(6) Loyd, E. J., Hiner, R. L., J. AGR. FOOD CHEM. 7, 860 (1959).
(7) Miller, M., Kastelic, J., *Ibid.*, 4, 357 (1956).
(8) Möhler, K., Kiermeier, F., *Z. Lebensm.-Untersuch. u. -Forsch.* 96, 90 (1953).
(9) Paul, P., Bratzler, L. J., *Food Research* 20, 635 (1955).
(10) Special Products Department, Rohm & Haas Co., personal communication.
(11) Valenzuela, M. de A., *Anales inst. farm. espan. (Madrid)* 2, 383 (1953).

Received for review August 7, 1959. Accepted November 19, 1959. Approved for publication by the director of Kansas Agricultural Experiment Station as Contribution No. 216, Department of Home Economics, and No. 588, Department of Chemistry.

The Separation of Organic and Inorganic Acid Anions in Filtered Tomato Purée by Partition Chromatography

DONALD B. BRADLEY
Agricultural Research Department, Campbell Soup Co., Riverton, N. J.

A method, employing silicic acid partition chromatographic techniques, is described for the separation of those organic and inorganic anions which contribute to the acidity of tomatoes. Acids were converted to the hydrogen form by passing filtered purée through the cation exchange resin, Dowex 50. Titratable and total acidity were determined by titrating an aliquot of filtered tomato purée before and after resin treatment, respectively, with 0.1*N* sodium hydroxide to a phenol red end point. Separation of acids was quantitative and total recovery was approximately 94% of the total acidity. Ten acids (acetic, lactic, fumaric, malic, pyrrolidone carboxylic, citric, phosphoric, hydrochloric, sulfuric, and galacturonic) were found to be present in the filtered tomato purée. The phosphate, chloride, sulfate, and galacturonate ions had not previously been separated from tomato purée by partition chromatography or reported as constituents of the total acidity.

PROCEDURES REPORTED for the separation of the organic acids of tomato fruits (*2*, *3*, *7*) were adaptations of the methods outlined by Isherwood (*4*) and Marvel and Rands (*6*). The Isherwood method, as employed by Bulen, Varner, and Burell (*2*) and Rice and Pederson (*7*), to isolate the organic acids in tomatoes required the use of sulfuric acid in the initial extraction of the organic acids as well as in the preparation of the silicic acid partition column. The author found in preliminary studies that the sulfuric acid was eluted in sufficiently high concentrations to mask the presence of acids eluted after citric acid.

The purpose of this paper is to report analytical procedures found adaptable for the separation and identification of organic and inorganic acids present in tomatoes. The data as presented can be used as a general guide in determining the content of individual acids in other fruits. The ratio of chloroform to 1-butanol as well as the quantity of solvent may have to be modified to meet the specific requirement of the fruit.

Experimental

Preparation and Preservation of Tomato Purée Samples. Twenty-five pounds of freshly picked tomatoes were used for each sample. The fruits were washed, trimmed, macerated, and heated to 155° F. in a steam-jacketed kettle and held at this temperature for 4 minutes with constant stirring. This material was then passed through a Langsenkamp laboratory pulper operating at approximately 1600 r.p.m. and equipped with a 0.027-inch finishing screen. This operation removed skins and seeds and reduced the pulp to a purée. The resultant purée was heated to 200° F., placed in cans, sealed, processed in boiling water for 10 minutes, and then cooled immediately with water.

Removal of Cations by Ion Exchange. Approximately 150 ml. of canned purée were centrifuged to separate the red pulp from the clear amber liquid. The supernatant liquid was filtered to remove traces of pulp. Fifty milliliters of the filtrate were passed through a 30-ml. volume of the cation exchange resin, Dowex 50 in the hydrogen ion cycle, in a 17 × 0.75-inch glass tube. The eluent containing acids, sugars, and neutral material not attracted to cation exchange resin was collected in a 100-ml. volumetric flask at the bottom of the ion exchange tube. Five- to 10-ml. increments of distilled water were added to the top of the column to ensure a quantitative removal of the acids. Sugars present after cation exchange did not hinder the separation of acids on the silicic acid partition column.

The resin was regenerated by treating with four bed volumes of 2.5*N* hydrochloric acid and washing with distilled water (with occasional back flushing) until the eluent was free of chloride (silver nitrate test).

