Varietal Differences in Tomatoes: A Study of Alpha-Keto Acids, Alpha-Amino Compounds, and Citric Acid in Eight Tomato Varieties before and after Processing

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The effects of processing on α-keto acids, α-amino compounds, and citric acid were studied qualitatively and quantitatively in eight tomato varieties. Dihydroxytartaric acid was identified in four unprocessed varieties with pyruvic and α-keto glutaric acids. Processing resulted in variable decreases in pyruvic acid concentrations, disappearance of dihydroxytartaric acid, and increases of α-keto glutaric acid contents. Glutamic acid and glutamine were recognized generally in the highest concentrations among the 16 identified α-amino compounds, and while processing caused decreases in the amide, the acid concentration increased in most cases. Ratios of pyruvic to α-keto glutaric, and α-amino nitrogen and α-keto glutaric to citric acid were analyzed for use as presumptive indices for flavor discrimination.

Spenser and Stanley (*22*) found in the vacuum distillate of fresh and canned tomato juice: volatile acids, carbonyl compounds, and esters. They recognized in the typical tomato odor fraction 12 carbonyl compounds, of which only acetaldehyde and isovaleraldehyde were identified. Bulen *et al.* (*5*) separated and identified pyruvic and α-keto glutaric acids in fresh tomato juice. As far as could be determined, no work had been reported on the quantitative amounts of α-keto acids in tomatoes and their probable significance in tomato flavor. The acidity in tomato juice has been studied by many investigators (*1, 2, 5, 18, 23-25*). Citric acid concentrations were 0.621 to 0.634% (*23*) and 0.336% (*18*) in fresh and canned juices, respectively. These concentrations represented about 73 to 80% (*24*) and 65% (*18*) of the total acidity in fresh and canned tomatoes, respectively. Beidler (*4*) explained the significance of citric acid in stimulating the maximum flavor response (sourness) at the lowest molar concentrations.

Buogo's study of glutamic acid content in several commercial tomato pastes led to the conclusion that unadulterated tomato pastes contained not more than 10% glutamic acid (*6*). Ito (*12*) and Safina (*20*) determined the amino acids present in tomato juice and tomato puree, respectively. Later, Carangal *et al.* (*7*), Saravacos *et al.* (*21*), and Airan *et al.* (*1*) reported on the amino acids in tomato fruits which did not agree completely with Safina's results.

This work was designed to study the acidic carbonyl compounds (keto acids), citric acid, and α-amino compounds qualitatively and quantitatively in fresh and processed tomato samples from eight varieties.

Experimental

Samples from eight tomato varieties were harvested from the 1959 crop at the Ohio State University farm in Columbus, Ohio. These varieties were Rutgers, Wisconsin 55, KC 135, KC 146, Glamour, Early Bird F_2, Moreton Hybrid, and Cardinal Hybrid. Ten fruits from both Rutgers and Glamour varieties were frozen at 0° F. directly after being harvested. All varieties were processed in the pilot-scale laboratory at the Horticulture and Forestry Department 1 day after harvesting. Tomato juice was canned in plain-tin 303 cans after emerging from the extractor (extractor samples), and after being treated with High-Temperature, Short-Time process (HTST) for 2.5 minutes at 240° F. in a plate heat exchanger (American Plate Vacuum Co.). Canned tomato juice samples were frozen at 0° F. until they were analyzed. All experiments were run in duplicate.

Analysis of Keto Acids. Extraction from Tomato Juice and Preparation of 2,4-DNPH-one. Adaptations of the method of Bassett and Harper (*3*) were as follows: 10 grams of tomato juice were centrifuged at 5000 r.p.m. for 10 minutes. The supernatent liquid (serum) was decanted into a 150-ml. beaker, and 5 ml. of distilled H_2O were used to aid in the transfer. The pH of the serum was determined and the serum was deproteinized by adjusting the pH to 7.0 with 10% sodium tungstate solution; then to pH 4.0 with 0.667*N* H_2SO_4 solution. This combination of sodium tungstate and sulfuric acid was found to be the best method to precipitate the proteins from tomato serum. Suspended proteins were centrifuged out at 5000 r.p.m. for 10 minutes.

