ALUMINUM CLARIFICATION OF SUGARBEET BREI EXTRACTS

S. S. Martin, R. J. Hecker, and G. A. Smith*

INTRODUCTION

Many different methods have been explored and described for analyzing sucrose content of sugarbeet roots (7, 20). For rapid analysis of sucrose, most laboratories use polarimetry of aqueous root extracts clarified by a lead salt. Now, because of the growing concern about lead as an environmental pollutant, an alternative clarificant is needed. Aluminum, being both environmentally non-toxic and inexpensive, would be an ideal substitute if it were shown to be satisfactory as a clarificant. Along with many other metals and salts, aluminum salts were examined and used long ago for clarification of sugarbeet extracts [e.g., (2, 24)], and the use of aluminum salt, particularly "alumina cream" (aluminum hydroxide), either alone or in conjunction with lead salts, was and still is recommended for specific purposes [e.g., (9)]. However, aluminum-clarified extracts, although clear, are more colored than comparable lead-clarified extracts; in the past this resulted in interference both to sucrose analysis by means of manual, long-cell polarimeters, and to analysis of other extract chemical constituents which for convenience often were determined in the same clarified samples used for sucrose analysis. In particular, aluminum-clarified extracts were not satisfactory for the widely used "blue number" method of amino nitrogen determination (4, 6). Recent instrumentation develop-

ment, however, has led to the use of automatic, short-pathlength polarimeters and of amino nitrogen analysis by techniques such as the ninhydrin colorimetric procedure or its modifications (22, 23) or fluorometric analysis (1, 3, 8, 28).

Realizing that some of the former objections to the use of aluminum salts as sugarbeet extract clarificants no longer exist, we began a re-evaluation of their utility for that purpose. Partial reports of some of these studies have appeared elsewhere ("Sugarbeet Research," 1976-1979). The objective of the experiment reported here was to make a detailed comparison between aluminum chloride and lead subacetate as sugarbeet brei extract clarifi-cants, drawing the samples from an experimental design intended to provide a wide range in both sucrose and other extract chemical component concentrations. We thus sought to examine the efficacy of aluminum chloride as a sugarbeet extract clarificant over a more extensive range of variation than would be encountered in any normal commercial growing situation.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The experiment was a randomized complete block design with four cultivars, two nitrogen fertilization levels, and 18 repli-cations. The cultivars were of varied genetic constitution, including a sugarbeet/fodder beet hybrid, a commercial variety, a high-sucrose obsolete variety, and an experimental hybrid:

Identifier	Description
A	52-305 CMS X Ovana, F_1; HALF FODDER BEET
B	Polish AJ-ZZ
C	GW 'Mono Hy D2'
D	[(FC 504 CMS X FC 502/2) x 662119s1] X FC 702/5; Experimental hybrid

The entire experiment received preplant ammonium nitrate at 118 kg N/ha (subsequently designed as N level 1). The "high N" treatment (N level 2) received a subsequent application of an additional 112 kg N/ha, as ammonium nitrate side-dressed at 10 weeks after planting. The experimental area had about 112 kg N/ha as residual nitrate in the top 60 cm of soil. N level 1 was optimum; N level 2 provided excessive N. Each plot

consisted of four rows, each 7 m long. The two center rows of each plot were harvested separately and the roots were composited for a single sample.

EXTRACT PREPARATION AND CLARIFICATION

Composited brei from each root sample was thoroughly hand-mixed to obtain as homogeneous a mixture as possible. From this mixture, two portions of brei were taken, one to be clarified by means of lead subacetate solution (Baker 2290, 11.5 g/L)[+] and one to be clarified by means of aluminum chloride solution (Baker 0498, 1.81 g/L, corresponding to 1.0 g/L of the anhydrous salt). Each clarificant was added by a proportioning balance in the ratio of 177.0 ml solution to 26.0 g brei. Each of the paired samples subsequently was treated as nearly identically as possible. The brei plus clarifying solution was mechanically blended for 2 min at ca. 400 rpm in a household-type blender with Mason jar container. The homogenate was filtered through Whatman No. 1 paper and the polarization of the filtrate was determined in an automatic polarimeter fitted with the 20 mm cell. A portion of each filtrate was promptly frozen and held at -20 C until further analysis.