Separation of Acids by Partition Chromatography. The partition column was set up in a manner similar to that reported by Marvel and Rands (*6*). Only the coarser fraction of the silicic acid was used for the partition column. Six samples were run at one time using a battery of six ion exchange tubes and six partition chromatographic tubes.

Resin-treated filtrate, containing approximately 1 meq. of total acid (determined by titrating to phenol red end point) was pipetted into a 50-ml. beaker and concentrated on a water bath to 1.5 to 2 ml. at temperatures below 40° C. Two grams of oven-dried silicic acid were mixed thoroughly with the sample in a manner similar to that reported by Wise (*9*) until a free-flowing powder was obtained. The mixture was placed on top of the column in a dry form. Five milliliters of chloroform were used to remove any residue in the beaker and to suspend the sample at the top of the tube. A wad of cotton was placed on top of the sample to prevent disturbance of the sample or partition column as solvents were added.

The nine solvents used to elute the acids were composed of different concentrations of 1-butanol and chloroform

saturated with distilled water, with the exception of the last solvent which was methanol. Techniques employing a continuously changing mixture of solvents were not adopted because of problems involved in saturating the solvent mixture with water. It also appeared simpler to add the desired quantity of each specific solvent directly to each column. The sequence, composition, and quantity of each of the solvents used are shown in the following table.

Sequence	Volume, % Chloroform	1-Butanol	Total Solvent, Ml.
1	85	15	250
2	75	25	50
3	65	35	200
4	50	50	150
5	40	60	50
6	35	65	150
7	30	70	100
8	20	80	100
9	0	100	200
10	100% methanol		200

Air pressure up to 20 p.s.i. was required to force the solvents through the silicic acid column.

Eluted fractions were collected manually from the partition column in 10- or 50-ml. portions, into 50- or 125-ml. Erlenmeyer flasks, respectively. The 10-ml. fractions were collected between the elution of acids to determine completeness of separation. Preliminary studies had shown that each acid was eluted consistently within specific fractions of the total volume. The citric acid was eluted by this system at elution volumes from 540 to 850 ml. Peak elution of this acid occurred at 610 ml. (Figure 1). Pure samples of each acid were used for qualitative comparisons except for pyrrolidone carboxylic acid which was unavailable commercially. Identification of pyrrolidone carboxylic acid was assumed to be as reported by Rice and Pederson (*7*). The phosphate, chloride, and sulfate from the purée were identified by standard inorganic methods.

Eluted fractions were titrated with standard 0.02*N* sodium hydroxide using a phenol red indicator. Titer and indicator were blended with the nonaqueous organic solvent through addition of 10 to 25 ml. of acid-free methanol with vigorous stirring of the sample during titration. Blank corrections were required for each solution volume, because of impurities in the organic solvents.

Where the concentration of chloride exceeded 8 meq. per liter of filtered purée, there was danger of incomplete separation of the phosphate and chloride ions. When concentrations were too high, fractions containing these acids were combined and separated by other means. For routine samples, phosphorus was determined colorimetrically by the method of Sherman (*8*) and the chloride determined by difference.

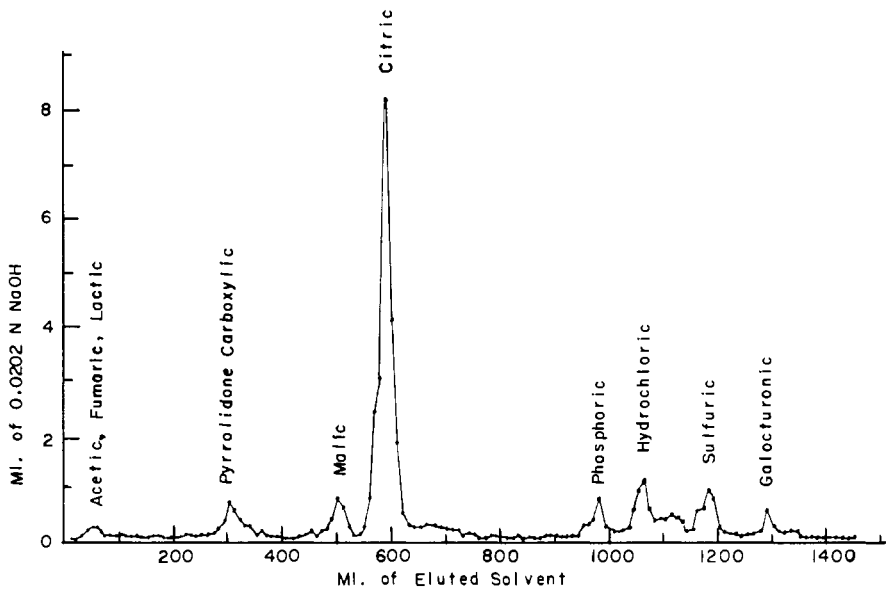

Figure 1. Titration of acids from tomato purée as eluted from the partition column