The deproteinized serum was returned to the same beaker with 0.5 ml. of 1% 2,4-dinitrophenylhydrazine (2,4 DNPH) in 2*N* HCl. The mixture was left for 1 hour at room temperature (72° F.); then the 2,4 DNPH-ones were extracted with anhydrous ether. When the ether layer was light yellow, the extraction was stopped, and the ether extract amounted to about 150 ml. Ether was evaporated in a ventilated hood by a stream of air at room temperature. The dried 2,4 DNPH-ones were dissolved in the least volume of 1*N* NH_4OH (1 to 3 ml.). The nonacidic 2,4 DNPH-ones were extracted from the acidic 2,4 DNPH-ones with chloroform in a separatory funnel. For each 1.0 ml. of NH_4OH, approximately 50 ml. of chloroform were needed to extract the nonacidic 2,4 DNPH-ones. Any chloroform–NH_4OH colloidal suspension was broken by centrifuging at 1000 r.p.m. for 2 minutes. The 2,4 DNPH-ones layer (NH_4OH) was transferred to a 10-ml. vial which was stoppered and refrigerated at 35° F. until the chromatography analysis.

Chromatography and Identification of 2,4 DNPH-ones of Alpha-Keto Acids. The method developed by El-Hawary and Thompson (*9*) was used in chromatographing the acidic 2,4 DNPH-ones. Spots were identified by developing for 24 hours known 2,4 DNPH-ones of: α-keto glutaric (KG), pyruvic (P), and dihydroxytartaric acid (DHTA). Also, color reactions between 1*N* NaOH and the 2,4 DNPH-ones were used in identifying the monocarbonyls and the dicarbonyls. Ultraviolet and infrared spectra were used in establishing the presence of DHTA in tomato juice.

Preparation of Known 2,4 DNPH-ones. Alpha-keto glutaric and pyruvic acids were obtained from the Sigma

Chemical Co. Dihydroxytartaric acid was prepared from nitrotartaric acid by Lachman's method (*14*). This acid was crystallized as the sodium salt which is slightly water-soluble.

Ten milligrams of both KG and P were weighed and dissolved separately in 5.0 ml. of distilled water. One milliliter of 1% 2,4 DNPH was added to each solution, and the mixtures were left to react at room temperature (72° F.) for 1 hour. The acidic hydrazones were extracted in anhydrous ether which was evaporated to dryness in a stream of air. Exactly 10.0 ml. of 1*N* NH_4OH were used to dissolve the dried hydrazones, which were transferred to labeled vials and refrigerated until chromatographed.

The bis-2,4 DNPH-one of DHTA was prepared by suspending 0.5 gram of the DHTA salt in 10.0 ml. of distilled water, and acidifying with 2*N* HCl until all the salt was dissolved. Ten milliliters of 2,4 DNPH solution were added to the DHTA solution, and the mixture was left for 1 hour. The hydrazone began to form after 5 minutes with a semigel structure. After 1 hour in reaction, the bis-hydrazone was extracted in ether which was evaporated in a stream of air at room temperature. The bis-hydrazone was kept over night in a desiccator over $CaCl_2$. Fifty three and nine-tenths milligrams were weighed, corresponding to 20 mg. of DHTA, and dissolved in 200 ml. of 1*N* NH_4OH. This solution was used for chromatographic separation, and for preparing a standard curve for the quantitative analysis of DHTA.

The 2,4 DNPH-one spots which were separated from the tomato serum samples were compared with the known 2,4 DNPH-ones, and were compared with those reported in the literature. Infrared spectra of DHTA-one and the unknown sample were scanned from a KBr pellet in a Baird-Atomic infrared spectrograph.

Semiquantitative Determination of Keto Acids. A standard curve for pyruvic and α-keto glutaric hydrazones was prepared by applying 5, 15, 25, and 40 μg. of the keto acids to a chromatostrip, which was developed for 24 hours as were the other chromatographs. The dried spots were cut off and put in 1 × 6 inch test tubes, as well as a blank which was a colorless area of the chromatostrip approximately the same size as the hydrazone spots. All spots were eluted in 8.0 ml. of 1*N* NaOH, and absorbances were measured at 430 mμ and 445 mμ for KG and P, respectively (*3*). The fast moving isomer of P was not found in any measurable amounts, and only the slow moving isomer was measured at 445 mμ.

