
(3) Moritsugu, T., *Proc. Intern. Soc. Sugar-Cane Technologists* **1959** (Pub. 1960), 315.
(4) Obara, J., Suzuki, K., *Proc. Res. Soc. Japan Sugar Refineries' Technologists* **7,** 26 (1958).
(5) Powers, H. E. C., *Intern. Sugar J.* **61,** 17 (1959).
(6) Sutton, W. J., Almy, E. F., *J. Dairy Sci.* **36,** 1248 (1953).

Received for review March 5, 1962. Accepted December 17, 1962. Published with the approval of the Director as Paper No. 122 in the Journal Series of the Experiment Station of the Hawaiian Sugar Planters' Association, Honolulu, Hawaii.

GRAPE JUICE FLAVOR

Determination of Methyl Anthranilate in Grape Juice by Electron Affinity–Gas Chromatography

LEONARD R. MATTICK, WILLARD B. ROBINSON, LAVERNE D. WEIRS, and DAVID L. BARRY

New York State Agricultural Experiment Station, Cornell University, Geneva, N. Y.

A quick, accurate determination for methyl anthranilate in grape juice is presented. The method uses an extraction of the methyl anthranilate with benzene, separation by gas chromatography, and detection with an electron affinity detector. Recoveries of methyl anthranilate from grape juice averaged 98 ± 1.7%. A concentration of less than 0.1 p.p.m. methyl anthranilate can be determined. Comparison of the diazotization and gas chromatographic methods showed that the results of the diazotization method may be expected to be high relative to the gas chromatographic method by an amount between 0.04 and 0.11 p.p.m.

METHYL ANTHRANILATE has been considered a major flavoring constituent of Concord grape juice and is the basis for evaluating grape juice products. The presence and concentration of this compound is of importance to the processors of Concord juice and the plant breeders concerned with Concord-type grapes. The present method for determining methyl anthranilate in grape juice involves steam distillation, diazotization, and coupling with α-naphthol-2-sulfonate as described by A.O.A.C. (*1*) and the modification of Shaulis and Robinson (*7*). This method appears to be satisfactory for higher concentrations of methyl anthranilate, but is not particularly sensitive at lower concentrations. The method is empirical in the time requirements for the addition of reagents, color development, and collected volume.

Lovelock and Lipsky (*5*) recently described a gas chromatographic detector which is exceptionally sensitive to certain classes of compounds. This device has been designated as the election affinity detector. Lovelock (*4*) employed this method of detection for the determination of tetraethyl lead in gasoline, while Goodwin *et al.* (*3*), Clark (*2*), and Mattick *et al.* (*6*) applied this technique to the determination of residues of chlorinated hydrocarbons employed as pesticides. Methyl anthranilate showed a good response to this type of detection.

Materials and Methods

Gas Chromatographic System. A Barber-Colman Model 10 gas chromatographic instrument was employed in the analysis. The electrometer circuit was modified by the addition of a 9×10^{10} ohm "Victoreen" (type RX-1, Hi Meg) resistor. This resistor was placed in the circuit to change the sensitivity of the electrometer from 10^{-9} to 3×10^{-10} amp. full scale. The normal high voltage power supply was disconnected from the cell and replaced by a 67.5-

Table I. Recovery of Methyl Anthranilate from Grape Juice

P.P.M. Added	P.P.M. Recovered	Corrected P.P.M.	Per Cent Recovery
5.0	5.0	4.9	98
1.0	1.06	0.96	96
0.5	0.60	0.50	100
0.1	0.203	0.099	99
0.0	0.104	0.00	...

