Thin-Layer Chromatography of Sugar Beet Carbohydrates

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The use of thin-layer chromatography (TLC) for analysis of sugars in simple mixtures has been reported by several authors (3,5,6a,8). This new technique has been found to be extremely useful for qualitative analysis of the carbohydrates in crude sugar beet juices. As an alternative to paper chromatography, TLC offers comparable information in a shorter time without sacrificing the simplicity of the older method. We believe that this new technique merits the consideration of all in the beet industry who have occasion to run paper chromatograms.

Several books are now available that describe the general techniques and equipment of TLC (1,9,11). This paper will describe the TLC analysis of carbohydrates in sugar beet materials that we have found simplest. In our experience the techniques described by other workers did not give a completely satisfactory qualitative picture of the carbohydrate content of complex beet mixtures. TLC analysis of other beet constituents such as amino acids or organic acids has not been investigated in this laboratory, but would probably not be difficult.

Materials and Methods

The thin-layer applicator, 8 X 8-inch glass plates, plate holders, and tanks used were standard commercial items.

A variety of materials were tested as thin layer absorbents. Neither commercial silica gel G nor Kieselguhr G performed as well as a mixture containing equal parts of Celite Analytical Filter Aid® and anhydrous calcium sulfate (analytical grade) buffered with sodium acetate. A slurry made from 10 g each of Celite and calcium sulfate mixed with 50 ml of 0.02 M sodium acetate gave enough material to coat five glass plates with a layer 250 μ thick. After coating, the plates were air dried and then activated by heating at 125°C for one-half hour.

Solutions to be chromatographed were adjusted to 8-10% solids (RDS) without other purification or treatment. One or

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2 Numbers in parentheses refer to Literature Cited.

3 Brinkman Instruments, Inc., Westbury, N. Y.; Research Specialties Co., Richmond, California.

4 Johns Manville, New York, N. Y.
two microliters of each sample was placed at the origin line as in paper chromatography. Less disturbance of the absorbent layer resulted when a microsyringe with a capillary wire needle was used to apply the droplet than when a glass-tipped micropipette was used.

Chromatograms were developed by the ascending method in tightly closed rectangular tanks lined with filter-paper wicks for improved vapor equilibration. As long as the tank covers fit tightly, the same solvent could be used for several days. Results were better when equilibrating wicks were present than when they were absent. A double development technique gave maximum resolution of the components of interest. Plates were developed first in solvent A (ethyl acetate, 65; 2-propanol, 23; water, 12) (8) and then, after air drying, in solvent B (ethyl acetate, 55; 2-propanol, 30; water, 15). Solvent migration in each case was 15 cm instead of the usual 10 cm. Total time for the double development with intervening drying was about three hours.

After development, plates were dried and sprayed with Stahl's indicator (8) composed of 0.5 ml anisaldehyde, 0.5 ml concentrated sulfuric acid, and 9.0 ml 95% ethanol. Color development was brought about by heating at 125° C for 10-15 minutes.

Results and Discussion

Although other workers have suggested different conditions, we found that the above procedure gave optimum separation of the carbohydrates in beet materials. With the solvents chosen, only the carbohydrate materials move to any extent. Spraying irrigated TLC plates with other indicators (ninhydrin or bromocresol blue) did not reveal migration of any amino acid or organic acid components to the carbohydrate area. Figures 1 and 2 show typical chromatograms. Figure 1 shows that a mixture of pure beet carbohydrates, namely fructose, glucose, sucrose, raffinose, and galactinol distinctly separated into its component parts. We omitted kestose for lack of a suitable standard; it would have fallen in the gap between sucrose and raffinose. The separation was especially distinct when the components were all present in about equal quantities as in D, E, F of Figure 1. As the concentration of sucrose increased to 200-300 times that of the other components, the resolution of the reducing sugars became less distinct, but the other sugars remained unaffected. This trend can be seen in Figure 1, A, B, C.

Figure 2 shows a thin layer chromatogram of actual beet juices. Colloidal material in raw juice (B), high pH in intermediate juices (C and D), or high ash (E and F) in final
Figure 1.—TLC separation of pure sugars normally found in beet molasses, (1) fructose, (2) glucose, (3) sucrose, (4) raffinose, (5) galactinol. A, B, and C contain 50, 100, 150 μg respectively, of sucrose. Fructose, glucose, raffinose, and galactinol are all constant at the 0.6 μg level. In D, E, and F, 0.6 μg of each carbohydrate is present.