The maximum absorption for DHTA bis-hydrazone was established between 575 mμ and 585 mμ. Since the blank absorption values were found to increase at the longer wavelength (585 mμ), the shorter wavelength of the peak (575 mμ) was chosen to establish the standard curve for DHTA. This curve was prepared by applying 2.5, 5.0, 7.5, and 10.0 μg. of DHTA to a chromatostrip and proceeding with the method as mentioned for the KG and P curves.

The amounts of keto acids in the tomato samples were measured by eluting the bands in 8.0 or 10.0 ml. of 1*N* NaOH, and measuring absorbances at the specific wavelength. Concentrations were calculated as μg. of keto acid per 100 grams of juice. All measurements were carried out on a Bausch & Lomb Spectronic 20.

Citric Acid Analysis. Isolation of Nonamino Organic Acids. Ten grams of tomato juice were centrifuged as mentioned under the keto acids. Dowex 50-X4 (200–400 mesh) was prepared in the acidic form by washing several times with dilute HCl of pH 2.0, and used in 1-inch diameter columns. The tomato serum was passed through the resin, followed by a 10.0-ml. water washing of pulp. Columns were washed with 20 to 30 ml. of distilled water to verify that all organic acids were washed out of the resin. Perculates were concentrated in vacuo to volumes of 2.0 to 8.0 ml. for chromatography.

Separation of Citric Acid by Paper Chromatography. Ten to 25 μl. were applied in spots on 5.5 × 17.0 inch chromatostrips which were developed in butanol–acetic acid–water (120:30:50) solvent for 18 hours. Known citric acid spots were applied on each chromatostrip as guides for the distance and area of citric acid spots that separated from the mixtures. An alcoholic 0.1% bromcresol blue indicator (shifted to the blue color with concentrated NaOH) was used to locate the acidic spots on the chromatograms.

Quantitative Estimation of Citric Acid. Citric acid spots (parallel to the known) were cut off and put in 50-ml. Erlenmeyer flasks. Ten milliliters of distilled water were added to elute the acid which was titrated with 0.01*N* NaOH using a phenolphthalein indicator. Results were recorded as per cent of citric acid in tomato juice samples. Spots containing less than 0.15 μg. of citric acid resulted in approximately 85% accuracy.

Extraction and Identification of Alpha-Amino Acids and Amides. Amino compounds were eluted from the column with 30 ml. of 1*N* NH_4OH and dried in vacuo in a water bath at 40° to 50° C. The residue was treated with exactly 5.0 ml. of 80% ethanol which dissolved only amino acids and light peptides, while proteins and polypeptides were precipitated.

Two-dimensional paper chromatography was used to separate the amino acids as developed by Levy and Chung (*15*). Some modifications, however, were introduced as follows: 15 μl. containing between 2 to 3 μg. of amino nitrogen were applied to a spot approximately 4 inches away from the corner of an 18.5 × 22 inch Whatman No. 1 chromatography sheet. The chromatograms were developed in a chromatocab, which was described by Edwards *et al.* (*8*), for 22 to 24 hours in a solvent that was prepared from *n* - butanol – acetic acid – water in the ratio of 120:30:50. The second solvent was prepared with liquid phenol (88%), and borate buffer of pH 9.3 in the ratio of 9 to 1 with traces of 8-quinolinol. Phenol required from 20 to 23 hours to travel about 17 inches of the second dimension. After the chromatograms were dried at room temperature, they were sprayed with ninhydrin indicator (*15*), and heated in a ventilated oven at 90° C. for 3 minutes. The spots were demarcated under ultraviolet light, and compared with known amino acids (*15*).

Quantitative Determination of Alpha-Amino Nitrogen. Moore and Stein's method (*16*) was used in estimating α-amino nitrogen in tomato extracts. One milliliter of the 80% ethanol extract was diluted to 500 ml. in a volumetric flask with distilled water. Aliquots of 1.0 ml. were used in each determination, and results were calculated from a standard curve prepared by using known concentrations of glutamic acid.

Results and Discussion

Keto Acids. Three α-keto acids separated from fresh samples of the eight tomato varieties which were identified as (I) α-keto glutaric acid, (II) dihydroxytartaric acid, and (III) pyruvic acid (slow moving isomer) in Figure 1. The fast moving isomer of pyruvic was detected only in traces under ultraviolet light. All compounds were identified with knowns, and with the changes in color in 1*N* NaOH (*3*, *13*). Ultraviolet absorption of DHTA samples in acidified chloroform and distilled H_2O were compared with the absorption curve of known DHTA (Figure 2). The peak in water solution was found at 270 mμ which was shifted to 275 to 276 mμ in the chloroform solution. This shift was caused mainly by the acidic conditions in the chloroform solution.

