

Some Details of the Hot Water Digestion Method For the Determination of Sugar in Cossettes

S. J. OSBORN¹

Credit is due to Pellet. (13)² for championing the method of hot water digestion which is usually given his name and which is the generally accepted method in all countries for determining the percentage of sugar in cossettes. The purpose of this paper is to present, in summarized form, a description of some of the details which have been found to be important.

Preparation of Sample.—Cossette samples are usually ground in some kind of meat chopper. This allows a large sample to be ground rapidly to a fineness sufficient to secure a well mixed sample suitable for analysis by hot water digestion.

Concentration.—The use of half-normal (26 grams of ground beets per 200 ml. of solution) concentration is general. The use of full normal concentration is not advisable because the proportion of pulp to liquid is so great that there is risk of incomplete diffusion. With the use of half-normal concentration the reading of the filtrate in a 400-mm. tube gives directly the percentage of sugar.

Temperature of Digestion. Variations of temperature seem to be unimportant, at least within reasonable limits. A bath temperature of 80° C, which insures complete diffusion after 30 minutes immersion of the digestion flask, is generally standard. There is nothing gained by using any higher temperature.

Supplementary Digestion.—In the digestion as first practiced the flask was filled about two-thirds full, and the volume was completed after cooling. "Weisberg (16) pointed out that under this condition results were too low on account of incomplete equalization of concentration in the surrounding liquid with that of the liquid in the cells of the pulp. This error was found to increase, as would be expected, with increased coarseness of pulp.

It is advantageous to carry out the principal digestion with an incompletely filled flask, both to allow⁷ air bubbles to escape readily and to permit the contents to be mixed by swirling at intervals during the digestion. Weisberg accordingly prescribed a supplementary digestion, now generally standard, which involves completing the volume approximately to the mark with hot water and then digesting for

¹General Chemist, Great Western Sugar Company, Denver, Colo.

²Italic numbers in parentheses refer to literature cited.

10 minutes longer. After cooling, the necessary small amount of Writer is added to complete the final exact volume.

Evacuation to Remove Air.—Not always recognized is the error caused by the presence in the ground pulp of air which may not be completely removed during digestion. This causes high results because the true volume of liquid is less than 200 ml. if air is present.

Any one who has tried to run a hot water digestion on finely rasped pulp produced by a Keil disc knows that results are as much as 0.5 percent high if the air occluded in large amounts in the pulp is not removed.

Although the amount of occluded air in cossettes which have been reduced to a pulp in a meat chopper is much less than in rasped pulp, it is sufficient to produce a definite error in the Pellet digestion method, at least, under some conditions. We have found differences, with and without the removal of air, varying from .03 when the flask was shaken four times during the digestion up to .12 (in one case .15) when the flask was not shaken at all.

The air bubbles can largely be removed by evacuation and the best time to do this is after the normal weight of cossettes has been rinsed into the flask with water, with the use of about 100 ml., so that the flask will be only half full. This allows room for the bubbles to break without loss of liquid.

Another great advantage of the removal of the air is that the particles of pulp tend to sink instead of float, and at the end of the digestion the solution is so free from foam that it can usually be made up to the mark accurately without the use of ether to break the foam.

Because the removal of air is in every way advantageous, it should be a part of the standard hot water digestion method.

Volume Allowance for Marc.—In the volumetric method it is customary to use a flask graduated at a volume which will exceed 200 ml. by the amount considered to represent the proper allowance for the marc and lead precipitate. Investigators of various countries have reported a great amount of work on the subject (3) which is too extensive to permit any review in this paper. It is important that the volume of the lead precipitate, at one time neglected, be taken into consideration as well as the volume of the marc.

Although there is no complete agreement, on a standard volume allowance for marc and lead precipitate, and possibly there should not be because of variation of beets in different localities, it is now generally believed that the old figure of 0.6 ml. (for the volume occupied by the marc and lead precipitate from 26 grams of beets) is too low.

The preponderance of opinion now favors a value of 1.0 ml., but some believe it to be 1.5 ml., or a little more.

As our own results (11, 12) have indicated a value of 1.0 ml., we graduate the cossette flask at 201.0 ml.

Adsorptive Effects of Marc.—One other feature in connection with mare needs consideration—its possible influence on polarization caused by its adsorptive power. Some investigators (3) claim that marc adsorbs water, the so-called "colloid water" or "water of imbibition," and that results are therefore too high on account of the increased volume of "marc hydrate" and the decreased volume of solution.

