Determination of Saponins in Refined Beet Sugars

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The flocculent precipitate which sometimes forms in acidified sirups made from either beet or cane sugars has created a problem for both the bottling and the refined-sugar-producing industries. The floc from beet sugar is composed of sugar-beet saponins, fat, colloidal coloring matter, and inorganic materials (1, 4)[2]. The acid-insoluble beet saponins appear to act primarily as scavenging agents, adsorbing other colloidal matter in the sirups. In this laboratory, the nature of the cane floc has not been investigated, other than to ascertain that it does not contain saponin, so that the work to be described applies only to beet sugars.

At present there is no satisfactory small-scale, rapid method for predicting the floc performance of a refined beet sugar. A gravimetric floc determination reported by Eis, Clark, McGinnis, and Alston (1) requires about five pounds of sugar and a minimum analysis time of about six to eight hours. A qualitative analysis for total floc is frequently used for control work: heated, acidified sirups are scored visually for floc content after standing for varying periods of time, usually 24 hours (2).

This paper reports the development of a method suitable for control work which is capable of estimating the few parts per million of saponins in refined beet sugars. The analysis measures the saponins and not total floc, because, in our experience, no additional floc is produced in sugar sirups from which saponin has been removed. The saponins are removed from an acidified sirup by filtration and are determined colorimetrically in glacial acetic acid with a reagent composed of 10 percent antimony pentachloride in chloroform. The elapsed time for an analysis is about two hours.

Reagents.

Chloroform: Reagent grade.

Glacial acetic acid: Reagent grade.

Antimony pentachloride: Reagent grade, as purchased, usually contains too much free chlorine to produce color with saponin (2). Vacuum distillation in an all-glass system gives a purified product satisfactory for color development. A suitable reagent can also be made by adding up to 10 mole percent of $SbCl_3$ or by blowing dry air through commercial antimony pentachloride (2).

A 10 percent (V/V) solution of $SbCl_5$ in chloroform is prepared. The reagent should be handled in a fume hood.

Standard Sugar Beet Saponin. This reference material can be isolated from factory juices or lime muds (1, 4). A solution containing 0.04 mg. of standard per ml. of glacial acetic acid makes a suitable colorimetric standard and appears to keep well in a glass-stoppered flask.

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[2] Numbers in parentheses refer to literature cited.

Procedure. Dissolve 12.5 gm. of refined beet sugar in 250 ml. of water and add 2.5 ml. of concentrated HCl. Add 3.75 gm. of filter aid to the solution and filter with suction through a small Buchner funnel (6 cm.) using Whatman[a] No. 5 paper precoated with 1.25 gm. of Celite analytical filter-aid. Wash the sides of the funnel and the filtered material portion-wise with acidified wash water until the wash contains no more sucrose. Dry the funnel and contents in an oven for 20 minutes at 105°. Elute saponin from filter-aid with about 8 ml. of hot glacial acetic acid, collecting the eluate in a 10-ml. beaker. If necessary the acetic acid solution can be refiltered with suction through a Whatman No. 50 paper on a small funnel, collecting the clear filtrate in a graduated tube or a 10-ml. volumetric flask. After making to definite volume (as necessary) with acetic acid, pipette a 1-ml. aliquot into a colorimeter tube (19 x 105 mm.) and add exactly 7 ml. of antimony pentachloride reagent. Shake tube to mix, stopper tightly, and allow to stand at room temperature. After 10 minutes, read the absorbance at 535 $m\mu$ on a colorimeter. Calculate the mg. of saponins in the unknown sample from the absorbance of the known standard saponin reagent and a reagent blank determined at the same time. The antimony pentachloride hydrolyzes enough in moist air to coat glassware badly unless it is rinsed immediately after use with chloroform. Although the reagent attacks rubber and cork stoppers, rubber stoppers covered with Teflon tape are satisfactory. Silicone grease is a suitable stopcock lubricant for the reagent dispensing buret.

Discussion and Results

The method is based on the reaction of sugar beet saponins with antimony pentachloride to produce a red-colored complex with an absorption maximum at 535 $m\mu$, determined on a Beckman model B spectrophotometer. Sucrose and antimony pentachloride also react to give a brownish complex with no maximum in the visible spectrum. Since, however, the sugar complex could contribute background absorbance for the saponin determination, it must be completely washed out of the saponin sample prior to color development. Fatty material in refined beet sugar does not seem to interfere, because floc samples, with or without washing by fat solvents, give identical saponin values.

Color formation depends on several variables. The red complex represents an intermediate stage of reaction, because, after long standing, the solutions become a grayish blue. Heating increases the formation rate and intensity of the red color; however, it also accelerates the fading process. When antimony pentachloride reagent is added to an acetic acid solution of saponin, enough heat is evolved to produce a color in 10 minutes at room temperature which is essentially stable for another 35 minutes. Contrary to the report of Steinle and Kahlenberg (3), light is not necessary for development of the color.

