Millipore-Antimony Pentachloride Colorimetric Method for the Rapid Determination of Saponin in Refined Beet Sugar

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In the past 16 years or so, methods have been devised for the characterization and determination of floc (3,7,8,10,13)[2] in refined beet sugar. Positive correlations were generally found to exist between saponin content and relative floc content (5,12,14). Walker (12) introduced the first reliable quantitative method for determination of saponin content in refined beet sugar. He utilized an antimony pentachloride reagent. Later, others (14,1) followed with adaptations of his method.

Gaddie and West (4) revealed the secret for the production of floc free sugar: Maintenance of a high pH in the white pan. Others (5,6) confirmed their findings. Thus the rule of thumb for production of floc free sugar was to maintain a pH in the white pan that was high enough to take care of the saponin level present in the massecuite

Even with the general ability to produce floc-free sugar and to confirm that fact by relative floc tests or by quantitative saponin tests, problems still persist. Strikes of floc-free sugar lose their identity in the company of floc sugar in bulk sugar bins. The problem was lack of time to get a floc grading or saponin analysis of bulk or liquid sugar at time of shipment. Thus a method had to be developed which would be simple to perform and would determine if a sugar is floc-free, in less than 10 to 15 minutes.

Over the years we have filtered the saponin from a sugar solution acidified to pH 1.0 with HCl onto a F-fritted glass Buchner funnel disk of 4-5.5 microns porosity, washed out the sugar with dilute acid, and then after drying, extracted the saponin with methanol followed by color development with concentrated sulfuric acid (1). It usually required about 4 hours for an acidified solution of 100 grams of sugar to filter through such a funnel. In tests with various filter media, the 1.2 micron Millipore filter proved to be very retentive and required less than 2 minutes for complete filtration of an acidified solution of 100 grams of beet sugar.

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[2] Numbers in parentheses refer to literature cited.

Stansbury and Hoffpauir (11) retained cane floc from an acidified cane sugar solution on a Millipore filter. They eventually extracted the floc from the filter for further analysis. Our thoughts were directed originally to the idea of extracting the saponin from the Millipore filter with methanol, and eventually developing the saponin color with concentrated sulfuric acid. This plan was readily abandoned when it became apparent that the Millipore filter was dissolved by methanol which in turn gave a color development with concentrated sulfuric acid.

The Millipore technical literature (9) indicated that qualitative spot tests could be performed on the Millipore filter surface. It was noted in the Millipore Chemical Resistance Table (9) that the Millipore filter was not affected by chloroform. We were able to demonstrate that a chloroform solution containing antimony pentachloride gave the characteristic pink color test for saponin on the Millipore filter surface without destroying the filter.

Grab samples of sugar from each of our mills were evaluated for floc and for relative saponin content as listed in Table 1. In the American Crystal Sugar Company adaptation of the Spreckels' floc method, an acidified 31 Brix sugar solution of pH 2.0 with HCl, is brought to boil and allowed to simmer for 5 minutes. A floc grade, by The Seven-Up grading system, not exceeding 2 at the end of 72 hours is considered to be Bottlers sugar.

Table 1 points out the excellent agreement of the floc method and of the saponin Millipore-$SbCl_5$ method.

The Saponin Millipore-$SbCl_5$ test was run on these same sugars several times by different individuals. Each time the results duplicated those listed in Table 1. By determining the relative saponin contents by the Millipore-$SbCl_5$ method (10-15 minutes), we were able to predict the floc performance (at the end of 72 hours) of those sugars.

Table 1.—Original evaluation of saponin by Millipore-$SbCl_5$ method.

Factory	Floc grade	Saponin by Millipore-$SbCl_5$	
		Rank	Color intensity
A	4	7	Intense Pink
B	4	7	Intense Pink
C	4	7	Intense Pink
D	2	6	Mildly Pink
E	1	1	Barely Pink
F	1	1	Barely Pink
G	1	1	Barely Pink
H	1	1	Barely Pink
I	1	1	Barely Pink

Since a sugar with a floc grade no greater than a plus 2 at the end of 72 hours will meet the Bottlers standard, such a sugar should then serve adequately as a control. Thus if the developed saponin color of the unknown strike of sugar is less than or equal to that of the control sugar then it is considered to be floc free and thus acceptable to the beverage industry. By running the control at the same time or just prior to the unknown, then the parameters of the test are the same for the control and the unknown. Great care should be exercised in selecting the control. It is possible that the control may allow some floc sugars to be graded as floc free sugar by the Millipore-$SbCl_5$ method. Historically we have found this rule of thumb, that if a sugar contains 1 ppm saponin or less it will produce a floc not greater than a plus 2. Thus if the control contains 1 ppm saponin or less it should reject all floc sugar. This rule of thumb may not exist for all companies, due to the purity of the saponin standard, to the kind of floc test and to the final time of observation of the acidified sugar solution for floc grading.

If the chemist is satisfied that an appropriate control has been obtained, then a sufficient quantity of that lot of sugar should be set aside.

Antimony Pentachloride Reagent

The following antimony pentachloride solution (2) after aging 3 to 4 days serves quite adequately for producing a pink color with saponin on a Millipore filter:

5 ml $SbCl_5$
95 ml $CHCl_3$
1 g $SbCl_3$

Antimony pentachloride as it appears commercially contains free chlorine. The free chlorine inhibits the saponin-antimony pentachloride reaction. One gram of antimony trichloride added to 100 ml of 5% antimony pentachloride-chloroform solution will tie up the chlorine to make antimony pentachloride. An excess of antimony trichloride will impede the color reaction of saponin with antimony pentachloride.