On the other hand Mintz et al. (10) and Martraire (9) claim that marc adsorbs sugar and thereby causes low results.

Obviously we cannot say that results are high on account of adsorbed water and that, they are at the same time low on account of adsorbed sugar. We have to make some choice or, at any rate if both effects exist, the important point is which one predominates.

Some of our own work, which is submitted with no finality, confirms in at least some degree the effect found by Martraire. We repeated his procedure, consisting of a series of cossette digestions to each of which a weighed amount of sugar was added (approximately enough to double the polarization), and a parallel series made without the addition of sugar, all tests being made in duplicate. The difference in polarization was compared to that calculated as equivalent to the added sugar (table 1).

Our results, in agreement with those of Martraire (9) and also of Bonnet (2), show no determinable adsorptive effect for finely divided, rasped pulp, but with pulp ground in a meat chopper there was a distinct failure to "recover" the added sugar by .09 percent on beets in one series and .05 percent in another. This indicates that with coarsely ground pulp an adsorptive effect exists, but no one seems to be able to figure out exactly to what extent the polarization of the original cossettes is affected.

Martraire says that meat choppers, which give a coarse pulp, should not be used for the analysis of beets. However, the writer knows of no machine which will satisfactorily reduce a cossette sample of several pounds, a desirable size, to fine pulp in a reasonable length of time.

This question can probably be further studied to advantage.

Use of Acetic Acid.—The addition of one or several drops of acetic acid just before the solution is polarized is recommended in

Table 1.—Polarization effect of sugar added to hot water digestions, campaign 1939-37.

Reported by	Kind of pulp	Percentage sugar by hot digestion	g Sugar added	Pol. of digestion with added sugar		Loss of polarization attributed to adsorption
				Calcd.	Actual	
Bair	Rasped	15.22	3.9	30.22	30.20	.02
Bair	Rasped	14.32	3.9	29.32	29.30	.02
Bair	Rasped	17.40	3.9	32.40	32.40	.00
					Avg.	.013
Hartman	Rasped	19.06	4.0	34.38	34.35	.03
Hartman	Rasped	17.05	4.0	32.43	32.45	-.02
					Avg.	.005
Bair	Meat chopper	15.25	3.9	31.25	31.15	.10
Bair	Meat chopper	15.55	3.9	30.55	30.50	.05
Bair	Meat chopper	15.75	3.9	30.75	30.70	.05
Bair	Meat chopper	10.45	3.9	31.45	31.40	.05
Bair	Meat chopper	13.55	3.9	30.55	30.55	.00
Bair	Meat chopper	10.00	3.9	31.00	30.85	.15
Bair	Meat chopper	15.95	3.9	30.95	30.88	.07
Bair	Meat chopper	11.00	3.9	29.90	29.75	.15
Bair	Meat chopper	15.15	3.9	30.15	29.95	.20
					Avg.	.09
Hartman	Meat chopper	15.45	4.5	32.74	32.60	.14
Hartman	Meat chopper	16.25	4.7	33.33	33.30	.03
Hartman	Meat chopper	13.55	4.1	31.32	31.35	-.03
Hartman	Meat chopper	14.28	4.5	33.39	33.40	.01
Hartman	Meat chopper	16.37	4.5	33.34	33.32	.02
Hartman	Meat chopper	15.30	4.6	32.99	32.80	.19
Hartman	Meat chopper	15.45	4.02	30.91	30.75	.16
Hartman	Meat chopper	14.45	4.2	30.60	30.78	-.18
Hartman	Meat chopper	16.12	4.5	32.43	32.55	-.12
					Avg.	.05

some methods. The purpose evidently is either to prevent the formation of lead fructosate (4) or to correct the effect of lead subacetate on the polarizing power of asparagine (5).

We have made a number of tests with and without the addition of various amounts of acetic acid without finding any difference in polarization. We have therefore not included the use of acetic acid in our method.

From the standpoint of pH it may be noted that lead subacetate is not so alkaline as is commonly supposed. The standard solution, of density 55° Brix, is a 6.6 pH determined electrometrically. (Colorimetric determination shows a wide difference, viz., 7.5 pH.) We

have found "digestion juice" in the absence of lead subacetate to be within the range of about 6.0-6.8 pH and to be *but little* affected in pH by the addition of lead subacetate. The use of a little acetic acid could hardly be expected to make much change in pH.

