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.

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MEASUREMENT OF PLANT ACIDS

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

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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-

umn with Dowex 50 and one with Duolite A-4. The third column, 30 cm. long and 3 cm. in diameter, is filled to contain 100 ml. of wet Dowex 50.

Procedure

Use carbon dioxide–free water for dilution of samples and washing solutions through the resin columns. Dilute 50 grams of molasses, containing about 50 to 100 meq. of anions, to 200 ml., and pass the solution through the column containing 400 ml. of wet, 20- to 50-mesh, Dowex 50 resin in the hydrogen form. This weight of molasses is greater than is necessary for the determination of equivalent weight and total weight of acids, but other analytical values are determined on the excess.

When the solution has completely passed into the resin, wash and collect 1500 ml. of effluent. Mix the effluent thoroughly and titrate 50-ml. aliquots to pH 8.3 with 0.1*N* sodium hydroxide. Calculate the equivalents of acid and use this value to calculate total weight of acids present (*1*). Pass 1000 ml. of the acidic effluent through the column of 400 ml. of Duolite A-4 resin (base form). Wash the column with 2 liters of distilled water. Discard the effluent. Elute the resin with 10% ammonium hydroxide (prepared by diluting 600 ml. of 28% ammonia to 3 liters with water). Add sodium hydroxide to the effluent equivalent to the anions present as calculated from the preceding titration and concentrate the solution on a hot plate to approximately 200 ml. Cool the solution and pass it through a column of Dowex 50(H) containing 100 ml. of resin. Wash and continue washing until the final volume of the effluent is 1000 ml. Mix the contents and measure three 50-ml. aliquots into tared 150-ml. beakers. Titrate each aliquot to pH 8.3 with 0.1*N* sodium hydroxide. Calculate the equivalents of acid present after subtracting the titration blank. Evaporate the titrated solution in a forced-draft air oven or by other suitable means, dry the sample at 105° C. for 2 hours, cool the beakers, weigh, and subtract the weight blank. From the corrected weight of the sodium salts and the equivalents of acid present, calculate the average equivalent weight. From this value and the equivalents of acid found after passage of the sample through the first column of Dowex 50, calculate the total weight of acid present in the sample.

Determination of Blank Values

The titration blank which arises from acidic resin decomposition products is obtained by using distilled water as the load in place of molasses and carrying out the regular procedure for equivalent weight determinations. In the determination of the titration blank, the final weight consists of the salts of acidic and neutral resin decomposition products. The weight blank consists of acidic and neutral resin decomposition products and neutral molasses substances. The solids due to neutral molasses constituents are determined by using molasses as the load on the cation exchange resin and carrying out the regular procedure up to the step of eluting the anion resin column with ammonium hydroxide. After the regular wash of 2 liters of water, 3 more liters of water are collected and treated as if it were the ammonium hydroxide solution obtained as the eluate from the anion exchange column. The final weight blank is the sum of weight due to these neutral constituents and that of acidic and neutral resin decomposition products obtained in the determination of the titration blank as outlined above. The titration blank was estimated at 0.13 ml. of 0.1*N* sodium hydroxide. The weight blank was determined to be 0.0023 gram. Blank values should be determined for each lot of resin.

Results and Discussion

Carbon dioxide–free distilled water was used to dilute samples and wash resin columns. Small amounts of carbonate salts introduced by the sodium and ammonium hydroxide solutions are mostly decomposed to sodium hydroxide, ammonia, and carbon dioxide during concentration of the ammonium hydroxide effluent (*2*). The carbon dioxide and ammonia will be volatilized while boiling during evaporation. Carbonate introduced in other steps of the procedure will interfere by giving acid titration values and increasing the weight of the salts.

Sodium hydroxide equivalent to the anions present is added to the ammonium hydroxide effluent to convert ammonium salts to sodium salts and thus decrease losses of acids during concentration.

Sodium sulfate and chloride are dried to constant weight over phosphorus pentoxide in vacuum in 3 days. Sodium salts of molasses acids could not be dried by this procedure and were dried to constant weight at 105° C. (in an air oven) in 2 hours. The difficulty in drying the salts of molasses acids is probably due to the presence of sodium lactate. Equivalent weight determinations of lactate derived from calcium lactate gave a value of 94.9 after the sodium salts were dried over phosphorus pentoxide for 16 days. Continued drying at 105° C. reduced the value to 90.6.

An error of 0.001 gram in 0.250 gram of salt equals 0.5 equivalent weight unit and a difference of 0.01 meq. of acid in a titration of 2.4 meq. in the 50-ml. aliquot equals 0.3 equivalent weight unit. The equivalent weight error is increased if either the weight of the salt or the titration is decreased by use of smaller samples.

Two experiments demonstrate the recovery of acid (added to molasses) and the determination of its equivalent weight. Approximately 50 meq. of oxalic acid were added to 50 grams of molasses, with acids of an average equivalent weight of 82.1, and the equivalent weight of the mixture was determined (found, 66.2, calculated, 66.6). The equivalent weight of oxalate was determined to be 42.9 (theoretical, 44.0). The experiment was repeated with acetic acid; the average equivalent weight was determined as 73.8 (calculated, 74.4). The equivalent weight of acetate was determined to be 57.2 (theoretical, 59.1).

