False Polarization: Quantitation and Characterization in Sugarbeet Processing Juices[1]

G. W. MAAG and G. H. SISLER[2]

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Sugarbeets rank high among the important agricultural products in the United States and are grown in 23 of the 50 states. In recent years, sugarbeet companies have attempted to perfect a system by which the growers could be paid on recoverable sucrose rather than on sucrose content or tonnage of factory delivered beets.

Generally, sucrose values are based on polarizing saccharimeter readings made in factory tare laboratories on representative samplings of delivered sugarbeets (7)[3]. Sucrose measured by this means is frequently termed "sugar" or "polarimetric sugar." Estimated recoverable sucrose can be calculated from the polarimetric (pol) readings and a correction factor based on the assessed quality of the sugarbeets. The assessed quality is determined by the concentration of certain nonsucrose components that cannot be removed during factory juice purification. These materials prevent the optimum extraction of sucrose during crystallization (1, 2, 7, 8). Several classes of compounds make up the bulk of the nonremovable impurities. Nitrogen components, especially those containing *alpha* amino nitrogen, rank high among these deleterious compounds (1, 2, 8).

Pol sucrose readings are subject to inaccuracies caused by the presence, in varying amounts, of other optically active compounds, such as other saccharides, and amino acids, amides, and related nitrogen (N) compounds (3, 7). Although gas liquid chromatography (GLC) provides accurate, reliable values in sucrose analysis, it is not applicable to the tare laboratory, where utmost analytical speed is required on a large number of samples (6).

The purpose of this experiment was to compare pol and GLC sucrose readings in sugarbeet factory processing juices and to quantitatively assess the effect of some optically active nonsucrose components on the pol readings.

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[2]Research Chemist, Agricultural Research Service, U.S. Department of Agriculture, Colorado State University, Fort Collins, Colorado 80523, and Analytical Supervisor, Research and Development, The Great Western Sugar Company, P.O. Box 149, Loveland, Colorado 80537, respectively.

[3]Numbers in parentheses refer to literature cited.

Materials and Methods

Nine random samples of five factory processing juices were collected in late October 1972 at the Great Western Sugar Company factory at Greeley, Colorado. The five processing juices were: diffusion or raw juice, thin juice, thick juice, standard liquor, and molasses (7).

Frozen immediately after collection, the samples were transported later to the Crops Research Laboratory, Fort Collins, Colorado, where they were stored at −30° C until prepared for analysis.

Pol sucrose and refractometer dry substance (RDS) were determined on the samples at the Great Western Research Laboratory, Loveland, Colorado. The nonsucrose dry substance was calculated by subtraction of the pol sucrose from the corresponding RDS reading for each sample. GLC sucrose, glucose, and amino acids, amides, and related N compounds were determined at the Crops Research Laboratory, at Fort Collins.

The thick juice, standard liquor, and molasses samples were brought to room temperature and diluted gravimetrically with glass distilled water to adjust the sucrose content to about 10-12% by weight. The approximate dilution required was ascertained from the pol sucrose and specific gravity (from RDS) readings made on the original samples. The diffusion and thin juice samples were prepared with no dilution.

GLC sucrose samples were prepared by weighing, in a tared small glass vial, about 130 mg of D(+) trehalose dihydrate (internal standard). About 0.9 ml of the sample (containing about 100 mg sucrose) was added and weighed. Each weight was recorded to the nearest 0.00001 g. After solution was complete, 0.2 ml of the sample-trehalose mixture was placed in a 2-ml Hewlett Packard[4] sampler vial and thoroughly dried by addition of about 1 ml of acetone to each vial. With a small jet of air topping each vial to aid evaporation and prevent bumping, the samples were heated to 40° C. Two or three additions of acetone were usually required to rid the samples of all moisture via the volatile acetone-water azeotrope. The silylating agent, N-trimethylimidazole (TMSI), was mixed with a drying agent, dimethylformamide (DMF), in the ratio 2:1 (v/v), and 1.5 ml was added to each dried sample vial. The vials were capped and placed in an oven at 60° C for 1 hour or until silylation was complete.

Duplicate GLC sucrose determinations were made on a dual column Hewlett Packard gas chromatograph, Model No. 5712A[4], fitted with a thermal conductivity detector, an automatic sampler, an integrator, and a recorder. Helium gas was used as the carrier gas. The ⅛ inch stainless steel columns were packed with 10% OV 17 (phenylmethyl-silicone) liquid phase on 80-100 mesh Chromosorb W-AW-DMCS (acid washed and treated with dimethyldichlorosilane).

[4]Mention of a trademark or proprietary product 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 that may also be suitable.