Destructible Polarization.—Stanek and Vondrak (14) have made an interesting contribution to cossette analysis in their method for determining "destructible polarization." They measure the change (usually a decrease) in polarization produced by heating digestion juice made alkaline with sodium hydroxide, this being considered to correspond to the change in the polarization of non-sugars produced by the action of lime in the factory earbonation process. The procedure is somewhat involved and is more suited to special investigations rather than as a routine method in the determination of sugar in cossettes.

We have made many determinations of destructible polarization at various times and on various conditions of beets. These have quite uniformly shown values of 0.1 to 0.2, beets of the best quality having shown the former value, or sometimes slightly less. We have not been successful in explaining high unaccountable losses by the presence of high destructible polarization in beets. In these respects our results have confirmed those of Stanek and Vondrak.

Addition of Lead Subacetate Before vs. After Digestion.—When the method of hot water digestion was first developed, the basic lead acetate reagent was added after digesting and cooling. When it was found that the reagent could be added before digestion without apparently affecting the results, this procedure then became customary because it was thought that, by precipitating impurities at the start, the extraction of the sugar and the clarification of the solution were facilitated.

During a campaign when the beets were abnormal and unaccountable losses were unusually high, Le Docte (8) reinvestigated this question. He found the results by hot water digestion, when the lead acetate was added *after* digestion, agreed with those obtained by cold water digestion, but, if the lead was added *before* digestion as was then customary, the hot water results might, in case of thawed and unsound beets, be much higher. This he attributed to the invert sugar present, on the ground that the levulose component would be rapidly attacked during digestion by the PbO of the basic lead acetate, while the dextrose would be unaffected, thus giving too high a polarization.

Le Docte's theory appeared plausible as far as it went, and we have at various times investigated the same question. Our work has involved on the one hand the behavior of cossette digestions to which

small amounts of invert sugar had been added, and on the other hand comparative determinations on cossette samples.

The results given in table 2 are typical. The amount of added invert sugar corresponds to the maximum amount that we have found in factory cossette samples—about 4 percent on dry substance..

These results offer strong confirmation of the Le Docte theory. It will be noted that the amount of added invert sugar is not enough to affect the polarization to a marked degree when the lead is added after digestion (compare 1 b and 2 b of table 2), but digestion in the presence of basic lead acetate of the solutions containing the added invert sugar elevates the polarization to the extent of about $+30^\circ$. This happens to be just what the increase of polarization (rotation of 0.1 gram of dextrose) would theoretically be if the levulose were destroyed by digestion in the presence of basic lead acetate and the dextrose component of the invert sugar were left unaltered.

Invert sugare (both the dextrose and levulose) is completely destroyed by heating with strong alkalies. With milder alkali treatment levulose is more readily attacked than dextrose. Digestion with basic lead might therefore be expected to have a preferential destructive action on levulose. However, in view of the large effects found with little change in pH, it seems probable that some specific action of lead subacetate on the levorotation of levulose is involved. Lobry de Bruyn and van Ekenstein (6) and Schneller (6) are reported to have observed, such an effect. Stanek and Vondrak (15) found that heating of digestion juice containing added invert sugar in the pres-

Table 2.—Effect of added invert sugar in cossette digestions.

	Brighton		
	Early in campn.	Jan- 8 1931	Jan. 15 1931
Campaign 1930-31			
1. Polarization determined:			
(a) Adding load before digestion	15.30	15.62	15.55
(b) Adding load after digestion	15.40	15.65	15.59
2. Same as 1, adding 0.2 gram invert sugar (in form of the calculated amount of the prepared solution) to 26 g cossettes in a 201 ml. flask, before digestion and lead addition. Polarizations:			
(a) Adding lead before digestion	15.60	16.00	15.85
(b) Adding lead after digestion	15.35	15.60	15.60
Campaign 1931-32			
	Billings	Lovell	Lovell
3. (a) Same as 1 (a) above	16.62	19.13	18.30
(b) Same as 1 (b) above	16.68		
4. (a) Same as 2 (a) above	16.92	19.30	18.52
(b) Same as 2 (b) above	16.68	19.03	18.22

ence of lead subacetate caused a plus polarization with little change in the copper-reducing power.

