Citrate and Malate Concentrations in Tomato Fruits: Genetic Control and Maturational Effects[1]

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Abstract. There is continual change in the acidity of tomato fruits during development and maturation. The concn increases during development and reaches a maximum near incipient color, then decreases until well beyond maturity. The inheritance of citrate and malate concn in 'Tondo Liscio' (TL) and PI 263713 (PI) is controlled by a single gene for each compound. The dominant alleles, which were linked in the coupling phase in PI, condition a high concn of citrate and a low concn of malate. Recombination was about 18%. Study of divergent tomato accessions indicated that there are factors which condition higher and lower concn of citrate than the range delimited by TL and PI. There appears to be more than one malate factor controlling higher concn, but many tomato accessions are similar to the dominant low parent (PI). Current evidence indicates that there is no practical reason to breed for a specific citrate/malate ratio.

Citric is usually the predominant acid in tomato fruits. Depending upon cv., environment, fruit maturity and postharvest treatment (3, 4, 5, 13, 16) it comprises 40 to 90% of the organic acids. The concn of malic, the other major organic acid, ranges from 10 to 60% of that of citrate, depending largely on the cv. (5). Galacturonic and pyrollidonecarboxylic acids also may occur in tomatoes. Concn of free galacturonate is relatively low in prime fruits, but increases with advancing maturity (13), probably as a result of the breakdown of pectins. Pyrollidonecarboxylate appears to be a decomposition product of glutamine and possibly of glutamic acid (16), and its concn depends primarily on postharvest treatments. Lactate, fumarate, oxalate, aconitate, acetate, and formate are sometimes present in tomatoes, but only in small quantities.

Organic acids are crucial to the flavor and processing characteristics of tomatoes. High solids and a favorable solids/acid ratio are essential to good quality tomatoes (6, 19, 20). Acid concn is an important processing characteristic, as it must be high enough to give a pH less than 4.4 to avoid problems with thermophilic organisms (17). Higher pH values necessitate longer processing times, increasing the difficulty of obtaining a high quality product.

With one exception, previous inheritance studies on tomato acidity have involved pH or titratable acidity rather than individual acids. A previous study (21) indicated that inheritance of malate concn is under monofactorial control, with dominance for low concn. Walkof and Hyde (22) concluded that tomato acidity was simply inherited with dominance for high acidity. Lower and Thompson (12) reported that inheritance of acidity is largely quantitative. They detected a major factor conditioning high acidity in some populations.

This study was undertaken to define inheritance of concn of citrate, to clarify understanding of the relationships between citrate and malate and to investigate the effects of maturity on concn of acids.

Materials and Methods

The cv. VF 145-7879 was used to study the effects of maturity on acid concn. Flowers on greenhouse-grown plants were tagged at anthesis, and fruits were harvested 2, 4, 6, 7, 8, 9, 10, 11, and 12 weeks later. After they were washed and packed in Scotchpak bags, they were frozen and stored at -40°C. Titratable acidity was determined by titrating 5 ml of serum to pH 8.1 with 0.1 N NaOH. The reported data are the means of 5 analyses from each of 3 replications. Each replication contained at least 10 fruits.

Single plants of the cv. Tondo Liscio (Italy) and the accession PI 263713 (Puerto Rico), selected on the basis of their citrate and malate concn, were used as parents. Evaluation of samples grown in New Jersey and California indicated that these accessions have heritable concn differences. Parents and progeny were field grown at Davis, California in 1971. A modified randomized complete block design with 4 blocks, each containing 24 plots was used. The number of 10 plant plot entries per population in each block were: parents and F_1-2; the 2 backcrosses and F_2-6. Fruits from each plant were harvested at incipient color, washed, dried, packed in Scotchpak bags, frozen, and stored at -40°C until analyzed. Only blocks 1 and 2 were analyzed because it became apparent that the inheritance of both compounds was simple. For statistical analysis the data were treated as random observations from a population.

