densitometer (Photovolt Corp., N. Y., Model 52-C) was used to determine the concentration of the amido black on the electrophoresis strips.

The location of the zinc-65 on the paper strips was detected by cutting the paper into 1-cm. segments, placing them into plastic test tubes, and counting the radioactivity of each segment by means of the scintillation detector.

Typical results, presented in Figure 3, show that the zinc in the plant extract subjected to electrophoresis migrated with the amido black stain of the protein fraction. The free zinc in the control strips did not migrate appreciably. A definite association of the radiozinc with some constituent in the plant extracts was observed. Since zinc-65 correlated with the amido black stain for proteins, a zinc-protein binding was evident.

The results of this investigation led to the conclusion that zinc in plant extract is both free and bound, and that zinc is associated with a protein. The following equilibrium may help to explain the experimental results:

$$Zn^{+2} + \text{protein} \rightleftarrows \text{zinc-protein} + 2H^{+}$$

If this equilibrium exists, an increase in the hydrogen ion concentration would dissociate the zinc-protein complex. This was observed in the buffered dialysis experiments (Table I) and in the hydrogen sulfide precipitation (Table III), in which the removal of zinc increased as pH of the extract was lowered. Acidic conditions caused the complex to dissociate more readily, shifting the above equilibrium to the left to form free zinc(II) ions. Equilibrium dialysis experiments (Figure 2) also presented evidence of greater zinc binding at higher pH values. Evidently the zinc-protein complex is more stable at higher pH.

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Received for review May 13, 1963. Accepted November 7, 1963. Division of Agricultural and Food Chemistry, 144th Meeting, ACS, Los Angeles, Calif., April 1963. Contribution No. 623, Kansas Agricultural Experiment Station, Manhattan, Kansas. From a dissertation by W. J. Johnson in partial fulfillment of the requirements of the Doctor of Philosophy degree.

TOMATO COMPOSITION

Varietal and Location Influence on Acid Composition of Tomato Fruit

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In tomato fruits from farms located in New Jersey, Pennsylvania, and Ontario, Canada, acidity levels and acid composition varied among varieties and locations. Results from replicated trials indicated major differences between varieties in percentage concentration of citric acid and quantity of acid anion in a titratable form or as a neutral salt. A high acid variety contained a higher percentage of potassium than a low acid variety. No differences were found in the root cation exchange capacities of varieties with low, medium, and high titratable acidity levels. Rootstocks of tomato plants had no significant influence on acidity of fruit from the scion.

THE PURPOSE of this investigation was to determine the acid composition of tomato fruit from varieties having low, medium, and high titratable acidities; the cation content of the vines and fruit; and the influence of rootstock and root cation exchange capacities on acid metabolism.

Reynard (*10*) reported that the titratable acidity of fruit from four tomato varieties maintained the same relative rank when grown at Chicago, Ill.; Davis, Calif.; Riverton, N.J.; and New Toronto, Ontario, Canada. However, levels of titratable acidity varied considerably between locations.

Anderson and Thompson (*1*) found threefold differences in acid content between a number of varieties and strains. Citric acid was reported by Manunta and Lafon (*7*) to be the principal acid in tomatoes. A high correlation was found between total acidity and citric acid content, but no correlation was noted between total acidity and either malic or succinic acid. Manunta and Lafon (*8*) found that F_1 hybrids of crosses between high and low acid varieties had citric acid levels intermediate between the parents but with a tendency toward the low acid parent.

Several investigators (*5, 6, 9*) have observed that potassium fertilization increases tomato acidity. Using soils with exchange capacities up to 13 meq. per 100 grams, Bradley (*3*) found that K fertilizers increased titratable acidity, total acidity, citric acid, and K content of vines and fruit. Potassium comprised up to 85% of the total cations associated with the acid anions as salts in tomato puree.

Experimental

Tomato Acidity as Related to Variety and Location. Seventeen nonreplicated tomato fruit samples, each weighing approximately 25 pounds, were collected in August at a uniform stage of ripeness from fields in New Jersey, Pennsylvania, and eastern Canada. Fruit samples represented varieties Ace, Improved Garden State (IGS), Rutgers, John Baer, and strain Kc54. The samples were pureed and canned, and the acid composition was determined by ion exchange and silica gel partition chromatography (*2*). Inorganic anions eluted from the silica gel partition column were titrated and reported as a group. Titratable and total acidity were determined by titrating a 10-ml. aliquot of filtered puree before and after resin treatment, respectively, with NaOH to a phenol red end point. Results are reported as milliequivalents per liter.

