In conclusion, this investigation gives a clear picture of the nonvolatile chemical constituents primarily responsible for the taste of fresh carrots. Work is in progress to assess the flavor notes resulting from heating the essential oils—free 80% ethanol extract and evaluate the contribution of the resulting volatile and nonvolatile compounds to carrot flavor.

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Nonvolatile Acids in Pineapple Juice

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The nonvolatile organic acids were extracted from summer and winter pineapple juice, separated by tlc, and identified as citric, malic, malonic, glycolic, tartaric, and galacturonic. Gas–liquid chromatography of methyl esters of the acids confirmed the presence of citric, malic, and malonic acids and detected, in addition, succinic acid; glc of TMS derivatives revealed the presence also of phosphoric acid. Seasonal variations in total acidity and relative amounts of citric, malic, and succinic acids were determined.

Pineapple juice has become one of the important processed products of pineapple. In 1970, over 8 million cases of pineapple juice were produced (Hawaii Department of Agriculture, 1972).

The acidity of the juice has been noted to vary with the season of harvest; fruit harvested in the winter has higher acidity and that harvested in summer has lower acidity (Mehrlich, 1961). The nature and amounts of organic acids in pineapple have been studied by a number of workers. Using the method of ester distillation, Nelson (1925) found that the acids in pineapple were 87% citric and 13% l-malic. In "The Pineapple," by Collins (1960), it was stated that the ratios of citric, malic, and ascorbic were 80:20:2; he reported the amounts were 10.87–13.98 mequiv of citric acid/100 g, 2.93 mequiv of malic acid/100 g, and 0.045–0.114 mequiv of ascorbic acid/100 g. Mehrlich (1961) described the 1949–1950 pineapple juice pack as averaging 14.60 mequiv of acid/100 g of juice and that the total acids varied from 12.8 to 28.4 mequiv. Citing unpublished data by Clark from 1939, Mehrlich stated that citric acid accounted for 28–66% of the total, with malic averaging 18–27% and unknown acids accounting for 12–52%. Gortner (1963) and Singleton and Gortner (1965), in

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studying compositional changes during development of the pineapple fruit, found that the malic acid content was affected by the environment; it appeared to be metabolized during periods of high light intensity and accumulated when sunlight and evapotranspiration were low. Citric acid content varied with the stage of fruit development. Employing gas-liquid chromatography (glc) and thin-layer chromatography (tlc), a comprehensive study of the nonvolatile acids of pineapple juice was done in this laboratory on samples representing the pack of an entire year.

MATERIALS AND METHODS

Pineapple Juice. Pineapple juice samples representing the summer and winter crop of 1970–1971 were obtained from a commercial canner. The juice had been packed by the canner in 404 × 700 cans with bright tin lining and fortified with ascorbic acid.

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

Extraction of Organic Acids. Pineapple juice (100 g) was mixed with 800 ml of 95% ethanol and filtered *in vacuo* through Whatman No. 2 filter paper. The filtrate was concentrated in a rotary flash evaporator at 40° until the ethanol was removed. This concentrate was percolated through a regenerated column of Amberlite IR 120 (H-form) cationic resin, and then through a 32 × 333 mm column of Amberlite IRA 400 (formate form) anionic resin. The anionic column was rinsed with water until the effluent was negative to Benedict's test for reducing sugars.

The acids were eluted from the anionic column with 225 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°. Benzene (3 ml) was added to remove formic acid as an azeotrope and the evaporation was continued until a dry residue was obtained. The residue was prepared for tlc and glc of methyl esters as previously described by Chan *et al.* (1971). Quantitative data were calculated from the ratio of the peak areas with reference to the internal standard (Dal Nogare and Juvet, 1962) in nine or more replications. From the ratio of areas, a ratio of weights was determined from a calibration curve. The calibration was done previously for each of the acids to correct for differences in detector response.

Preparation of Silyl Ether Derivatives. Acids isolated by ion exchange as described above were further dried in a desiccator over anhydrous $CaSO_4$. The acids were silylated by the method of Fernandez-Flores *et al.* (1970).