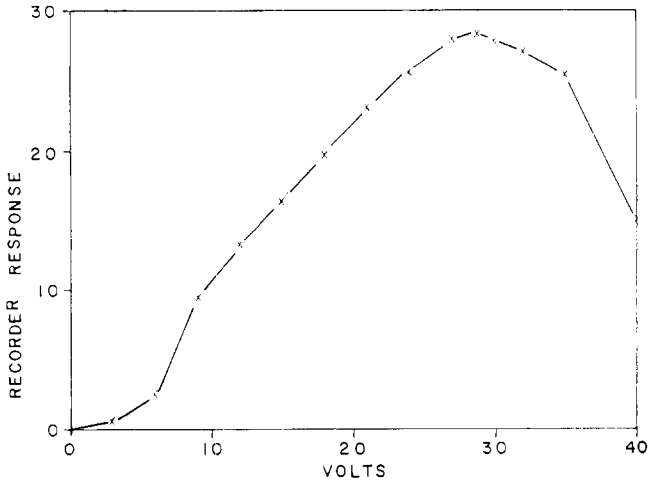

Figure 1. Effect of applied potential on the response of methyl anthranilate

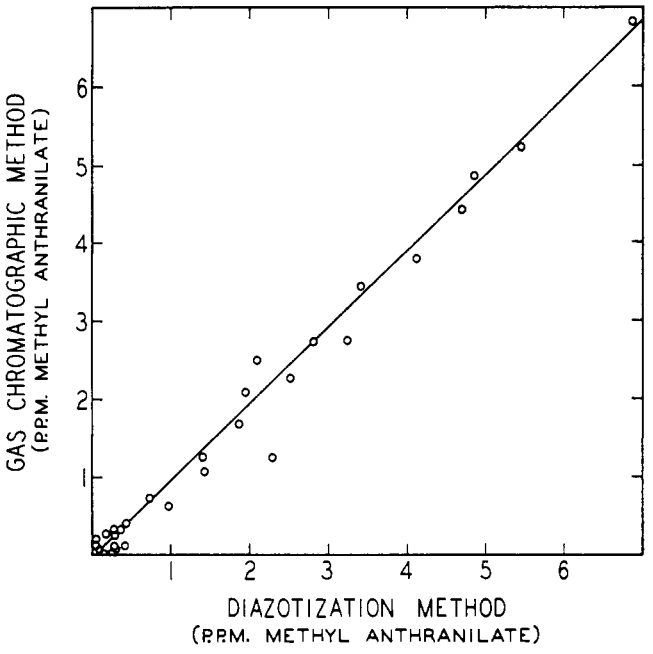

Figure 2. Comparison of the gas chromatographic method and the diazotization method for methyl anthranilate

[G. C. Method = 0.975 (diazo method) − 0.057]

volt, dry-cell battery with a wire-wound, 10,000-ohm potentiometer to vary the voltage to the detector between 0 and 67.5 volts. The battery was connected positive to the ground. The polarity switch of the electrometer was used in the positive rather than the usual negative position employed in β-ray detection.

The U-shaped column consisted of heavy walled, borosilicate glass tubing 5 mm. i.d. and 6 feet long. The partitioning medium was the ethyl acetate soluble fraction of Dow Corning high vacuum stopcock grease in a ratio of 1:5 on Chromosorb W 80–100 mesh. After packing, the column was preconditioned by heating to 230° C. with a flow rate of nitrogen at 60 ml. per minute. The column was baked until the bleed was at a minimum and at a constant rate. The progress was followed by employing the β-ray detection system. A voltage current curve (*9*) was run, and the column was considered conditioned when a well-defined plateau was evident between the voltages of 0 and 750 volts. The baking procedure usually took approximately 3 days for this substrate.

The operating parameters employed were: column temperature, 170° C.; cell temperature, 235° C.; flash heater, 265° C.; nitrogen pressure, 18 p.s.i.; flow rate of nitrogen, 60 ml. per minute.

The detector employed was the Barber-Colman Model No. A-4071 detector containing 56 microcuries of radium 226.

Optimum Voltage to the Detector. A 5-μl. sample of a benzene solution of methyl anthranilate (Eastman Organic Chemicals, No. 159) containing 1 μg. per ml. was injected into the gas chromatographic system. The voltage across the detector was varied betwen 0 and 40 volts by increments of 3 volts. Peak height measurements were determined and plotted against the voltage.

