BETAINE CONTENT OF SUGARBEET VARIETIES

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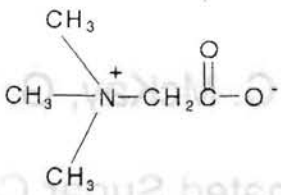
I. INTRODUCTION

Betaine (structure shown below) is one of the major nitrogen compounds in sugarbeets and has always been important with



respect to factory purification since it is not removed in liming and carbonation. Betaine has become an even more important constituent of molasses with the advent of molasses desugarization by ion exclusion since the betaine in molasses (typically present at levels of 5.5 to 7.0 g/100 g RDS) is one of the non-sugars less easily removed by ion exclusion. In addition, interest is increasing in the production of purified betaine or materials enriched in betaine as by-products of the ion exclusion process.

Because of the increased interest in betaine levels and the lack of information on how betaine levels might vary with sugarbeet variety in the Northwest growing area, a study of betaine levels in samples from a standard sugarbeet variety test was undertaken.

II. RESULTS AND DISCUSSION

A. Experimental Methods

Betaine was determined in samples from a 1990-91 variety test conducted by the Nyssa, Oregon Beet Seed Research Laboratory (then

operated by Amalgamated Sugar Company and now owned by Hilleshog Mono-Hy). Twenty-five sugarbeet varieties were planted in a 5×5 balanced lattice with 12 replications per plot. Sugarbeet brei samples from Rupert and Grandview test plots were produced and frozen at the Nyssa Laboratory and transported to Twin Falls for betaine analysis. Nitrate, sugar, and conductivity were measured at the Beet Seed Laboratory.

Betaine was determined by the following high performance liquid chromatography (HPLC) procedure:

> Brei samples were thawed, mixed, and two 25.00 g portions of brei were rinsed into separate 100 ml volumetric flasks with deionized water. Water was added to bring the liquid level just below the flask neck. Samples were heated at 80°C for 40 minutes, cooled, and one of each sample pair was clarified by addition of 1.5 ml of 20% $ZnCl_2$ and 1 ml of 3$\bar{N}$ KOH. Both samples were made up to the 100 ml mark with water and mixed. The unclarified sample was used for determination of refractometric dissolved solids. The clarified sample was filtered through filter paper followed by a 0.45μ membrane filter prior to betaine analysis.
>
> Betaine was determined by HPLC on a 30 cm HPX-87N cation exchange column at 85°C with 0.015 M Na_2SO_4 as eluent. Flowrate was 0.6 ml/min and injection volume was 10 μl. Three standards (0.25, 0.50, and 1.00 mg betaine/ml) were injected with each daily series of samples. Betaine levels in mg/ml were converted to both g/100 g beet and g/100 g RDS (using the measured RDS value).

B. Results

Betaine content values were stored in computer data files and mean values were calculated for each variety. Table 1 shows mean betaine levels (in both g/100 g beet and g/100 RDS) along with sugar, nitrate, and conductivity at both plots.

Mean betaine levels for varieties at a particular plot vary over a range of about 25% relative to the lowest values. This is a higher range of variation than that for sucrose (which varies

TABLE 1

Variety	Rupert					Grandview				
	Betaine g/100 g beet	Betaine g/100 RDS	Sugar g/100 g beet	Nitrate ppm/beet	Cond. (μS)	Betaine g/100 g beet	Betaine g/100 RDS	Sugar g/100 g beet	Nitrate ppm/beet	Cond. (μS)
A	0.154	0.837	16.0	569	859	0.218	1.197	15.1	898	860
B	0.156	0.866	15.8	529	822	0.208	1.140	14.8	980	881
C	0.142	0.778	15.9	530	877	0.211	1.135	15.2	1073	905
D	0.168	0.901	16.5	531	834	0.228	1.210	15.4	1039	868
E	0.157	0.870	15.6	553	864	0.222	1.186	14.9	1095	897
F	0.146	0.817	15.7	557	829	0.204	1.122	14.8	1037	910
G	0.179	0.956	16.3	555	878	0.246	1.280	15.4	1057	911
H	0.157	0.872	15.9	570	833	0.226	1.225	15.2	1123	955
I	0.154	0.844	15.8	522	852	0.211	1.186	14.7	833	850
J	0.146	0.811	15.7	546	920	0.225	1.199	15.3	1159	898
K	0.160	0.881	16.1	566	824	0.219	1.185	14.9	1150	891
L	0.163	0.902	16.0	520	810	0.234	1.225	15.1	1962	913
M	0.155	0.860	16.0	517	809	0.224	1.203	15.2	1127	919
N	0.149	0.826	15.9	520	887	0.222	1.214	15.4	1137	1002
O	0.145	0.807	15.7	499	807	0.198	1.073	15.2	1001	933
P	0.165	0.884	16.1	519	884	0.227	1.209	15.3	1128	887
Q	0.149	0.837	15.6	495	896	0.212	1.159	14.9	1089	911
R	0.150	0.830	15.7	559	867	0.218	1.173	14.9	1062	893
S	0.146	0.821	15.8	540	799	0.199	1.131	14.9	1794	903
T	0.161	0.892	16.0	560	808	0.235	1.268	15.1	894	926
U	0.147	0.814	15.7	522	876	0.210	1.143	14.7	1115	983
V	0.165	0.863	16.7	553	829	0.247	1.268	15.9	1103	804
W	0.143	0.791	15.6	495	882	0.214	1.176	14.8	670	858
X	0.150	0.846	15.6	560	896	0.232	1.272	15.0	1133	870
Y	0.153	0.830	16.1	505	784	0.209	1.125	15.2	1070	872