The infrared spectrograph of a DHTA-one known, and a DHTA-one isolated from a tomato sample is shown in Figure 3. Transmittance at 4.3 microns was greater than 100% because the infrared machine was adjusted for maximum sensitivity to compensate for the small sample of unknown which amounted to approximately 2.5 mg. This, however, did not contribute to better resolution

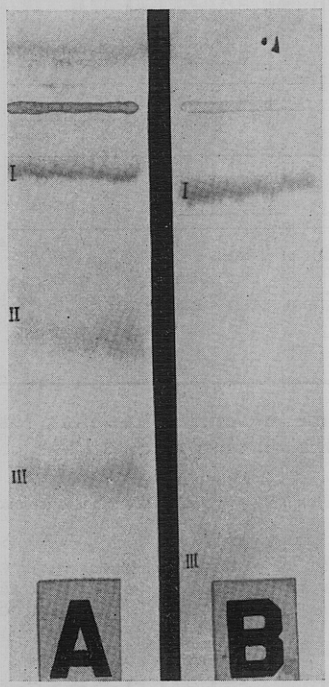

Figure 1. Alpha-keto acid hydrazones separated from (A) fresh and (B) processed Rutgers samples

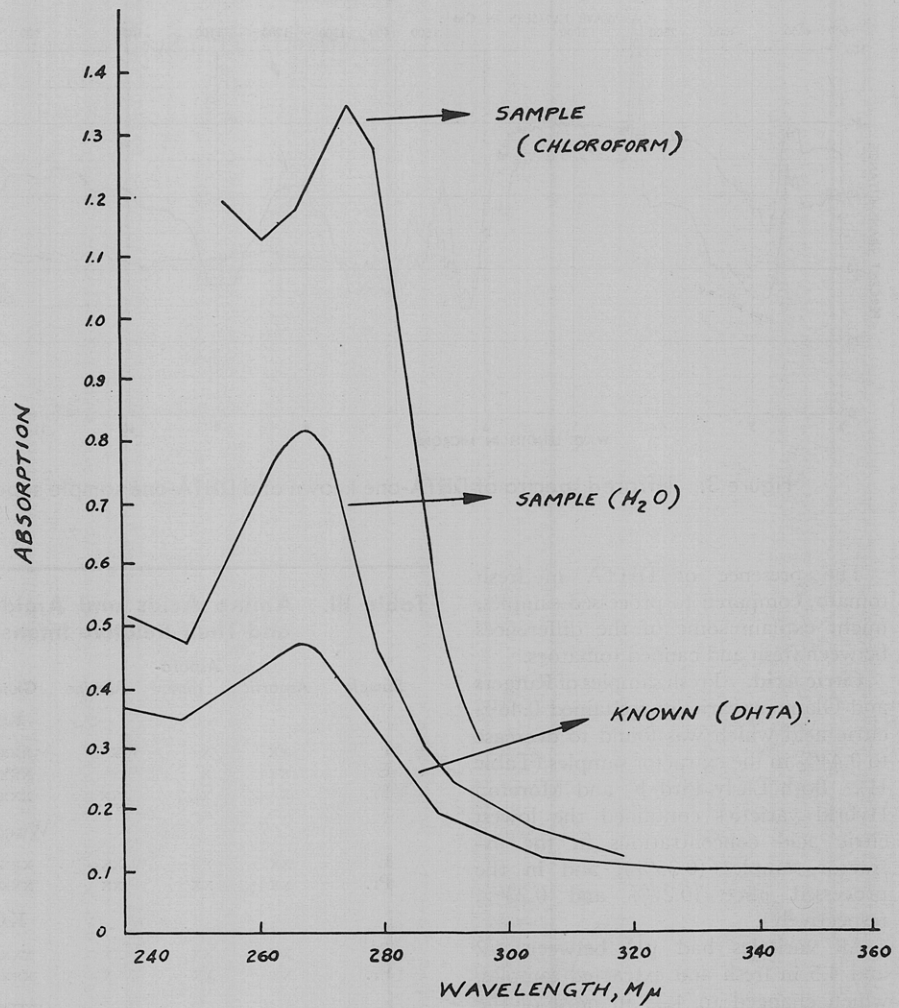

Figure 2. Ultraviolet absorption spectra of DHTA-one in chloroform and water solutions

in the spectra of both the sample and DHTA-one beyond 7 microns. The incomplete band(s) of DHTA-one spectrum at 8.8 to 9.5 microns probably represented the substituted positions on the benzene rings.

