THE NONVOLATILE ACIDS OF CONCORD GRAPE JUICE

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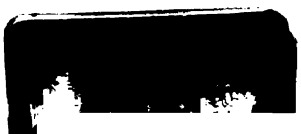

ABSTRACT

THE NONVOLATILE ACIDS OF CONCORD GRAPE JUICE

by Anton Amon

Juice and juice concentrate from Concord grapes (Vitis labrusca) of the 1967 season, grown in Michigan, were analyzed for nonvolatile acids. The following acids were identified by ion exchange column chromatography, paper chromatography, thin-layer chromatography and special tests: aspartic, citric, fumaric, galacturonic, glucuronic, glutamic, glyceric, lactic, malic, oxalic, phosphoric, succinic and tartaric. All except the amino acids, aspartic and glutamic, were determined quantitatively by titration after separation. Malic was the dominant acid followed by tartaric. These two acids accounted for approximately 90% of the total acidity of Concord grape juice.

In grape juice concentrate produced in 1966 and stored for one year at 38°F two more acids were identified: glycolic and alpha-pyrrolidonecarboxylic. No glyceric acid was detected in this concentrate.

The malic acid to tartaric acid ratio was 1:0.98 in the juice concentrate of 1967, but 1:0.75 in the year-old concentrate. This is due to the settling of potassium hydrogen tartrate (cream of tartar) during storage.

THE NONVOLATILE ACIDS OF CONCORD GRAPE JUICE

By

Anton Amon

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INTRODUCTION

The cells of higher plants, in contrast with those of other organisms contain relatively large amounts of organic acids. Their occurence is widespread, and they play a central role in cellular metabolism. These acids are early products of photosynthesis and serve as precursors for the synthesis of other compounds. They may act as growth regulators or germination inhibitors (61, 90). Their close metabolic relationship to fats, carbohydrates, and proteins emphasizes their key role in plants. The presence and importance of the Kreb's cycle in mature plant tissues remains still doubtful, although a number of workers have shown the functioning of this cycle in certain seedlings (13).

Early investigations have been primarily concerned with the organic acids present in large amounts. The acids present in small quantities were only determined in case of special interest. However, the minor acids may play an important role in the overall metabolism of the tissues.

From the practical point of view, the acids are important because they significantly contribute to the taste of foods, they participate in the enzymatic and non-enzymatic browning reactions (43, 53) and they may cause off-flavors (56, 73, 77).

The object of this study was to identify and quantitatively determine as many as possible of the nonvolatile acids of grape juice, to assess the effect of concentrating on the acid composition of

this juice and to study the change in the acids in the concentrate after one year storage.

This research was conducted on Concord grapes, not only because Michigan is a principal area of the United States in the growing of this variety of grapes, but also because no other grape type has rivalled the Concord variety for grape juice quality. The balance of sugars, acids, flavoring substances and astringend characteristics of this grape result in a very palatable and wholly satisfactory juice (85).

REVIEW OF LITERATURE

A. The acids of grapes

The acids of the European type grapes (Vitis vinifera) have been studied very extensively. There are few studies, however, on the acids of the American type grapes (V. labrusca).

1. The acids of V. vinifera

Charles (16) separated the organic acids of leaves, petioles, shoots, stalks, and berries of European grapes and presented quantitative results for malic, tartaric, citric, succinic, fumaric, glyceric, quinic, and shikimic acids. Davtyan (19) analyzed 44 varieties of grapes and confirmed by chromatography the presence of tartaric, mostly in the form of potassium tartrate (0.70-1.25%), malic, citric, oxalic, succinic and fumaric. Quantitative and qualitative determination of organic acids in grape juice was carried out by Colagrande (17); the following acids were reported: fumaric, succinic, oxalsuccinic, isocitric, glycolic, oxalic, mandelic, malic, citric, cis-and trans-aconitic, maleic, and tartaric. Alquier-Bouffard et al. (5) reported on the citric acid content of grapevine and its variations. Kushida (48) found succinic, lactic, malic, citric and tartaric acid in Japanese grapes and wines.

Changes in acids during ripening of the grape (6, 32, 3, 31, 27, 47, 83) and the influence of environment on the metabolism of organic acids (45, 55) have been discussed.

The storage life of grape juice held at -5.5 to -2.5°F was

extended for several months by addition of small quantities of organic acids (66). Chemical changes, including acid changes, in wine grapes during storage were pointed out by Dzheneev (24).

Amerine (1) mentioned in his review that Semichon and Flanzy (79) verified earlier work that glyoxylic acid is a constituent of grapes. Ventre (87) found up to 0.13% glucuronic and 1.0% gluconic acid in musts of diseased grapes. Sound vintages contained none or no more than 0.03% of glucuronic acid. Rodopulo (75) reported 0.0085% oxalic acid in must and 0.0087% in the wine. Peynaud and Charpentiè (68) developed a colorimetric procedure for glucuronic acid. In a review about the inorganic constituents of wines Amerine (2) showed that the phosphorus content in grapes increased during maturation to 150-350 mg per liter.

Hale (35) indicated that the berry is an important site of synthesis of organic acids. He obsrved that the acid content of the berry increases until cell division ceases.