Gas-Liquid Chromatography. The separation and identification of the organic acids as their methyl esters by glc were done by the method descibed by Chan *et al.* (1972); however, with pineapple juice, 100–700 mg of adipic acid were added as the internal standard. For the separation and identification of the TMS derivatives, a 7-ft, 0.093-in. i.d. stainless steel column packed with 3% OV-17 (Applied Science Laboratory) on Chromosorb G AW DMCS 60/80 was used. The OV-17 column was operated isothermally at both 180 and 140°, with injection port temperature of 190° and detector temperature of 220°. The nitrogen carrier gas flow rate was 25 cm^3 per min and the hydrogen gas flow rate was 44 cm^3 per min.

Thin-Layer Chromatography. The following sorbents were used for thin-layer chromatography: microcrystalline cellulose, Avicel PH-105 (FMC Corp.), coated plates (15 g of cellulose to 90 ml of water, 250 μ thick); precoated sheets of cellulose (Eastman Chromatogram sheet 6064) and silica gel (Polygram Sil N-HR Brinkmann Instruments, Inc.); 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, Avicel PH-105, and Silica Gel-Cellulose mixture were left to dry and set overnight at room temperature, and were then dried at 110° 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 BFW (*n*-butyl alchol-formic acid-water) 8:5:10 (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 (0.4% 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-IV each represent averages of nine or more replications.

RESULTS AND DISCUSSION

Pineapple acids chromatographed on cellulose and developed in solvent I (EFW) showed five spots (Table I). Four had R_f values corresponding to the known acids malonic, glycolic, malic, and citric. The R_f value for phosphoric acid corresponded closely to that of citric. The remaining acid was unidentified.

Acids chromatographed on microcrystalline cellulose (Avicel PH-105) and developed in solvent I (EFW) showed six spots with R_f values corresponding closely to malonic, glycolic, malic, citric, tartaric, and galacturonic acids. Phosphoric acid in mixtures with known acids was not resolved from citric. When chromatographed singly, it had R_f values very close to citric acid (Table I).

Acids chromatographed in solvents I and II with a mixture of silica gel-cellulose as the sorbent showed three spots whose R_f values matched those of malic, citric, and galacturonic acids (Table II).

Pineapple acids chromatographed on silica gel showed four spots, with two of the acids identified as citric and malic. One of the spots had an R_f value which corresponded to both tartaric and phosphoric acids. The remaining acid was unidentified (Table II).

The relative retention times for the gas chromatography of the methyl esters of known and pineapple acids on DEGS and FFAP columns are shown in Table III. Methyl esters chromatographed on the DEGS column showed the presence of four acids; three were identified as succinic, malic, and citric. The fourth peak was unidentified. Methyl esters chromatographed on the FFAP column at 145 and 170° confirmed the presence of malonic, succinic, malic, and citric acids and indicated the presence of possibly two unidentified acids. The presence of tartaric, galac-

Table I. R_f Values (×100) of Acids; Cellulose (Eastman Chromatogram 6064) and Avicel PH-105 Developed in Solvent I (EFW[a])

	Eastman Chromatogram 6064		Avicel PH-105	
Compound	Authentic	Pineapple	Authentic	Pineapple
Malonic	81	79	85	85
Glycolic	72	71	79	78
Malic	62	62	71	70
Citric	53	53	63	61
Phosphoric	52		64	
Unknown		34		
Tartaric	37		51	49
Ascorbic	38			
Galacturonic			18	16

[a] EFW (anhydrous ethyl ether–formic acid–water), 2:5:3 (v/v).

Table II. R_f Values (×100) of Acids on Silica Gel–Cellulose Developed in Two Solvent Systems and on Silica Gel (Polygrams Sil N-HR) Developed in Solvent II (BFW[b])

	Silica Gel–Cellulose				Silica gel (Polygram Sil N-HR)	
	Solvent I (EFW[a])		Solvent II (BFW[b])		Solvent II (BFW[b])	
Compound	Authentic	Pineapple	Authentic	Pineapple	Authentic	Pineapple
Malic	61	60	44	44	54	53
Citric	52	52	32	34	47	45
Tartaric					36	34
Phosphoric	55		28		37	
Unknown						19
Galacturonic	14	14	03	04		

[a] EFW (anhydrous ethyl ether–formic acid–water), 20:5:3 (v/v). [b] BFW (*n*-butyl alcohol–formic acid–water), 8:5:10 (v/v).