The amounts of KG, P, and DHTA found in the eight tomato varieties are shown in Table I. Alpha-keto glutaric acid concentrations were higher in the processed samples than in both fresh and extractor samples. This increase was caused mostly by the deamination of glutamic acid. Pyruvic acid concentrations were higher in the extractor samples than in the fresh samples because of enzymatic activities in the juice before sterilization. Loss of pyruvic acid in all of the processed samples was probably caused by a decarboxylation reaction, which resulted in the formation of acetaldehyde; this, upon exposure to air, was oxidized to acetic acid (*10*). Only four varieties contained DHTA in the extractor samples; moreover, the fresh samples of Rutgers and Glamour were richer in DHTA than the extractor samples. Processed samples generally did not contain DHTA, and it was suggested that DHTA was oxidized in the presence of heat and Fe^{+3} to oxalic acid, or carbon dioxide and water (*11, 19*).

Table I. Amounts of Alpha-Keto Glutaric (KG), Pyruvic (P), and Dihydroxytartaric Acids (DHTA) in Eight Tomato Varieties

	Micrograms/100 Grams of Juice								
	KG			P			DHTA		
Variety	F[a]	E	Pr.	F	E	Pr.	F	E	Pr.
Rutgers	1525	1200	1800	325	400	400	450–540	175	...
Wisconsin 55	...	1800	2200	...	400	100	...	...	...
KC 135	...	1050	1100	...	150	5	...	...	...
KC 146	...	1150	1200	...	250	100	...	270–360	...
Glamour	1350	1350	1600	400	600	600	666	250–360	...
Early Bird F_2	...	1800	1800	...	500	133	...	...	...
Moreton Hybrid	...	1300	1800	...	400	200	...	...	...
Cardinal Hybrid	...	1500	1700	...	100	5	...	175–180	...

[a] F: fresh, E: extractor, Pr: processed.

Table II. pH, Citric Acid, and Amino Nitrogen in Eight Tomato Varieties before and after Processing

	Fresh			Extracted			Processed		
Variety	pH	% Citric acid	Mg. amino N/100 gm. juice	pH	% Citric acid	Mg. amino N/100 gm. juice	pH	% Citric acid	Mg. amino N/100 gm. juice
Rutgers	4.30	0.46	123.0	4.30	0.44	136.7	4.25	0.27	190.0
Wisconsin 55	...	...	...	4.50	0.37	108.3	4.30	0.34	128.3
KC 135	...	...	...	4.30	0.44	118.3	4.25	0.34	120.0
KC 146	...	...	...	4.50	0.54	102.5	4.25	0.46	111.7
Glamour	4.45	0.46	114.3	4.50	0.44	121.3	4.30	0.36	169.8
Early Bird F_2	...	...	...	4.20	0.35	100.0	(4.25)	0.27	102.5
Moreton Hybrid	...	...	...	4.40	0.35	98.0	4.25	0.23	106.7
Cardinal Hybrid	...	...	...	4.45	0.46	106.7	4.25	0.35	118.3