Analyses were made at the following conditions:

Column temperature: 265° C
Injection port temperature: 250° C
Detector temperature: 300° C
Integrator sensitivity setting: position 3
Detector sensitivity setting: position 6
Helium flow rate: 30 ml/min
Sample size: 1-1.5 μl
Analysis and integration time: 12 min

Standard sucrose-trehalose readings were made after each set of four dupicate samples, and GLC sucrose percentage was calculated by weight on original beet.

Glucose was determined colorimetrically on duplicate samples by the glucose oxidase method (4), after clarification of the samples with lead acetate. Glucose was not determined on diffusion juice, because the lead acetate did not completely remove interfering substances.

Twenty amino acids, two amides, and ammonia were measured quantitatively on duplicate samples, before and after basic hydrolysis, by a Technicon Amino Acid Analyzer[4] (5). During basic hydrolysis, the amides, glutamine (GLN), and asparagine (ASN), along with any pyrrolidone carboxylic acid (PCA) present, were converted to their respective amino acids, GLN and PCA to glutamic acid (GLU), and the ASN to aspartic acid (ASP).

Before basic hydrolysis, the serine (SER), GLN, and ASN were eluted on the amino acid chromatogram as occluded peaks. The resulting occluded peak was measured quantitatively with SER as the standard. After hydrolysis, the SER peak was only SER, because the GLN had been converted to GLU, and ASN had been converted to ASP. Any PCA that had been present before the basic hydrolysis was hydrolyzed to GLU. Therefore, the GLU peak, after hydrolysis, resulted from the original GLU, plus the original GLN and PCA.

The amino acids, amides, PCA, ammonia, and glucose were calculated to milligrams per 100 pol sucrose.

Results and Discussion

In each factory processing juice, the pol sucrose was higher than the GLC sucrose (Table 1), but only the differences for standard liquor and molasses were significant, 1% and 5%, respectively. The pol readings were higher, possibly because of the effect of other optically active compounds in the juices. Sucrose is dextrorotary, $[\alpha]_D^{20} = +66.5°$, as are glucose, $[\alpha]_D^{20} = +52.7°$, raffinose $[\alpha]_D^{20} = +104.5°$, and a few other sugars that may be present (7). Some other juice components, such as amino acids, amides, and PCA, are either dextrorotary or levorotary, as shown in Table 2. From comparison of the pol and GLC sucrose readings, it appears that there was a greater effect from the dextrorotary than from the levorotary compounds on the pol readings. Glucose is mostly destroyed during carbonation. The other invert sugar,

Table 1.—Polarimetric and GLC sucrose, RDS (total solids), and nonsucrose solids means (9 samples) for five factory processing juices.

Component	Diffusion J.	Thin J.	Thick J.	Standard liquor	Molasses
% Sucrose					
Pol	12.72	11.56	59.68	64.14**	52.51*
GLC	12.27	11.10	58.63	61.49**	49.76*
Solids					
RDS (total)	14.58	12.59	64.98	67.99	80.08
Nonsucrose	1.86	1.03	5.30	3.85	27.57

**, * = significant difference, 1% and 5% levels, respectively.

Table 2.—Five factory processing juice means (9 samples) for amino acids, amides, PCA, ammonia, and glucose in milligrams per 100 pol sucrose, and the specific rotation of each component.

Component*	Specific rotation	Diffusion J.	Thin J.	Thick J.	Standard liquor	Molasses
1. ASP	+ 4.7°	158	140	179	146	861
2. ASN	− 9.3	112	67	101	37	165
3. THR	−28.3	12	10	10	6	10
4. SER	− 6.8	96	87	115	74	557
5. HOMOSER	−27.0	4	3	4	3	35
6. GLU	+31.4	70	205	284	188	1,108
7. GLN	+ 6.1	383	150	85	36	114
8. PCA	−11.9	816	920	1,319	918	3,880
9. PRO	−85.0	16	29	22	18	169
10. GLY	None	7	16	21	19	154
11. ALA	+ 8.5	72	68	90	65	492
12. VAL	+22.9	39	36	48	36	312
13. MET	− 8.2	16	9	17	12	63
14. ILE	+11.3	71	66	88	64	482
15. LEU	+15.1	72	64	87	62	460
16. TYR	−10.6	52	127	192	116	651
17. PHE	−35.1	8	7	10	8	81
18. GABA	None	304	204	254	174	1,031
19. ORN	−11.5	3	1	1	1	3
20. LYS	+14.6	10	8	11	8	60
21. HIS	39.7	13	6	8	4	5
22. TRP	−31.5	24	14	28	21	150
23. ARG	+12.5	14	7	8	5	32
24. NH_3	None	14	65	4	15	11
25. GLUCOSE	+52.7	Not det'm	227	150	98	250

