

Nonvolatile Acids of Papaya

Harvey T. Chan, Jr., Thomas S. K. Chang, Allan E. Stafford, and John E. Brekke*

The nonvolatile acids were extracted from papaya, separated by thin-layer chromatography, and identified as α -ketoglutaric, citric, malic, tartaric, ascorbic, and galacturonic. Quantitative determinations by

glc showed citric and malic to be present in approximately equal amounts, with α -ketoglutaric in much lesser amount; these three acids and ascorbic acid accounted for 85% of the total acid in papaya.

The papaya (*Carica papaya*), native to tropical America, is widely distributed throughout the Tropics. It is consumed mainly as fresh fruit, but some processed papaya products have been marketed for many years (Elliot, 1950; Heid and Curl, 1944; Ochse *et al.*, 1961). There is growing interest in processed papaya as the acreage of this fruit crop has increased in Hawaii and elsewhere. The *Solo* cultivar of Hawaii, a yellow-fleshed type, is said to be one of the best presently available (Purseglove, 1968).

Among fruits, papaya is notably low in acid, and the edible portion has a pH range of 4.5 to 6.0 (Pulley and von Loesecke, 1941). Data acquired in this laboratory over the past several years show the *Solo* papaya to be at pH 5.0 to 5.5. Chen (1964) studied some of the chemical changes in papaya during ripening and noted the presence of citric and malic acids, with malic appearing to decrease as the fruit ripened; no quantitative data were reported. Other references to the acids in papaya are general in nature, usually consisting only of data on total titratable acidity (Stahl, 1935; Heid and Curl, 1944). To preserve the delicate flavor of papaya in heat processed products, it is desirable to use the comparatively mild heat treatment recommended for high acid foods. The naturally occurring acids of papaya must therefore be supplemented by edible organic acids to adjust the pH to below 4.5. More comprehensive knowledge of the organic acids found naturally in papaya is necessary for making judgments on acidification of papaya products. It may be desirable to acidify with an edible acid not found in papaya so that the amount of true papaya solids in a product can be more accurately determined.

MATERIALS AND METHODS

Puree Papaya. Papaya of the common commercial variety (*Solo*) was processed to puree by mechanical separation of peels and seeds and heat inactivation of enzymes in

the pulp (Stafford *et al.*, 1966). The puree was stored in hermetically sealed cans at 0° F.

Total Acidity, Total Volatile Acidity, and Ascorbic Acid Analyses. 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. One kilogram of papaya puree was mixed with 2 l. of methanol and filtered *in vacuo* through Whatman No. 1 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 \times 4 cationic resin, and then through a column of Amberlite IRA 400 anionic resin. The column was rinsed successively with 1500 ml of water to remove the sugars, then with 20 ml of methanol followed by 200 ml of water.

The acids were eluted from the anionic column with 100 ml of 6 N formic acid followed by water, until approximately 250 ml of eluate was obtained. The eluate was concentrated to about 10 ml *in vacuo* at 59° C, and further evaporated to a syrupy consistency under a stream of nitrogen for 16 hr at room temperature to remove formic acid.

For thin-layer chromatography (tlc), the extract was taken up in 2 ml of ethanol; for gas-liquid chromatography (glc), the sample was taken up in 2 ml of methanol.

Methylation. The acids were esterified to their corresponding methyl esters using the method described by Mazliak and Salsac (1965). Five milliliters of BF₃-methanol 14% (w/v) was added to 2 ml papaya extract in methanol and allowed to react for 16 hr at room temperature in tightly capped vials. One-microliter samples of the reaction mixtures were injected directly into the gas chromatograph.

Gas-Liquid Chromatography. A Varian Aerograph Model 200 gas chromatograph with a flame ionization detector was used. Nitrogen partially saturated with water was the carrier gas at a flow rate of 25 cm³ per min. The hydrogen gas flow rate was 44 cm³ 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 (diethyleneglycol succinate) on Chromosorb W HMDS 60/80, and a 2.5-ft, 0.093-in. i.d., stainless steel column packed with 5% NPGS

Hawaii Fruit Laboratory, Western Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture; in cooperation with the Hawaii Agricultural Experiment Station, University of Hawaii, Honolulu, Hawaii 96822

Table I. R_f Values ($\times 100$) of Acids on Cellulose MN 300 Developed in Three Solvents

Solvent Compound	I(EFW) ^a		II(BFW) ^b		III(BBIFW) ^c	
	Known	Papaya	Known	Papaya	Known	Papaya
α -Ketoglutaric	76	75	77	77	72	73
Malic	61	61	55	55	62	61
Citric	56	52	45	45	55	57
Ascorbic	43	41	27	...	49	...
Quinic	40	...	22	...	36	...
Tartaric	39	...	26	26	39	38
Oxalic	27	24	25	...	20	...
Gluconic	27	...	13	...	20	...
Galacturonic	14	15	11	10	22	21

^a EFW (ethyl ether-formic acid-water) 20:5:3. ^b BFW (*n*-butyl alcohol-formic acid-water) 4:1:5. ^c BBIFW (benzyl alcohol-*tert*-butyl alcohol-isopropyl alcohol-formic acid-water) 24:8:1:8.