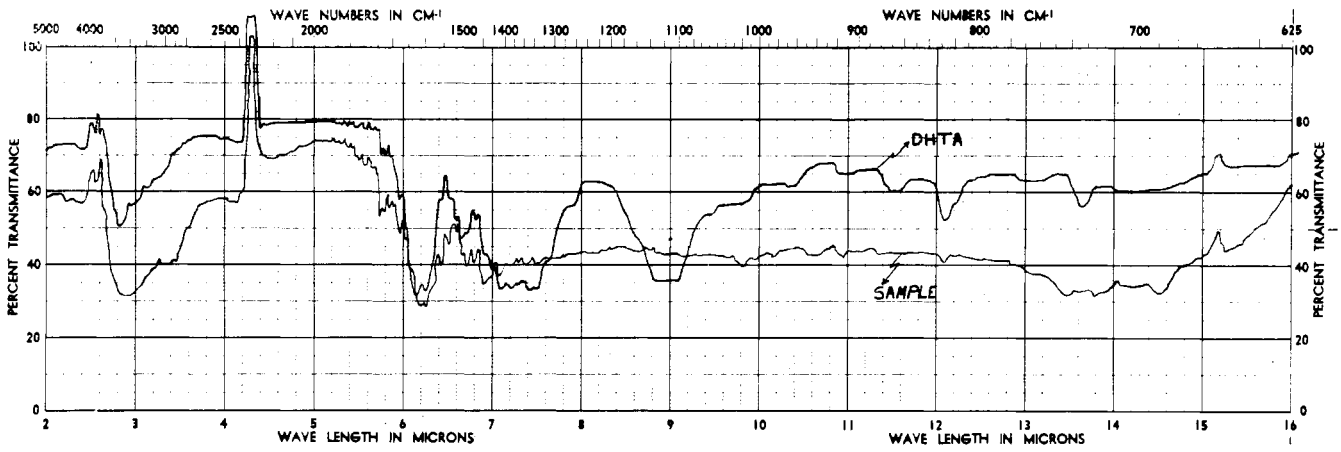

Figure 3. Infrared spectra of DHTA-one known and DHTA-one sample separated from fresh Rutgers samples

The presence of DHTA in fresh tomato, compared to processed samples, might explain some of the differences between fresh and canned tomatoes.

Citric Acid. Fresh samples of Rutgers and Glamour varieties contained 0.46% citric acid which was found to decrease to 0.44% in the extractor samples (Table II). Both Early Bird F_2 and Moreton Hybrid varieties contained the lowest citric acid concentrations in the extractor samples (0.35%), and in the processed juice (0.27% and 0.23%, respectively).

All varieties had pH between 4.2 and 4.5 in fresh and extracted samples, which changed to 4.25 in most of the processed samples (Table II). This drop in pH could have been caused by the lower concentrations of citric acid, which increased the degree of its ionization, or by developing reactions between the tomato acids and the tin plating of the cans.

Alpha-Amino Acids and Amides. Various quantities of 14 amino acids and two amides were found in all tomato varieties used in this work (Table III). The degree of intensity of each spot was marked with an *X*. Glutamine and asparagine were the two amides that were present invariably in all fresh, extracted, and processed samples. Visually, glutamine was three to four times as high in concentration as asparagine in fresh and extractor samples. Processing apparently did not affect asparagine as much as glutamine, which was detected in lower concentrations than in fresh samples. Rice *et al.* (*18*) suggested that glutamine was one of the compounds which changed to pyrrolidone carboxylic acid (PCA) in canned tomatoes. Glutamic acid concentrations increased in the processed samples of Rutgers, Wisconsin 55, KC 146, Glamour, Early Bird F_2, and Cardinal Hybrid. Glutamic acid increase may have been caused by the heat treatment which resulted in partial deamination of glutamine. Proline

Table III. Amino Acids and Amides Found in Tomato Varieties Tested and Their Relative Intensity on the Chromatograms

Sample	Aspartic	Asparagine	Alanine	Glutamic	Glutamine	Glycine	Leucine	Isoleucine
				Rutgers				
F	xx	x	x	xxxx	xxx	trace	trace	trace
E	x	x	x	xxxx	xxxx	trace	x	trace
Pr.	x	x	xx	xxxxx	xxxx	trace	x	trace
				Wisconsin 55				
E	xx	xx	xx	xxxx	xxx	trace	x	x
Pr.	xx	xx	xx	xxxxx	xxx	trace	trace	trace
				KC 135				
E	xx	xx	xx	xxxx	xxx	trace	xx	trace
Pr.	xx	xx	xx	xxxx	xxx	trace	xx	trace
				KC 146				
E	x	x	x	xxxx	xxx	trace	trace	trace
Pr.	x	x	xx	xxxxx	xxx	trace	trace	trace
				Glamour				
F	x	x	x	xxxx	xxx	...	trace	trace
E	xx	x	x	xxxx	xxx	trace	x	trace
Pr.	x	x	xx	xxxxx	xx	trace	x	...
				Early Bird F_2				
E	xx	x	x	xxx	xxx	trace	x	trace
Pr.	xx	x	x	xxxx	xx	...	x	trace
				Moreton Hybrid				
E	xx	x	xx	xxxx	xxxx	trace	x	trace
Pr.	xx	x	x	xxxx	xxx	...	x	...
				Cardinal Hybrid				
E	xx	x	xx	xxxx	xxx	trace	x	trace
Pr.	xx	x	x	xxxxx	xx	trace	trace	trace

