Organic Acids in the Ripening Banana Fruit

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The nonvolatile organic acids undoubtedly play a role in fruit ripening as they do in many other metabolic processes. As an approach to defining this role in the banana fruit, information is needed on the nature of these acids and on the changes which occur during ripening.

Earlier reports (23) indicate the presence of malic, citric, oxalic, and tartaric acids in the banana fruit, with malic acid being the principal one. Lulla and Johar (13) found only malic and citric acids in 9 varieties of bananas from India. Wolf (25) found the same 2 acids plus an unknown fraction in Colombian bananas. Steward et al. (20) have recently reported the presence of 30 organic acids in alcoholic extracts of the banana fruit. Malic and citric acids were predominant; the other acids occurred in trace amounts. These workers also detected 22 keto acids by extraction of the phenylhydrazones.

There is little quantitative information available on the organic acids in bananas or on the changes which occur during ripening. The malic acid concentration has been reported to vary between 0.8 and 7.5 meq/100 g fresh weight (23) and to increase three to sevenfold during ripening (2.8). Pyruvic, a-ketoglutaric, and oxaloacetic acids also increase during the ripening (2).

The present study was undertaken to confirm the identity of the nonvolatile organic acids of the banana fruit and to determine the concentration of these acids throughout ripening.

Materials and Methods

Plant Material. Three stems of mature, green banana fruit (Musa acuminata var. Gros Michel) were obtained from Central America over a period of approximately 4 months. A blemish-free hand from each stem was ripened at 14° and 90% relative humidity, after brief treatment with 1000 ppm of ethylene (22). Single bananas were detached at several stages of ripening, as determined by the peel color, for measurement of CO₂ production and for subsequent determination of the organic acids. To make certain that the ripening of the chosen banana was neither greatly retarded nor greatly advanced, its CO₂ production was compared at each stage with that of at least one other banana from the hand. CO₂ production was determined on the Beckman infra-red analyzer in a system similar to that of Brun (3).

Isolation of Acids. The fruit pulp (80-100 g) was homogenized 5 minutes in 100 ml of 95% ethanol in a Waring blender. After filtration of the mixture, the residue was extracted twice more with 100 ml of 95% ethanol and the filtrates were combined. The alcoholic extract was added directly to an anion exchange column and the organic acids were determined as described earlier (17). Larger quantities of the acids were obtained by displacement chromatography on coupled columns, as described by Anet and Reynolds (1), but with only 4 columns (2.2 x 15 cm, 1.9 x 9 cm, 1.2 x 6 cm, 0.9 x 5 cm). The acids were located in the effluent by paper chromatography in ether: 88% formic acid: water in the ratio of 5:2:1 (12, 17).

A gradient elution procedure similar to that of DeKock and Morrison (5) was used to separate the acids on silica gel columns. The acids were added to the column as described by Wager and Isherwood (24). It was necessary to maintain a continuous equilibration of the t-aryl alcohol-chloroform mixture entering the silica gel column by means of a thin supernatant layer of 0.1 M H₂SO₄ in the mixing flask.

Oxalic acid was determined by a modification of a procedure described earlier (17). Calcium oxalate was precipitated and collected as usual, but it was then redissolved in 4 N perchloric acid and titrated with 0.1 N perchlorato-cerate in 4 N HClO₄ to the nitroferroin endpoint (19). A micro-pipette (Micro-pipettic Instrument Company) was used for the titration, and the mixture was stirred continuously. The cerate solution was standardized with sodium oxalate (19) and maintained its titer for several months when stored in the dark.

The keto acids were extracted as the 2,4 dinitrophenylhydrazone derivatives (21) which were separated on paper chromatograms (16). The amino acid derivatives of the keto acids were prepared by the procedure of Towers et al. (21) and were separated both on paper chromatograms (7) and on the Spincaco amino acid analyzer.

Results

Identification of the Acids. Figure 1 shows a typical separation of the organic acids from the pulp of a single banana. The acids were tentatively identi-
1-

Oxalic

Glycolic

Quinic

from

through fraction 130, I.

Shikimic

Glutamic

by

were

extract

alcoholic

of

chromatography.

FIG. 1. Ion exchange separation of the organic acids of the banana fruit. Dowex 1 X 10, acetate form, 1.0 X 18 cm. Gradient elution, 2.5 N acetic acid in reservoir through fraction 130, 6 N formic acid thereafter; flow rate, 2 ml/minute; fraction volume 2.0 ml. Sample: alcoholic extract representing 90 g fresh weight of unripe banana pulp.

ified from their positions on the gradient elution chromatogram and by paper chromatography of aliquots from each fraction. In most cases, the identities were confirmed both by specific tests (Table I) and by comparison with authentic acids in silica gel chromatography. Glutaric acid was identified only

by comparison with authentic glutaric acid in the 3 chromatographic systems. Glutamic and aspartic acids were not separated on silica gel columns. Sufficient quantities for these tests were obtained by displacement chromatography of the acids from 1 kg of ripe fruit. Oxalic acid was also isolated directly from alcoholic extracts by precipitation as calcium oxalate at pH 4.5.

