Colorimetric Determination of Aconitic Acid in Sorgo

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In a rapid colorimetric method for the determination of aconitic acid in sorgo juice, the acid is extracted from clarified starch-free juice with 2-butanone after lowering the juice pH to 1.3. One extraction removes 99% of the aconitic acid. Other organic acids, at the level found in sorgo juice, do not interfere with color development. Acetic anhydride and pyridine, when added to an aliquot of the 2-butanone layer, produce a purple color which is read on a spectrophotometer at 550 mμ.

Aconitic acid (1,2,3-propenetricarboxylic acid) is found in sorgo juice in amounts ranging from 0.2 to 1.0%. Its presence is reported to give the characteristic bitter taste of sorgo molasses. However, aconitic acid, in the quantities found in sorgo, will prevent the formation of crystalline sugar. The acid can be removed from the juice by the addition of lime and calcium chloride, producing calcium aconitate which can be removed by centrifugation. However, a surplus of calcium chloride will prevent the crystallization of sucrose. A rapid quantitative method was therefore needed for the determination of aconitic acid, to enhance crystallization of sucrose from sorgo.

Several methods for the determination of aconitic acid have been reported, most of which are time-consuming and plagued with interferences. Mader and Webster (1954) described a system for measuring the organic acids of sirup by partition chromatography. Ambler and Roberts (1947a, b, c) produced a decarboxylation method using potassium acetate–acetic acid solution; many organic and inorganic substances interfere. A quantitative extraction with ethyl ether, used by McCalip and Seibert (1941), is too time-consuming to be practical.

Regna and Bruins (1956) described a method for the extraction of aconitic acid from molasses by using 2-butanone. They reported 93% recovery of the acid by using six extraction cycles in a three-stage unit. Final analysis was by the decarboxylation method of Ambler and Roberts (1947a). Taylor (1919) developed a qualitative test for aconitic acid by adding acetic anhydride and heating. The red color formed was unstable, finally turning brown.

This paper describes a method for extraction of aconitic acid from sorgo juice and a rapid method for measurement of the concentration.

MATERIALS

Samples of sorgo juices were obtained from Otto Coleman, Sugar Crops Field Station, U.S.D.A., Meridian, Miss. The varieties received were Rio, Wiley, Hodo, Sart, Brandes, and Mn 1056.

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Reagents used were acetic anhydride (99 to 100%), Eastman Organic Chemicals; 2-butanone, Matheson, Coleman and Bell; pyridine, J. T. Baker, reagent grade; and *cis*- and *trans*-aconitic acid, reagent grade, K & K Laboratories, Plainview, N. Y.

METHOD

Separation of Aconitic Acid from Sorgo Juice. Approximately 100 ml. of juice was centrifuged to separate the starch. The starch-free juice was shaken with Celite filter aid and filtered with suction. The clarified juice, which has a pH of 5.0 to 5.5, was lowered to pH 1.3 with sulfuric acid solution to furnish cations to convert aconitates to aconitic acid. Ten milliliters of the juice was shaken 4 minutes with 20 ml. of 2-butanone in a centrifuge tube and centrifuged 1 minute to separate the layers.

Color Development. An aliquot of the 2-butanone layer (0.1 or 0.2 ml. is usually sufficient for sorgo juices) was evaporated to dryness using a steam bath and a stream of air. Ten milliliters of acetic anhydride and 0.01 ml. of pyridine were added. The sample was mixed and after 45 minutes color was read on a Coleman Model 14 spectrophotometer at 550 mμ.

Standard Aconitic Acid Curve. Solutions of *trans*-aconitic acid were prepared in acetic anhydride to produce final concentrations ranging from 2 to 20 μg. per ml. in a volume of 10 ml. Pyridine (0.01 ml.) was added and the solution mixed and read after 45 minutes on the spectrophotometer at 550 mμ. Concentration *vs.* absorbance was plotted to give a straight line, accurate between 4 and 20 μg. per ml.

RESULTS AND DISCUSSION

Prior to the extraction of aconitic acid from sorgo juice, separate water solutions of aconitic, tartaric, citric, and l-malic acids were extracted with 2-butanone. The 2-butanone layer was evaporated to dryness and titrated with NaOH, using phenol red indicator. One extraction with 2-butanone removed 80% of the aconitic, 28% of the l-malic, 23% of the citric, and 16% of the tartaric acid. This extraction was repeated using the described colorimetric method for quantitation. Tartaric and l-malic acid gave clear solutions with acetic anhydride and pyridine, while citric acid gave a light pink color only when the concentration was at least ten times the level of that found

in sorgo juice. The only compound found which seems to interfere with the color development is water.