[1] Present address: Campbell Soup Co., Davis, Calif.

Table I. Influence of Variety and Location on Titratable and Total Acidity and Acid Composition of Tomato Puree

Variety	Titratable Acidity	Total Acidity	Lactic Fumaric Acetic	Pyrrolidone Carboxylic	Malic	Citric	Sulfuric Phosphoric Hydrochloric	Galacturonic
			MEQ. PER LITER OF FILTERED PUREE					
			RIVERTON, N. J.					
Ace	51	117	1.5	9.3	9.5	58.0	23.5	11.6
	55	124	3.6	10.7	9.1	62.0	27.7	9.9
IGS	63	134	1.9	13.7	5.1	71.0	30.1	6.1
	67	134	T[a]	11.9	5.8	76.6	24.7	7.7
Rutgers	58	126	1.8	11.3	2.5	62.7	36.7	6.4
Strain Kc54	67	129	T	12.9	2.9	85.2	19.5	T
	80	150	3.0	16.0	3.7	96.0	22.5	3.8
John Baer	76	144	T	14.9	4.9	77.9	29.1	15.5
	76	142	T	15.2	4.9	80.0	28.0	10.4
			LUMBERTON, N. J.					
IGS	58	124	2.5	7.5	10.3	61.4	22.1	11.9
	58	134	1.5	14.4	5.3	68.3	26.5	9.7
Rutgers	54	119	1.8	11.5	4.1	59.4	24.8	9.4
	62	130	2.5	13.2	4.5	69.9	23.6	9.0
	66	144	2.3	14.1	7.2	74.2	23.9	11.1
			CHALFONT, PA.					
Ace	34	91	1.5	5.9	5.2	41.6	22.4	5.6
			NEW TORONTO, ONTARIO, CANADA					
Ace	35	87	1.5	13.5	3.3	40.9	24.9	9.4
John Baer	68	128	1.6	11.5	4.7	75.9	21.4	7.4

[a] T = trace.

Table II. Percentage of Acids in Tomato Puree as Influenced by Location and Variety

Variety	Lactic Fumaric Acetic	Pyrrolidone Carboxylic	Malic	Citric	Sulfuric Phosphoric Hydrochloric	Galacturonic
		PERCENTAGE OF TOTAL ACIDITY[a]				
		RIVERTON, N. J.				
Ace	1.3	7.8	6.3	49.4	20.0	9.6
	2.9	8.6	7.3	50.0	22.3	7.9
IGS	T[b]	10.2	3.8	53.0	22.5	4.6
	T	8.9	4.3	57.2	18.4	5.8
Rutgers	1.4	9.0	2.0	49.7	29.1	5.1
Strain Kc54	T	10.0	2.3	66.0	15.1	T
	2.0	10.7	2.4	64.2	15.1	2.5
John Baer	T	10.4	3.4	54.1	20.2	10.8
	T	10.7	3.5	56.3	19.7	7.3
		LUMBERTON, N. J.				
IGS	1.9	6.1	8.4	49.7	17.9	9.7
	1.1	11.1	4.0	51.0	19.7	7.2
Rutgers	1.6	9.7	3.4	50.1	20.9	7.9
	1.9	10.3	3.5	54.1	18.3	6.9
	1.6	9.8	4.8	51.5	16.7	7.7
		CHALFONT, Pa.				
Ace	1.4	6.5	5.0	46.0	25.6	6.1
		NEW TORONTO, ONTARIO, CANADA				
Ace	1.5	13.5	3.3	40.9	24.8	9.4
John Baer	1.3	9.0	3.7	59.7	16.9	5.8

[a] Recovery averaged 94% of total acidity.
[b] T = trace.

