# Malic and Citric Acids in Pickling Cucumbers

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## ABSTRACT

The content of major organic acids in pickling cucumbers has been determined. Malic acid was found to be the major organic acid in commercial size pickling cucumbers. The concentration among a group of six cultivars ranged from 0.2–0.3% on a fresh weight basis. Malic acid was present in all parts of the fruit. The highest concentration was in the outer 3 mm of the mesocarp, followed by the endocarp, the inner mesocarp, and the exocarp. During enlargement and maturation of cucumbers, citric acid became the principal organic acid, reaching levels in excess of 1% on a wet weight basis in the endocarp. This large accumulation of citric acid is the probable cause of the decline in endocarp pH during fruit maturation.

## INTRODUCTION

RECENTLY, we found that the major source of $CO_2$ during cucumber juice fermentation is the degradation of malic acid by a malo-lactic fermentation (McFeeters et al., 1982). As a result, we wanted to know the amount and distribution of malic acid in pickling cucumbers. In addition, during cucumber maturation there is a large decrease in endocarp pH (Bell, 1951; Saltveit and McFeeters, 1980). This suggested that a major change in the acids may occur during maturation.

Hirose (1976), in an investigation of chilling injury during refrigerated storage, found malic acid to be the major organic acid in cucumbers of an unspecified cultivar. These fruit also contained a considerable amount of citric acid and low levels of pyruvic, α-ketoglutaric and oxalacetic acids. Based upon the fact that 120g fruit were 30 cm in length, it is probable that the cucumbers were a slicer-type cultivar. These cultivars are genetically quite different from commercial pickling varieties.

The objectives of this investigation were: (1) to characterize the malic acid concentration and distribution in pickling cucumbers, and (2) to explain the reason for the decline in endocarp pH during cucumber ripening.

## MATERIALS & METHODS

CUCUMBERS were obtained from the North Carolina State University Horticultural Science Department farms. They were grown under spray irrigation using standard cultural practices. For whole fruit samples of different cultivars, three lots of four fruit each (3.8–5.1 cm diameter) were blended for analysis. To investigate the effect of fruit size on malic acid content, eight to ten fruit of each size range were blended in a single sample. 'Chipper' cultivar was sampled from the same location in both 1980 and 1981. To investigate the distribution of malic acid within the cucumber, single fruit were dissected into endocarp, inner mesocarp, outer mesocarp, and exocarp (Fig. 1).

Cucumbers were analyzed to relate the changes in endocarp pH during maturation to changes in organic acids. Fruit weights ranged from 422–952g for 'Chipper' and from 335–720g for 'Calypso' cultivars. The color of the fruit varied from green to light yellow. When only endocarp samples were analyzed, the fruit were cut longitudinally, the pH measured and the endocarp tissue was removed. Sixty grams of endocarp were heated to boiling on a hot plate, deionized water was added to replace any water lost during heating, and the samples were filtered through glass wool and frozen.

In order to measure the distribution of organic acids between the mesocarp and endocarp of the fruit during maturation, special care was taken to prevent contamination of the mesocarp tissue samples by juice from the endocarp. The fruit were cut transversely into three sections. Five plugs of mesocarp were taken from each section with a cork borer. The ends of each mesocarp cylinder were trimmed to remove peel tissue and any tissue that may have been contaminated with liquid from the endocarp when the fruit were cut. The endocarp pH was measured, and the endocarp tissue was prepared as described above.

Fresh, whole cucumbers or mesocarp tissue were blended in a Waring or Tekmar blender (Tekmar Co., Cincinnati, OH). The blended samples were filtered through glass wool to remove insoluble material. About 10–20 ml of filtrate were frozen. For analysis, the samples were thawed, centrifuged briefly in a clinical centrifuge, and the supernatant filtered through a 0.45μ Gelman Acrodisc filter (Gelman Science Inc., Ann Arbor, MI) prior to injection into the liquid chromatograph.