(neopentylglycol succinate) on Chromosorb W HMDS 60/80 (Gee, 1965). The DEGS and NPGS columns were operated isothermally at 170° and 145° C, respectively, with an injection port temperature of 180° C and a detector temperature of 220° C.

The NPGS column was used for the quantitative determination of the acids with succinic acid as the internal standard. Known quantities of succinic acid (0.1 to 0.5 g) were added to 1 kg of papaya puree. The extraction, methylation, and gas chromatography of the acids were performed as described above. Quantitative data were calculated from the peak area and weight relationships in ten or more replications.

Thin-Layer Chromatography. Silica gel (Eastman Chromagram sheet 6060), cellulose (Eastman Chromagram sheet 6064), and cellulose powder MN 300 (Macherey, Nagel and Co.) coated plates (15 g of cellulose to 90 ml of water, 250 μ thick) were used. The plates were activated at 110° C for 30 min and stored in a desiccator. The organic acid extract in ethanol (1 to 5 μ l) was applied directly to the plates with a micropipette. The plates were developed in the organic phases of the following solvent systems: I. EFW (anhydrous ethyl ether-formic acid-water) 20:5:3 (v/v); II. BFW (*n*-butyl alcohol-formic acid-water) 4:1:5 (v/v); and III. BBIFW (benzyl alcohol-*tert*-butyl alcohol-isopropyl alcohol-formic acid-water) 24:8:1:8 (v/v).

After development the plates were dried overnight. The yellow acid spots were located on a blue-green background by spraying first with bromophenol blue (0.04% w/v with 0.05% sodium acetate in 95% ethanol) followed by bromocresol green (0.4% w/v in 95% ethanol) adjusted to pH 5.5. The R_f values in Tables I to III each represent averages of seven or more replications.

RESULTS AND DISCUSSION

Papaya acids chromatographed on cellulose MN 300 and developed in solvent I (EFW) showed the presence of six acids (Table I). The acids had R_f values corresponding to those for the known acids α -ketoglutaric, malic, citric, oxalic, gluconic, and D-galacturonic. One of the papaya acids had an R_f value corresponding to either tartaric, quinic, or ascorbic acid. When the acids were chromatographed on cellulose MN 300 in solvent II, five spots appeared with R_f values, indicating the presence of α -ketoglutaric, malic, citric, and galacturonic acids. One spot had an R_f value corresponding to either ascorbic or tartaric acid. The acids chromatographed on the same absorbent in Solvent III

Table II. R_f Values ($\times 100$) of Organic Acids on Cellulose 6064 Developed in Solvent II (BFW)^a

Compound	Known	Papaya
α -Ketoglutaric	78	80
Malic	61	63
Citric	56	53
Ascorbic	41	45
Tartaric	36	35
Galacturonic	08	09
Quinic	30	...
Gluconic	38	...
Oxalic	72	...

^a BFW (*n*-butyl alcohol-formic acid-water) 4:1:5.

Table III. R_f Values ($\times 100$) of Organic Acids on Silica Gel 6060 Developed in Two Solvents

Solvent Compound	II(BFW) ^a		III(BBIFW) ^b	
	Known	Papaya	Known	Papaya
α -Ketoglutaric	45	41	68	68
Malic	45	...	52	50
Citric	32	29	40	39
Galacturonic	10	12	12	14
Quinic	10	...	31	...
Tartaric	20	...	26	...
Oxalic	52	...	68	...
Ascorbic	26	...	13	...

^a BFW (*n*-butyl alcohol-formic acid-water) 4:1:5. ^b BBIFW (benzyl alcohol-*tert*-butyl alcohol-isopropyl alcohol-formic acid-water) 24:8:1:8.

Table IV. Relative Retention Time of Methyl Esters of Known Organic Acids and Papaya Acids—Two Columns

Compound	5% NPGS at 145° C		15% DEGS at 170° C	
	Known	Papaya	Known	Papaya
Dimethyl succinate	1.00	1.00	1.00	1.00
Dimethyl malate	4.57	4.38	5.24	5.28
Dimethyl α -keto-glutarate	5.82	5.82	6.75	6.82
Trimethyl citrate	23.62	24.16	25.86	25.91

indicated the presence of five acids with R_f values corresponding to α -ketoglutaric, malic, citric, tartaric, and galacturonic acids.