ANALYSIS OF SELECTED NON-SUCROSE EXTRACT COMPONENTS

Frozen clarified extracts were thawed in batches in a microwave oven until ice just remained, then allowed to stand briefly at room temperature until thawing was complete. Each filtrate was analyzed for sodium and potassium by flame photometry with lithium internal standard. Amino nitrogen was determined by two methods: (1) spectrophotometrically, with ninhydrin; and (2) spectrofluorimetrically, after reaction with o-phthalaldehyde in 0.05 M borate buffer at pH 9.0 (excitation 340 nm, emission 455 nm). In each case the data were expressed as mg N in glutamic acid per 100 ml extract. Total nitrogen was determined by direct nesslerization of acid-digested samples (21), and betaine was determined spectrophotometrically as the reineckate.

[+]Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

RESULTS

The data were analyzed initially by analysis of variance. As anticipated by the experimental design, there were significant differences (in each of the two filtrate types) among cultivars for every component studied: sucrose percentage, and filtrate concentrations of sodium, potassium, nitrate, amino N, total N, and betaine (Table 1). Similarly, levels of most analyzed components differed significantly between the two nitrogen fertilization levels (Table 1). In either extract type, the interaction of cultivar with N-level was significant only for nitrate. Inspection of the nitrate data showed that this interaction was a result of the magnitudes of the data for each cultivar-nitrogen level combination, not of any underlying change in rank order of means for each genotype within each N-level.

Table 1. Summary of main effect and interaction mean squares from analysis of variance for aluminum-clarified and lead-clarified extract components.

Extract Component	Source of Variance[+]		
	Cultivar	N-Level	C X N
ALUMINUM-CLARIFIED			
Sucrose	382**	10**	1.9
Amino N	141**	8**	0.5
Total N	467**	51**	4.0
Nitrate	5252**	523**	191.*
Sodium	758**	59*	15.7
Potassium	2344**	26	0.9
Betaine	163**	7	11.2
LEAD-CLARIFIED			
Sucrose	356**	11**	1.1
Amino N	119**	11**	0.4
Total N	325**	11	6.1
Nitrate	3830**	458**	130.*
Sodium	786**	66*	17.
Potassium	2383**	40*	3.4
Betaine	23899**	570	567.

*, ** Significant at the 5% and 1% level of probability, respectively.

[+] The degrees of freedom associated with the F-tests were 3 and 119, 1 and 119, and 3 and 119 for cultivar, N-level, and their interaction, respectively.

SUCROSE

Because this experiment was designed primarily to examine the ability of aluminum chloride to clarify sugarbeet extracts for polarimetric sucrose determination, these data will be presented in greater detail than those for other extract components. Table 2 summarizes the comparative sucrose means for lead-clarified and aluminum-clarified extracts, both for each combination of cultivar and nitrogen fertilization, and across each of those two factors. For the overall comparison, across both cultivars and N-levels, the polarimetrically determined sucrose means were nearly identical. The lead-clarified extracts were very slightly higher in sucrose, on average, than the comparable aluminum-clarified extracts (14.73 and 14.70, respectively), but there was no statistically significant difference by paired-sample t-test (Table 2). The correlation coefficient of 0.99 confirmed the high degree of correspondence between the two extract types. Because both variables were functions of an underlying cause, neither is correctly a function of the other and a simple regression description would be inappropriate. Instead, the method of principal axes was used to characterize the bivariate data. When plotted as a bivariate scattergram, the data are described by an ellipse having principal axis given by:

$$ALSUC = -0.53 + 1.03(PBSUC)$$

where ALSUC = pol sucrose of aluminum-clarified filtrates, and PBSUC = pol sucrose of lead-clarified filtrates. The minor axis (at right angles to the principal axis if data are plotted on the same scale) is described by:

$$ALSUC = 29.0 - 0.97(PBSUC).$$

The two eigenvalues (characteristic roots) are λ_1 = 9.19 and λ_2 = 0.032; the high ratio of eigenvalues (λ_1/λ_2 = 285) indicates the extreme elongation and narrowness of the ellipse encompassing the data.

