Nonvolatile Acids of Passion Fruit Juice

Harvey T. Chan, Jr.,* Thomas S. K. Chang, and Eduardo Chenchin

The nonvolatile acids were extracted from yellow and purple passion fruit, separated by tlc, and identified as lactic, malonic, malic, citric, ascorbic, and galacturonic. Glc of the methyl esters of the acids confirmed the presence of lactic, malonic, malic, and citric and revealed the presence of succinic acid. Quantitative analysis by glc using adipic acid as an internal standard showed yellow and purple passion fruit differed in both total acid content and in the relative proportions of each of the acids.

Passion fruit juice is growing steadily in popularity as a component in beverages, syrups, bakery, and dairy products. Commercially produced passion fruit in Hawaii is mainly the yellow type (*Passiflora edulis f. flavicarpa*) also known locally as yellow lilikoi (Univ. of Hawaii, 1956). Purple passion fruit (*P. edulis f. edulis*) is produced commercially in Australia, New Zealand, South Africa, and elsewhere in the tropics (Purseglove, 1968; Mott, 1969). Passion fruit juice is prepared by slicing the whole fruit and then mechanically separating the seeds and peel from the pulpy juice (Boyle *et al.*, 1955). The juice is reported to be a good to excellent source of provitamin A, niacin, riboflavin, and ascorbic acid; there are some differences between yellow and purple passion fruits in these nutritional factors (Wenkam and Miller, 1965; Pruthi, 1963). The juice of yellow passion fruit is orange–yellow in color, highly aromatic and flavorful, and very acid; juice of purple passion fruit is deeper orange in color and is said to be somewhat more aromatic and less acid than the yellow. Aside from its unique flavor, the comparatively high acid content of passion fruit juice is its most distinctive characteristic and is important in processing and formulation of products containing this fruit. Total acid content values (expressed as citric acid w/w) ranging from 2.4 to 7.6% have been reported (Pruthi, 1963); yellow passion fruit juice in Hawaii averages about 4% acid (Boyle *et al.*, 1955). The presence of citric and malic acids, ubiquitous to fruits, has been reported (Pruthi, 1958); Anet and Reynolds (1954) stated that passion fruit juice probably contained mucic acid in the free state. A comprehensive study of the nonvolatile organic acids of passion fruit juice was done in this laboratory employing thin-layer chromatography (tlc) and gas–liquid chromatography (glc).

MATERIALS AND METHODS

Passion Fruit Juice. Yellow passion fruit (*Passiflora edulis f. flavicarpa*) and purple passion fruit (*Passiflora edulis f. edulis*) were harvested from the Hawaii Agricultural Experiment Station farm. The juice was expressed from passion fruit pulp through four layers of cheesecloth and stored at 0° F.

Total Acidity, Total Volatile Acidity, and Ascorbic Acid. The colorimetric method of Loeffler and Ponting (1942) with slight modifications was used for ascorbic acid assay. Total titratable acidity and total volatile acidity were determined by the methods of the AOAC (1960).

Extraction of Organic Acids. Passion fruit juice (200 g) was mixed with 1300 ml of 70% methanol in a Waring Blendor for 1 min and filtered *in vacuo* through Whatman No. 2 filter paper. The filtrate was concentrated in a rotary flash evaporator at 40° C until the methanol was removed. This concentrate was percolated through a regenerated column of Dowex 50W × 4 (H- form) cationic resin, and then through a column of Amberlite IRA 400 (OH form) anionic resin. The Amberlite column was rinsed with 1500 ml of water to remove the sugars.

The acids were eluted from the anionic column with 100 ml of 6 *N* HCl followed by water until approximately 250 ml of eluate was obtained. The eluate was concentrated and further prepared for tlc and glc as stated previously (Chan *et al.*, 1971).

Gas–Liquid Chromatography. A Varian Aerograph Model 200 gas chromatograph with a flame ionization detector was used. The carrier gas, nitrogen, at a flow rate of 25 cm^3 per min, was partially saturated with water to reduce tailing (Mon *et al.*, 1966). The hydrogen gas flow rate was 44 cm^3 per min. Two different columns were used for retention time studies: a 9-ft, 0.093-in. i.d. stainless steel column packed with 15% DEGS (diethylene glycol succinate) on Chromosorb W HMDS 60/80, and a 9-ft, 0.093-in. i.d. stainless steel column with 5% FFAP on Chromosorb G A/W DMCS 60/80. The DEGS column was operated isothermally at 170° C with detector temperature at 220° C and injector temperature at 180° C. The FFAP column was operated isothermally at both 170° C and 145° C with injection port temperature of 180° C and detector temperature of 220° C.

The FFAP column was used for the quantitative determination of the acids with adipic acid as the internal standard. Known quantities of adipic acid (0.1–0.2 g) were added to 100 g of passion fruit juice. The extraction, methylation, and gas chromatography of the acids were performed as described above. Quantitative data were calculated from the ratio of the peak areas with reference to the internal standard in nine or more replications (Dal Nogare and Juvet, 1962).