Recovery of Methyl Anthranilate from Grape Juice. By using the voltage across the detector which gave the maximum response to methyl anthranilate, a standard curve was constructed with a benzene solution of methyl anthranilate containing 1 μg. per ml.

A grape juice known to be low in methyl anthranilate was employed as a check sample. To this grape juice, 0, 0.1, 0.5, 1.0, and 5.0 p.p.m. methyl anthranilate were added. Five milliliters of grape juice were placed in a 12-ml. conical centrifuge tube. Five milliliters of benzene were added and the tube was stoppered and shaken for a period of approximately 30 seconds. The tube was then centrifuged in an International Chemical Centrifuge Model CL at 3450 r.p.m. for approximately 30 seconds. An aliquot between 1 and 10 μl. of the supernatant benzene layer was placed in the gas chromatographic system. The resulting peak height was compared to the standard curve and the p.p.m. methyl anthranilate calculated. The corrected p.p.m. methyl anthranilate was obtained by subtracting the value obtained from the check sample from the p.p.m. methyl anthranilate found in the sample.

Recoveries of added methyl anthranilate from a grape juice known to have a low concentration of methyl anthranilate can be seen in Table I. The range of recovery was between 96 and 100% with an average of 98%. Standard deviation for the recovery was calculated to be ± 1.7.

Comparison of the Gas Chromatographic Methods with the Diazotization Method for Methyl Anthranilate. Forty-seven samples of grape juice obtained from grape seedlings were analyzed for their methyl anthranilate concentration using both the gas chromatographic–electron affinity method and the method of Shaulis and Robinson (*7*).

Results and Discussion

The optimum voltage across the detector for maximum response to methyl anthranilate was found to be 29 volts (Figure 1). This optimum voltage was determined for this specific detector under the operating conditions described. A similar detector produced by the same manufacturer and bearing the same model number was subjected to the same determination. A similar applied voltage response curve resulted except the optimum voltage for this detector was 26 volts. The voltage to the detector for the compound in question will not only depend upon the electron affinity of the compound, but also on the shape of the detector, temperature, and pressure of the gas within the chamber (*5*).

Following the determination of the optimum voltage to the detector, a standard curve was constructed. The peak height was plotted against the micrograms of methyl anthranilate injected into the gas chromatographic system. A straight line relationship between 0 and 9×10^{-3} μg. resulted. The standard error for this relationship was calculated to be 2×10^{-4} μg. In this standard curve, the peak height was employed rather than the peak area. Retention time for the methyl anthranite

was 4 minutes. This resulted in a sharp peak.

A 10-μl. Hamilton microsyringe was employed in the injection of the samples. Over-injection occurred owing to the "flashing-off" of a portion of the solution in the syringe needle. This laboratory, as well as Goodwin *et al.* (*3*), has found that a standardized procedure makes this increment "flashing off" reproducible, so that allowance can be made when the syringe is filled to obtain an accurate aliquot.

The gas chromatographic method was compared with the diazotization method. Forty-seven samples of grape juice obtained from grape seedlings were analyzed by both methods, and results are shown in Figure 2. The range of concentration was between 0 and 6.8 p.p.m. The data were subjected to the student's t test (*8*). The value for t ($t_{92d.f.}$ = 4.368) was larger than the critical 1% value of t. It was concluded that the observed difference of means, 0.078 p.p.m. methyl anthranilate, is not reconcilable with the zero difference one would expect if both analyses have the same bias. These analyses show that the results of the diazotization method may be expected to be high relative to those of the gas chromatographic method by an amount between 0.04 and 0.11 p.p.m.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Methods of Analysis," 9th ed., p. 101, 1960.
(2) Clark, S. J., Abstracts, 140th Meeting ACS, Chicago, Ill., 1961.
(3) Goodwin, E. S., Goulden, R., Reynolds, J. G., *Analyst* **86,** 697 (1961).
(4) Lovelock, J. E., *Anal. Chem.* **33,** 1958 (1961).
(5) Lovelock, J. E., Lipsky, S. R., *J. Am. Chem. Soc.* **82,** 431 (1960).
(6) Mattick, L. R., Barry, D. L., Antenucci, F. M., Avens, A. W., J. AGR. FOOD CHEM. **11,** 54 (1963).
(7) Shaulis, N., Robinson, W. B., *Proc. Am. Soc. Hort. Sci.* **62,** 214 (1953).
(8) Snedecor, G. W., "Statistical Methods," 5th ed., p. 45, Iowa State College Press, Ames, Iowa, 1956.
(9) Wheelco Instrument Manual, "Model 10 Gas Chromatography Ionization Detection System," p. 29, Barber-Colman Co., Rockford, Ill., 1959.