Comparative studies of tartaric, succinic, citric, oxalic, and gluconic acids were carried out on white wine grapes by Ivanov (41). Chlorogenic and isochlorogenic acids were found to be polyphenols typical of grape juice (37). Nechaev (62) studied the accumulation of sugars and the changes in acidity of six commercial varieties of grapes during three consecutive years (62).

The importance of the relative proportions of acids and sugars in the determination of the desirability of grapes was discussed by Shoemaker (80) and Caldwell (15). Delmas et al. studied the acids

of grape leaves (21, 22) and found that 90-95% of the leaf acidity is due to tartaric, maleic and oxalic acids.

Statistical tables on pH and titratable acidity of grape juice are given by Baker (8).

Deibner et al. (20) separated the dinitrophenylhydrazones of the ketonic acids, alpha-ketoglutaric and pyruvic, in grape juice by cellulose thin-layer chromatography. Popper (71) wrote about ion exchange treatment of Thompson seedless grape juice. Succinic acid in grape juice was determined by a manometric method (60), in which succinic dehydrogenase was used. Mayer et al. (59) described an enzymatic determination of malic acid in wine and grape juice, which is based on the dehydrogenation of L-malic acid and the corresponding reduction of diphosphopyridine nucleotide. The tartaric acid content of grape juices and wine was also determined polarographically (88). Saulnier-Blache (76) determined the esters formed during stabilization of grape juices with boiling alcohol.

2. The acids of V. labrusca

Hartman (36) reported the acid content of Concord grape juice to be as follows: tartaric, 1.07%; malic, 0.31%; citric, 0.02%.

Analytical data for Concord grape juice after treatment with various resins were given by Zubeckis (96). The changes in malic and tartaric acids in New York grown Concord grapes over a period of years were studied by Robinson (74).

Biochemical studies of grapes grown in Minnesota were undertaken by Barnes (9).

The acidity of grapes has generally been reported either as titratable acidity (29, 30) or as total acidity (29, 46, 4) or as bound acidity (29, 67, 69).

B. Methods of qualitative and quantitative determination of organic acids

Much of the early work on the occurence of organic acids in fruit was unreliable because the acids were not usually isolated and the analytical methods lacked specificity. In the relatively few cases where acids were isolated, the methods used were of low sensitivity. Nelson (63) successfully applied the ester distillation technique to the study of fruit acids, but this method is unsatisfactory for esters of low volatility or for the investigation of minor acids. Curl and Nelson (18) isolated citric, isocitric, and malic acids by destillation of their ethyl esters and characterized them as hydrazides. Later, Pollard et al. (70) and Prigot and Pollard (71) identified organic acids as their piperazonium salts. Mixtures of dicarboxylic acids were also separated by preparing their amides or imides which were then distilled (10, 23).

Oxalic acid was isolated and identified by crystallographic methods after precipitation as calcium oxalate.

The uronic acids, specially galacturonic and glucuronic, have been separated by anion exchange column chromatography (49) and determined on thin silica gel layers with naphthoresorcinol solution as a specific detecting reagent (65). D-galacturonic acid can also

be determined colorimetrically with 2-thiobarbituric acid (95). Uronic acids have also been separated by circular chromatography (84), and using anthrone (38).

Lactic acid has been determined spectrophotometrically (28). Highvoltage paper electrophoresis has been applied to the study of organic acids (34). Following Isherwood's introduction (40) of column chromatography for the separation of organic acids on silica gel, the method has been adapted and modified by a number of investigators(12). Columns containing alumina-Al_2O_3- (33) and cellulose (89) have also been used for the separation of organic acids.

Gas chromatography is widely used to determine volatile acids(42,51).

Paper chromatography has become a very popular procedure for the separation of various substances in mixtures and biological fluids. This method was first applied to the separation of organic acids by Lugg and Overell (54). One- and two-dimensional chromatography has been used to determine mono- and dibasic acids (58), di- and tri-carboxylic acids (81), and mixture of organic acids (93, 97).

Recently organic acids have been separated by means of elution chromatography using ion-exchange resins (64). Anion exchangers were used for the separation of organic anions from cations and uncharged molecules present in biological extracts (50). Ion exchange resins have been used by many investigators (14, 78, 91, 39) to determine fruit acids.

MATERIALS AND METHODS

Approximately twenty kilograms of Concord grapes were collected randomly from the fruit which arrived at the A. F. Murch Company in Paw Paw, Michigan on October 6, 1967. From the same day's 70° Brix grape juice concentrate four eight ounce cans were taken. Four eight ounce cans of the 1966 pack that had been stored at 38°F were also obtained. The fresh grapes were removed from the stems, washed, and pressed by hand through four folds of cheesecloth. The filtrate was mixed and two fifty-gram aliquots were removed for acid analysis. The contents of the four juice concentrate cans for each year were mixed separately and two twentyfive-gram aliquots were removed for acid analysis.

The principal analytical procedure used in this work is a modification of a previously described (39, 57) ion exchange column chromatographic method.

A. Preparation of the acid concentrate

To 50 grams of the fresh unconcentrated grape juice 120 ml 95% ethanol were added, and to 25 grams of juice concentrate 70 ml of 95% ethanol, for the precipitation of the pectins. The mixture was blended in a Waring Blendor for three minutes at high speed. It was then cooled to room temperature, filtered through sharkskin paper, and the residue washed thoroughly with 50-60 ml of 80% ethanol. Filtrate and washings were combined and concentrated to 60-80 ml in vacuo in a flash evaporator, with the water bath at 40°C.