	Lysine	Histidine	Phenylalanine	Serine	Threonine	Valine	Proline	Tyrosine
				Rutgers				
F	x	x	trace	x	x	xx	xx	x
E	x	x	trace	xx	xx	xxx	xx	trace
Pr.	x	x	trace	xx	xx	xxx	x	trace
				Wisconsin 55				
E	xx	xx	x	x	xx	xx	xx	trace
Pr.	x	x	trace	x	xx	xx	x	...
				KC 135				
E	x	x	trace	xx	x	xxx	x	trace
Pr.	x	x	trace	xx	x	xxx	x	trace
				KC 146				
E	x	x	trace	x	x	xxx	...	trace
Pr.	x	x	trace	x	x	xxx	xx	trace

(*Continued on page 503*)

was detected after processing samples of KC 146, Glamour, and Early Bird F_2. No difference was detected between fresh and processed samples in the concentrations of aspartic, asparagine, valine, serine, threonine, lysine, and histidine. Ito (*12*) did not detect any difference in amino acids between raw and canned tomato juice. Alanine concentrations varied between varieties so that no trend could be established from these results. Processed samples of Wisconsin 55 and Cardinal Hybrid contained lower concentrations of leucine than the fresh or extractor samples. Generally speaking, leucine concentration varied with variety from a trace to relatively high concentrations.

Isoleucine, glycine, phenylalanine, and tyrosine were detected in all samples as traces. Pure amino acids in solution were proved to exert different flavors (*17*), and it should be expected that tomato juice flavor would be affected by the relative concentrations of each amino acid present.

Alpha-Amino Nitrogen Content. The quantitative changes in amino nitrogen varied from one variety to another, as shown in Table II. The increases of amino nitrogen in the extractor samples over the fresh samples of Rutgers and Glamour varieties were caused by the mechanical effects of the comminuter and enzymatic activities, which resulted in releasing more peptides and amino acids from the proteins. The high contents of amino nitrogen in processed samples might be attributed to the hydrolytic effects of the sterilization temperature (240° F.) on proteins. All canned samples developed the typical cooked flavor and aroma of tomato juice.

Ratios of P:KG, KG:C, and N:C. These ratios were suggested to give the food technologist simple figures which would be objectively associated with the quality of tomatoes; furthermore, they would help tomato breeders in evaluating new varieties. The suggested ratio of P:KG as a scale for the qualitative difference between fresh and processed tomatoes did not show any promising results since the ratio in the fresh Rutgers variety was almost the same as that of the processed samples; however, ratios between KG and citric, and α-amino nitrogen and citric acid indicated a positive relationship between processing and the ratios (Table IV). Generally, higher ratios of KG:C and N:C were found in processed samples because concentrations of α-keto glutaric and amino nitrogen increased while citric acid amounts decreased. The significance of these ratios could be determined after correlating them with the organoleptic qualities of each tomato variety.

Table III. *(continued)*

Sample	Aspartic	Asparagine	Alanine	Glutamic	Glutamine	Glycine	Leucine	Isoleucine
				Glamour				
F	x	x	trace	x	x	xxx	x	...
E	x	x	trace	x	x	xxx	...	...
Pr.	x	x	trace	x	x	xxx	xx	...
				Early Bird F_2				
E	x	x	trace	x	x	xxx	...	trace
Pr.	x	x	trace	x	x	xxx	xx	trace
				Moreton Hybrid				
E	x	x	trace	x	x	xxx	xx	trace
Pr.	x	x	trace	x	x	xxx	xx	trace
				Cardinal Hybrid				
E	x	trace	trace	x	x	xxx	x	trace
Pr.	x	...	...	x	x	xxx	xx	trace

Table IV. Ratios of Pyruvic Acid (P) to Alpha-Keto Glutaric Acid (KG), Alpha-Keto Glutaric Acid (KG) to Citric Acid (C), and Amino Nitrogen (N) to Citric Acid