We have made many hundred determinations on factory cossettes comparing the addition of lead before and after digestion. With normal beets there is no determinable difference. With deteriorated beets there is often a difference of as much as 0.2 percent sugar, the polarization being lower when the lead is added after digestion. On individually selected rotten beets still greater differences have been obtained. There is evidence that the difference tends to increase with progress of the campaign as deterioration of stored beets takes place.

Fortunately then the point is not of importance for normal beets on which most determinations of sugar are made, and in such cases the lead may be added either before or after digestion. However, since there is evidence that the presence of lead during digestion causes a false plus polarization in deteriorated beets containing invert sugar, it is the opinion of the writer that the results under such conditions are more accurate when the lead is added after digestion, and this procedure has been incorporated in our standard method of analysis.

There is one other point in this connection that may deserve consideration: Whether any destruction of sucrose by inversion may take place under the conditions of the hot water digestion. Since it has been pointed out that the pH of the digestion juice is little affected by the addition of lead subacetate, the question is almost equally pertinent whether the lead is added before or after digestion. At 6.0 pH, which according to our experience is probably the usual Lower limit of a cossette digestion, the destruction of sucrose in 40 minutes at 80° should not exceed .01 percent on beets. It is still possible that the effect might exist in measurable amount in the case of badly deteriorated beets.

If the point is important, it can readily be taken account of by adding 0.1-0.2 gram of calcium carbonate to the digestion before heating. Enough of this will go into solution to raise the pH almost to 7 or slightly above, but the amount undissolved will not noticeably affect the volume when the solution is made to the mark. We have made many tests with and without the use of calcium carbonate, but, since we have never found any difference in results, we have not incorporated its use into our method.

Bachler, who omitted the lead addition in order to obtain an unclarified digestion juice for the determination of refractometer dry substance, has said (1) "From many hundreds of control tests we

know that no appreciable destruction of sucrose occurs during the digestion without lead acetate."

It also occurred to the writer to investigate whether the addition of lead subacetate after digestion causes any difference in the volume of marc and lead precipitate, all tests previously reported having been made with the addition of lead before digestion (11, 12). Determinations by the Brown method (11) are reported in table 3. The hot digestions show a slightly lower average value when the lead is added after digestion, although the difference is small and hardly significant. The results incidentally confirm the value of 1.0 ml.

Table 3.-----Determinations of the volume of marc and lead precipitate, campaign 1933-34.

Test No.	Percentage sugar	Lead addition	Material	Volume of ppt. in milliliters calculated to 28 grams
1	15.3	Before	Cosettes, hot digestion	.96
2	14.9	Before	Cosettes, hot digestion	.94
3	17.9	Before	Cosettes, hot digestion	.98
4 a	16.0	Before	Cosettes, hot digestion	.96
4 b	10.0	After	Cosettes, hot digestion	.90
5 a	14.8	Before	Cosettes, hot digestion	.92
5 b	14.8	After	Cosettes, hot digestion	.90
6 a	15.4	After	Cosettes, hot digestion	.90
6 b	15.4	After	Cosettes, hot digestion	.94
6 c	15.4	After	Cosettes, hot digestion	.92
		Average, lead before digestion		.95
		Average, lead after digestion		.91
7	18.1	Before	Rasped pulp, cold digestion	.88
8	15.8	Before	Rasped pulp, cold digestion	.78
9 a	17.0	Before	Rasped pulp, cold digestion	.78
9 b	17.0	Before	Rasped pulp, cold digestion	.70
10 a	17.5	Before	Rasped pulp, cold digestion	.80
10 b	17.5	Before	Rasped pulp, cold digestion	.82
11 a	16.8	Before	Rasped pulp, cold digestion	.74
11 b	16.8	Before	Rasped pulp, cold digestion	.62
12	16.4	Before	Rasped pulp, cold digestion	.74
		Average		.77
13	17.5	After	Rasped pulp, hot digestion	.98
14 a	17.0	After	Rasped pulp, hot digestion	.92
14 b	17.0	After	Rasped pulp, hot digestion	.90
15	18.1	Before	Rasped pulp, hot digestion	.92
16	15.8	Before	Rasped pulp, hot digestion	.88
17 a	16.4	Before	Rasped pulp, hot digestion	1.04
17 b	16.4	Before	Rasped pulp, hot digestion	1.07
		Average, lead before digestion		.98
		Average, lead after digestion		.93

previously established (11, 12). It is of interest that the determinations by cold water digestion are distinctly lower; this may deserve consideration in the cold water method, with which this paper is not concerned.