The concentrated grape filtrate was applied on a water washed Dowex 50W-X8 cation exchange resin (H^+form, 200-400 mesh) column, 15 cm long by 0.7 cm in diameter. The organic acids were eluted by means of water. The column retained the amino acids and cations. The eluate from the column was concentrated in the flash evaporator to a volume of 40-60 ml, and aliquots were titrated with 0.1N NaOH using phenolphthalein as an indicator to determine the acid content. Sample portions corresponding to a total acidity of 1.0 milli-equivalent (meq.) were used for fractionation. The rest of the acid extract was stored at -10°F.

B. Column chromatography

Dowex 1-X8 acetate, anion exchange resin, 200-400 mesh was used for fractionating the mixture of the acids on a column 32 cm long and 0.7 cm in diameter. The commercial Dowex 1-X8 (chloride form) was converted to the acetate form by passing 1N sodium acetate solution through the column until the effluent was free from chloride as tested with silver nitrate. The resin in the column was then washed with 0.1N acetic acid to remove the excess sodium acetate. Finally the resin was washed with demineralized water.

The sample containing one meq. of acidity was applied to the column and 20 ml water were passed through the column to wash out the sugars. Then, the column was connected to the concentration gradient elution system. The elution system consisted of an eluant reservoir, a mixing flask, and a pressure regulator. A 250 ml separator funnel, to the tip of which was fused a capillary glass tube reaching close

to the bottom of the mixing flask served as the reservoir for the eluant. A 125 ml suction flask, the side arm of which was connected to the column by means of a capillary tube and tygon sleeves was used as a mixing vessel. An air pressure regulator (Moore Products Co., Philadelphia 24, Penna.) was connected to the separatory funnel by means of a rubber tubing to keep the pressure in the system at 160 inches of water. Mixing was accomplished by a Teflon-coated magnet in the flask and stirring by a magnetic stirrer beneath the flask. The level of the liquid in the mixing flask was kept just above the side arm. The first eluant consisted of 100 ml 3N acetic acid, the second of 50 ml 6N acetic acid and the third of 300 ml 6N formic acid. One hundred ten fractions of 4.0 ml volume were collected using an automatic fraction collector (Rinco Instruments, Greenville, Illinois).

The fractions were dried in a vacuum oven at 40°C. For the quantitative determination the fractions were redissolved in hot water and titrated with 0.02N NaOH, using phenolphthalein as indicator. The fractions corresponding to each peak were pooled, treated with Dowex 50W-X8 cation exchange resin to remove the sodium, filtered, and dried for qualitative determination. For the determination of unknown peaks, 22 known acids were passed and eluted through the anion exchange column to determine the effluent volume of each and the order of their emergence. To obtain sharper peaks only 6 acids were passed through the column at a time. Percent recoveries of the known acids were also calculated.

C. Paper chromatography

The dried fractions were dissolved in 80% ethanol and chromatographed on Whatman No. 1 paper (46 cm x 57 cm). The spots were placed 2.5 cm apart and 7.0 cm away from the long edge of the paper. Twenty-eight known acids were spotted in between the unknown fractions from grape. two different solvents, one acidic and one alkaline, were used: (a) n-Butanol and 3N formic acid, 1:1 by volume; (b) ethanol, ammonium hydroxide and water, 20:1:4 by volume (11). The lower phase of the mixture (a) or the mixture (b) was used for vapor equilibration. The spotted papers were irrigated descendingly by the upper phase of the mixture (a) or, the mixture (b) for 20 and 12 hours respectively at a temperature of 22° ± 0.5°C. At the end of this time the solvent front was marked and the papers were dried in an air draft for at least 2 hours and sprayed with a 0.04% solution of bromphenolblue (BPB-Na salt) in 95% ethanol. The acids showed as yellow spots against a blue background.

The Rf values were determined. The grape acids were tentatively identified with the known acids on the basis of having similar Rf values.

Thin layer chromatography (82) and spot tests or color reactions were used for the various acids to confirm the identity of these acids.

Pure acids were obtained from commercial sources for the identification of the acids from grape and to determine the quantitative recovery using column chromatography.

D. Confirming tests

1. Lactic acid: Test with p-hydroxydiphenyl and sulfuric acid

0.5 ml of test solution and 1 ml concentrated sulfuric acid were treated for two minutes in a dry test tube in a water bath at 85°C and cooled to 28°C. A pinch of p-hydroxydiphenyl (p-phenylphenol) was added, the mixture was swirled several times and allowed to stand for 10-30 minutes. A violet color appeared and indicated the presence of lactic acid. Limit of identification: 1.5 γ lactic acid (26).

2. Galacturonic acid: A drop of saturated basic lead acetate was placed on a test spot on a paper and heated for one minute on life steam. Brick red color appeared in the presence of galacturonic acid.

3. Glyceric acid: Test with naphthoresorcinol and sulfuric acid

0.5-1 ml of test solution was heated with 0.75 ml concentrated sulfuric acid containing naphthoresorcinol (1 mg/10 ml), for 30-50 minutes in a water bath at 90°C. A blue color appeared in the presence of 10 γ or more of the acid (26).

