Determination of Organic Acids

chromatography several times as sensitive as standard method in detecting acids in wine, juices, plant extracts

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The nonvolatile organic acids of fruit, vegetable and plant extracts can be determined by a simple, sensitive, and comparatively rapid method.

The procedure is based on *a*, ion exchange techniques to separate the organic acids in a relatively pure form, and *b*, paper chromatography to identify the acids.

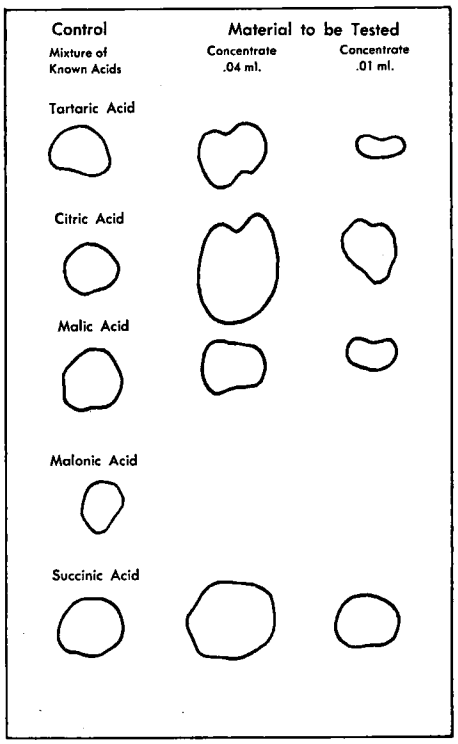
A sample of the solution to be tested—wine, fruit juice, plant extract—is passed over a column of anion resin which adsorbs all organic acids. After washing the column to remove residual sugars and cations the organic acids are eluted with dilute ammonium hydroxide. This solution containing the ammonium salts of the organic acids is passed over a column of cation resin which converts the material to free organic acids.

This acid fraction is concentrated by heating until a test paper chromatogram indicates the concentration giving the best resolution. Two spots containing different concentrations of the material to be tested are placed on a sheet of Whatman No. 1 filter paper next to a mixture of known acids.

The papers are suspended in a closed chamber, and the butanol phase of a butanol-formic acid-water solvent is allowed to flow down the paper by capillary action. After 10 to 14 hours the papers are removed from the chamber, air dried for three or more hours, and sprayed with a solution of Bromphenol Blue in 95% ethyl alcohol adjusted to pH 6.5 with dilute sodium hydroxide. The organic acids appear as yellow spots on a blue background.

The new method was used in determining the tartaric acid contents in berry wines. The presence of tartaric acid in berry wines indicates adulteration with grape wine. By adjusting the concentration of the acid fraction and the amount of material placed on a spot, as little as 50 parts per million—ppm—of tartaric acid can be detected in the wine.

Several commercial berry wines were selected which had been analyzed for tartrate by the two commonly used procedures, the official standard procedure of the Association of Agricultural Chemists —AOAC—and the Mathers method. The same wines were subjected to chromatography. The results obtained by all three methods are compared in the table.



Chromatogram of one of the berry wines tested. The control column—left—shows position of acids. The appearance of spots in the center and right columns indicates presence of the various acids in the tested material.

Wine	AOAC	Mathers	Chromatography
	gram tart. acid/100 ml.	indicated by shade of red	
N	.097	++ dark red	++
Q	.098	++ dark red	++
S	.091	++ dark red	++
I	.056	— trace pink	—
W	.017	+ faint red	—
M	.040	— trace pale red	—
B	.047	++ dark red	—

N, Q, and S wines were definitely positive for tartrate by all three procedures while I, W, M, and B wines were negative by paper chromatography and questionable by the other methods. Since the chromatographic method would have been able to detect any concentration above .005 grams per 100 milliliters the standard AOAC method appears to be not sensitive enough in the lower concentrations.

In the wines M and B the chromatographic method indicated the presence of some material that appeared just above the tartaric acid spot. The position of this interfering material varied with its concentration and even went below the tartaric spot as was demonstrated in certain berry juices. This material was found in the juice of boysenberries, loganberries, youngberries, and raspberries but not in blackberries. The only known material that behaves in a similar manner is oxalic acid. Preliminary isolation of this material and spot tests also tend to point to oxalic acid but it has not been definitely identified.

To check the possibility that tartaric acid does not come from grapes but is synthesized by yeast or other organisms in the musts, laboratory fermentations were carried out under different conditions in glass containers. The yeast used for the fermentations was *Saccharomyces cerevisiae* var. *champagne*.

In one portion of the container youngberries were crushed and fermented without any further treatment at room temperature and at 100° F. In another portion the youngberry must was treated as is done commercially. It was diluted with water until the acid concentration was approximately .6% as tartaric acid, and sucrose was added to bring the must to about 23° Balling. This second portion, too, was fermented at room temperature and at 100° F. No tartaric acid was detected in any of these fermentations.

Control of only a few of the many variables was attempted in these trials. Soundness and variety of fruit, area where grown, organism used for inoculation, and many other variables have not been tried and may be far more important in tartaric acid synthesis.

In addition to determining tartrate, the chromatographic method can be used to identify some 20 organic acids including citric acid. The method may aid in checking the effect of processing by comparing the acid contents of fresh and processed juices; finding the steps in processing that cause the destruction of acids; and determining the time when the acids of a fruit have dropped sufficiently for harvesting.

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