Identification of Acids by Paper Chromatography. The organic acids were separated and identified by paper chromatographic techniques employing the method described by Lugg and Overell (*5*) and Buch, Montgomery, and Porter (*1*). The developing solvent, 1-pentanol saturated with an equal volume of 5*M* formic acid, was used in the descending technique. The papers were sprayed with bromophenol blue, ammoniacal silver nitrate, acetic anhydride in pyridine, ammonium vanadate, or ceric ammonium nitrate. Papers were viewed both in daylight and ultraviolet light.

Results

The procedure as developed proved to have great utility. Acid constituents in over 100 samples of tomato purée were determined. Differences in the content of acid constituents between various tomato varieties were determined, as well as those induced in the content of individual acids by potassium fertilization. Recovery of acids averaged 94% of the total acidity.

Ten acids were determined qualitatively. The group included seven organic acids (lactic, fumaric, acetic, pyrrolidone carboxylic, malic, citric, and galacturonic) and three inorganic acids (phosphoric, hydrochloric, and

Table I. Volumes of Solvent Required to Elute Acid Constituents from Partition Column

Successive Volumes Required for Complete Elution of Acids, Ml.	Acid or Acids Contained in Eluted Fractions
0–250	Acetic, fumaric, lactic
250–400	Pyrrolidone carboxylic
400–540	Malic
540–850	Citric
850–1020	Phosphoric
1020–1135	Hydrochloric
1135–1250	Sulfuric
1250–1350	Galacturonic

Table II. Acid Composition Determined by Partition Chromatography, Titratable and Total Acidity, and Percentage Acid Recovery of Three Tomato Purée Samples

Acids	Concentration, Meq./Liter, Sample 1	Sample 2	Sample 3	Percentage of Total Acidity, Sample 1	Sample 2	Sample 3
Acetic, lactic, fumaric	1.1	3.2	2.1	1.2	2.7	1.7
Pyrrolidone carboxylic	6.4	6.5	8.6	6.7	5.5	6.7
Malic	8.3	6.3	5.6	8.8	5.2	4.4
Citric	52.5	62.7	65.8	55.5	52.9	51.5
Phosphoric	9.4	6.3	5.6	8.9	5.3	4.4
Hydrochloric	2.3	14.9	20.3	2.4	12.6	15.8
Sulfuric	4.4	7.3	7.3	4.6	6.2	5.7
Galacturonic	6.8	5.5	4.7	7.1	4.6	3.7
Titratable acidity	44.1[a]	54.1	58.1			
Total acidity	95.1[a]	118.5	127.8			
Recovery, %	96	95	94			

[a]Milliequivalents/liter of titratable and total acidity based on titration of 10 ml. of filtered purée to a phenol red end point before and after resin treatment, respectively.

sulfuric). All acids except lactic, fumaric, and acetic were separated quantitatively by silicic acid partition chromatography. These three acids were eluted and titrated as a group and together comprised 1 to 3% of the total acidity.

Analyses of some tomato purée samples not shown in this paper indicated that the sulfate ion was absent entirely, or present only in trace amounts.

The successive volumes of solvent required for the elution of each acid or acids are listed in Table I.

Figure 1 is a titration curve of a typical tomato purée sample showing the volume of 0.02*N* sodium hydroxide required to neutralize each fraction as it was eluted from the partition column.

The concentrations (milliequivalents per liter and percentage) of the individual acids from three typical tomato purée samples having a relatively wide range in titratable acidity are given in Table II. The titratable acidity, total acidity, and per cent recovery are also shown.