4. Glucuronic acid: Test with naphthoresorcinol

Same test as for glyceric acid. A yellow color with a greenish fluorescence indicated the presence of glucuronic acid in the test solution (26).

5. Glycolic acid test with 2,7-dihydroxynaphthalene (2,7-naphthalenediol) and sufuric acid

A mixture of test solution (0.5-1.0 ml) and 2 ml of concentrated sulfuric acid containing 2,7-naphthalenediol (1 mg/10 ml) was heated for 10-15 minutes in a water bath. A red-violet to red appeared

according to the amount of glycolic acid present. At least 0.2 γ glycolic acid are necessary to give this color reaction (26).

6. Tartaric acid: Test with β , β '-dinaphthol and sulfuric acid 0.5-1.0 ml of the test solution was treated with a little solid β , β '-dinaphthol in concentrated sulfuric acid and heated for half an hour in a water bath at 85°C. When tartaric acid was present, a luminous green fluorescence gradually appeared during the heating and deepened on cooling. As little as 10 γ of this acid can be detected(26).

7. Phosphoric acid: Test with ammonium molybdate and benzidine A drop of the test solution was placed on quantitative filter paper, followed by a drop of molybdate and a drop of benzidine solution. Then the paper was held over ammonia. When most of the free mineralized acid has been neutralized, a blue stain appeared.

Ammonium molybdate solution: 5 g salt in 100 ml water and poured into 35 ml nitric acid (sp.gr.=1.2)

Benzidine solution: 0.05 g base in 10 ml concentrated acetic acid, diluted with water to 100 ml (25).

For the determination of the degree Brix an Abbé refractometer was used.

RESULTS AND DISCUSSION

A. Fresh grape juice and grape juice concentrate 1967 harvest:

1. Qualitative analysis: The following acids in order of their emergence from the anion exchange column were identified in the grape juice and its concentrate of the 1967 season: glutamic, aspartic, lactic, galacturonic, glucuronic, glyceric, succinic, malic, tartaric, citric, fumaric and phosphoric (Figure 1). The citric acid titration line appears as a shoulder of the tartaric acid peak, although these two acids separated well when they were tested alone in small quantities (Figure 2).

The fraction containing tartaric and citric acids were subsequently subjected to paper chromatography and silica gel chromatography (94). Both of these methods separated citric and tartaric well as shown in Table 1 and Figure 3. The silica gel chromatography also showed the absence of isocitric acid since citric and isocitric are not separable by the Dowex 1-X8 and paper chromatography methods used in this work.

The identity of the acids was established as follows:

(a) The effluent volumen (Figure 1) from the Dowex 1-X8 column chromatography was the same with those of the known acids.

(b) The Rf values of all of the acids which were paper chromatographed with two different solvents after they had been separated by column chromatography agreed with those of known acids (Table 1).