Samples were removed from the freezer the evening prior to the preparation for analysis, and allowed to thaw in the Scotchpak bags. After thawing, the serum in the bag was thoroughly mixed and then filtered through Whatman 90 paper. The fruits were not macerated, as preliminary work had indicated that the serum present after freezing and thawing contained a representative sample of soluble fruit constituents.

A 10 ml sample of the filtered serum was mixed with the internal standard (0.20 mmoles tartartic acid), and placed on a 10 x 200 mm AGI-X8 column. The column was washed with 60 ml of water to remove sugars, and the acids were eluted with 60 ml of 6N formic acid. The eluate was evaporated to dryness under vacuum, and the acids were redissolved in 20 ml of water. Two ml of this solution were added to a 5 ml serum bottle and lypholized.

Trimethylsilyl (TMS) derivatives were prepared by adding 1 ml of a mixture of hexamethyldisilazane, trimethylchlorosilane, and pyridine (4:1:5 v/v/v) to each dried sample. The serum bottle was sealed with a sleeve-type septum, shaken 20 min and stored at -10°C until analysis.

The TMS ethers were separated on a 12.5′ x 1/8″ O.D. stainless steel column packed with 2% OV-210 on 80-100 mesh AW-DMCS treated Chromsorb G. A dual flame-ionization Hewlett-Packard 5750 gas chromatograph was used for the analyses. Each sample was analyzed on both columns simultaneously. If the data from both detectors were not in close agreement (<1% difference) the analysis was repeated. The temp program was 150 to 250°C @ 15°/min. Injector and detector temp were 250° and 270°, respectively. Gas flow rates were 20, 30, and 200 ml/min for H_2, N_2, and air, respectively. Peak ht of citrate and malate relative to tartrate was used to estimate concn. Standard curves were obtained with known quantities of citrate and malate, using the same procedures

Twenty-five divergent tomato accessions selected from a

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previous study (20) were used to investigate the among-line distributions of citrate and malate. The plants were field grown at Davis, California, in randomized plots in 1970. Five 10-plant plots of each line were harvested twice. The fruits were picked at full maturity, then washed, frozen, and stored at -20°C. Analytical procedures were as described above.

Results and Discussion

The acid concn in tomato fruits changes continually during development and maturation (Fig. 1). It increases to incipient color, then decreases to well beyond maturity. These results agree with those in previous investigations (9, 18, 23). Fluctuations in concn due to degree of maturity can therefore easily overshadow genetic differences and complicate a genetic analysis. Incipient color is the only easily definable stage of fruit maturity. The acid concn in 'Tondo Liscio' (TL) and 'PI 263713' (PI) varied greatly between the genetic study and the comparison of tomato accessions study because of differences in fruit maturity. The fruits used in the genetic study were at incipient color, whereas mature fruits were used in the other study.