The phosphates in Peaks VII and VIII of figure 1 are under further study. Although much of the P1 appears to be in Peak VIII, Peak VII always contains some P1 plus an unidentified organic monophosphate.

Eight keto acids were detected in an extract of 40 g of ripe pulp. Pyruvic, α-ketoglutaric, oxaloacetic and glyoxylic acids were tentatively identified by chromatography of the phenylhydrazones and the identities were then confirmed by hydrogenation of the hydrazones and chromatographic identification of the resulting amino acids. Beta-hydroxypropionic, α-ketoisocaproic and/or α-keto-β-methylvaleric acids and 2 unknown compounds were detected only as amino acid derivatives.

Changes during Ripening. Peel color is a convenient guide to the ripeness of bananas, but it is difficult to correlate with physiological processes such as respiration or volatiles production (15). The banana samples were therefore all classified as pre-climacteric, climacteric, or post-climacteric, based on their CO₂ production. Table II shows the CO₂ production, peel colors and organic acid content of the bananas at these 3 stages. The data showed good agreement for the 3 independent experiments. Aliquots from the various peaks were checked at each stage of ripening via paper chromatography to verify the identity of the acids. The "citric peak" (Peak VII, fig 1) always contained significant amounts of phosphates and unknown C.

The acids in Peaks I through V, figure 1, have been pooled as "other acids," since the individual acids all occurred in trace quantities (0.005 to 0.1 meq/100 g fr wt) and showed little or no change during ripening.

Oxalic acid was determined in separate aliquots of the alcoholic extract. Identical results were obtained at all stages of ripening when comparable samples were extracted with 1 N HCl, thus indicating that the oxalic acid was present in some form other than calcium oxalate.

No attempt was made to obtain quantitative data on the keto acids.

Discussion

Our results indicate that there are 14 organic acids, other than keto acids, which occur in the banana fruit pulp at concentrations greater than 0.005 meq/100 g fresh weight. Presumably many of the acids detected by Steward et al. (20) were present in concentrations below this lower limit. The methods used in the earlier studies (13, 23, 26) were generally not sensitive enough to detect the minor acids.

Table I. Identifying Tests Applied to the Organic Acids of Banana Fruit Pulp.

All melting and sublimation points were determined on the Fisher-Johns apparatus and are uncorrected.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic and Aspartic Quinic</td>
<td>Two dimensional chromatography and color test with ninhydrin-dicyclohexylamine (7)</td>
</tr>
<tr>
<td>Glyceral</td>
<td>Green color with naphthoresorcinol-H₂SO₄ (6)</td>
</tr>
<tr>
<td>Glycolic</td>
<td>Blue color with naphthoresorcinol-H₂SO₄ (6); calcium glycolate mp: 137° with no depression of mixed mp</td>
</tr>
<tr>
<td>Shikimic</td>
<td>Pink color with 2,7-dihydroxynaphthalene (6)</td>
</tr>
<tr>
<td>Succinic</td>
<td>Red color with aniline (27)</td>
</tr>
<tr>
<td>Malic</td>
<td>mp: 101°; mp authentic acid: 101°; mixed mp: 99°</td>
</tr>
<tr>
<td>Citric</td>
<td>mp: 151° with no depression of mixed mp</td>
</tr>
<tr>
<td>Oxalic</td>
<td>Pink color with 2,7-dihydroxynaphthalene after conversion to glycolic acid (6); methyl oxalate derivative mp: 51.5° (18); isolated and authentic acid sublimed rapidly above 95°, completely sublimed at about 140°</td>
</tr>
</tbody>
</table>
Table II. CO₂ Production, Peel Color and Organic Acid Content of Bananas during Ripening