(c) The Rf values of glutamic, aspartic, galacturonic, glucuronic,

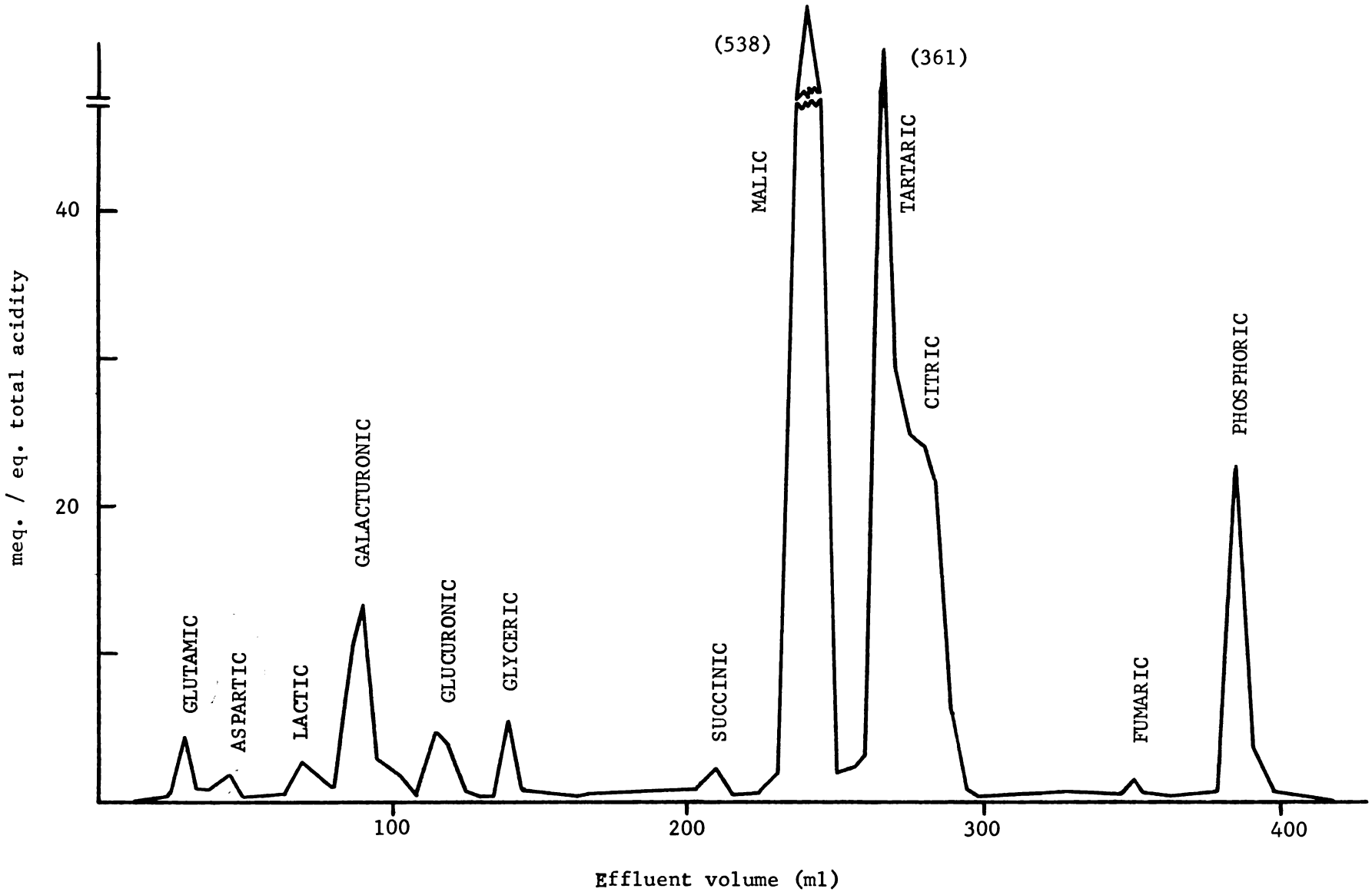

FIGURE 1: TITRATION OF COLUMN CHROMATOGRAPHIC FRACTIONS OF ACIDS OF CONCORD GRAPE JUICE (17.8° BRIX)

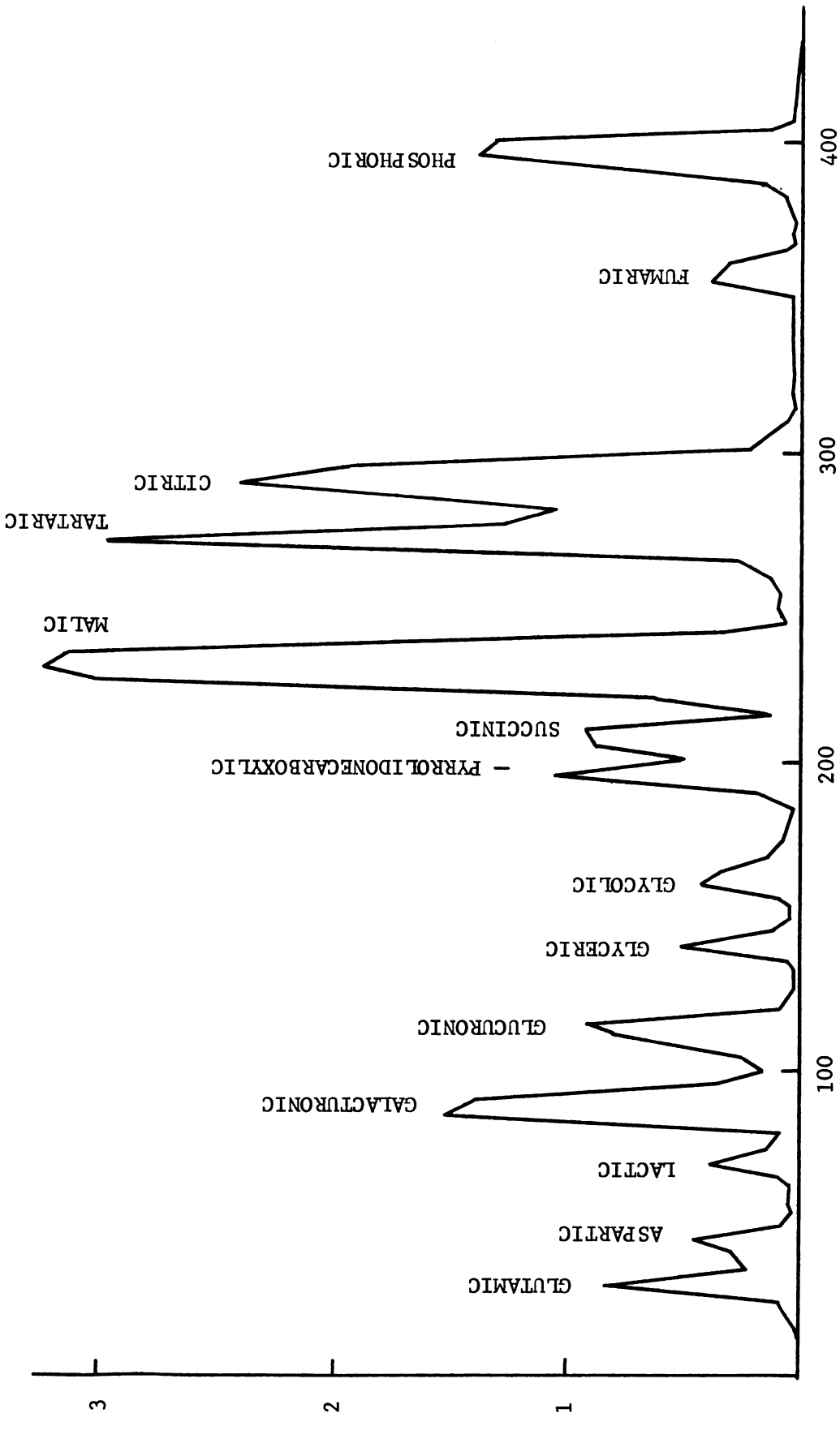

FIGURE 2: FLOWING CHROMATOGRAM OF PURE ACIDS

TABLE 1. Rf values of grape acids chromatographed on paper after fractionation by column chromatography and percent recovery of these acids from Dowex resin columns.