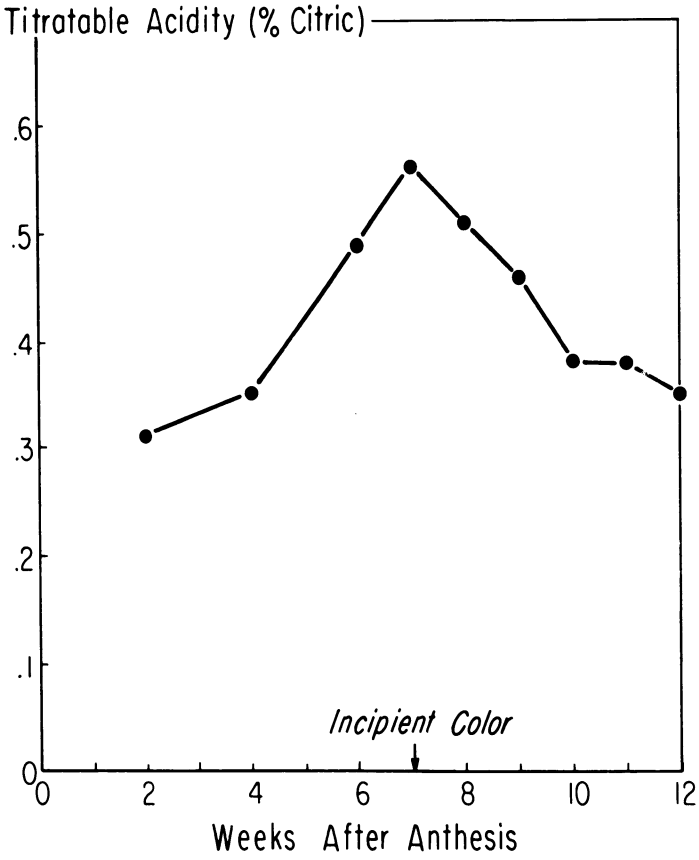

Fig. 1. Change in acid concn of fruits of cv. VF 145-7879 during development and maturation.

The regression equations for citrate and malate were y = 26.54(x) - 1.12 and y = 22.92(x) - 0.43, respectively, in which y was mM and x was citrate or malate peak ht relative to peak ht of the internal standard (tartrate).

Differences in concn of citrate and malate between 'TL' and 'PI' are controlled by a single gene for each compound (Fig. 2 and 3). The PI alleles, which condition a high concn of citrate and a low concn of malate were dominant. Goodness of fit tests indicated that the segregating populations fit the expected ratios (Table 1). The distributions of segregating populations were continuous, probably because of maturity effects. Consequently, based on parental concn, 37.5 mM citrate and 15 mM malate were chosen as the dividing points between 'TL' and 'PI' types.

The genes controlling the differences in concn appear to be linked in coupling phase. Since both compounds are formed by a similar metabolic pathway, the possibility exists that competition for a common precursor could give the appearance of coupling phase linkage. The recombinants with high concn of both compounds (*ctr*^{D}/*mlt*) had mean concn of 50.4 mM citrate and 19.8 mM malate. These compare favorably with 51.9 mM citrate for the *ctr*^{D} parent and 22.6 mM malate for the *mlt* parent. The high recombinants ranged from 57.5 to 87.3 mM citrate + malate with a mean of 66.7 mM which is less than the theoretical high recombinant concn of 74.5 mM. The low recombinants (*ctr*/*mlt*^{D}) had mean concn (30.2 mM citrate and 8.8 mM malate) very similar to the appropriate mean parental concn (29.3 mM citrate and 8.8 mM malate). The reduced mean value of the high recombinants may indicate some competitive effects. However, since 30% of the high recombinants equalled or exceeded the theoretical high mean and because the mean values for the 2 compounds were close to parental values, it is likely that linkage accounts for most of the aberrant ratios. Recombination was estimated to be 17.6 ± 3.9% in the B_1 population and 17.9 ± 4.0% in the F_2 population (Table 2). The good agreement between recombination estimates for the 2 segregating populations gives credence to the dividing points between 'TL' and 'PI' types.

It is proposed that the gene controlling citrate differences be designated *ctr* and the one controlling malate, *mlt*. The superscript D will be used to denote dominance, thus the genes in 'PI' are *ctr*^{D} and *mlt*^{D}.

Table 1. Goodness of fit tests for citrate and malate concns in segregating populations from crosses between 'Tondo Liscio' (P_1) and PI 263713 (P_2).