Measurement of pH was with an Orion 901 pH meter (Orion Research Inc., Cambridge, MA). Organic acids were measured by HPLC. The HPLC system consisted of a Waters 6000A pump and model 401 refractive index detector (Waters Associates, Milford, MA), a Rheodyne 7125 injector (Rheodyne Inc., Berkeley, CA), and a Spectra-Physics 4100 computing integrator (Spectra-Physics Autolab Div., San Jose, CA). The acids were separated by reverse phase using Dupont Zorbax $C_{18}$ or $C_8$ columns (Dupont Company, Wilmington, DE) or Waters 5 or 10μ Radial-Pak $C_{18}$ columns. For all columns the elution solvent was 0.05M phosphoric acid with the pH adjusted to 2.5 with concentrated $NH_4OH$. Concentrations were

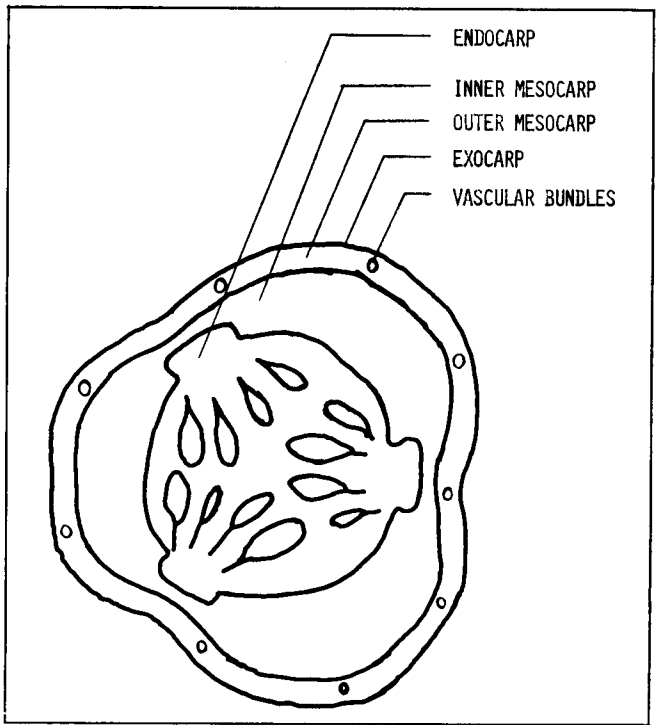


*Fig. 1—Cucumber cross section showing the parts sampled for malic acid analysis.*

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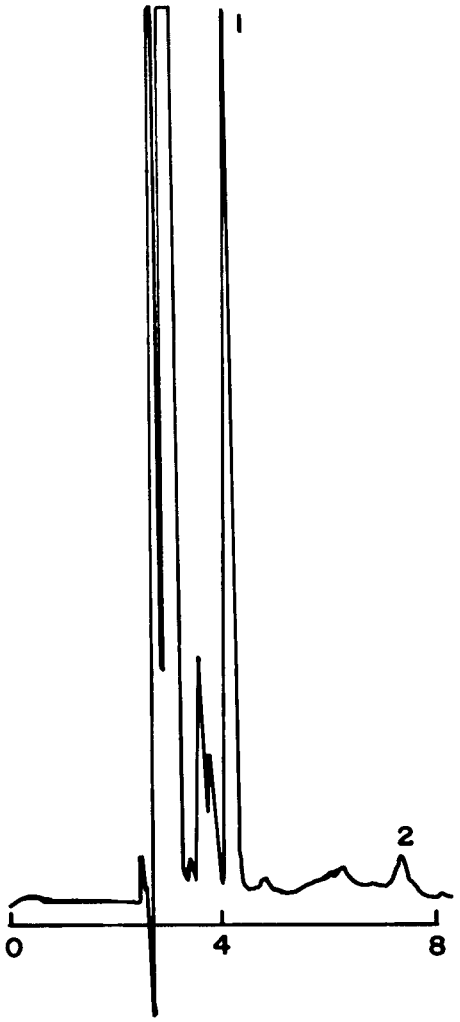


*Fig. 2—Chromatogram of the organic acids in cucumber fruit ('Chipper' cultivar). $C_{18}$, 5μ, Waters Radial-Pak column with pH 2.5, 0.05M ammonium phosphate eluant. Flow rate of 1.0 ml/min. RI detector set at 8X. Malic acid, 1; citric acid, 2.*

estimated based upon comparison to the peak heights of standard compounds.