<table>
<thead>
<tr>
<th>Stages of ripening</th>
<th>Pre-climacteric</th>
<th>Climacteric</th>
<th>Post-climacteric</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ production, mg/100 g fr wt x hour</td>
<td>2.0–4.0 (steady)</td>
<td>10.0–17.5 (rising)</td>
<td>9.0–11.0 (erratic fluctuations)</td>
</tr>
<tr>
<td>Peel colors</td>
<td>Green</td>
<td>Yellow-green through yellow with green tips</td>
<td>Fully yellow through yellow flecked with brown</td>
</tr>
<tr>
<td>Organic acids, meq/100 g fr wt:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malic</td>
<td>1.36* (1.11–1.61)</td>
<td>5.37 (4.95–5.80)</td>
<td>6.20 (5.59–6.90)</td>
</tr>
<tr>
<td>“Citric peak”</td>
<td>0.68 (0.43–0.93)</td>
<td>1.70 (1.49–2.10)</td>
<td>2.17 (1.84–2.50)</td>
</tr>
<tr>
<td>Oxalic</td>
<td>2.33 (2.32–2.33)</td>
<td>1.32 (1.20–1.44)</td>
<td>1.37 (1.20–1.54)</td>
</tr>
<tr>
<td>“Other acids”</td>
<td>0.19 (0.11–0.28)</td>
<td>0.16 (0.07–0.26)</td>
<td>0.17 (0.11–0.24)</td>
</tr>
<tr>
<td>Total organic acidity**</td>
<td>4.43 (3.96–4.90)</td>
<td>8.74 (8.46–9.05)</td>
<td>10.90 (9.00–12.00)</td>
</tr>
</tbody>
</table>

* Mean values for at least 6 samples from the 3 independent experiments. The ranges are shown in the parentheses. ** Includes the phosphate of Peak VIII, figure 1.

With a few exceptions, we have identified the same acids reported by Steward et al. (20). They reported lactic, tartaric, pyroglutamic and citramalic acids in addition to those we identified; we have added glutaric acid to the list. We have also confirmed the fact that malic and citric are major acids. Our results differ from all the earlier studies in finding oxalic acid to be a major acid at all stages of ripeness. The oxalic acid occurs entirely in soluble form, probably as potassium or sodium salts.

We have detected only 8 keto acids in ripe pulp in contrast to the 22 keto acids reported by Steward et al. (20). Here again the difference probably resides in the fact that Steward et al. (20) extracted 9 kilograms of pulp and hence detected acids which occurred at exceedingly low concentrations. More refined methods will be required to obtain quantitative data on the keto acids and to note any changes during ripening.

The concentration of the major acids changes markedly during the early stages of ripening at about the time of the climacteric and then tends to remain constant or increase slowly thereafter. In the unripe fruit, oxalic acid is the predominant acid. As the fruit ripens, malic acid and “citric peak” acidity increase 3 to 4-fold, while oxalic acid drops to about 60% of its original value. The net result is a doubling of the organic acidity during ripening with malic acid becoming the predominant acid in ripe bananas.

The concentrations of malic acid at each stage of ripening show good agreement with those reported by Harris and Poland (8). Barker and Solomos (2) also found a marked increase in malic acid during banana ripening. In other fruits, malic acid concentrations tend to fall during ripening and storage (10, 11, 14), although there is one report that the malic acid concentration increases several fold during the ripening of apricots and cherries (26).

Since so little is known about the overall process of fruit ripening, it is difficult to discuss the organic acid results in any meaningful biochemical or physiological terms. However, there are certain aspects of our results which deserve some comment. First, the absence of many of the acids of the Krebs cycle and the almost negligible amounts of most of those detected, would imply either an exceptionally rapid turnover of these acids in the banana fruit or perhaps that some or all of the Krebs cycle reactions are not operative. Second, the considerable increase in malic acid during ripening of bananas suggests that this fruit would be interesting material for testing Hulme’s hypothesis (9) that some special utilization of malic acid is an important feature of the ripening process. Finally, it would be interesting to know something more about the role of oxalic acid in banana ripening. Although oxalate is generally metabolically inert in plants (4), the ripening banana evidently is capable of metabolizing this acid. However, the total drop in oxalate content during ripening is small (ca. 1 meq/100 g fr wt) and, even if direct oxidation is assumed, would not significantly increase the CO₂ production. The more pertinent question is whether oxalate is being turned over at an appreciable rate, and if so, via what mechanism. It would also be of interest to know if oxalate is metabolized during the ripening of other fruits.
Summary

The nonvolatile organic acids of the banana fruit pulp have been determined at various stages of ripening. Unripe fruit contained approximately 4.5 meq of total organic acidity per 100 g fresh weight. Oxalic acid made up about 50% of this total, malic acid 35%, and "citric peak" acidity (citric acid plus certain phosphates) 10%. During ripening, both malic and "citric peak" acidity increased three to four-fold and oxalic acid dropped to about 60% of its original value. The net result was a doubling of the organic acidity of the ripe fruit, with malic acid comprising about 65% of the total, "citric peak" 20%, and oxalic acid 10%. The remaining acidity at all stages of ripeness consisted of a series of acids, each present in trace quantities. These included glutamic, aspartic, glutaric, quinic, glyceric, glycolic, and succinic acids plus a number of keto acids. The oxalic acid in bananas is present in some form other than calcium oxalate.

Acknowledgment

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Literature Cited