Acid Composition of Tomato Fruit and Cation Content of Fruits and Vines (Replicated Plots). Tomato fruit samples were obtained from two replicated variety tests at Riverton, N. J. The 25-pound samples drawn from Field 1 represented strain 874 and varieties IGS and Ace. Those from Field 2 represented strains 874, 109-F29 and the variety IGS. The 25-pound samples were obtained from three replications of each variety from both Fields 1 and 2.

Acid composition was determined as cited above (*2*). The Ca, K, and Na determinations were made by flame photometry. Phosphorus was determined by a modification of Sherman's method (*11*).

Root Cation Exchange Capacity. Cation exchange capacities were determined (*4*) for roots of $1^1/_2$-month old plants of Ace, IGS, and 874. Tomato plants, 10 days after germination, were set in coarse sand in $^1/_2$-gallon pots. Each variety was replicated three times.

Grafted Plants. Plants of Ace and 874 were selected as representing lines having low and high titratable acidities, respectively. In the hot-beds, scions from 874 were grafted on Ace rootstocks, and, conversely, Ace scions were grafted on 874 rootstocks. In mid-May, 2 weeks after grafting, the plants were transplanted into field plots. Ace and 874 control plants were included. Plots of control or grafted plants were replicated four times. Spacing between rows was 5 feet and in the row 2 feet. A uniform fertilizer treatment of 1000 pounds of 10–4–8 (10–10–10) was applied over the test area. Plants were supported by wire cages. A random fruit sample of approximately 12 pounds was collected from the seven plants in each plot for laboratory analyses. Titratable acidity was determined on filtrate of ripe fruit from the August 10 and September 3 pickings by titrating a 10-ml. aliquot with 0.1*N* NaOH to a phenolphthalein end point.

Results

Tomato Acidity as Related to Variety and Location. Substantial differences in acidity levels were found between varieties grown at Riverton, N. J. (Tables I and II). The varieties in Tables I and II are arranged in ascending order of their titratable acid content.

Average titratable acidity values for Ace, IGS, Rutgers, Kc54, and John Baer were 53, 55, 58, 74, and 76, respectively. Titratable acidity refers to that quantity of acid anion normally present in tomato puree in the hydrogen form, and total acidity indicates that quantity of acid present after removal of cations by resin treatment. Acid anions normally present in the hydrogen form (calculated from Table I) comprise 44, 49, 46, 53, and 54%, respectively, of the total acidity for the above varieties.

Ace had a greater percentage of its total acid normally combined with bases than any of the other varieties. As the acidity level increased due to variety, the percentage of total acid combined with bases decreased.

The concentration of citric acid consistently increased as the titratable acidity of the variety increased.

Milliequivalent per liter concentrations of pyrrolidone carboxylic tended to increase and malic decrease as the titratable acidity level of the variety increased. Changes in concentration of

Table III. Titratable and Total Acidity and Acid Composition of Tomato Puree from Two Varieties and Two Strains from Replicated Field Plots

Variety	Titratable Acidity	Total Acidity	Lactic Fumaric Acetic	Pyrrolidone Carboxylic	Malic	Citric	Phosphoric	Hydrochloric	Sulfuric	Galacturonic
Field 1				MEQ. PER LITER OF FILTERED PUREE						
874	86.8	156.4	3.9	15.6	6.0	89.4	5.2	18.7	4.8	9.1
IGS	79.4	143.2	3.0	11.9	12.2	75.6	9.0	13.0	5.7	8.2
Ace	70.1	131.7	3.7	13.3	13.7	61.5	11.5	11.3	5.2	9.3
L. S. D. 5%	8.4	12.6	0.6	1.9	2.8	12.5	N. S.	4.7	N. S.	N. S.
1%	13.9	20.9		3.1	4.6	20.7				
Field 2				MEQ. PER LITER OF FILTERED PUREE						
874	77.0	147.5	2.9	10.8	5.1	80.4	22.5	17.2	1.3	3.8
IGS	57.6	119.5	3.7	6.5	6.8	61.4	20.6	14.0	2.4	3.1
109-F29	53.3	110.5	2.0	8.6	6.8	52.8	11.1	11.5	2.9	4.8
L. S. D. 5%	5.1	13.4	0.4	0.3	N. S.	10.0	6.0	3.0	N. S.	1.3
1%	8.5	22.2	0.6	0.5		16.6	9.8	4.3		

