Investigation of Factors Influencing the Sachs-Le Docte Method

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The Sachs-Le Docte (SLD) method of analysis is one of the simplest, most rapid analytical methods ever devised for sucrose. There are two factors which may prevent the determination of a statistically reliable volume of basic lead acetate to be used with a normal weight of beet **pulp.** These are optically active non-sucrose constituents and the volume of marc. Because of changes in beet varieties and harvesting practices in California during the past 20 years, a technical committee composed of representatives of California growers and processors, the University of California and of this laboratory undertook studies of the determination of the accuracy of the SLD method and the SLD volume, which is now 179.1 ml. for California beets. The first phase of this work was a study of factors influencing the Sachs-Le Docte method. It was carried out by personnel of the Western Utilization Research Branch.

The plan of work for the investigation was to examine the effect of lead acetate on each of the optically active constituents in California beets and then each of the incidental factors, such as heating, solubilization of pulp, etc., which might influence the results. These variables are discussed in the following paragraphs.

Effect of Basic Lead Acetate on Rotation of Sucrose

Bates and Blake (2)[2] measured the change in rotation of a sucrose solution in the presence of increasing concentrations of basic lead actate. Their results are shown in the curve in Figure 1. Our results are similar as shown in the same figure. In measurements of beet juices with a rotation of 13° to 20° S to which are added only the slight excesses of basic lead acetate ordinarily used, the lead error is so small that it is not measurable in the saccharimeter used in this work.

Rotation of Amino Acid Solutions in Presence of Basic Lead Acetate

The marked effect of certain salts on the rotation of many amino acid solutions has been noted in the literature, but no systematic study of the effect of lead acetate on amino acids which occur in beet juice was found. The composition study under way at this laboratory has divulged the amino acids present in California beet juice. The rotations of the more abundant acids in different concentrations of lead acetate are shown in Figure 2. L-asparagine and L-aspartic acid are dextrorotatory in lead acetate while L-glutamic acid, pyrrolidone carboxylic acid, glutamine, leucine, valine, isoleucine and alanine are levorotatory. The combinations of amino acids shown in Table 1 are based upon analyses of diffusion juices from Brighton, Colorado, and Manteca, California (3) . The dextrorotatory amino acids are

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[2] Numbers in parentheses refer to literature cited.

practically nullified by the levoratatory acids as reported in Table 2, so that the ionic optically active compounds in beet juice have only a small effect in the SLD volume.

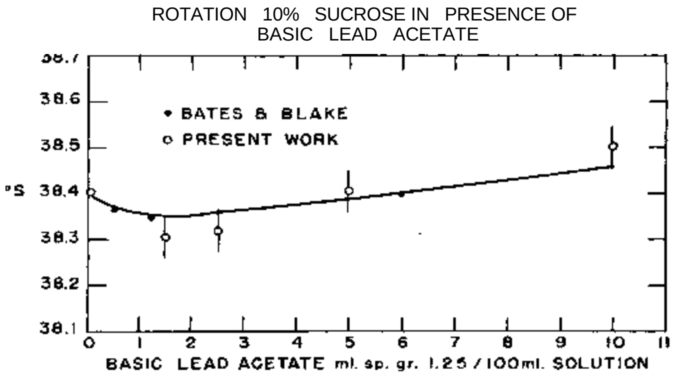

Figure 1.—Effect of basic lead acetate on rotation of sucrose solutions. (10 ml. of 1.25 sp. gr. lead solution per 100 ml. is equvialent to a 5° Brix concentration of basic lead acetate.)

Table 1.—Amino Acid Mixtures Typical of Sugar Beet Juices.

Solution	I (Calif.)	II (Colo.)
	(mg./liter, 10% sugar basis)	
L-alanine	75	40
L-aspartic acid	80	10
L-glutamic acid	130	60
L-glutamine	970	485
L-isoleucine	60	30
L-leucine	50	30
L-serine	50	25
L-threonine	50	25
L-valine	40	25
L-malic[1]	200	200

[1] Optically active nonamino organic acid.

Table 2.—Effect of Basic Lead Acetate on Rotation of Amino Acid Mixtures[1] in 10 Per-cent Sucrose Solution.

Vol. of the lead acetate solution	0	1	2	5	10	20
I Δ° S[2]	—0.05	+0.18	+0.15	—0.12	—0.10	—0.20
σ	0.05	0.09	0.04	0.11	0.10	0.18
II Δ° S	—0.03	+0.08	—0.10	—0.16	—0.13	—0.10
σ	0.05	0.06	0.09	0.13	0.08	0.11

[1] Amino acid mixtures are shown in Table 1.
[2] S = 76.8° for 10 percent sugar solution.

Inasmuch as the SLD method as applied in California calls for hot digestion, both sucrose and sucrose plus amino acid solutions were heated at 80° C. for 30 minutes with basic lead acetate and the optical rotation measured. The unheated solutions like those in Table 2 had an optical rotation of 76.9° S in 5° Brix basic lead acetate solution and one of 76.9° S in heated 5° Brix lead solution. No difference was obtained.

Rotation of Beet Marc Constituents

Of the water-soluble constituents in beet marc, pectin has a specific rotation sufficiently high to effect the SLD method. To test this hypothesis one gram each of marcs previously extracted free of sucrose (No. 123, 170 in Ref. 4) was heated in 100 ml. of water at 80° C. for 30 minutes. The average rotation of the lead-free solution was + 0.8° S. In the presence of 5° Brix lead acetate under the same conditions, the rotation was + 0.04° S. These results can be explained by assuming that a small amount of pectin is extracted by water but is precipitated by the basic lead ion. Saponin, present in fresh pulp, would act like pectin.

