Nonvolatile Acids in Pineapple Juice

Harvey T. Chan, Jr.,* Eduardo Chenchin, and Paul Vonnahme

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% 1-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 Citing unpublished data by Clark from 1939, Mehrlich stated that citric acid accounted for 28–66% of the total, with malic averaging 18–27% and unknown acids accounting for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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Platenius, H., Plant Physiol. 9, 671 (1934).

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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 metabolicized during periods of high light intensity and accumulated when sunlight and evaportranspiration were low. Citric acid content varied with the stage of fruit development and the climatological changes (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°C 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°C. Benzene (3 ml) was added to remove formic acid as an azetrope 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 Negro and Juven, 1962) in nine or more replications. From the ratio of areas, a ratio of weights was determined 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 CaSO₄. 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 described 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 comparison of the 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. 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 II. 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°C confirmed the presence of malonic, succinic, malic, and citric acids and indicated the presence of possibly two unidentified acids. The presence of tartric, galacturonic,

**RESULTS AND DISCUSSION**

Pineapple acids chromatographed on cellulose and developed in solvent I (EFW) showed five spots (Table I). Four had Rf values corresponding to the known acids malonic, glycolic, malic, and citric. The Rf 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 Rf 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 Rf 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 Rf values matched those of maleic, 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 Rf 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°C confirmed the presence of malonic, succinic, malic, and citric acids and indicated the presence of possibly two unidentified acids. The presence of tartaric, galacturonic acids, and pectic substances were confirmed in some samples.

**Table I.** Rf Values (×100) of Acids; Cellulose (Eastman Chromatogram 6064) and Avicel PH-105 Developed in Solvent I (EFW)²

<table>
<thead>
<tr>
<th>Compound</th>
<th>Eastman Chromatogram 6064</th>
<th>Avicel PH-105</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Authentic</td>
<td>Pineapple</td>
</tr>
<tr>
<td>Malonic</td>
<td>81</td>
<td>79</td>
</tr>
<tr>
<td>Glycolic</td>
<td>72</td>
<td>71</td>
</tr>
<tr>
<td>Malic</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Citric</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>Phosphoric</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tartaric</td>
<td>37</td>
<td>51</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Galacturonic</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

² EFW (anhydrous ethyl ether-formic acid-water), 2:5:3 (v/v/v).
Table II. Relative Retention Time of Methyl Esters of Authentic Organic Acids and Pineapple Acids on Two Columns

<table>
<thead>
<tr>
<th>Compound</th>
<th>Authentic</th>
<th>Pineapple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malic</td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td>Citric</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Tartaric</td>
<td>55</td>
<td>28</td>
</tr>
<tr>
<td>Phosphoric</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Unknown</td>
<td>55</td>
<td>28</td>
</tr>
<tr>
<td>Galacturonic</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Authentic</th>
<th>Pineapple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl succinate</td>
<td>0.48</td>
<td>0.50</td>
</tr>
<tr>
<td>Dimethyl adipate</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dimethyl malate</td>
<td>2.48</td>
<td>2.42</td>
</tr>
<tr>
<td>Trimethyl citrate</td>
<td>10.85</td>
<td>10.83</td>
</tr>
<tr>
<td>Unknown</td>
<td>14.1</td>
<td>14.1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Authentic</th>
<th>Pineapple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malic</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Citric</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Tartaric</td>
<td>145</td>
<td>145</td>
</tr>
<tr>
<td>Phosphoric</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>Unknown</td>
<td>170</td>
<td>170</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Acid</th>
<th>Winter crop, mequiv/100 g</th>
<th>Summer crop, mequiv/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric</td>
<td>7.31</td>
<td>6.09</td>
</tr>
<tr>
<td>Malic</td>
<td>3.82</td>
<td>3.91</td>
</tr>
<tr>
<td>Succinic</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Volatile acids</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>Total</td>
<td>11.50</td>
<td>10.47</td>
</tr>
<tr>
<td>Total titratable acids</td>
<td>15.30</td>
<td>12.54</td>
</tr>
</tbody>
</table>

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

LITERATURE CITED


Carbohydrate and Cyclitol Content of Cannabis

John W. Groce1 and Louis A. Jones*

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-glucose, and sucrose and the cyclitols (+)-quebrachitol, α(-)-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 Δ2-tetrahydrocannabinol, and other cannabinoid isomers have been reported (Gaoni and Levine, 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 Salemkir, 1969; Salemkir 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 cannabinooids 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 quantitatively and qualitatively the carbohydrate content of this plant material. Additionally, since cyclitols are polyhydroxycyclohexanes, degradation 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 x 1/8 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-α-arabinofuranoside, penta-TMS-β-D-fructofuranoside, penta-TMS-D-galactofuranoside, octa-TMS-D-glucose, octa-TMS-D-mannose, tetra-TMS-c-ribose, penta-TMS-L-arabinofuranoside, 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 D-fucose, D-glucose, N-acetyl-D-galactosamine, and N-acetyl-D-glucosamine; from Calbiochem, Los Angeles, Calif., 3-O-methyl-D-glucose and β-D-galactosamine; from Mann Research Laboratories, New York, N. Y., (+)-quebrachitol; from Pfanziehl Laboratories, Inc., Waukegan, Ill., β-fructose, ribitol, and meso-erythritol. Supplied from other sources were L-rhamnose, myo-inositol, and other sugars.

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