Table III. Relative Retention Time of Methyl Esters of Authentic Organic Acids and Pineapple Acids on Two Columns

Compound	Authentic	Pineapple
	15% DEGS at 170°	
Dimethyl succinate	0.48	0.50
Dimethyl adipate[a]	1.00	1.00
Dimethyl malate	2.48	2.42
Trimethyl citrate	10.85	10.83
Unknown		4.93
	5% FFAP at 145°	
Dimethyl malonate	0.27	0.25
Dimethyl succinate	0.38	0.37
Dimethyl adipate[a]	1.00	1.00
Unknown		0.49
	5% FFAP at 170°	
Dimethyl adipate[a]	1.00	1.00
Dimethyl malate	2.16	2.16
Trimethyl citrate	8.58	8.56

[a] Internal standard.

turonic, saccharic, and ascorbic acids could not be confirmed by glc; galacturonic, saccharic, and ascorbic acids are so hydroxylated that these methods are unsatisfactory (Chan *et al.*, 1971). The glc of organic acids as their trimethylsilyl ethers has been reported to overcome the difficulties of glc separation of highly hydroxylated compounds by Fernandez-Flores *et al.* (1970), Brunelle *et al.* (1967), and Martin *et al.* (1971). An attempt was made to isolate the organic acids from pineapple juice as lead salts and separate them by glc as their TMS derivatives, according to Fernandez-Flores' methods. This approach did not yield reliable quantitative data, which has also been shown to be the case by Weissberger *et al.* (1971) and Wagener *et al.* (1971). In lieu of the above method, the acids were isolated by ion-exchange chromatography and converted to their corresponding TMS derivatives. The relative retention times for the known and pineapple acids on the OV-17 column are shown in Table IV.

Table IV. Relative Retention Time of TMS Derivatives of Authentic Organic Acids and Pineapple Acids on a 3% OV-17 Column at Two Temperatures

Compound	Authentic	Pineapple
	140°	
TMS phosphoric	0.27	0.28
TMS succinic	0.35	0.36
TMS malic	0.76	0.76
TMS adipic[a]	1.00	1.00
	180°	
TMS adipic[a]	1.00	1.00
TMS citric	3.29	3.29
TMS saccharic	5.56	

[a] Internal standard.

The presence of saccharic acid in pineapple juice as reported by Thimann and Bonner (1950) was not confirmed by this method. The absence of its TMS derivative in the chromatogram indicated that the acid was not present in pineapple as a free acid.

The trimethylsilylated derivatives of pineapple acids chromatographed on OV-17 at 140 and 180° revealed the possible presence of phosphoric acid and confirmed the presence of succinic, malic, and citric acids.

The quantitative data obtained by this method yielded total fixed acid values much higher than the total acids obtained for pineapple juice by titration. This anomaly appears also in the work reported by Martin *et al.* (1971), where the TMS acid values for some wine samples were much higher than those determined titrimetrically.

The quantitative data obtained from the methyl esters on the FFAP column are shown in Table V. Citric acid was the predominant acid in both summer and winter pineapple. In winter pineapple juice, citric acid accounted for 63.5% of the acids, followed by malic (33.2%) and succinic (0.52%). Although pineapple juice from the summer crops contained the same acids as the winter crop, there were some slight differences in the abundance of each of the acids. Citric acid was found to account for 58.2% of the acids, followed by malic (37.4%) and succinic (0.57%).

The total acid content for summer and winter pineapple juice as determined by glc plus titration of ascorbic and volatile acids was 10.47 and 11.50 mequiv/100 g for summer and winter pineapple juice, respectively. The total acids obtained by titration were 12.54 and 15.30 mequiv/100 g for summer and winter pineapple juice, respectively. Total titratable acids were higher than those obtained by the summation of the individual acids determined by glc plus the volatile and ascorbic acids. The higher values obtained by titration were attributed to the presence of galacturonic, tartaric, phosphoric, malonic, glycolic, pectic, and unidentified nonvolatile acids.

Table V. Quantitative Determination of Organic Acids in Summer and Winter Pineapple Juice

Acid	Winter crop, mequiv/100 g	Summer crop, mequiv/100 g
Citric	7.31	6.09
Malic	3.82	3.91
Succinic	0.06	0.06
Ascorbic	0.22	0.25
Volatile acids	0.09	0.16
Total	11.50	10.47
Total titratable acids	15.30	12.54

The relative proportions of citric to malic acid determined in this study differ considerably from those stated by Nelson (1925) and Collins (1960). The differences are attributed to the greater precision and accuracy of the newer methods employed in this study. The use of these newer methods has also led to the elucidation of previously unreported acids in pineapple.