The data indicate that increases in the titratable and total acidity were accompanied by increases in the content of the citrate and chloride ions. Major increases in the titratable acidity are due to increases in the citric acid content. The pH of tomato purées is 4.5; therefore, the chloride ion would be present as a salt and would contribute little to the titratable acidity. In spite of the substantial differences in the titratable and total acidity, the content of citric acid for the three samples remained at approximately 53% of the total. The difference in the citric acid content between samples 1 and 3 was 13.3 meq. per liter. On a percentage basis, the difference was only 4.1%. The data indicate that regardless of changes in either the titratable acidity, total acidity, or in the concentration of the chloride ion, the citrate ion consistently made up approximately 53% of the total acidity. The difference in the chloride ion content between samples 1 and 3 was 17.9 meq. per liter.

This was the first time, as far as could be determined from the literature, that the three inorganic acids (phosphoric, hydrochloric, and sulfuric) and galacturonic acid have been separated from tomato purée by silicic acid partition chromatography. These acids were responsible for 20 to 30% of the total acidity of the tomato. As a result, a greater percentage of the total acidity was recovered than heretofore. The three tomato purée samples listed in Table II show a total recovery of 95.8, 95.0, and 93.9% of the total acidity.

A primary advantage of this procedure was the ability to convert the acid salts in the tomato purée to their respective acids through ion exchange techniques without the addition of foreign materials. Procedures employing adaptations of the Isherwood method (*4*) required the addition of sulfuric acid to the plant material in order to lower the pH value to 2 for conversion of the acid salts to acids. As the added sulfuric acid was eluted from the partition column it masked the presence of the inorganic acids normally present in tomatoes.

Literature Cited

(1) Buch, M. C., Montgomery, R., Porter, W. L., *Anal. Chem.* **24,** 489–91 (1952).
(2) Bulen, W. A., Varner, J. E., Burell, R. C., *Ibid.*, **24,** 187–90 (1952).
(3) Carangal, A. R., Jr., Alban, E. K., Varner, J. E., Burell, R. C., *Plant Physiol.*, **29,** 355–60 (1954).
(4) Isherwood, F. A., *Biochem. J.* **40,** 688–95 (1946).
(5) Lugg, J. W. H., Overell, B. T., *Australian J. Sci. Research, Ser. A* **1,** 98–111 (1948).
(6) Marvel, C. S., Rands, R. D., *J. Am. Chem. Soc.* **72,** 2642–6 (1950).
(7) Rice, A. C., Pederson, C. S., *Food Research* **19,** 106–14 (1954).
(8) Sherman, M. S., *Ind. Eng. Chem., Anal. Ed.* **14,** 182–5 (1942).
(9) Wise, W. S., *Analyst* **76,** 316 (1951).

Received for review March 25, 1958. Accepted December 28, 1959.

MEASUREMENT OF PLANT ACIDS

Determination of Average Equivalent Weight and Total Weight of Plant Acids by Ion Exchange Resins Applied to Sugar Beet Molasses

J. B. STARK

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Albany, Calif.

Ion exchange resins can be used to separate the anions in plant extracts and determine the average equivalent weight and total weight present. The equivalent weight of a California straight house sugar beet molasses was determined to be 82.1, with a standard deviation of 0.4.

As a contribution to increased knowledge of plant composition, a method is presented for the concomitant determination of average equivalent weight and total weight per cent of free and/or combined acids. The method has been applied to sugar beet molasses but should be applicable to other plant extracts with little or no modification. The procedure requires only the determination of the equivalents of anions as sodium or potassium salts.

Preparation of Ion Exchange Columns

The cation exchanger, Dowex 50–X8 (Dow Chemical Co., Midland, Mich.), is regenerated in large lots in a column with 3 liters of 5% hydrochloric acid per liter of wet resin. After regeneration, the resin is washed with distilled water until the effluent is chloride-free.

Initially the lot of anion exchanger, Duolite A-4 (Chemical Process Co., Redwood City, Calif.), is regenerated in a column with 3 liters of 4% sodium hydroxide per liter of wet resin and washed with distilled water until the effluent is colorless to phenolphthalein. The anion resin column used in the equivalent weight determinations is regenerated during use by the passage of ammonium hydroxide used to elute the acids and is ready for re-use after washing with 9 to 12 liters of distilled water.

Three different ion exchange columns are used for the separation of the acids in molasses from the remainder of the compounds. Two of the columns are glass tubes 60 cm. long and 4 cm. in diameter. One end is stoppered with a one-hole rubber stopper containing an outlet tube which can be closed with a screw clamp. A circle of fine-mesh nylon bolting cloth held in by the stopper is used to cover the outlet tube to prevent loss of resin. The columns are loaded to contain 400 ml. of wet resin, one col-