Table I. Determination of Equivalent Weights of Anions by Ion Exchange Methods

Anion	Found	Equivalent Weight Av.	Equivalent Weight Calcd.
Chloride	35.6		
	35.9		
	37.0		
	36.3		
	36.1	36.2[a]	35.46
Acetate	59.4		
	59.4		
	58.7		
	59.6		
	59.3	59.3[a]	59.05
Sulfate	47.4		
	48.2	47.8	48.03
Pyrrolidone carboxylate	128.5		
	128.7	128.6	128.11

[a] Standard deviation. Chloride 0.53, acetate 0.35.

Five determinations of the equivalent weight of chloride and acetate were made with solutions of either the salts or the free acids as the load on the first Dowex 50 column. Two determinations each were made with solutions of sulfuric and pyrrolidone carboxylic acids. These results, presented in Table I, are slightly above theoretical, possibly because of the use of an insufficient weight blank.

The average equivalent weight of the acids present in a sample of California straight house sugar beet molasses from eight determinations was found to be 82.1, with a standard deviation of 0.4. Equivalent weight determinations of molasses acids made with potassium salts were 81.9 and 81.8, which compare favorably with the average of 82.1 found with sodium salts.

Compositional information in addition to the equivalent weight and total weight of acids can be obtained by further manipulation of the resins and their eluates (*3*, *4*). Basic and amphoteric nitrogen compounds relatively free from metallic cations can be obtained from the first column of Dowex 50 by elution with ammonium hydroxide. After evaporation of the ammonia, this fraction can be further fractionated into the more basic nitrogen compounds such as betaine and basic amino acids by passing the solution through a column of Dowex 1(OH) (Dow Chemical Co., Midland, Mich.). The neutral and acidic amino acids are retained on the resin, while the basic

amino acids and betaine pass into the effluent. The Dowex 1 column can be eluted with acetic or hydrochloric acid to recover the amino acids or can be eluted first with aqueous carbon dioxide to recover the neutral amino acids and other compounds having similar ion exchange properties.

The fraction containing acids can be used for identifying and determining specific acids. The effluent from the column of Duolite A-4 contains carbohydrates and other neutral substances.

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CITRUS BY-PRODUCTS

Lactic Acid Production by Fermentation of Citrus Peel Juice

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Citrus peel juice is produced in large quantities during the manufacture of canned citrus juices. A study was undertaken to determine the possibility of producing lactic acid by fermentation of this waste juice. Fermentations were conducted at 45° C. in the presence of excess calcium carbonate to neutralize continuously the acid formed. A naturally occurring lactobacillus isolated from a fermenting grapefruit juice was able to accomplish a 90%-efficient conversion of sugars to lactic acid in a period of 4½ to 5 days only in the presence of accessory nutrients. Growth factors supplied by yeast autolyzate or malt sprouts were particularly beneficial. Analyses of the fermentation runs were considerably simplified by the use of a cation exchange method for determining the lactic acid produced during fermentation in the presence of calcium carbonate.

IN THE CITRUS FRUIT processing industry, utilization of peel juice is becoming a major factor in maintaining economical plant operations. Preparation, composition, and current uses of peel juice have been adequately covered by Braverman (*2*). This report deals with the selection of a suitable lactobacillus organism which, in the presence of added growth factors, is capable of vigorous and efficient fermentation of peel juice. Furthermore, the need was felt for a simple and rapid method adaptable to routine determinations of lactic acid produced during fermentation in the presence of calcium carbonate. A cation exchange method was developed for this purpose; the principle involves the exchange of calcium ions from the calcium lactate in the clarified fermentor liquor for the hydrogen ions of the exchange resin. The acid thus formed is titrated with a standard alkali solution and calculated as lactic acid.

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Materials

Peel Juice. Citrus peel juice was obtained directly from the canning line, adjusted to pH 6.0 by the addition of phosphoric acid and filtered to remove insolubles. During the off-season, citrus molasses was diluted with tap water to a Brix density of approximately 13°, adjusted to pH 6.0 with a 10% sodium phosphate solution, and filtered.

Yeast Autolyzate. Ground yeast cakes, 1 kg., were autolyzed with 100 ml. of ethyl acetate at room temperature. After complete liquefaction, the mixture was stirred mechanically for 1 hour, during which time it was maintained neutral to litmus by the addition of a 10% sodium phosphate solution. Toluene, 80 ml., was vigorously stirred into the mixture which was then incubated for 24 hours at 35° C. Neutralization and incubation were repeated always making certain of an excess of toluene to prevent putrefaction. The autolyzate was clarified by the addition of egg white and after being heated on a steam bath for 30 minutes, the mixture was filtered hot through a Büchner funnel with the aid of kieselguhr. The solids were extracted with an equal volume of water and filtered. The combined extracts were concentrated on a steam bath to a Brix density of 25° and stored under toluene in the refrigerator.

Tomato Extract. A quantity of tomatoes was macerated in the Waring Blendor and filtered through a Büchner funnel with the aid of kieselguhr. The extract was vacuum concentrated to a Brix density of 15° and stored, under toluene, in the refrigerator.

Culture Maintenance Medium. All cultures were maintained in a nutrient agar composed of glucose (2%), peptone (0.5%, Difco-Bacto), yeast autolyzate (10% v./v.), and agar (1%). Ten-milliliter portions were poured into lipless test tubes (150 × 15 mm.) containing a pinch of calcium carbonate, plugged with cotton, and sterilized at 15 pounds for 20 minutes. Before setting, the contents of the tubes were shaken to disperse the finely suspended calcium carbonate through the medium. In this way, growth of the lactobacillus cultures was easily detected by clearing of the medium. The cultures were maintained by weekly stab transfers followed by incubation at 45° C. until good growth oc-