Table IV. Acid Composition Expressed as Per Cent of Total Acidity[a] of Filtered Tomato Puree (Replicated Tests)

Variety or Strain	Acetic Lactic Fumaric	Pyrrolidone Carboxylic	Malic	Citric	Phosphoric	Hydrochloric	Sulfuric	Galacturonic
Field 1			PERCENTAGE OF TOTAL ACIDITY					
874	2.4	9.9	3.8	57.1	3.4	11.9	3.0	5.8
IGS	2.1	8.3	8.5	52.7	6.3	9.1	3.9	5.7
Ace	2.8	10.1	10.7	46.8	8.7	8.6	3.9	7.1
L. S. D. 5%	0.49	N. S.	2.2	3.5	N. S.	N. S.	N. S.	N. S.
1%			3.6	5.9				
Field 2			PERCENTAGE OF TOTAL ACIDITY					
874	1.9	7.2	3.4	53.3	14.9	11.4	0.9	2.5
IGS	3.1	5.3	5.6	50.1	16.8	11.5	1.9	2.5
109-F29	1.8	7.6	6.0	46.9	8.7	10.4	2.9	4.8
L. S. D. 5%	0.4	N. S.	1.2	3.3	2.3	N. S.	N. S.	1.0
1%	0.6		2.0	5.5	4.0			1.6

[a] Recovery averaged 96% of the total acidity.

Table V. Content of Ca, Na, K, and P in Tomato Vines and Puree

Variety or Strain	Ca	Na	K	P
Field 1		PERCENTAGE DRY WEIGHT OF VINES		
874	2.59	0.59	4.07	0.25
IGS	2.65	0.60	3.26	0.22
Ace	2.61	0.43	2.50	0.17
L. S. D. 5%	N. S.	N. S.	1.02	N. S.
1%			1.68	
Field 2		MEQ. PER LITER OF PUREE		
874	2.4	0.59	83.5	31.9
IGS	2.1	0.55	74.2	26.1
109-F29	2.6	0.60	72.5	23.6
L. S. D. 5%	N. S.	N. S.	10.9	N. S.

Table VI. Root Cation Exchange Capacities of Seedlings

Variety	Meq. per 100 Grams of Dry Roots
Ace	27.90
IGS	30.43
Strain 874	31.00
L. S D.	N. S.

Table VII. Titratable Acidity of Control and Grafted Tomato Plants

Variety	Titratable Acidity (Season Mean) (Ml. of 0.1N NaOH[a])
Ace	4.90
874	6.10
Ace scion on 874 rootstock	4.80
874 Scion on Ace rootstock	6.60
L. S. D. 5%	0.55
1%	0.79

[a] 10 ml. of filtered puree titrated to phenolphthalein end point.

other acids followed no specific pattern.

Ace samples were obtained from Riverton, N. J.; Chalfont, Pa.; and New Toronto, Ontario, Canada. The titratable acidity, total acidity, and citric acid content of samples from Riverton were considerably higher than found in samples from the other two geographic locations. The New Jersey samples contained about 50% more titratable acid than the Ontario or Pennsylvania samples.

For John Baer variety, titratable acidity, total acidity, and citric acid concentration was higher for the samples produced at Riverton, N. J., than for the New Toronto samples (Table I). Calculations show that 54 and 53% of the total acid was present in a hydrogen form, respectively, for the two geographical locations.

Rutgers samples obtained from fields in the Lumberton, N. J., area had titratable acidity levels ranging from 54 to 66. Citric acid and total acidity increased as the titratable acidity increased. Concentrations for acids other than citric displayed no consistent pattern in relation to the titratable acidity.

Data in Table II show the percentage that each acid or combination of acids contributed to the total acidity. Samples from Riverton, N.J., indicated that as the acidity level of the variety increased, there was a pronounced increase in the percentage that citric acid contributed to the total acidity. The citric acid percentage in the Ace sample from NewToronto was considerably lower than that found in the Ace sample from Chalfont or Riverton. Acids other than citric displayed less consistent and less pronounced differences between varieties.