by only 7.1% relative to the lowest value) even though betaine levels are much lower (under 0.3%/beet). Analysis of variance on the original values shows differences between varieties to be significant at the 95% level for both plots.

The betaine data given in Table 1 is easier to visualize in the form of the bar graph shown in Figure 1. This shows mean betaine levels (in %/beet) for the 25 varieties at both plots. Note that some varieties (labelled G and V) are relatively high in betaine at both locations while others (labelled O and S) are low at both locations. In other words, betaine content seems to be definitely related to variety. This is also illustrated in the linear regression relationship of Rupert with Grandview betaine content (Figure 2). In this graph, and subsequent linear regression calculations, each point represents a sugarbeet variety. The fairly high correlation coefficient (0.7467) indicates that varieties higher in betaine at one location are likely to be high at the other location. A similar relationship is true for betaine based on RDS although the correlation coefficient is lower (0.6572). For comparison, correlation coefficients between plots are given in Table 2 for other measurements in addition to betaine. Note that conductivity and nitrate show no correlation of variety means between plots while betaine, sucrose, and dissolved solids correlate reasonably well.

Also note from Figure 1 that the most significant difference in betaine levels is not between varieties but between plots,

FIGURE 1

BETAINE VARIETY TEST
1990-91
RUPERT
GRANDVIEW
BETAINE (g/100g BEET)
0.26
0.21
0.16
0.10
0.05
0.00
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y

FIGURE 2

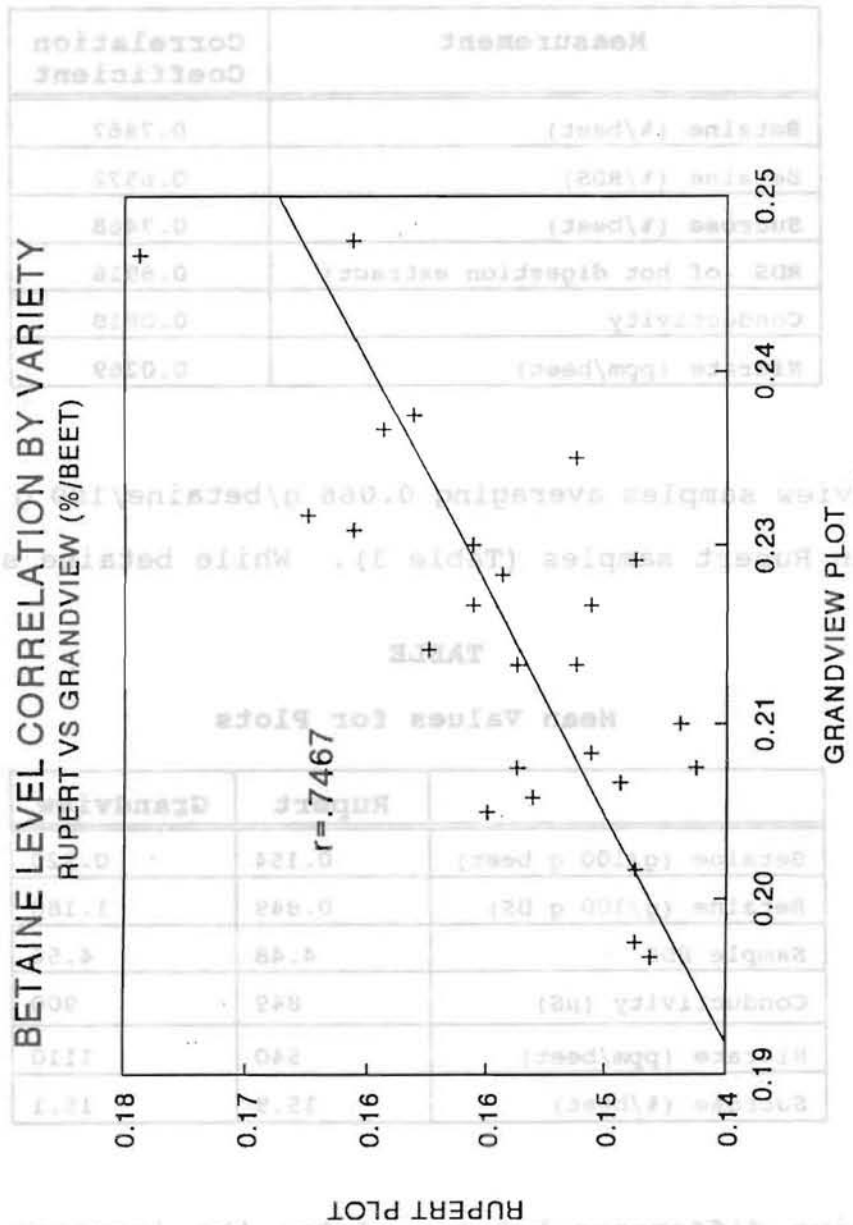

BETAINE LEVEL CORRELATION BY VARIETY
RUPERT VS GRANDVIEW (%/BEET)
r=.7467
RUPERT PLOT
GRANDVIEW PLOT
0.18
0.17
0.16
0.16
0.15
0.14
0.19
0.20
0.21
0.23
0.24
0.25