*1. aspartic, 2. asparagine, 3. threonine, 4. serine, 5. homoserine, 6. glutamic, 7. glutamine, 8. pyrrolidone carboxylic acid, 9. proline, 10. glycine, 11. alanine, 12. valine, 13. methionine, 14. isoleucine, 15. leucine, 16. tyrosine, 17. phenylalanine, 18. *gamma* aminobutyric acid, 19. ornithine, 20. lysine, 21. histidine, 22. tryptophan, 23. arginine, 24. ammonia.

fructose, $[\alpha]_D^{20} = -92.3°$, which is more unstable than glucose under high temperature and alkalinity, is essentially eliminated during processing. Raffinose is quite stable and is carried through the manufacturing process and concentrates in the molasses. This saccharide accumulates in sugarbeets that have been stockpiled at low temperatures for long periods. Raffinose is especially troublesome in sugar manufacture because of its adverse effect on sucrose crystallization

(7). Because these samples were collected in late October, the raffinose content probably was not as high as in beets processed later in the campaign.

Table 2 lists the amino acids, amides, PCA, ammonia, and glucose in milligrams per 100 pol sucrose and the specific rotation of each component. GLY, GABA, and ammonia contain no asymmetric carbon and, therefore, are not optically active.

Glutamic acid, GABA, aspartic acid, serine, and tyrosine were the amino acids present in the largest quantities in all juices. The two amides, glutamine and asparagine, also ranked high in relative amount, but each decreased as the juices progressed through the factory processing. Asparagine converted to its respective amino acid, aspartic. Glutamine was partially converted to glutamic acid, but mainly to PCA, thus causing the PCA to increase in quantity during processing. When the components were calculated to milligrams per 100 pol sucrose (Table 2), the relative quantities appeared to be very high in molasses. This larger amount was caused partly by concentration after water evaporated during processing, but mainly by the extraction of sucrose via crystallization and, therefore, a parallel increase in the relative concentration of the nonsucrose constituents.

Glucose decreased in the thick juice and standard liquor, but increased in molasses (Table 2). This increase was caused by relative concentration of nonsucrose constituents after sucrose crystallization, as above, as well as by the continued slow rate of sucrose inversion during the factory processing (7).

By definition, specific rotation, $[\alpha]_D^{20}$, of a substance is the rotation, expressed in degrees, that is afforded by 1 gram of that substance dissolved in 1 ml of water (20° C) in a tube 1 dm long. Table 3 gives a comparative estimate, expressed in whole numbers, of the algebraic sum of the dextrorotary and the levorotory effect of the optically active measured amino acids, amides, and PCA in each processing juice. These values are based on the optical activity times the grams per unit volume of each when calculated to 100 pol sucrose. Likewise, the estimated dextrorotary effect of glucose for each juice is given. Specific rotation was assumed to be the same as it is for the individual component in a pure component-water solution.

Table 3.—Estimated specific rotation effect of the optically active amino acids, amides, PCA, and glucose measured in the factory processing juices.

Component-rotation	Estimated specific rotation				
	Diffusion	Thin	Thick	Standard liquor	Molasses
Amino acids, etc.					
Levorotary	−15	−17	−23	−16	−82
Dextrorotary	+ 9	+11	+15	+10	+64
Glucose					
Dextrorotary	Not determined	+12	+ 8	+ 5	+15
Net effect	?	+ 6	0	− 1	− 3

The amino acids, amides, and PCA in each processing juice result in a levorotary effect, mainly because PCA, formed in large amounts during processing, is levorotary. However, the glucose, which is dextrorotary, overcomes the levorotary effect, except in the standard liquor and molasses where the excess levorotation was minimal. Because glucose was not determined in the diffusion juice, the net effect could not be calculated. The net effect of the measured optically active compounds did not completely account for the disparity in polarization and GLC values, particularly in standard liquor and molasses. By accounting for the effect of raffinose, most of the disparity could probably be explained (7). Other compounds that might be expected to contribute to false pol sucrose values would be galactinol, $[\alpha]_D^{20} = +130.6°$, and the several kestoses with an average specific rotation, $[\alpha]_D^{20} = +25°$. Further study of the optically active compounds in the processing juices, especially in the standard liquor and molasses, is needed for the complete reason for the higher polarimetric readings.

Summary

In nine samples of five sugarbeet factory processing juices, the polarimetric sucrose readings consistently were higher than the corresponding GLC readings, but the differences were significant only in standard liquor and molasses samples. Glucose decreased quantitatively during processing, but its dextrorotary effect overcame most of the levorotary effect of the 20 measured amino acids, two amides, and the hydrolyzed product of glutamine, pyrrolidone carboxylic acid, some of which have dextro and some levo specific rotation. Results indicate that other dextrorotary compounds in the samples, besides those measured, cause the significantly higher pol sucrose readings in the standard liquor and molasses.

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