Population	Ratio of P_1 to P_2 types		Chi Square	P
	Observed	Expected		
Citrate				
B_1	57:62	1:1	.210	0.7-0.6
F_2	33:85	1:3	.402	0.6-0.5
Malate				
B_1	58:61	1:1	.076	0.8-0.7
F_2	31:87	1:3	.047	0.9-0.8

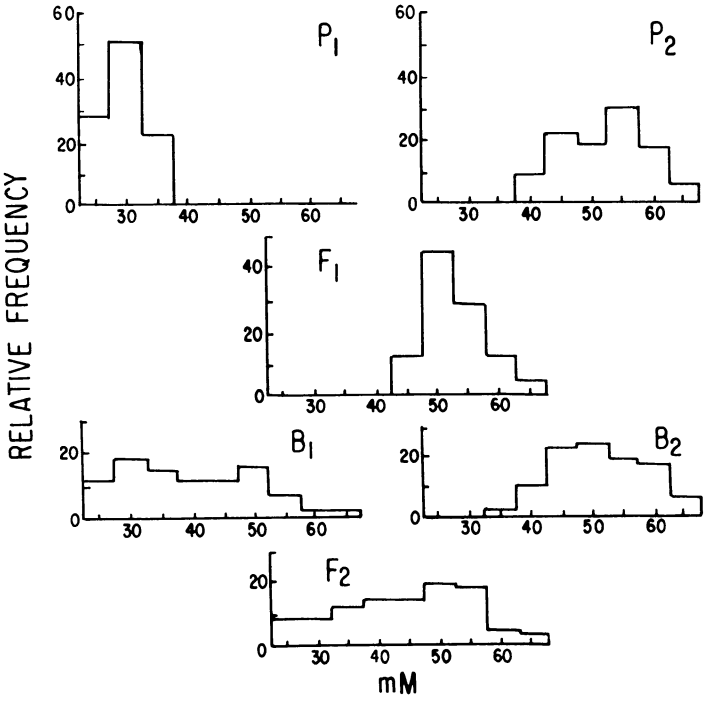

Fig. 2. Relative frequency distribution for citrate concn in 'Tondo Liscio' (P_1), PI 263713 (P_2), and F_1, B_1, B_2 and F_2 progeny.

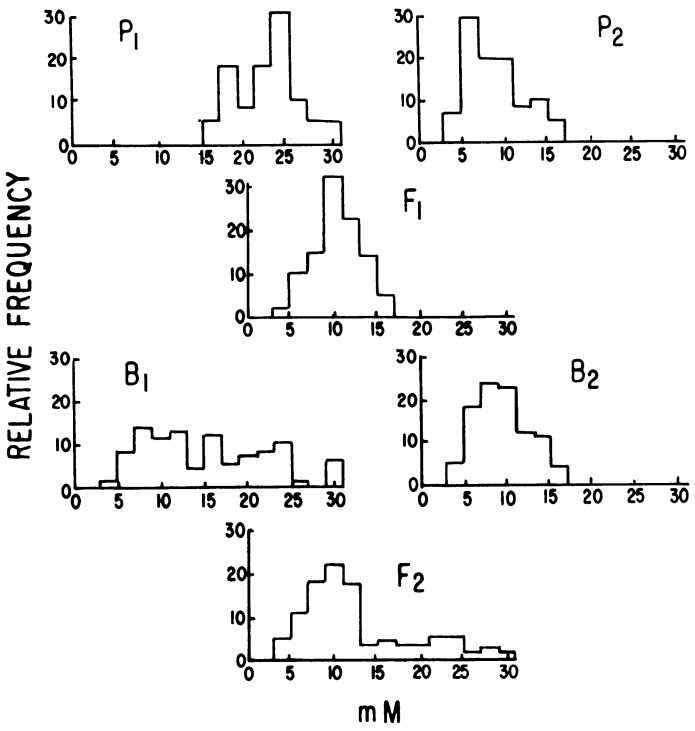

Fig. 3. Relative frequency distribution for malate concn in 'Tondo Liscio' (P_1), PI 263713 (P_2), and F_1, B_1, B_2 and F_2 progeny.

There were no significant differences between reciprocal crosses (Table 3), indicating little or no extrachromosomal effects. The mean concn of the recessive parent (TL) was significantly different from that of all progeny populations. The dominant parent (PI) resembled the backcrosses to it and the F_1's for both citrate and malate concn.

Table 2. Genotypic distribution and recombination frequency for genes controlling citrate and malate concns in tomato fruits.