To confirm the identity of citric acid in cucumber samples, its degradation by citrate lyase from *Aerobacter aerogenes* (Sigma Chemical Co., St. Louis, MO) was demonstrated. The reaction conditions of Dagley and Dawes (1955) were used.

## RESULTS & DISCUSSION

A CHROMATOGRAM of an extract of a fresh, immature cucumber is shown in Fig. 2. Only malic and citric acids, among the common acids found in plants, were observed by HPLC. The identity of malic acid was verified by the fact that the retention time of malic acid matched that of authentic malic acid on four different reverse phase HPLC columns. The peak height ratio between a refractive index detector and ultraviolet detector at 215 nm was the same for the compound in cucumber extracts as for authentic malic acid. Finally, both the natural compound and authentic L-malic acid were degraded rapidly during cucumber juice fermentations by lactic acid bacteria (McFeeters et al., 1982).

Table 1 shows the effect of cucumber size on the malic and citric acid content of 'Chipper' cultivar. Only the small fruit had a measurable amount of citric acid. In contrast,

*Table 1—Effect of fruit size on citric and malic acid concentrations and fruit pH in 'Chipper' cucumbers*[a]

| Fruit weight (g) | Fruit diameter (mm) | pH | Malic acid (μ moles/g fruit wt) | Citric acid[b] |
|---|---|---|---|---|
| 10 | 14 | 6.05 | 29.4 ± 7.6 | 1.6 |
| 25 | 20 | 6.19 | 29.5 ± 1.5 | 1.8 |
| 50 | 29 | 5.96 | 26.2 ± 8.1 | 1.2 |
| 100 | 37 | 5.86 | 24.0 ± 2.2 | —[c] |
| 150 | 44 | 5.75 | 22.1 ± 1.8 | — |
| 250 | 55 | 5.61 | 21.1 ± 0.4 | — |

[a] Cucumbers were grown at the same location in two crop years.
[b] Determined only in 1981.
[c] Not detectable.

*Table 2—Malic acid distribution within immature cucumbers*[a]

| Cucumber weight (g) | Endocarp | Inner mesocarb | Outer mesocarp | Exocarp |
|---|---|---|---|---|
| 42 | 25.7 | 20.1 | 30.2 | 14.6 |
| 139 | 23.9 | 16.6 | 32.3 | 16.0 |
| 280 | 19.4 | 16.5 | 27.2 | 15.5 |

[a] 'Calypso' cultivar. Concentrations are expressed as μmoles malic acid/g fresh weight.

*Table 3—Malic acid content of size no. 3*[a] *cucumbers of different cultivars*

| Cultivar | Malic acid (μmoles/g) |
|---|---|
| 'Calypso' | 21.7 ± 1.2 |
| 'Chipper' | 23.4 ± 2.0 |
| 'Addis' | 14.2 ± 0.4 |
| 'Green Pak' | 14.7 ± 1.7 |
| 'Pixie' | 15.4 ± 1.7 |
| 'G29' | 14.7 ± 0.3 |

[a] 3.8—5.1 cm diameter.

Hirose (1976) reported 5 μmoles/g citric acid in 120g fruit. A small decline in the concentration of malic acid was observed as fruit size increased. This pattern of malic acid concentration was also observed for 'Calypso,' but citric acid was not detected (data not shown). The pH of the blended cucumbers delcined as the fruit size increased.

The distribution of malic acid in different areas of the cucumber fruit was also determined. Fig. 1 shows the fruit sections analyzed. Table 2 shows the malic acid concentrations in three fruit of different size. The highest malic level was in the mesocarp area just under the exocarp. This outer mesocarp contains the major vascular bundles that lead from the peduncle to the blossom end of the fruit and into the endocarp (Judson, 1929). The endocarp, including the placentae, has a lower level and the inner mesocarp and exocarp still lower conentrations of malic acid.