Hot Water Digestion Method of the Great Western Sugar Company.—The detailed procedure of the Great Western Sugar Company is on record elsewhere (7).

Summary

Various details of the hot water digestion method for the determination of sugar in cossettes have been discussed. The supplementary digestion to equalize concentrations, evacuation to remove air, and addition of the lead subacetate after digestion are recommended as standard procedure. Some questions are not finally resolved.

Acknowledgment

For numerous investigations, the results of which are described in this paper largely in summarized form, the writer is indebted to many chemists of the Great Western Sugar Company, particularly to C. B. Milieu for his investigations on the effect of invert sugar, to G. E. Stevens for establishing the effect of removing air by evacuation, to J. A. Bair for the marc determinations reported in this paper, and to J. A. Bair and V. V. Hartman for their work on the polarization effect of sugar added to hot water digestions.

Literature Cited

1. Bachler, F. R. The Sachs-Le Docte Method. Its Application to the Determination of Sugar Beets under Conditions in Southern California. *Facts About Sugar* 29 : 194. 1934.
2. Bonnet, J. Do Cossettes Retain Sugar? *Facts About Sugar* 33, no. 4: 36. (1938) from *Bull. Inst. Agron. Gembloux* 6 : 196. 1937.
3. Browne, C. A. and Zerban, F. W. *Physical and Chemical Methods of Sugar Analysis* (John Wiley & Sons, Inc.) 3d ed. 360-3.
4. Browne, C. A. and Zerban, F. W. Chapter IX: Methods of Simple Polarization. *Physical and Chemical Methods of Sugar Analysis* (John Wiley & Sons, Inc.) 3d ed. 323.
5. Browne, C. A. and Zerban, F. W. Chapter IX: Methods of Simple Polarization. *Physical and Chemical Methods of Sugar Analysis* (John Wiley & Sons, Inc.) 3d ed. 372.
6. Brown, C. A. and Zerban, F. W. Chapter XI : Special Methods of Saccharimetry. *Physical and Chemical Methods of Sugar Analysis* (John Wiley & Sons, Inc.) 3d ed. 493.

7. Browne, C. A. and Zerban, F. W. Chapter IX: Methods of Simple Polarization. Physical and Chemical Methods of Sugar Analysis (John Wiley & Sons, Inc.) 3d ed. 363.
8. Le Docte, A. Influence du sous-acetate de plomb sur la polarisation dans la digestion aqueuse a chaud. *Sucr. Beige* 40: 130. Influence de la composition et de la quantite du reactif clarifiant. *Ibid.*, 40:221. Notes pratiques de Chimie analytique sucriere. *Ibid.*, 40:252, 274. 1912.
9. Martraire, M. Sucrose, Beet Pulp, and Adsorption. *Bull. Assoc. Chim. Sucr. Dist.* 52:775. 1935. Determination of Sugar in the Beet. *Ibid.*, 53 :609, 617. 1936. The Adsorptive Power of Sugar Beet Marc. *Ibid.*, 55 :441. 1938.
10. Mintz, I. B., Kartashov, A. K., and Trofimovskii, N. S. The Volume of Marc of Normal Weight of Beets. *Nauk. Zapiski Tzukrovoi Prom.* 14:483. 1931.
11. Osborn, S. J. and Brown, R. J. The Marc Volume of Sugar Beets. *Facts About Sugar* 27:434. 1932.
12. Osborn, S. J. Volume of Sugar Beet Marc and Its Adsorptive Effect. *Ind. Erig. Chem., Anal. Ed.* 6:37. 1934.
13. Pellet, H. Numerous papers in *Bull. Assoc. Chim. Sucr. Dist.*
14. Stanek, V. and Vondrak, J. Eine schnelle Methode zur Bestimmung der zerstorbaren Polarisation bei Rubenanalysen. *Z. Zuckerind. Cechoclovak Rep.* 50:257. 1926.
15. Stanek, V. and Vondrak, J. Ube den durch die Anwesenheit von Invertzucker bei der Bestimmung der Saccharose in der Rube nach der wasserigen Digestion verursachten Fehler. *Ibid.*, 51:220. 1927.
16. Weisberg, J. Sur une cause d'erreur dans la dosage du sucre de la betterave par la digestion aqueuse a chaud. La digestion rationnelle. *Bull. Assoc. Chim. Sucr. Dist.* 25 :600. 1907.