If the data are examined by cultivar, either separately at each nitrogen level or across nitrogen levels, the source of the slightly lower aluminum-clarified extract mean relative to the lead-clarified mean for the overall comparison can be seen. For entry A, the half fodder beet, sucrose determined in aluminum-

Table 2. Comparison of sucrose means in aluminum-clarified (Al) and lead-clarified (Pb) extracts. (Sucrose was determined by polarimetry and is expressed as percentage of root fresh weight.)

| | N-Level 1 | | | |
| | Mean | | Paired | |
Cultivar	Al	Pb	t	r^+
A	11.83	11.95	2.89**	0.97
B	16.87	16.90	1.22	0.96
C	15.54	15.53	0.53	0.96
D	15.35	15.33	0.65	0.96
Across cultivars	14.89	14.93	1.86	0.99

| | N-Level 2 | | | |
| | Mean | | Paired | |
Cultivar	Al	Pb	t	r
A	11.04	11.21	3.94**	0.98
B	16.83	16.73	1.44	0.87
C	15.28	15.29	0.21	0.93
D	14.92	14.90	0.54	0.97
Across cultivars	14.52	14.53	0.73	0.99

| | Across N-Levels | | | |
| | Mean | | Paired | |
Cultivar	Al	Pb	t	r
A	11.44	11.58	4.86**	0.98
B	16.84	16.81	0.53	0.91
C	15.41	15.41	0.16	0.95
D	15.13	15.12	0.85	0.97
Across cultivars	14.70	14.73	1.72	0.99
Across cultivars, A excluded	15.79	15.78	0.86	0.97

$^+$Correlation coefficient.
*, **Significant at the 5% and 1% level of probability, respectively.

clarified extracts was significantly lower than sucrose determined in lead-clarified extracts; the sucrose percentage difference was about 0.15. Correlation coefficients, however, between the two sample types within cultivar A were as high or higher than those for any other cultivar. If cultivar A were excluded from the comparison, pol sucrose in the fully sugarbeet samples clarified by the two methods differed in mean by only 0.01, and paired-sample analysis showed no significant difference at the 0.001 level of probability (Table 2.)

SODIUM AND POTASSIUM

Both sodium and potassium means in the two extract types were in close agreement (Table 3) and correlation coefficients were high. If the two sets of samples were statistically analyzed as groups, no significant difference between the extract types was found for these components. Paired comparisons analysis, however, showed a slightly but consistently greater concentration of sodium and potassium in the lead-clarified samples relative to the corresponding aluminum-clarified ones (see the paired t-test values in Table 3). The cultivar means for each component also illustrate that both sodium and potassium concentrations in the lead filtrates were slightly greater than those in the corresponding aluminum filtrates. Correlation coefficients between sodium or potassium determined in pressed raw juice and the corresponding values in either clarified extract type were similar (0.74 and 0.75 for sodium in raw juice with Al and Pb extracts, respectively; 0.61 and 0.64 for potassium in raw juice with Al and Pb extracts, respectively). Thus, although it might be expected that the two extracting and clarifying solutions would differ in sodium or potassium extraction, the similarity of means and correlation coefficients indicates this was not the case.

Table 3. Comparison of non-sucrose component concentrations[+] in sugarbeet extracts clarified by aluminum (Al) or lead (Pb) solutions.

Component	Clarificant	Cultivar				Mean	r[‡]	Paired t
		A	B	C	D			
Sodium	Al	14.3	8.8	6.6	10.1	10.1	0.97	2.46*
	Pb	14.5	9.0	6.7	10.2	10.2		
Potassium	Al	28.8	16.5	19.5	17.1	20.5	0.95	6.8**
	Pb	29.4	17.1	20.4	17.7	21.2		
Amino N	Al	8.4	5.2	6.2	5.7	6.4	0.88	13.4**
(Ninhydrin)	Pb	7.6	4.7	5.8	5.1	5.8		
Amino N	Al	10.2	6.3	7.7	6.9	7.8	0.95	19.4**
(o-Phthal.)	Pb	9.4	5.6	7.2	6.2	7.1		
Total N	Al	25	19	20	20	21	0.69	15.0**
	Pb	26	22	23	22	23		
Betaine	Al	35	38	35	35	36	0.22	91**
	Pb	137	179	170	159	161		
Nitrate	Al	29	12	10	16	17	0.96	27**
	Pb	23	9	7	12	13		

[+]Data are in mg/100 ml of extract.

[‡]Correlation coefficient.

AMINO N

Amino acid nitrogen in the two extract types was proportion-
ally similar by each of the two analytical methods used. Amino N
concentration was greater in the aluminum-clarified extracts than
in the comparable lead-clarified samples (Table 3). Because of
the known differences in reagent sensitivity to different amino
acids, the o-phthalaldehyde fluorescence method yielded higher
values for amino N in both extract types than did the ninhydrin
spectrophotometric method. By either analytical method, the
correlation coefficients again confirmed the high degree of associ-
ation between the data for each filtrate type. The correlation
coefficient between the two analytical methods was 0.94 for both
extract types.