Thin-Layer Chromatography. The following sorbents were used for thin-layer chromatography: Cellulose powder

Hawaii Fruit Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Honolulu, Hawaii, in cooperation with the Hawaii Agricultural Experiment Station, University of Hawaii, Honolulu, Hawaii.

Table I. R_f Values (×100) of Acids; Cellulose (Eastman Chromatogram 6064) Developed in Solvent I. EFW[a]

Compound	Known	*P. edulis*	*P. flavicarpa*
Lactic	90	89	
Malonic	82	82	
Malic	66	65	65
Citric	58	56	57
Tartaric	45	45	44
Ascorbic	43		
Unknown		21	21
Galacturonic	10	11	12

[a] EFW (anhydrous ethyl ether–formic acid–water) 20:5:3 (v/v).

MN 300 (Macherey, Nagel and Co.) coated plates (15 g of cellulose to 90 ml of water, 250 μ thick); precoated sheets of cellulose (Eastman Chromagram sheet 6064); Silica Gel–Cellulose coated plates (24 g of MN Silica Gel G and 16 g of Cellulose MN 300 (Macherey, Nagel and Co., to 144 ml of water, 300 μ thick). The coated plates, Cellulose MN 300, and Silica Gel–Cellulose mixture were left to dry and set overnight at room temperature, then dried at 110° C for 1 hr. Plates were stored in a desiccator until used (Chang and Chan, 1971).

The organic acid extract in ethanol (1 to 15 μl) was applied directly to the plates with a micropipette. The plates were developed in the following solvent systems: EFW (anhydrous ethyl ether–formic acid–water) 20:5:3 (v/v) and BBIFW (benzyl alcohol–*tert*-butyl alcohol–isopropyl alcohol–formic acid–water) 24:8:8:1:8 (v/v).

After development the plates and sheets were dried overnight at room temperature. The acid spots were located as previously stated (Chan *et al.*, 1971). In addition, when sprayed with 2,6-dichlorophenolindophenol (0.1% in 95% ethanol) the white ascorbic acid spots were differentiated from the other pink acid spots on a violet background. The R_f values in Tables I and II each represent averages of nine or more replications.

RESULTS AND DISCUSSION

Purple passion fruit (*P. edulis*) acids chromatographed on cellulose (Eastman chromagram 6064) and developed in solvent I (EFW) showed the presence of seven acids (Table I). Five of the acids had R_f values corresponding to the known acids lactic, malonic, malic, citric, and galacturonic. Malonic acid was detected only when large amounts of acid extract (10–15 μl) were spotted. Of the remaining two acids one of the acid spots corresponded closely to that of both ascorbic and tartaric; the remaining spot was unidentified. When yellow passion fruit acids were chromatographed in the same system, only five acids were detected. Three of the acids had R_f values corresponding to malic, citric, and galacturonic acids. Of the two remaining acids one was unidentified and the other corresponded closely to that of both ascorbic and tartaric acids.

Eastman chromagram 6064 sheets developed in solvent II (BBIFW) flaked, and further attempts to utilize this particular system were abandoned.

Purple passion fruit acids chromatographed in solvents I and II with a mixture of silica gel–cellulose as the sorbent showed the presence of five acids, four of whose R_f values matched those of lactic, malic, citric, and galacturonic acids (Table II). Tartaric and ascorbic acids were unresolved in solvent I and appeared as a long single spot. In solvent II ascorbic acid coincided with the citric acid spot and its presence was detected with 2,6-dichlorophenolindophenol. Chromatography of yellow passion fruit acids on silica gel cellulose in solvents I and II showed the same acids as purple passion fruit except that lactic was absent. It was later shown by glc that lactic acid is present in very low concentration in yellow passion fruit. Mucic acid was not detected in the tlc systems, confirming the observation of Pruthi (1963).

The relative retention times for the gas chromatography of the methyl esters of known and passion fruit acids on DEGS and FFAP columns are shown in Table III. Passion fruit acids tentatively identified by comparison of their relative retention times with those of known acids were lactic, malonic, succinic, malic, and citric. Methyl lactate and dimethyl malonate did not appear as separate peaks on the DEGS column (Figure 1) but did appear as separate distinct peaks on FFAP (Figure 2 and 3). The presence of tartaric, galacturonic, and ascorbic acids could not be confirmed by glc methods; galacturonic and ascorbic acids are so highly hydroxylated that these methods are unsatisfactory, as was also observed by Li and Woodroof (1968) and Mazliak and Salsac (1965).

The quantitative determination data are shown in Table IV. For yellow passion fruit citric acid was found to be the predominant acid, constituting about 83% of the acids, followed by malic, which constituted about 15.9% of the acid content. Pruthi (1963), using paper chromatography, estimated citric to be 95% and malic to be 5% of the total acid. The other acids shown (Table IV) to be present in yellow passion fruit but in much lesser amounts were lactic (0.87%),

Table II. R_f Values (×100) of Acids on Silica Gel–Cellulose Developed in Two Solvents

	Solvent I. EFW[a]			Solvent II. BBIFW[b]		
Compound	Known	*P. edulis*	*P. flavicarpa*	Known	*P. edulis*	*P. flavicarpa*
Lactic	82	82		74	74	
Malic	60	62	60	59	59	60
Citric	52	55	55	52	53	55
Tartaric	36			34	38	
Ascorbic	31	29	31	52		
Galacturonic	15	14	18	17	15	15

[a] EFW (anhydrous ethyl ether–formic acid–water) 20:5:3 (v/v). [b] BBIFW (benzyl alcohol–*tert*-butyl alcohol–isopropyl alcohol–formic acid–water) 24:8:8:1:8 (v/v).

