Gas Chromatographic Resolution of Nonvolatile Organic Acids in Peaches

Organic acids in peaches were analyzed by gas-liquid chromatography. The acids were extracted with methanol, isolated as lead salts, and regenerated with hydrogen sulfide. Dried acids were methylated with boron trifluoride–methanol reagent, and their methyl esters separated on DEGA and SE-52 columns using a temperature-programmed gas chromatograph equipped with a flame ionization detector. The acids were predominantly malic acid and citric acid. Succinic acid was tentatively identified, and was present in small amounts in peaches.

Organic acids in peaches have been extensively studied by research workers, but the statements regarding the component acids are often contradictory. Bigelow and Dunbar (1917) reported that probably only malic acid was present in peaches, as did Ito and Sakasegawa (1952). Nelson (1924) stated that peach acids consisted principally of a mixture of malic acid and citric acid in almost equal proportions. Nuccorini and Cerri (1930) found varying concentrations of malic, citric, and tartaric acids in peaches with tartaric acid predominating. In contrast, Schenker and Rieman (1953) have shown that citric acid was the predominant acid in peach juice, followed by malic acid and tartaric acid. Ryugo (1964), using the method of Schenker and Rieman, showed that malic acid was present in amounts greater than citric acid in ripe peaches, but no tartaric acid was detected. Other acids reported in peaches were quinic acid (David *et al.*, 1956), chlorogenic acid and its isomers (Corse, 1953; Sondheimer, 1958), D-galacturonic acid (Anet and Reynolds, 1955), and some unidentified acids.

The advent of gas-liquid chromatography (GLC) appeared to provide a powerful tool to study the peach acids anew. In recent years, a number of publications on the esterification of the Krebs cycle acids for analysis and identification by GLC have appeared; however, the application of this technique to fruits has been conspicuously scarce. Gee (1965) analyzed the organic acids of tomato powder as their methyl esters by GLC, but no details were given. Mazliak and Salsac (1965) reported the determination of organic acids in apples by GLC.

The purpose of the present study was to explore the gas chromatographic technique in the determination of the organic acids in peaches. This communication reports preliminary results that may stimulate further studies.

EXPERIMENTAL

Three varieties of peaches—Coronet, Southland, and Sullivan Elberta—grown at Fort Valley, Ga., at three stages of ripeness—shipping ripe, firm ripe, and soft ripe—were used. In addition, Coronet peaches at the shipping ripe stage were allowed to ripen off the tree at room temperature to provide firm ripe and soft ripe fruit for comparison. Commercial reagent grade organic acids were used as reference standards. Boron trifluoride–methanol reagent was obtained from Applied Science Laboratories, State College, Pa.

The procedure for isolation of organic acids consisted of mixing at least 10 peeled and pitted peaches at the same stage of ripeness with absolute methanol to give a final alcoholic concentration of 70%, macerating the mixture for 2 minutes in a Waring Blendor, and filtering the slurry to remove precipitated pectins and other insoluble solids. The acids in the extract were precipitated with lead acetate and centrifuged. The precipitates were washed free of sugars with 70% methanol, and the acids regenerated with H_2S. The above procedure was essentially that of the AOAC method of analysis (1960) for malic acid, citric acid, and tartaric acid in fruits. The acid solution was concentrated in a rotary vacuum evaporator to remove the H_2S and acetic acid, and a portion was further dried under vacuum at room temperature for GLC.

The organic acid standards and acids isolated from peaches were converted to their methyl esters using BF_3–methanol reagent, as described by Alcock (1965). About 10 to 20 mg. of a known acid or peach extractives were dissolved in 1 ml. of the methylating reagent, and the mixture was allowed to stand 16 hours in a small, tightly capped vial at room temperature. The reacted mixture was used directly for gas chromatography.

The gas chromatograph was an F & M Model 720 with a flame ionization detector. Two liquid phases on Diatoport-S (60- to 80-mesh) were packed in 6-foot by 5-mm. I.D. stainless steel columns: 20% DEGA (diethyleneglycol adipate polyester) and 3% SE-52. Conditions of operation for DEGA columns were: injection at column temperature at 155° C. for 2 minutes, then programmed at 7.5° C. per minute to 215° C.; for SE-52 columns, injection at 80° C. for 2 minutes, then programmed at 2.5° C. per minute to 225° C. Helium was used as carrier gas at a flow rate of 90 ml. per minute. Injection port temperature was 170° C., and detector temperature was 235° C. Quantitative measurements of the individual peach acids were made by comparing the peak heights or areas obtained with those of known amounts of the standard acids. Composition of the acids was determined in duplicate on each sample. Acid content was determined by titration to pH 8.2 with 0.1*N* NaOH.