The acids chromatographed on cellulose (Eastman Chromagram sheet 6064) and developed with Solvent II (BFW) showed six spots with R_f values corresponding to those of

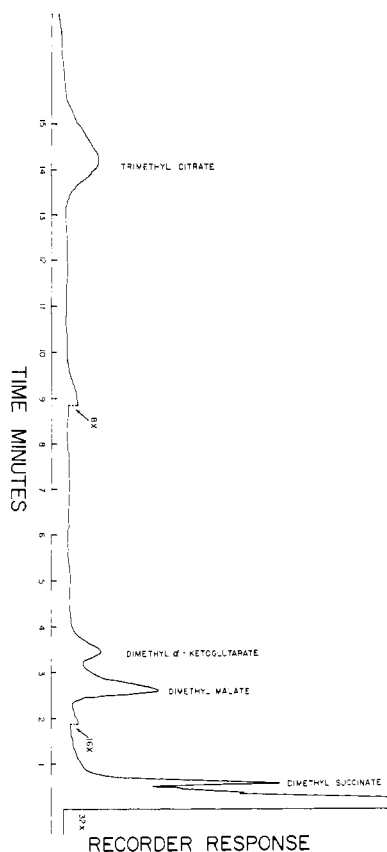


Figure 1. Chromatogram of methyl esters of organic acids from papaya on NPGS

α -ketoglutaric, malic, citric, ascorbic, tartaric, and galacturonic (Table II). This adsorbent and solvent system was preferred over others employed. Solvent I on this adsorbent gave unsatisfactory resolution. Solvent III caused extensive flaking of the adsorbent (this subsequently was corrected).

Acids applied to silica gel (Eastman Chromagram sheet 6060) and developed in Solvent II (BFW) showed only three spots, with two corresponding to galacturonic and citric acids; the R_f of the third spot was close to the value for α -ketoglutaric and malic (Table III). With this same adsorbent and Solvent III, four spots appeared with the R_f values of α -ketoglutaric, malic, citric and galacturonic. Solvent I caused flaking and separation of this adsorbent.

There were indications of the presence of quinic, oxalic, and gluconic acids (Table I) on cellulose MN 300 plates developed in Solvent I (EFW). Their presence was not confirmed with the other adsorbent-solvent systems.

Typical chromatograms of the methyl esters of organic acids from papaya chromatographed on NPGS and DEGS columns are shown in Figures 1 and 2. The glc retention times of known organic acids and of acids extracted from papaya are shown for DEGS and NPGS columns in Table IV. These data confirm the presence of α -ketoglutaric, malic, and citric acids. 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).

The results of the quantitative determinations by glc are as follows. Malic and citric acids were found in papayas in approximately equal amounts, 0.464 and 0.525 mequiv per 100 g wet weight, respectively. They are about ten times

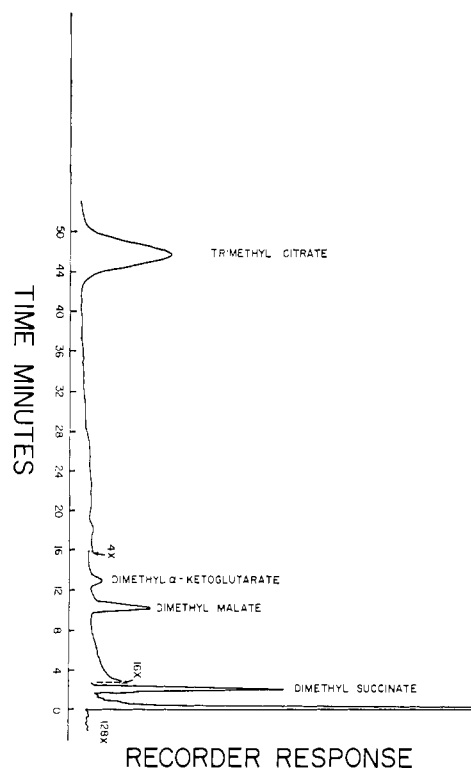


Figure 2. Chromatogram of methyl esters of organic acids from papaya on DEGS

more abundant than α -ketoglutaric acid, of which there is only 0.042 mequiv per 100 g.

The total titratable acidity of papaya puree was 1.54 mequiv per 100 g. Papaya puree had 0.279 mequiv per 100 g of ascorbic acid (49.2 mg per 100 g) which, together with the malic, citric, and α -ketoglutaric acids, totaled 1.31 mequiv per 100 g, about 85% of the total titratable acidity. Total volatile acids in papaya were 0.123 mequiv per 100 g and contributed 8% to the total titratable acidity. The remaining 7% (0.11 mequiv per 100 g) was attributed to galacturonic, tartaric, pectic, and unidentified nonvolatile acids.

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