Table 3 shows the malic concentration in several pickling cucumber cultivars. It shows a rather narrow range of malic acid from 14–23 μmoles/g. During the past two seasons, lots of 3.8–5.1 cm diameter (size no. 3) commercial cucumbers analyzed for malic acid usually had about 20 μmoles/g of malic acid. The 120g fruit used by Hirose (1975) had 15 μmoles/g, which is within this range. This suggests that the variation in malic concentration may be rather small. However, further work will be required to properly assess both genetic and environmental effects upon the variability of malic acid in cucumbers.

The present results show that pickling cucumber cultivars contain sufficient malic acid to account for previous

observations of $CO_2$ production in brined cucumbers attributed to the activity of *L. plantarum* (Fleming et al., 1973). They found that 84 mg $CO_2$/100g of cucumbers was produced as a result of fermentation. The degradation of 19 μmoles/g malic acid by the malo-lactic reaction would result in the production of an equivalent amount of $CO_2$.

Further work is needed to demonstrate whether there is a direct relationship between malic disappearance and $CO_2$ formation during fermentation of whole cucumbers. Fleming et al. (1973) found that brined cucumbers also produce approximately an equal amount of $CO_2$ in the absence of any bacterial growth. The exact origin of this $CO_2$ remains to be determined. However, if cucumber cultivars could be developed with low levels of malic acid or if suitable lactic acid bacteria could be found which lacked the ability to degrade malic acid, it may be possible to reduce $CO_2$ production enough to prevent bloating without purging fermentations.

During maturation the pH of the cucumber endocarp can decrease to as low as 3.5 (Saltveit and McFeeters, 1980). Analysis of the endocarp of ripening 'Calypso' cucumbers showed a large accumulation of citric acid as the pH decreased (Fig. 3). In addition to identical retention times with known cirtic acid on reverse phase chromatography with $C_8$ and $C_{18}$ columns, the identity of citric acid was confirmed by showing that citrate lyase caused a decrease in the presumptive citric acid in an endocarp extract and formation of acetic acid. Oxalacetic acid was not determined because other components of the reaction mixture interfered with the peak during chromatography.

The changes in acid concentration of the endocarp and mesocarp of 'Chipper' cucumbers as the endocarp pH declines are shown in Fig. 4. Again, citric acid accumulated in the endocarp. Citric acid also accumulated in the mesocarp, but the levels remained much lower than in the seed area. The malic acid concentration declined in the endocarp, but did not show any clear pattern of change in the mesocarp tissue. Cucumbers with an endocarp pH near 3.5 have been observed to have citric acid concentrations as high as 73 μmoles/g or 1.4% on a wet weight basis. This concentration approaches the level of sugars in cucumber fruit (Pharr et al., 1977). These high concentrations of citric acid are thought to be the cause of the decline in endocarp pH of the cucumber during ripening. Since the accumulation of citric is so large, it would be of interest to determine whether it is synthesized in the fruit or if it is transported into the fruit. It would also be of interest to know the function of citric accumulation. Perhaps it could be involved in seed maturation or release, since the major accumulation is in the endocarp.

## CONCLUSIONS

IT HAS BEEN FOUND that malic acid is the predominant organic acid in commercial sizes of pickling cucumbers. The concentration appears to be great enough so that malic degradation by nongas-forming lactic acid bacteria during fermentation (McFeeters et al., 1982) may account for a large proportion of the $CO_2$ production that Fleming et al. (1973) attributed to bacterial action.

There is a large accumulation of citric acid in the endocarp during the maturation of cucumbers and a smaller increase in the mesocarp tissue. This accumulation of citric acid is the probable cause of the drop in endocarp pH which has been previously observed.