Population	Genotype: *ctr*^{D}/*mlt*^{D}	*ctr*/*mlt*^{D}	*ctr*^{D}/*mlt*	*ctr*/*mlt*	Recombination
	No. individuals				
B_1	51	10	11	47	.176 ± .039
F_2	76	11	9	22	.179 ± .040

There is great variation in concn of acids among tomato accessions. Titratable acidity from 0.31 to 0.91%, as citric acid, has been reported for *Lycopersicon esculentum* (11). Slightly higher values were reported for certain *L. esculentum* x *L. pimpinellifolium* accessions. In the present study, selection of progeny with combined low concn alleles or combined high concn alleles could result in more than a 100% difference in total acid, from about 35 mM to about 75 mM (at incipient color). It is unlikely however, that the major factors in 'TL' and 'PI' account for most of the variation that exists in tomatoes. Other alleles of these genes may exist and it is probable that an expanded search, particularly among primitive accessions, would uncover other major genes affecting citrate and malate concn. The likelihood of detecting other factors depends on the magnitude of mutations which have occurred in the genes controlling assimilation of these compounds. Unless a mutation conditions a substantial change in concn it is doubtful that it could be detected because of the great effect of stage of maturity on acid concn in tomato fruits (Fig. 1).

Table 3. Mean concns of citrate and malate of fruits of 'Tondo Liscio' (P_1), PI 263713 (P_2), and F_1, B_1, B_2 and F_2 progeny.

Population	Pedigree	Citrate	Malate
		Concn (mM)[z]	
P_1		29.3a	22.6a
P_2		51.9e	8.8d
F_{1a}	P_1 x P_2	53.4de	11.7cd
F_{1b}	P_2 x P_1	51.7de	9.1d
B_{1a}	P_1 x F_{1a}	38.9b	14.8bc
B_{1b}	P_1 x F_{1b}	38.1b	16.7b
B_{2a}	P_2 x F_{1a}	52.0de	9.3d
B_{2b}	P_2 x F_{1b}	50.0de	9.1d
F_{2a}	F_{1a} x F_{1a}	46.4cd	12.0cd
F_{2b}	F_{1b} x F_{1b}	42.1bc	12.5cd

[z]Means not followed by the same letter are significantly different at the 5% level, Duncan's Multiple Range Test.

The study of divergent tomato accessions indicated that there are genetic factors other than those in 'TL' and 'PI', which influence varying concn of citrate and malate (Table 4). The accessions with citrate concn significantly greater than in 'PI' (No. 21) are small-fruited wild types. Some of these have much greater concn than does 'PI', and it is probable that the genetic basis for citrate assimilation is different. The majority of the accessions studied have citrate concn not significantly different from those in 'TL' and 'PI'.

Table 4. Mean concns of citrate and malate of fruits of 28 tomato accessions.

No.	Accession	Concn (mM)[z] No. Citrate	No. Malate
1)	PI 190188	1) 57	5) 11.5
2)	PI 126436	2) 54	15) 8.7
3)	PI 129143	7) 48	16) 8.2
4)	PI 127810	6) 43	3) 7.4
5)	PI 272691	3) 40	22) 6.9
6)	PI 128299	8) 39	13) 6.5
7)	PI 128233	20) 35	10) 6.4
8)	PI 155369	5) 34	6) 5.7
9)	Crimson	21) 33	11) 5.7
10)	Walters	24) 33	8) 4.9
11)	Merbein Midseason	19) 32	4) 4.8
12)	Florida 1339-08-S3	9) 31	24) 4.6
13)	Campbell 22 (lutescent mutant)	18) 30	1) 4.4
14)	Epoch	14) 30	2) 4.3
15)	Tondo Liscio	25) 29	14) 4.3
16)	Best of All	12) 29	12) 4.1
17)	STEP 375	17) 27	7) 3.7
18)	Pizza	11) 25	20) 3.7
19)	Red Jacket	10) 24	17) 3.6
20)	Rutgers Ve	4) 23	9) 3.3
21)	PI 263713	15) 23	23) 3.3
22)	Delsher	16) 23	25) 3.1
23)	Manalucie	23) 22	21) 3.0
24)	PI 280597	22) 22	19) 2.9
25)	DX72 (Campbell)	13) 22	18) 2.8
26)	Campbell 146	22) 17[y]	22) 10.3[y]
27)	Campbell 1327	26) 18[y]	26) 2.5[y]
28)	PI 255842-S1	27) 10[y]	27) 5.5[y]
		28) 17[y]	28) 1.9[y]