OTHER NITROGENOUS COMPONENTS

Total nitrogen means were similar in the two extract types
(Table 3), but the correlation between data, although highly
significant, was only moderate. Betaine was largely destroyed
in the aluminum-clarified extracts, leading to highly significant
differences between paired samples and to almost totally unassoci-
ated data between the extract types. Nitrate, determined by
specific ion electrode without correction for the expected chloride
interference in aluminum chloride clarified extracts, differed
between extracts, but a high linear correlation existed. Thus,
either a linear correction applied to specific ion data obtained in
the usual way or calibration of the nitrate electrode in the
presence of the appropriate chloride interference would yield data
comparable to that of lead-clarified extracts. Alternatively,
clarification by another aluminum salt that would show less inter-
ference with the nitrate electrode could be used. For example,
the selectivity coefficient for sulfate is less than that for
chloride.

DISCUSSION

Aluminum-clarified extracts usually were slightly yellow to
yellow-black, but this caused no difficulty in a short-pathlength
polarimeter. Although no objective measurements were made,
both filtration rate and clarity of the filtrate appeared essentially

the same between the two extract types. The low pH of the aluminum chloride solution resulted in a sugarbeet brei extract filtrate of low pH. However, in a randomly selected group of ten aluminum-clarified samples that were read by polarimetry, allowed to stand at room temperature for one to two hours, and then reread, the second value was in every case within 0.02 of the original reading (in % sucrose). Thus, apparently no appreciable inversion of sucrose occurred.

In aluminum-clarified samples, two major factors might have been expected to influence the net optical rotation on which the estimated sucrose content of sugarbeet extracts is based: (1) a possible difference (relative to lead extracting solution) in proportions of various optically active compounds extracted, or (2) a different contribution of optically active compounds present to the net rotation, because of the recognized fact that specific rotations of several sugarbeet extract impurities vary with extract pH [e.g., (29)]. The results of this study, however, showed that even in quite diverse experimental material, the net result of such effects in the aluminum-clarified extracts was negligible, inasmuch as the determined sucrose means were nearly identical.

When one clarifying agent is substituted for another, no improvement is realized in several other sources of error in sucrose determination by polarimetry--e.g., the assumed constancy of marc volume, or the optical rotatory contributions of non-sucrose extract components. If these sources of error are considered to be acceptable in lead-clarified samples, however, they should be equally so in aluminum-clarified ones. Thus, we conclude that aluminum chloride clarified samples are fully satis-factory for polarimetric sucrose determination in any sugarbeet genotypes likely to be encountered in commercial practice in the U. S.

Lipets and Oleinik (12) in Russia examined aluminum sulfate and aluminum chloride as sugarbeet extract clarificants for pol sucrose determination, but their test consisted of only 10 samples and thus had no statistical foundation. They and their colleagues have reported extensively on the use of aluminum salts in sucrose extraction and juice clarification in the sugar factory (10, 11,

13-19, 25-27). Burba and Puscz (5) compared 68 pairs of samples, presumably of a single genotype, clarified by either lead subacetate or aluminum · chloride, and reported no significant difference between clarification methods for sucrose (by polari- metry), sodium or potassion (determined by flame photometry), or amino N (determined by the ninhydrin spectrophotometric method). With our more varied experimental material, small but significant differences between the two extract types were detected for sodium, potassium, and amino N. Nevertheless, as we have shown else- where in a preliminary experiment ("Sugarbeet Research," 1977), although the predictor equation coefficients are slightly changed, purity prediction from concentrations of sodium, potassium, and amino N in aluminum-clarified filtrates is equally as satisfactory as such prediction from lead-clarified filtrates. We shall discuss this further in a future paper on purity predictor models.

SUMMARY

Aluminum chloride was compared to lead subacetate as a sugarbeet extract clarificant. Samples analyzed ranged from half fodder beets to high-sucrose sugarbeets. Concentrations of both sodium and potassium were nearly identical in the two extract types. Amino N concentration in aluminum-clarified extracts was greater than, but highly correlated with, comparable data in lead-clarified extracts. Betaine was largely destroyed by aluminum clarification. Polarimetrically determined sucrose concentration means in the two extract types differed statistically only for the half fodder beet. For any sugarbeet genotype likely to be encountered in commercial practice, aluminum clarified filtrates are satisfactory for determination of sucrose and several common impurity components.

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