Acid Composition of Tomato Fruit and Cation Content of Fruits and Vines (Replicated Plots). Variety Ace and

strain 109-F29, IGS, and 874 were considered to be low, medium, and high, respectively, in titratable acidity. Their relative positions were maintained when the selections were grown in replicated plots at two locations (Table III). Titratable acidity comprised a greater percentage of total acidity in fruit samples from replicated plots than for varieties listed in Table I with similar acid ranges.

Citric acid exceeded all other acids in concentration and increased as the acidity level of the variety or strain increased (Table III). For example, from Field 1, citric acid levels for Ace, IGS, and 874 were 61.5, 75.6, and 89.4 meq. per liter, respectively, or 46.8, 52.7, and 57.1 per cent of the total acidity. Samples from Field 2 displayed essentially the same pattern.

The percentage that malic acid contributed to the total acidity decreased significantly in Field 1 as the acidity level of the strain or variety increased (Field 1, Table IV). No significant differences in malic acid content were found in samples from Field 2.

The combined concentration of acetic, lactic, and fumaric acids manifested no trends due to changes in acidity levels of varieties or strains. This was also the case with pyrrolidone carboxylic, sulfuric, and galacturonic acids. Phosphoric acid displayed an erratic pattern. The hydrochloric acid concentration was significantly greater in 874 than in Ace or 109-F29.

Analytical data for Ca, Na, K, and P in the tomato vines of Field 1 are summarized in Table V. Ace was significantly lower in K than 874. Differences in both K content and fruit acidity between Ace and 874 were significant. There were no differences between varieties in the content of Ca, Na, or P.

Data for Ca, Na, K, and P in tomato puree are summarized in Table V. The K content in the puree of 874 was significantly higher than for 109-F29.

Root Cation Exchange Capacity. No significant differences were noted in the cation exchange capacities of roots from three varieties of tomato seedlings with different acidity levels (Table VI).

Grafted Plants. Titratable acidities of tomato puree from control and grafted plants are shown in Table VII. Control 874 and Ace plants manifested their usual differences in titratable acidity. No significant differences in titratable acidity were noted between control Ace and Ace scions on 874 rootstocks. The titratable acidity of fruit from 874 scions on Ace rootstocks tended to be higher than the titratable acidity of control 874 plants.

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Received for review April 10, 1962. Accepted November 14, 1963.

PLANT TISSUE ANALYSIS

Colorimetric Determination of Glucose, Fructose, and Sucrose in Plant Materials Using a Combination of Enzymatic and Chemical Methods

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A combination of enzymatic and chemical colorimetric procedures for more specific determination of glucose, fructose, and sucrose in plant and food materials has been developed. These methods are simple and have a high degree of specificity. The usual clarification procedures often required for the removal of nonsugar-reducing substances are not necessary.

THE NEED for simpler and more specific methods for the quantitative determination of glucose, fructose, and sucrose in plant and food materials is well recognized. A specific enzymatic determination of glucose in serum, plasma, and urine was first reported in 1956 (*5, 13*). This method is based on the coupling of the two following enzymatic reactions:

$$\beta\text{-D-glucose} + \text{glucose oxidase} \rightarrow \text{D-glucono-}\delta\text{-lactone} + \text{hydrogen peroxide} \quad (1)$$

[1] National Science Foundation Research Participant.

$$\text{hydrogen peroxide} + \text{peroxidase} + o\text{-dianisidine} \rightarrow \text{color} \quad (2)$$

The color formed is measured colorimetrically.

Potter *et al.* (*8, 9*) used this method to determine the amount of fructose present in plant material. The difference between the amount of reducing sugar present before and after destruction of glucose by glucose oxidase was taken as a measure of fructose.

In the investigation reported in this paper, glucose, total fructose, and sucrose are determined directly. Free glucose is determined by the glucose oxidase method. After hydrolysis of the sucrose by invertase, the total glucose (free glucose + glucose in sucrose) is determined by the glucose oxidase method. The difference between total glucose and free glucose permits the calculation of the amount of sucrose. Then total fructose is determined by a modification of the method used by Wise *et al.* (*14*). Since the amount of sucrose is known from the glucose oxidase analysis, the amount of free fructose is readily obtained. For convenience, this method of analysis is called Method I.

An alternate procedure for the analysis of sugar in plant materials which does