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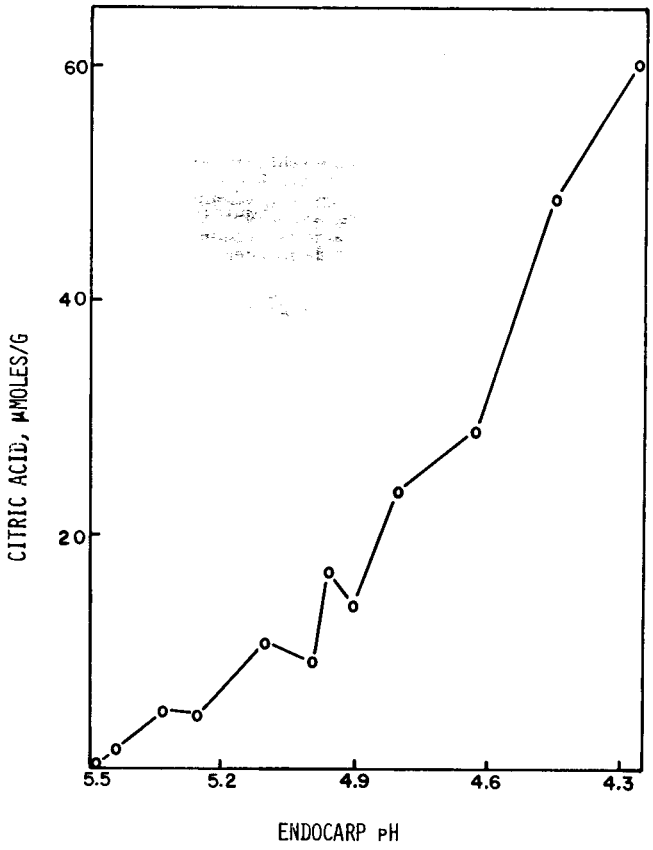


*Fig. 3—Citric acid concentration in the endocarp of ripening 'Calypso' cucumbers as a function of endocarp pH. Each point shows the analysis of a single fruit.*

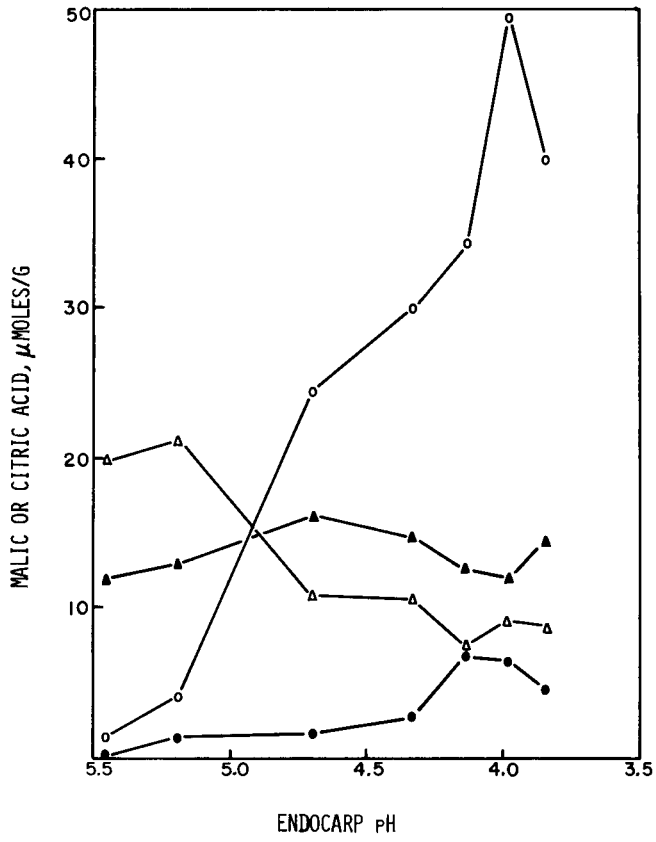


*Fig. 4—Citric acid and malic acid changes in the mesocarp and endocarp of ripening 'Chipper' cucumbers. Individual fruit were analyzed: △, malic acid in the endocarp; ▲, malic acid in the mesocarp; ○, citric acid in the endocarp; ●, citric acid in the mesocarp.*

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Ms received 3/24/82; revised 7/19/82; accepted 7/29/82.

Paper no. 8232 of the journal series of the North Carolina Agricultural Research Service, Raleigh, NC.
This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, IL.
The technical assistance of Mrs. Suzanne Armstrong in the course of this work is greatly appreciated.