	Rf x 100 values				% Recovery	
	Solvent 1 (a)		Solvent 2 (b)			
ACIDS	K	Unk	K	Unk	Dowex 1-X8	Dowex 50W-X8
Glutamic	11	11	36	36	89	11
Aspartic	9	10	26	25	91	8
Lactic	70	71	80	79	100	100
Galacturonic	16	16	40	40	88	100
Glucuronic	14	13	45	44	82	100
Glyceric	45	44	72	70	100	100
Succinic	70	72	52	54	100	100
Malic	53	53	44	44	100	100
Tartaric	33	34	14	15	97	100
Citric	47	46	22	22	100	100
Fumaric	84	84	50	51	100	100
Phosphoric	36	38	6	6	100	100

K = Known or pure acid

Unk = Unknown from grape

Solvent 1 (a) n-butanol + 3N Formic acid, 1:1

Solvent 2 (b) Ethanol (96%) + ammonium hydroxide (25%) + water, 20:1:4

Whatman #1 paper, descending run, with either solvent

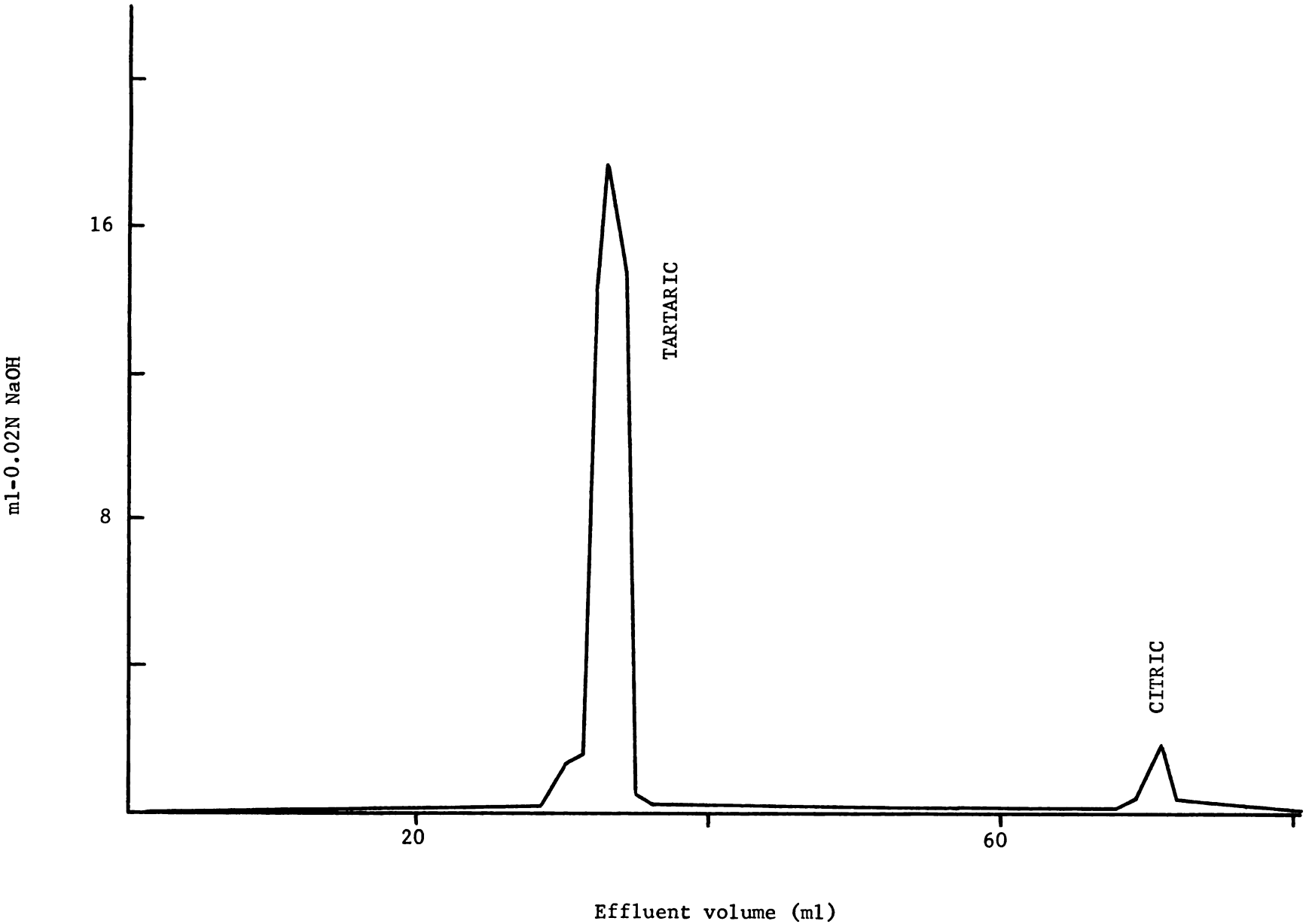

FIGURE 3: SEPARATION OF THE TARTARIC-CITRIC ACID PEAK ON SILICA GEL COLUMN

succinic, tartaric, citric and phosphoric acids which were chromatographed on thin-layer after they had been separated by column chromatography agreed with those of known acids (Table 2); the remaining acids did not chromatograph well on thin-layer.