When the pyridine is added to the acetic anhydride containing aconitic acid, the solution turns yellow. After 5 minutes the color becomes red, changing to purple after 30 minutes. Ninety minutes after the pyridine is added, the absorbance slowly decreases.

The extraction of aconitic acid from a 20% sucrose solution gave an 84% recovery. Higher concentrations of sucrose did not increase extractability. The recovery of added *trans*-aconitic acid from Wiley sorgo juice with a single 2-butanone extraction is shown in Table I. Average recovery was 99%, more than the amount recovered from water or water-sucrose solutions. A possible explanation is that the soluble constituents in the juice in addition to sugars cause the partition coefficient to favor the 2-butanone layer to a greater degree. Mader and Webster (1954) reported that aconitic, tartaric, citric, and l-malic acids account for 94% of the organic acids in sorgo juice. Of these four, only aconitic will produce a color at the concentrations found in sorgo juice.

The ratio of the volume of 2-butanone to the volume of juice was varied, with little difference in the amount of aconitic acid extracted. A 2 to 1 ratio of 2-butanone to juice was chosen because of convenience and because their mutual solubility gave lesser phase volume change.

If the pH of the juice is not lowered, no aconitic acid is extracted from the varieties tested by this procedure. This indicates the presence of soluble sodium and potassium aconitates, with no free aconitic acid in the juice. This is contrary to the report of Ventre *et al.* (1946), who report the presence of free aconitic acid.

The aconitic acid contents of Sart, Rio, Mn 1056, Brandes, Hodo, and Wiley varieties were 3.1, 7.2, 2.5, 4.5, 2.0, and 3.1 mg. per ml. of juice, respectively.

No attempt was made to determine whether the acid was *cis*- or *trans*-aconitic. Both acids develop similar colors in acetic anhydride and pyridine; therefore, the results here show total aconitic acid. Figure 1 shows the visible spectra for *cis*- and *trans*-aconitic acid and a spectrum for the color developed in a sample of sorgo juice. Of the two aconitate isomers, Stout *et al.* (1967) report that *cis*-aconitate was low in all species or range forages, whereas the *trans*-aconitate level was as high as 6% (dry basis) in some forages.

Ventre *et al.* (1944) patented a method for separation of calcium aconitate as a by-product in the manufacture of sugar from sorgo. Later they (1946) removed 82% of the aconitic acid from sorgo juice by the addition of lime and calcium chloride. The removal of this amount should be sufficient to allow sugar to crystallize. By use of the acetic anhydride–pyridine rapid colorimetric method, it is now possible to ascertain the proper quantity of lime and calcium chloride necessary for the removal of aconitates from sorgo. This would allow sucrose to crystallize without interference of aconitic acid and excess calcium chloride.

Table I. Recovery of Aconitic Acid from Wiley Sorgo

Aconitic Acid, Mg./Ml. Juice		Recovery,
Added	Found	%
0	3.13	
1.0	4.10	97
2.0	5.08	98
3.0	6.09	99
4.0	7.01	97
5.0	8.15	100
6.0	9.12	100
8.0	11.40	103
10.0	13.05	99
12.0	15.10	100

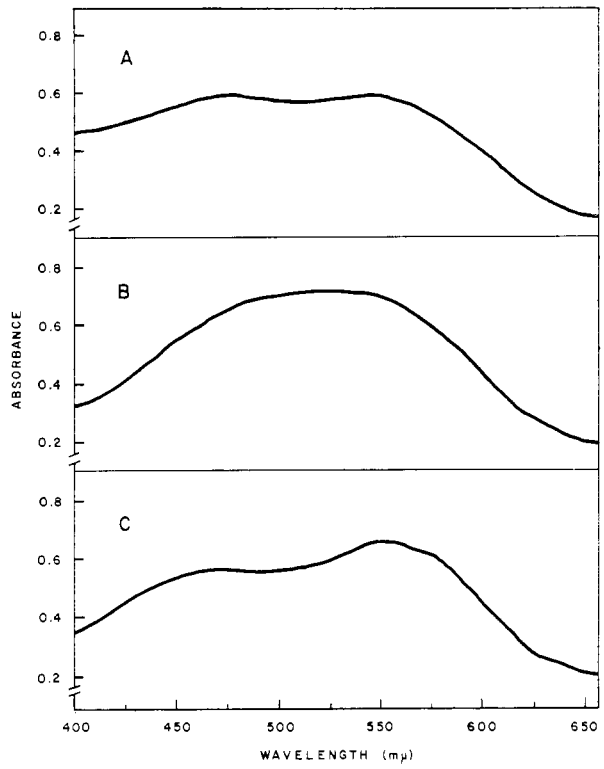

Figure 1. Visible spectra

A. Aconitic acid extracted from sorgo juice
B. *cis*-Aconitic acid
C. *trans*-Aconitic acid

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