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Carbohydrate and Cyclitol Content of Cannabis

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The carbohydrate and cyclitol content of *Cannabis sativa* grown in the United States (MS-13), Thailand, and Viet Nam was determined *via* silylation and gas chromatographic techniques, and the methods of isolation are described. MS-13 contained the carbohydrates ribitol, fructose, α- and β-D-glucose, and sucrose and the cyclitols (+)-quebrachitol, D(−)-bornesitol, and *myo*-inositol. Only the Thailand sample contained (+)-inositol, whereas only the Viet Nam sample contained erythritol. The carbohydrate–cyclitol content was MS-13 > Thailand > Viet Nam.

In the last decade, those compounds indigenous to *Cannabis sativa* have received much attention and elegant research on the isolation and identification of cannabinol, cannabidiol, the psychotomimetically active Δ^9-tetrahydrocannabinol, and other cannabinoid isomers have been reported (Gaoni and Mechoulam, 1971; Joyce and Curry, 1970). However, there is a paucity of information concerning the classification and amounts of other compounds present in this plant material. Numerous noncannabinoid terpenes have been identified by gas chromatography (gc) and constituted 0.1% of the leaf (Martin *et al.*, 1961; Nigram *et al.*, 1965). Muscarine, choline, and trigonelline have been isolated (Brecht and Salemink, 1969; Salemink *et al.*, 1965) and, more recently, several unknown alkaloids (0.003%) have been reported (Klein *et al.*, 1971). Qualitatively, Adams *et al.* (1940) isolated and identified the cyclitol, quebrachitol, in the steam distillate of an ethanolic extract of Cannabis. In all studies, however, the largest class of compounds to be isolated and identified is the cannabinoids themselves.

It has been suggested that 41% of the phenols found in the mainstream of cigarette smoke derive from the carbohydrate content of the flue-cured tobacco leaf (Bell *et al.*, 1966). In view of the fact that the common usage of Cannabis is *via* the smoking process, it was of interest to determine qualitatively and quantitatively the carbohydrate content of this plant material. Additionally, since cyclitols are polyhydroxylcyclohexanes, dehydration mechanisms can be proposed which would lead to the production of phenols, and knowledge of the cyclitol content would be similarly useful.

The present communication describes the separation techniques and analysis of three samples of Cannabis from different origins and their carbohydrate and cyclitol contents.

EXPERIMENTAL SECTION

A Beckman CC-4 equipped with a flame ionization detector was used as a single column instrument with a Model 3370A Hewlett-Packard electronic integrator. The injection block and detector line were maintained at 260°, detector block was at 350°, and all runs were programmed from 100 to 164° at 2°/min and then from 164 to 252° at 8°/min, with a helium flow of 15 ml/min at a pressure of 80 psi. A 10 ft × ⅛ in. stainless steel column containing 2% OV-17 on Gas Chrom Q (80–100 mesh) was employed.

Standard trimethylsilyl (TMS) sugar solutions of tetra-TMS-L-arabinose, penta-TMS-β-D-fructose, penta-TMS-D-galactose, penta-TMS-α-D-glucose, penta-TMS-β-D-glucose, octa-TMS-lactose, octa-TMS-maltose, penta-TMS-D-mannose, tetra-TMS-C-ribose, penta-TMS-L-sorbose, octa-TMS-sucrose, and tetra-TMS-D-xylose were obtained from Pierce Chemical Co., Rockford, Ill. Free sugars, obtained from Nutritional Biochemicals, Inc., Cleveland, Ohio, were L-fucose, L-glucose, *N*-acetyl-*O*-galactosamine, and *N*-acetyl-*O*-glucosamine; from Calbiochem, Los Angeles, Calif., 3-*O*-methyl-D-glucose and D-galactonolactone; from Mann Research Laboratories, New York, N. Y., (+)-quebrachitol; from Pfanstiehl Laboratories, Inc., Waukegan, Ill., D-fructose, ribitol, and *meso*-erythritol. Supplied from other sources were L-rhamnose, *myo*-inosi-

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