(d) The spot tests (25, 26) which were carried out for galacturonic, glucuronic, glyceric, lactic, tartaric and phosphoric acid were all positive.

2. Quantitative analysis:

The results of a quantitative analysis of the acids present in Concord grape juice is graphically presented in Figure 1.

The variuos fractions were titrated for total acidity and paper chromatograms of the titrated fractions were prepared for confirmation of the identity of the acids in each fraction. Solutions of pure acids (of the acids identified in grapes) were prepared and divided into two equal aliquots. One aliquot was titrated directly and the other put through the column and then titrated. Recovery experiments were first conducted to establish the extent to which the acids identified as present in Concord grape juice were not altered quantitatively by passing them through the Dowex 1-X8 and Dowex 50W-X8 columns. The results of this study are presented in Table 1. All the acids, except the amino acids, glutamic and aspartic acid, were recovered 100% from the Dowex 50W-X8 column. The amphoteric character of these amino acids may account for the low recoveries. Since the solution was acidic, the dissociation of the carboxylic groups of glutamic and aspartic acids was suppressed and the amino group was present

TABLE 2. Rf values of grape juice acids chromatographed on thin-layer after fractionation by column chromatography.

	Rf x 100 values					
	Solvent (a)		Solvent (b)		Solvent (c)	
ACIDS	K	Unk	K	Unk	K	Unk
Glutamic	-	-	63	61	-	-
Aspartic	-	-	55	55	-	-
Galacturonic	-		-	-	34	35
Glucuronic	-	-	-	-	49	50
Succinic	30	30	-	-	-	-
Tartaric	8	7	-	-	-	-
Citric	5	5	-	-	-	-
Phosphoric	0	0	-	-	-	-

K = Known or pure acids

Unk = Unknown from grape juice

Solvent (a) Ethanol (96%) + water + ammonium hydroxide (25%), 100:12:16

Solvent (b) Ethanol (96%) + water, 70:30

Solvent (c) Benzene + glacial acetic acid + methanol, 20:20:60

Kieselgel-G (according to Stahl) ascending run, with solvents (a) & (b)

Kieselgel-G with boric acid (according to Stahl), ascending run with solvent (c).

in its positive charged form, which resulted in binding the molecule partially to the cation exchange resin. The recoveries of these amino acids were not quantitative from the Dowex 1-X8 column either. For these reasons it was decided not to include quantitative estimations of the aspartic and glutamic acids of Concord grape juice in this study. For the remaining acids the proper recovery adjustments were made before their concentrations in the juice and the juice concentrate were reported.

The citric and tartaric acids were titrated after the fractions obtained from the Dowex 1-X8 chromatography were rechromatographed on silica gel column.

For the quantitative determination of the acids, the titer values (ml of alkali used), under each peak, were added together and the necessary correction from the recoveries was applied. Since the pK values of all acids were below 8.5, all carboxyls were titrated with NaOH using phenolphthalein as an indicator. The phosphoric acid titration values were multiplied by 3/2 because only two-thirds of this acid is titrated (57) with phenolphthalein as an indicator.

After the corrections were made for the recoveries, the volume of base used for the titration of each acid was converted to milli-equivalents (meq.) of acidity. This gave the meq. of acidity of each acid in the total acidity applied on the column. From this, the meq. of each acid per 100 g of grape juice was calculated. Using this last step and the equivalent weights of the acids, mg of each acid per 100 g of grape juice was obtained (Table 3).

TABLE 3. Nonvolatile acid content of hand-expressed juice and commercial juice concentrate of Concord grapes.

Calculated as mg/100g juice, 17.8° Brix		
ACIDS	Juice	Concentrate
Lactic	5.5	5.3
Galacturonic	55.0	54.0
Glucuronic	20.6	21.0
Glyceric	12.3	12.1
Succinic	2.8	2.8
Malic	746.2	857.4
Tartaric	639.8	844.0
Citric	39.7	44.2
Fumaric	2.2	2.2
Phosphoric	23.2	22.8
Oxalic	1.8	1.8

The total acidity (free and bound) was determined by titration to ph 8.1 after removal of all cations by Dowex 50-X8. It is expressed as grams of malic acid per 100 g of grape juice and is given in Table 4. Refractometry indicated that the total soluble solid content of the hand expressed grape juice was 17.8°Brix and that of the concentrated 70.0°Brix. To facilitate the comparison, the content in acids of the grape concentrate was recalculated to a 17.8°Brix level (Table 4).

Both the total acidity and the concentration of malic, tartaric and citric acids are greater in the juice concentrate than in the fresh juice at equal degrees Brix. The reason for the discrepancy is probably due to the hot-pressing method, which had been used in the juice processing plant, whereas the grapes were extracted in the laboratory by hand, without application of heat. It is known that the external layers of fruits contain less acidity than the deeper ones (86) and by low pressure more juice is extracted from the superficial layers than the deeper ones.