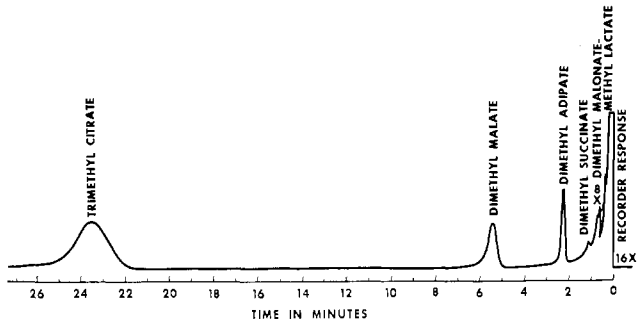

Figure 1. Chromatogram of methyl esters of organic acids from yellow passion fruit on DEGS at 170° C

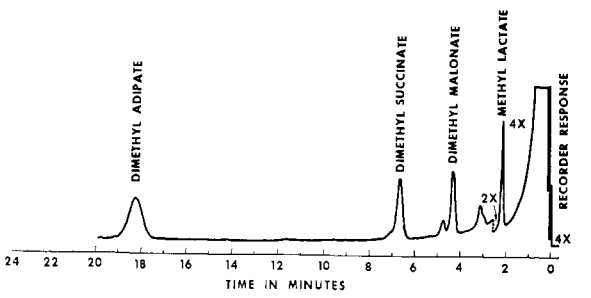

Figure 2. Chromatogram of methyl esters of organic acids from purple passion fruit on FFAP at 145° C

Table III. Relative Retention Time of Methyl Esters of Known Organic Acids and Passion Fruit Acids on Two Columns

Compound	Known	*P. flavicarpa*	*P. edulis*
	15% DEGS at 170° C		
Methyl lactate	0.32	0.32	0.34
Dimethyl malonate	0.34		
Dimethyl succinate	0.46	0.49	0.49
Dimethyl adipate	1.00	1.00	1.00
Dimethyl malate	2.35	2.37	2.42
Trimethyl citrate	10.91	10.40	11.19
	5% FFAP at 145° C		
Methyl lactate	0.13	0.13	0.13
Dimethyl malonate	0.26	0.26	0.24
Dimethyl succinate	0.38	0.38	0.37
Dimethyl adipate	1.00	1.00	1.00
	5% FFAP at 170° C		
Dimethyl adipate	1.00	1.00	1.00
Dimethyl malate	2.26	2.25	2.26
Trimethyl citrate	8.52	8.52	8.52

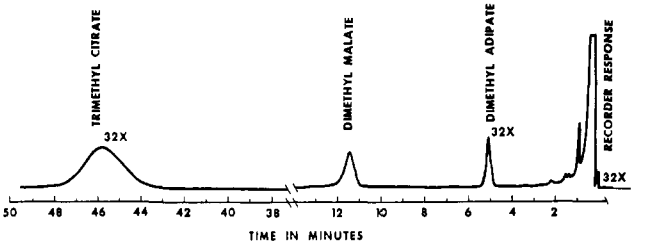

Figure 3. Chromatogram of methyl esters of organic acids from yellow passion fruit on FFAP at 170° C

Table IV. Quantitative Determination of Organic Acids in Yellow Passion Fruit (*P. flavicarpa*) and Purple Passion Fruit (*P. edulis*)

Acid	*P. flavicarpa* mequiv/100 g	*P. edulis* mequiv/100 g
Citric	55.00	13.10
Malic	10.55	3.86
Lactic	0.58	7.49
Malonic	0.13	4.95
Succinic	trace	2.42
Ascorbic	0.06	0.05
Volatile acids	0.11	0.12
	Total 66.43	31.99
Total titratable acids	65.83	32.01

malonic (0.20%), and succinic acids. These acids were not shown by tlc techniques due to their low concentrations in yellow passion fruit but were elucidated by glc due to its greater sensitivity. Although purple passion fruit was found to contain the same acids as yellow passion fruit, the relative abundance of each of the acids differed markedly as shown in Table IV. Citric acid was found to be the abundant, constituting 41.0% of the acids but the second most abundant acid was lactic (23.4%), followed by malonic (15.5%), malic (12.1%), and succinic acid (7.56%).

The total acid content for yellow and purple passion fruit as determined by both glc and titration of ascorbic and volatile acids was 66.43 and 31.99 mequiv/100 g, respectively. These values were within 1% of the total acids obtained by titration which were 65.83 and 32.01 mequiv/100 g for yellow and purple passion fruit, respectively.

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