RESULTS AND DISCUSSION

The GLC results of the retention times of acid standards and acids isolated from peaches on DEGA and SE-52 columns are shown in Table I. The peach acid mixture was resolved into four components on the DEGA column and three on SE-52. Considerable tailing, especially of the

Table I. Retention Time in Minutes of Methyl Esters of Known Organic Acids and Peach Acids on Two Columns

	20% DEGA[a]		3% SE-52[b]	
Compound	Known	Peach	Known	Peach
Fumaric	5.7	Absent	6.5	Absent
Succinic	6.7	6.7	7.0	7.0
Unknown	...	9.5	...	Absent
Malic	14.8	15.0	10.0	10.0
Tartaric	28.0	Absent	12.5	Absent
Citric	32.5	32.6	17.3	16.5

[a] 6-ft., 5-mm. I.D. columns; 60- to 80-mesh Diatoport-S; helium flow, 90 ml. per minute; column temperature, 155° C. for 2 minutes, then temp. program. 7.5° C./min. to 215° C. [b] 6-ft., 5-mm. I.D. columns; 60- to 80-mesh Diatoport-S; helium flow, 90 ml. per minute, column temperature, 80° C. for 2 minutes, then temp. program. 7.5° C./min. to 225° C.

Table II. Acid Content and GLC Acid Ratios in Three Peach Varieties at Different Stages of Maturity

Stage of Maturity	Titratable Acidity, Me/100 G.	Acid Ratios, % Total Acid		
		Succinic	Malic	Citric
	CORONET			
Shipping ripe	12.4	0.6	28.5	70.9
Firm ripe	11.8	0.9	54.0	45.1
Soft ripe	9.3	1.9	74.9	23.2
Firm (off-tree)	12.5	0.4	33.5	66.1
Soft (off-tree)	13.0	0.3	68.5	31.2
	SOUTHLAND			
Shipping ripe	15.9	0.8	37.5	61.7
Firm ripe	15.2	0.7	50.7	48.6
Soft ripe	12.9	0.7	63.6	35.6
	SULLIVAN ELBERTA			
Shipping ripe	15.3	0.9	41.1	58.0
Firm ripe	14.3	1.5	44.2	54.3
Soft ripe	11.0	1.3	52.5	46.2

malic peak, was observed when SE-52 was used. Consequently, quantitative measurements of the acid composition of the 11 samples of peaches were made using the DEGA column (Table II). The degrees of methylation of organic acids with BF_3–methanol were variously reported as complete or almost complete (Alcock, 1965; Kuksis and Prioreschi, 1967) and the yield of esters was assumed to be the same for every acid in the present studies. The data confirmed previous observations that the major acids of peaches are malic and citric acid. Minute amounts of an unidentified compound from the peach extractives appeared on the DEGA column at a retention time of 9.5 minutes but did not show up on the SE-52 columns. This peak at 9.5 minutes is therefore not necessarily an ester, but could be caused by an impurity or side product of the reaction mixture. A small amount of succinic acid was found in all peach samples. This acid was tentatively identified based on retention times on two GLC columns. Other analytical techniques such as infrared and mass spectroscopy would be useful for an unequivocal identification.

Tartaric acid was not detected in any sample, and was probably absent in peaches. Not all the organic acids reported to be present in peaches are determined by the present GLC method. For example, quinic acid and galacturonic acid, if present, were not amendable to analysis by the present procedure because of their highly hydroxylated nature. Mazliak and Salsac (1965) were not able to demonstrate the presence of quinic acid and shikimic acid in apples using similar GLC techniques. However, suitable columns and suitable derivatives such as trimethylsilyl ethers may be used to advantage.

Although statistical analyses were not attempted in the present studies, the quantitative results indicated that citric acid was predominant at the shipping ripe stage, and that the concentration decreased through firm ripe and soft ripe stages, while the reverse was noted for malic acid. For Coronet peaches ripened off the tree, the same relationship appeared, although to a lesser extent than in the fruit ripened on the tree.

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