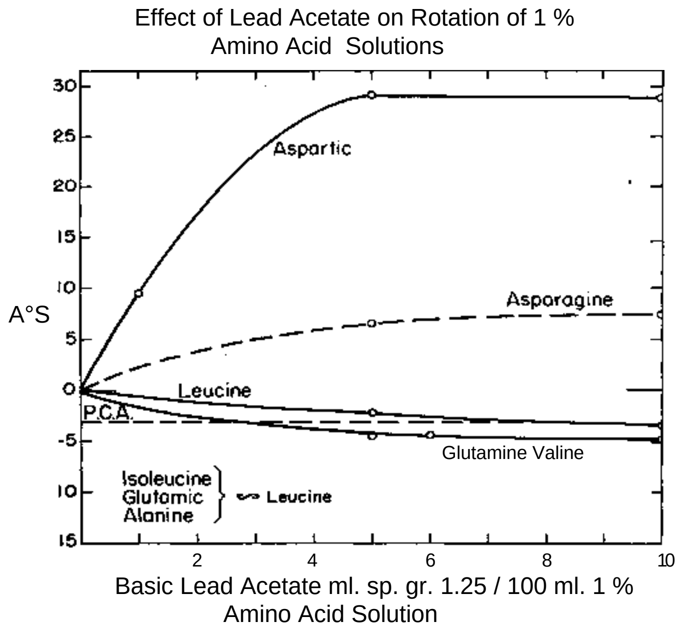

Figure 2.—Change in rotation of amino acids with increasing concen-trations of basic lead acetate.

Comparative Sugar Analyses

The results obtained to this point strongly suggest that the SLD method can provide a reliable measure of the sucrose content of beets. The next step was to compare the results obtained by the SLD method and an official AOAC method (1) when applied to a series of beet pulps. These pulps were regular samples prepared and weighed by factory chemists at various tare laboratories. The samples were weighed into friction top 8-ounce tin cans, frozen and transported in the frozen state to this laboratory. They were extracted with 179.1 ml. of 5° Brix basic lead acetate solution at 80° C. for 30 minutes without being removed from the cans. After filtration and adjustment to 20° C. the optical rotation was measured. A portion of the nitrate was deleaded, the sucrose hydrolyzed in acid solution, and total sugars measured by the Munson-Walker method (1) . Four of the samples were also analyzed for invert sugar before hydrolysis. There was only 0.04 percent invert in the samples analyzed. The average sucrose content of 36 samples of beets was 15.65 percent by polarization and 15.75 percent by the AOAC method. The agreement between the two methods is about as good as can be expected between different sugar methods. Another inference that can be drawn is that in California beets the raffinose and invert contents are small.

Volume of Marc

The other factor which must be evaluated to make the SLD valid is the volume of marc or volume of juice in the normal weight of beets. One of the most direct ways of doing this is to follow the Daeschner-Bauer modi-fication of Krocker's method (5) . The details of this method were pro-vided by Paul Alston of the Spreckels Sugar Company and are as follows.

The sawed or rasped pulp is carefully mixed and a normal weight of the mixed sample is placed in each of six friction top cans or SLD cru-cibles. To three of the samples is added 179.1 ml. (this may be any known volume near 180 ml.) of 5° Brix basic lead acetate solution and to three others 179.1 ml. of 5° Brix to which was added 10 percent sugar. The six samples are extracted in the usual way and filtered, and the optical rotations measured. The same volume of 10 percent sugar in basic lead acetate solu-tion used above (179.1 ml.) is diluted to 200 ml. and measured in the saccharimeter. The true SLD volume is given by the equation:

$$V = 379.1 - \frac{200\ P_1}{P_2 - P_3}$$

where P_1 is the rotation of the sugar solution, P_2 the rotation of the beet juice plus added sugar, and P_3 the rotation of the beet juice in basic lead acetate without added sugar.

Measurements by this method were undertaken during the past cam-paign in six tare laboratories as well as our own and will be continued during the next campaign. Results of this cooperative undertaking will be released at a later time when the effect of different seasons can be evaluated.

A second method is to measure the weight of marc in a normal weight of beets. If this weight is subtracted from 26 g., the weight of juice is obtained and that weight can be converted to volume of juice by dividing by its density. Marc determinations under way at this laboratory show an average of 4.7 percent for 85 inbred varieties. Use of this figure leads to an SJLD volume of 176.8 ml. The calculated average for the SLD volume in-cludes a correction for the fact that a low marc beet is likely to be low in sucrose (4), but it does not correct for water of hydration. Scatchard (6) measured hydration of sucrose in aqueous solution and concluded that about one water molecule per polar group in sucrose was held fairly firmly by the sucrose molecule. Most of the marc is carbohydrate in nature and if one molecule of water is "bound" by each polar group, the water of hydra-tion would be between 20 and 30 percent. This would require a correction of about 0.4 ml. in the SLD volume. We have been unable, however, to detect a change in the rotation of sucrose solutions when marc is added to them sufficient to require a correction of this magnitude.

Corrections for Neutral, Optically Active Constituents

California sugar beets generally are low in raffinose and no correction appears to be necessary for it at the present time. If, however, 0.05 per-cent of raffinose were present, a correction factor might become necessary, because the specific rotation for raffinose, in the absence of basic lead acetate, is 124°, while that for sucrose is 66.5°. This means that, for every 0.05 per-cent of raffinose, the SLD volume would be increased by 0.9 ml.

In summary, the factors which might affect the accuracy of the SLD method have been examined, and it is concluded that the method is accurate when applied to California sugar beets. The Daeschner-Bauer modification of Krocker's method appears satisfactory for the determination of a statis-tically reliable volume to be used in the SLD method.

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