TABLE 2

Between-Plot Correlation Coefficients for Variety Mean Values

Measurement	Correlation Coefficient
Betaine (%/beet)	0.7467
Betaine (%/RDS)	0.6572
Sucrose (%/beet)	0.7468
RDS (of hot digestion extract)	0.6916
Conductivity	0.0818
Nitrate (ppm/beet)	0.0269

with Grandview samples averaging 0.066 g/betaine/100 g beet or 46% higher than Rupert samples (Table 3). While betaine shows a very

TABLE 3

Mean Values for Plots

	Rupert	Grandview
Betaine (g/100 g beet)	0.154	0.220
Betaine (g/100 g DS)	0.849	1.188
Sample RDS	4.48	4.56
Conductivity (μS)	849	900
Nitrate (ppm/beet)	540	1110
Sucrose (%/beet)	15.9	15.1

high relative difference between plots; the increase in sucrose from Grandview to Rupert is only 5.3%. Among the quality factors of nitrate level, conductivity, sucrose content, and sample solids content (which may indicate the moisture level in original roots) the only one with a between-plot difference approaching those of

betaine is nitrate. This seems to suggest that betaine levels in this test may be related to available nitrogen sources. Betaine levels in sugarbeets have been shown to be increased by stressful conditions, particularly increasing soil salinity[1,2] but the mean conductivity levels here do not seem to show a major difference in total dissolved salts in sugarbeet samples. The two plots used in this test were different in general characteristics. The Rupert plot consisted of lighter, sandy soil and was sprinkler-irrigated. The Grandview plot was more alkaline soil with higher sodium and organic levels. The furrow irrigation generally used in the area would also lead to a higher concentration of salts. Soil characteristics seem to fit well with the theory of increased betaine production in response to increased soil salinity.

The possibility that betaine levels are related to nitrate or conductivity levels was investigated further using within-plot correlation of variety mean values. Table 4 shows correlation coefficients of betaine levels (in g/100 g beet) with other measurements. Note that essentially no correlation exists between betaine level and either nitrate level or conductivity. This seems to contradict the mean values for plots (Table 3) which shows high nitrate going along with high betaine. Possibly within plots the variety means for nitrate and conductivity are too uncertain and low in range to give significant correlations with betaine

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[1] Hanson, A.D. and R. Wyse, *Plant Physiol.*, 70 1191 (1982)

[2] Paleg, L.G., T.J. Douglas, A. van Daal, and D. B. Keech, *Aust. J. Plant Physiol.*, 8, 107 (1981).

TABLE 4

Correlation of Betaine Level with Other Analyses

Correlation	Correlation Coefficient	
	Rupert	Grandview
Betaine (g/100 g beet) vs. sucrose (%/beet)	0.7422	0.6702
Betaine (g/100 g beet) vs. sample RDS	0.6904	0.7762
Betaine (g/100 g beet) vs. conductivity	0.1711	-0.1881
Betaine (g/100 g beet) vs. nitrate (ppm/beet)	0.2996	0.0579
Betaine (g/100 RDS) vs. sucrose (%/beet)	0.5680	0.4910

levels. Nitrate also does not correlate with sugar content (r=.188 and .265 for Rupert and Grandview respectively) or conductivity (r=.0141 and .204). Betaine levels (as a percent by beet weight) seem to correlate well with sucrose content or sample RDS (which are related to each other since sucrose is the major dissolved solid). Furthermore betaine content as a percent on solids also correlates with sucrose content although the coefficients are lower (Table 4). This means that as sucrose content by weight increases, increasing betaine concentration based on solids may actually lower sucrose purity.

III. CONCLUSIONS

Overall conclusions drawn from these tests are:

(1) Sugarbeet varieties show significant differences in betaine level and the differences are maintained under different

soil conditions. Unfortunately higher sucrose content correlates strongly with higher betaine level so within the varieties covered in this test there are none with higher sucrose and lower betaine.

(2) Betaine level is more strongly affected by growing conditions than variety differences. Between the Rupert and Grandview plots, mean betaine level shows a 46% increase. This difference may be related to soil salts and nitrogen level (nitrate shows a 106% increase), since betaine production in beets is thought to be related to stress conditions, particularly higher levels of salts. Within plots no relationship between betaine and non-sugar related values (nitrate and conductivity) was observed. This lack of correlation may have been due to low ranges of variation relative to experimental error.