Figure 1 shows that there is a satisfactory straight-line relationship between saponin concentration and absorbance at 535 $m\mu$ for saponin values likely to be encountered in this analysis.

[a] Mention of trade names does not imply endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Tables 1 and 2 indicate that minor variations in the concentrations of antimony pentachloride in the reagent and water in the acetic acid-saponin eluate will not have a serious effect on the saponin values obtained

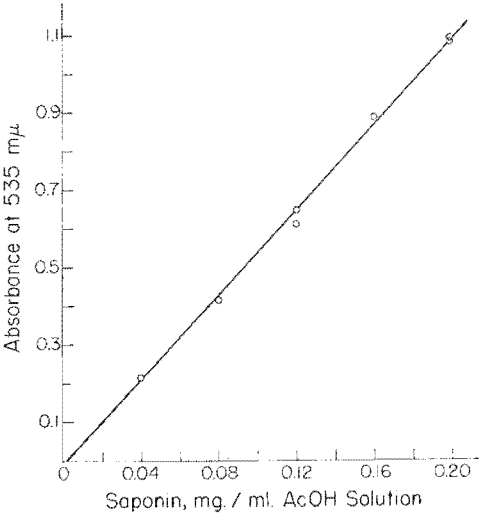

Figure 1.

Table 1.—Effect of $SbCl_5$ Concentration on Blank and Absorbance;[1] Saponin Concentration Constant.

$SbCl_5$ Concentration (%, V/V, in chloroform)	Absorbance	
	Blank	Saponin
5	0.028	0.135
10	.021	.512
15	.020	.505
20	.024	.495
25	.024	.445

[1] All absorbances are read at 535 mμ after color development has proceeded 10 minutes at room temperature.

Table 2.—Effect of Water in Saponin Acetic Acid Solution; Saponin Concentration Constant.

H_2O conc., vol. % in saponin-ACOH soln.	Absorbance[1]
0	0.459
10	.442
15	.402
20	.790[2]

[1] All absorbances are read at 535 mμ after color development has proceeded 10 minutes at room temperature.

[2] Turbid solution formed.

from the absorbance figures. The conditions outlined in the procedural section, therefore, represent an adequate way to measure beet saponins after isolation from beet sugar.

The quantitative isolation of beet saponin from the sugar sample is the most difficult problem encountered in the analysis. Clearly the task of separating and measuring an impurity present to the extent of a few parts per million is not easy. Solvent elution, distribution, or ion exchange methods were not satisfactory. It was found that filtration on sintered glass filters (1) was slower and no more efficient for floc removal than filter paper with filter aid. Table 3 shows that recovery of saponin is essentially quantitative from water, but that some loss occurs when 33 percent sirups are used.

Table 3.—Saponin Recovery from Acid Solutions, 250 ml.

Sugar (gm.)	Saponin added (mg.)	Saponin recovered (%)
0	0.20	98
0	.40	99
0	.60	93
0	.80	101
0	1.00	95
125	0.10	85, 63
125	.20	87, 63
125	.30	77, 75, 72
125	.40	76
125	.50	90, 88, 87
125	1.00	84, 83

Since, however, a reasonably constant amount of saponin can be recovered from the sirups at the various levels of saponin concentration, a correction factor could be determined, if necessary, to increase the accuracy of the method. Table 4 shows that replication of saponin values on a single sample of sugar is good, and that added amounts of saponin can be recovered from the sample. Table 5 lists the saponin analyses on a number of refined beet sugars. For comparison, floc values determined by the Spreckels method are included for some of the samples.

Table 4.—Replicate Analyses and Recovery of Added Saponin from a Beet Sugar Sample.

Saponin added (mg.)	Saponin found (mg.)
0	0.50, .56, .52 .53, .52, .47 .46, .52, .56, .52
.5	1.05, 0.95[1]
1.00	1.23, 1.28, 1.31

[1] Sample washed with chloroform before elution.

Table 5.—Saponin in Refined Beet Sugar Samples

Sample No.	p.p.m.	Spreckels analysis, p.p.m.
1	3.2	3, 4, 2
2	4.3	8
3	2.8	3
4	2.3	3
5	2.1	3
6	2.1	3
7	2.3	
8	5.4	
9	1.1	
10	3,6. 3.9, 1.9[1]	

[1] Sirup for analysis not acidified with HCl.

In general, the results obtained by this method show lower floc values because the oil content of the floc and other solids in the sirups are included in the gravimetric measurement but only the saponin is measured in the colorimetric method.

Although floc determination by the described procedure is not as accurate as might be desired, our limited experience has indicated a good correlation between floc content by visual estimation and by saponin analysis. The method should meet the needs for control work on floc, since large numbers of samples can be run easily in a fairly short time.

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References

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