Variety	P:KG			KG:C			N:C		
	F	E	Pr.	F	E	Pr.	F	E	Pr.
Rutgers	0.21	0.33	0.22	0.0033	0.0026	0.0066	0.266	0.304	0.764
Wisconsin 55	...	0.22	0.04	...	0.0048	0.0063	...	0.294	0.342
KC 135	...	0.14	0.004	...	0.0022	0.0031	...	0.256	0.346
KC 146	...	0.21	0.08	...	0.0021	0.0026	...	0.219	0.260
Glamour	0.29	0.44	0.37	0.0029	0.0030	0.0043	0.247	0.278	0.465
Early Bird F_2	...	0.28	0.074	...	0.0041	0.0066	...	0.278	0.381
Moreton Hybrid	...	0.30	0.11	...	0.0036	0.0077	...	0.273	0.463
Cardinal Hybrid	...	0.06	0.029	...	0.0032	0.0047	...	0.231	0.329

Acknowledgment

The authors express their gratitude to the Institute of Food Technology and Nutrition for supporting this research during the year 1959–60.

Literature Cited

(1) Airan, J. W., Barnabas, J., *J. Univ. Bombay* **22,** Pt. 3, No. 34 B, 29–31 (1953); *C.A.* **48,** 10132g (1954).
(2) Andreotti, R., Ceci, D., *Ind. Conserve Parma* **30,** 249–53 (1955); *C.A.* **50,** 5091g (1956).
(3) Bassett, E. W., Harper, W. J., *J. Dairy Sci.* **XLI,** 1206–17 (1958).
(4) Beidler, Lloyd, in Symp. "Chemistry of Natural Food Flavors," Quartermaster Food Container Inst., 1957.
(5) Bulen, W. A., Varner, J. E., Burrell, R. C., *Anal. Chem.* **24,** 187 (1952).
(6) Buogo, Giulio, *Ann. Chim. Applicata* **24,** 79–82 (1934).
(7) Carangal, A. R., Jr., Alban, E. K., Varner, J. E., Burrell, R. C., *Plant Physiol.* **29,** 355–60 (1954).
(8) Edwards, C. H., Gadsen, E. L., Carter, L. P., Edwards, G. A., *J. Chromatog.* **2,** 188 (1959).
(9) El-Hawary, M. F. S., Thompson, R. H. S., *Biochem. J.* **53,** 340 (1953).
(10) Fruton, J. S., Simmonds, S., "General Biochemistry," 2nd ed., p. 475, Wiley, New York, 1958.
(11) Gatet, L., Genevois, L., *Bull. Soc. Chim. France* **8,** 485–7 (1941).
(12) Ito, Saburo, *J. Util. Agr. Prod.* **1,** 203–4 (1954).
(13) Krupka, R. M., Towers, H. N., *Botan. Rev.* **25,** No. 1, 108–20 (1959).
(14) Lachman, Arthur, *J. Am. Chem. Soc.* **43,** 577 (1921).
(15) Levy, A. L., Chung, D., *Anal. Chem.* **25,** 396 (1953).
(16) Moore, S., Stein, W. H., *J. Biol. Chem.* **211,** 907 (1954).
(17) Mulder, H., *Neth. Milk Dairy J.* **6,** 157 (1952).
(18) Rice, A. C., Pederson, C. S., *Food Res.* **19,** 106 (1954).
(19) Rudopulo, A. K., *Biokhim. Vinodeliya, Sbornik* **5,** 199–212 (1957); *C.A.* **51,** 18042e (1957).
(20) Safina, G., *Conserve Deriv. Agrumari Palermo* **2,** 70–2 (1953).
(21) Saravacos, G., Luh, B. S., Leonard, S., *J. Food Res.* **23,** 329 (1958).
(22) Spenser, M. S., Stanley, W. L., J. Agr. Food Chem. **2,** 1113 (1954).
(23) Van Dame, H. C., *J. Assoc. Offic. Agr. Chem.* **36,** 580–5 (1953).
(24) Villarreal, F., Luh, B. S., Leonard, S. J., *Food Tech.* **14,** 176 (1960).
(25) Yamada, Koji, *Nosan Kako Gijutsu Kenkyu Kaishi* **3,** 1–4 (1956); *C.A.* **53,** 9509d (1959).

Received for review October 9, 1961. Accepted February 13, 1962.