Grapes are very high in pectic substances. The most likely way of formation of free galacturonic acid in fruit is the enzymatic degradation of pectin, which would require the presence of both pectin esterase and polygalacturonase (52). Both of these enzymes can be produced by microorganisms and pectin esterase is widely distributed in higher plants (52, 44). This would give an explanation for the relative high galacturonic acid content found (Table 3).

TABLE 4. Effect of processing on the total acidity of Concord grapes.

PRODUCT	Total acidity (free and bound) as g malic acid/100 g grape juice 17.8° Brix
Grapes	1.67
Grape Concentrate	2.12
Increase	0.45

TABLE 5. Rf values of grape acids chromatographed on paper after fractionation by column chromatography and percent recovery of these acids from Dowex resin columns.

	Rf x 100 values				% Recovery	% Recovery
	Solvent 1 (a)		Solvent 2 (b)		Dowex 1-X8	Dowex 50W-X8
ACIDS	K	Unk	K	Unk		
Glycolic	57	59	65	65	100	100
α-Pyrrolidone-carboxylic	53	53	60	62	80	100

(a) n-butanol + 3N Formic acid, 1:1

(b) Ethanol (96%) + ammonium hydroxide (25%) + water, 20:1:4

Whatman #1 paper, descending run with either solvent

B. Grape juice concentrate (70°Brix)-1966 harvest:

This juice concentrate had been stored for one year at 38°F.

The same procedure was used for qualitative and quantitative analysis as described for fresh grape juice and grape juice concentrate of the 1967 harvest.

The following acids in order of their emergence from the anion exchange column have been identified: glutamic, aspartic, lactic, galacturonic, glucuronic, glycolic, α-pyrrolidonecarboxylic, succinic, malic, tartaric, citric, fumaric, and phosphoric. Oxalic acid was determined by Baker's method (7), modified by Markakis et al.(57).

Two new acids were identified in the stored juice concentrate: glycolic and α-pyrrolidonecarboxylic acid. Glyceric acid was not present in the stored concentrate, although it was detected in the fresh juice and the concentrate which was not stored.

Table 5 shows the Rf values of the two new acids chromatographed on paper and the recovery of these acids from Dowex 1-X8 and Dowex 50W-X8 columns.

The mg of each acid per 100 g of grape juice, 17.8°Brix, corresponding to the 1966 concentrate are presented in Table 6. The malic to tartaric acid ratio was found to be 1:0.98 in the juice concentrate from 1967, whereas it occured in the relationship 1:0.75 in the stored juice concentrate. This appears to be due to the settling of excess cream of tartar, acid potassium tartrate, $KH(C_4H_4O_6)$, during storage. After storage a portion of the crystallized tartrates were centrifuged out before filling the cans. This resulted in the lower

TABLE 6. Nonvolatile acid content of Concord grape juice concentrate (harvest, 1966) after one year storage.

ACIDS	Expressed as mg/100 g grape juice 17.8° Brix
Lactic	4.3
Galacturonic	68.8
Glucuronic	14.1
Glycolic	6.2
ℓ-Pyrrolidonecarboxylic	11.9
Succinic	3.6
Malic	625.0
Tartaric	467.2
Citric	28.3
Fumaric	3.8
Phosphoric	12.5
Oxalic	1.4

tartaric acid content.

The occurence of α-pyrrolidonecarboxylic acid (PCA) has been observed previously in several processed fruits and vegetables (56, 77). In the cases investigated it has been shown to originate from glutamine

Since no PCA was detected in the processed fresh juice concentrate of the 1967 harvest, while it was present in the stored juice concentrate of the 1966 harvest, it may be assumed that this compound is formed in Concord grape juice concentrate either after long storage (one year) or its precursor varies in concentration from year to year. Further research will be necessary to provide a definite answer.

It is difficult to discuss the differences in glycolic, glyceric and other acids of grapes in biochemical or physiological terms, since so little is known about the acid metabolism in fruits genarally (92).

Climacteric conditions may play an important role, too.

SUMMARY AND CONCLUSIONS

1. Gradient elution ion exchange column chromatography, paper chromatography, silica gel chromatography, spot tests and titration were used for the separation, identification and quantitative determination of the nonvolatile acids of Concord grape juice concentrate.

2. Twelf nonvolatile organic acids and one nonvolatile inorganic acid were identified in the juice and its concentrate of the 1967 season: glutamic, aspartic, lactic, galacturonic, glucuronic, glyceric, succinic, malic, tartaric, citric, fumaric, phosphoric and oxalic; glycolic and α -pyrrolidonecarboxylic acid were additionally found in stored Concord grape juice concentrate of the 1966 harvest; no glyceric acid has been detected in the 1966 stored juice.

3. Malic acid was the dominant acid in all cases, followed by tartaric, galacturonic and citric. The other acids were present in very small relative quantities.

4. The hand expressed juice of Concord grapes indicated a lower total acidity as well as low content of malic, tartaric and citric acids in comparison to the juice expressed under industrial conditions.

5. The lower absolute and relative (to the other acids) concentration of tartaric acid in the stored juice concentrate must be a result of settling and separation of potassium hydrogen tartrate (cream of tartar).

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