Figure 2.—TLC separation of carbohydrates in actual beet juices; A, standard solution containing fructose, glucose, sucrose, raffinose, and galactinol; B, diffusion juice; C, first carbonation juice; D, phosphated thin juice; E, straight house molasses; and F, barium process molasses, 1.0 μl of each 10% RDS juice applied at origin. Sugars are (1) fructose, (2) glucose, (3) sucrose, (4) kestose, (5) raffinose, (6) galactinol, (7) and (8) unknowns.
molasses did not materially affect the resolution. Comparison of factory diffusion juice (B) and first carbonation juice (C) showed that processing did a good job in decreasing the levels of reducing sugars and, apparently, of kestose. D was a phosphated thin juice sample (2) prepared from stored beets showing abnormally high reducing sugars and kestose. E and F were molasses samples showing the contrast between straight house (E) and barium saccharate process products (F).

We do not list a table of Rf values for several reasons. Experience has shown that differences in plate preparation or changes in relative concentration of components influence the rate of migration of the components. The relative order of the components remains unchanged, however, and the overall pattern is recognizable regardless of the actual concentrations (Figures 1 and 2). A slight increase in Rf values occurred as plates aged after activation. The adsorbent properties of the calcium sulfate thin layer changed gradually with prolonged exposure to atmospheric moisture and reheating in the oven did not restore original activity. The changes in crystal size and shape associated with calcium sulfate aging are complicated (4), so slight changes in adsorbent properties were not unexpected. Nevertheless, TLC plates gave fairly consistent Rf values for several days after preparation and, regardless of the plate's age, the characteristic pattern of the various sugars was easily recognizable. Calcium sulfate without Celite was not a satisfactory thin layer material because it did not adhere to the glass very well.

The good separations obtained in this study seemed to depend on the use of extremely small quantities of carbohydrates. Optimum resolution was in the 0.5-3.0 µg range for each component and as little as 0.1 µg of most materials could be detected by Stahl's indicator. When amounts of sugars commonly used in paper chromatography were put on our TLC plates, it was impossible to obtain good separations. Because such small quantities of sugars were used in these TLC experiments, some of the common paper chromatography sprays (7) were not sensitive enough to locate the individual components. Nor was spraying with sulfuric acid followed by charring with heat sensitive enough. A silver nitrate (7) spray was as sensitive as Stahl's anisaldehyde-H2SO4, but produced more background color.

The double development technique (10) described in the experimental section was necessary for good resolution. Single development with either solvent A or solvent B was unsatisfactory as was reversing the order of development by using solvent
B first. In our opinion, the increased resolution from double development justified the more time-consuming technique.

Semi-quantitative analysis of beet sugar carbohydrates by visual comparison with known standards was not too accurate. In one instance, we hoped that minor quantity changes in a series of raffinose samples could be estimated by this method. Samples containing 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 µg of raffinose were developed and treated in the usual manner. A difference of 0.5 µg/µl of raffinose could be detected, but a difference of 0.25 µg/µl could not be seen with certainty. We concluded from this and other experiments that gross order of magnitudes could be established readily for various components by visual comparison, but that precise analysis was not feasible. Unfortunately, since we used considerably smaller quantities of material than Gee (3), it was not possible to employ her techniques for quantitative analysis of raffinose and sucrose. We contemplate further work on the use of TLC for quantitative analysis of beet carbohydrates.

Very recently, Prey and coworkers (6b) published a method for the quantitative determination of raffinose in beet molasses using TLC plates with silica gel G adsorbent and an acetone-water developer (9:1, vol/vol). In the beet juices we examined by his technique, the resolution of components was faster but not as good as we report. Quantitative results based on visual comparison of spots appeared to be similar to those from our method.

Summary

The use of thin layer chromatography to separate fructose, glucose, sucrose, kestose, raffinose, and galactinol in beet juices is described. The method is recommended as an alternative to paper chromatography for rapid qualitative analysis of the carbohydrates in sugar beet materials.

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

Literature Cited