Received for review June 15, 1962. Accepted August 29, 1962. Approved for publication by the Director of the New York State Agricultural Experiment Station, Cornell University, Geneva, N. Y.

MEAT FLAVOR

Components of the Flavor of Lamb

MARION JACOBSON and HELEN H. KOEHLER

Home Economics Experiment Station, Washington State University, Pullman, Wash.

Components of lamb flavor were studied in the volatile and water-soluble fractions. In the volatile materials from roasting lamb, carbonyl compounds were found to be important contributors to aroma. These components were further investigated by precipitation as 2,4-dinitrophenylhydrazones from vapors of simmering lamb. The 2,4-DNPHs were separated by column chromatography and characterized by infrared and melting-point analysis. Monocarbonyls present were identified as *n*-alkanals of two- to 10-carbon atoms, 2-alkanones of five- to 10-carbon atoms, and possibly 2-methylcyclopentanone. Polycarbonyls are undergoing fractionation and identification. Carbonyls collected from simmering lamb were saturated compounds. Water-soluble compounds of raw and cooked lamb were separated by dialysis and ion exchange. These included glucose, fructose, and inositol, and 19 amino-containing components. Among three breeds of sheep (Southdown, Hampshire, and Columbia), no differences were evident in analyses of volatile or soluble components.

RECENT STUDIES of the chemistry of meat flavor have included those of Pippen and coworkers (*19–21*) and Spencer (*22*) on chicken flavor; of Batzer *et al.* (*2*), Hornstein *et al.* (*11*), and Yueh and Strong (*25*) on beef; and of Hornstein and Crowe (*10*) and Witting and Schweigert (*24*) on pork. An abstract of a current study by Hornstein and Crowe (*9*) indicates that another report on the chemical nature of lamb flavor will be forthcoming.

The consumption of lamb and mutton has remained at a very low level. During the last 10 years, lamb has accounted for only 2.2 to 2.6% of the total meat used annually in the United States (*1*). The objective of this study was to determine the components of lamb flavor, the knowledge of which may suggest methods of making this meat more attractive to the consumer.

The experiments reported here were a part of a larger study including sensory evaluations of lamb varying in breed, age, sex, and feeding management (*12, 23*), and cookery methods (*7, 8*).

VOLATILE COMPONENTS FROM ROASTING LAMB

Experimental

Collection and Concentration. Methods of collection, concentration, and fractionation of the volatile components of roasting lamb were developed in this laboratory. The meat analyzed was obtained from the University flock of known breed and controlled management and feeding. Most of the meat came from animals 9 months of age. The ground samples were a mixture of the lean with its marbling and 1/4 inch or less of subcutaneous fat. The ground lamb was roasted for 7 hours under 20 inches of vacuum and at 80° C., the temperature of well-done lamb. Volatile materials were swept by air through specially constructed traps, two in ice-salt and four in dry ice–alcohol freezing mixtures (Figure 1). In each run, 7 pounds of meat were roasted in four pyrex pans. Condensates of all traps as collected from three runs, totaling about 2 liters, were concentrated for each analysis.