[z]Means not connected by a common line are significantly different at the 5% level, Duncan's Multiple Range Test.
[y]Data from Stevens and Long (21), not included in statistical analyses.

Table 4 also includes concn data for 'Delsher', 'Campbell 146', 'Campbell 1327' and PI 255842-Sl from a previous study (21). In that work there was a heritable difference in citrate between 'C 146' and 'C 1327'. It appears that 'C 146' has a concn similar to that of 'TL', the low parent in the present study, and that 'C 1327' has even less citrate. 'Delsher' was included in both studies, and can be used as a reference point. It was similar to 'C 146' in the earlier study and to 'TL' in this study. The concn range from the primitive accessions to 'C 1327', and the variance from 'TL' and 'PI', are good evidence for additional citrate factors.

About 40% of the accessions had malate concn similar to that in 'PI', but only 8% were like 'TL', the recessive parent. The malate concn of 'Delsher' differed greatly between this

study and the previous one. It is doubtful that maturity differences could account for this variation because of the techniques used and the similarity of citrate values obtained in the 2 studies. Rather, there may be a large genetic-environment interaction for this compound. Cultivars C 146, PI 255842-Sl, and PI had similar malate concn. The differences between 'C 146' and 'C 1327', and between PI 255842-Sl and 'Delsher' are controlled by single genes with dominance for low concn (21). The low concn of malate has consistently been conditioned by a dominant gene and most tomato accessions appear to be in this dominant class. The situation relative to higher concn is not clear, but there appears to be more than 1 factor involved. It is unlikely that 'TL', 'Delsher', and 'C 1327' fall into a single group for higher malate concn because of the differences observed between the former 2 in California and between the latter 2 in New Jersey.

It is possible to breed for various citrate/malate ratios. The question thus arises as to the merits of breeding for a specific ratio. Despite different disassociation constants, there is little difference in the pH obtained with equimolar concn of citric and malic acids (8). Greatly different citrate/malate ratios have no detectable effect on the relationship between pH and titratable acidity (20).

Several studies have involved the taste thresholds of citric and malic acids. Berg et al. (2) reported thresholds of 0.13 mM and 0.22 mM, respectively, for citric and malic acids. Fabian and Blum (7) found higher thresholds of 0.70 mM for citric acid and 0.75 mM for malic acid, and Pfaffman (14) reported similar thresholds of 0.79 mM for citric and 0.80 mM for malic acid. Powers et al. (15) found much lower difference, not recognition, threshold levels of 0.0024 mM for citric and 0.0029 mM for malic acid. Although the reported thresholds varied greatly among studies, generally the differences between citric and malic acids in any 1 study were small, indicating that there is little taste potency difference between the 2 compounds. Gardner (8), however, reported that malic acid has a greater (about 14%) sour intensity than does citric acid, and that the same general degree of tartness can be achieved with less malic acid. Harvey (10) showed that sourness is related to the total free-acid of a solution as well as to the H ion concn, and Beatty and Cragg (1) noted that sourness is proportional to the volume of buffer required to bring a given volume of an acid to pH 4.4. On a molar basis, the titer of citric acid exceeds that of malic acid.

As there is an easily managed genetic potential for citrate plus malate concn (total acidity), it is doubtful that attempts to control citrate/malate ratio would serve any practical purpose.

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