Antimony pentachloride has great affinity for water, thus producing pyroantimonic acid and hydrochloric acid. High relative humidities can have a quenching effect on the color development of the saponin. Glassware used to prepare the antimony pentachloride reagent can become coated with a residue of pyroantimonic acid that is practically impossible to remove. Glass stoppered bottles containing the antimony pentachloride reagent freeze shut requiring special efforts to unstopper.

We have found the use of glass bottles with Teflon lined screw caps to be quite successful for the preparation and storage

of reagent. The glass bottles are calibrated for various volumes with chloroform.

These unopened bottles of 5% antimony pentachloride reagent for saponin should last indefinitely. An 8-year old bottle of 5% antimony pentachloride reagent for saponin containing .5 grams of antimony trichloride per 100 ml of solution was found to have great sensitivity for saponin.

A 10% antimony pentachloride reagent gives a much stronger pink color for saponin than the 5% reagent. In our opinion, however, if fats are present with saponin, a brown color may form with the 10% reagent while the normal pink color of the saponin is developed with the 5% antimony pentachloride reagent.

Reagents and equipment:

1. 5% $SbCl_5$ solution
2. ph 2.0 HCl solution
3. Control sugar (plus 2 floc at end of 72 hours)
4. Millipore filter, Type RA White, 1.2 microns porosity, diameter 47 mm
5. Millipore filter holder
6. Glass pipette
7. Saponin from Beet Molasses (optional)
8. 1% NaOH solution (optional)
9. Schleicher & Schuell 507 filter paper (optional)

Glass pipette:

A suitable pipette can be made from 5-7 mm diameter glass tubing. The glass tubing opening at one end is drawn down to a diameter of about .5 mm or less. The pipette can dispense a drop of reagent that covers an area as small as 2 mm in diameter on the filter surface. By using a tall 2 oz screw cap glass bottle for the 5% antimony pentachloride reagent, it is possible to get enough reagent in the pipette without mouth pipetting to dispense at least 5-8 drops.

Another tall 2 oz bottle containing chloroform is used to rinse the reagent from the pipette. If the pipette is not cleaned after each use, it will become permanently plugged.

With practice, proper finger pipetting of the reagent may result in the dispensing of reagent at the rate of 1 drop per 1 to 2 seconds.

Saponin Millipore-$SbCl_5$ Control Method:

This method is used for rapid and routine analysis of refined beet sugar for saponin content and thus for floc forming potential. The following procedure is employed for the control sugar and the sugar of unknown saponin content.

Procedure:

1. Dissolve 100 grams of refined beet sugar in 200 ml of 2.0 HCl. (Time: 1 to 3 minutes)
2. Filter with vacuum through a 1.2 porosity Millipore filter. (Time: 1 to 2 minutes)
3. Rinse with 100 ml of pH 2.0 HCl. (Time: less than 1 minute)
4. Oven dry Millipore filter at about 105° C. (Time: 2 to 5 minutes)
5. Apply a drop of 5% $SbCl_5$ reagent to Millipore filter and to the control Millipore filter, compare at room temperature and/or at warmer temperatures.

Although high relative humidity may impede the saponin-antimony pentachloride pink color development at room temperature, this interference does not occur at temperatures as high as 105° C. If one is not satisfied with the color development, then he should apply reagent again to unused areas of the filters of the control and of the unknown and re-evaluate. These Millipore filters after evaluation have been retained enclosed as long as 4 months without any apparent decomposition and have still given a saponin test with antimony pentachloride reagent.

The Millipore $SbCl_5$ Control Method was tested at various factories this past campaign. The chief chemists provided their own control sugars. The results from four different factories are listed in Table 2.

The chief chemists from the four factories were impressed with the method. The chief chemist from D factory was not able to recheck the one sugar which failed the Millipore-$SbCl_5$ test. He thought that a control sugar with a slightly higher saponin content would have allowed more actual floc-free sugars to be graded floc free by the Millipore-$SbCl_5$ method. His control sugar was found to contain .8 ppm saponin.

The chief chemist from F factory reported that the Millipore-$SbCl_5$ method, graded as floc-free, 85 to 90% of those strikes of sugar found to be floc-free at the end of 72 hours by the Spreckels' floc method. He also thought that on several occasions the

Table 2.—Floc test versus Millipore-$SbCl_5$ control method at four factories.

Factory	No. of samples	Bottlers sugar	
		Floc test	Millipore-$SbCl_5$
D	60	37	20[1]
E	30	16	16[2]
F	60		"85 to 90% of floc test"
G	16	14	12

[1] On one occasion floc-free by Millipore-$SbCl_5$ became floc sugar by Spreckels' floc method at the end of 72 hours.

[2] On two occasions floc-free by Millipore-$SbCl_5$ became floc sugar by Spreckels' floc method at the end of 72 hours.

Millipore-$SbCl_5$ method graded as floc-free, sugars that became floc sugar at the end of 72 hours by the Spreckels' floc method.

It should be pointed out that in the original instructions to the chief chemists they were to use pH 1.0 HCl for solution of the 100 grams of sugar for testing. At pH 1.0, partial charring and brittleness of the Millipore filter on oven drying, and depressed color development of the saponin-$SbCl_5$ reaction occurred. Subsequently it was found that at pH 2.0 these problems disappeared. The data from D, E and F factories are composed mostly of pH 1.0 results. The results of G factory represent pH 2.0.

It may be that less saponin is filtered out at pH 2.0 than at ph 1.0; however, this should have no bearing on the method as long as the control sugar or standards are also made up at pH 2.0.

Weekly composites of sugar from each of our mills were sent to the Research Laboratory for analysis. Table 3 compares the floc test and the Millipore-$SbCl_5$ method.

The Millipore-$SbCl_5$ method rejected all of the floc sugars. The control sugar at the Research Laboratory contained .8 ppm saponin.

Saponin Millipore-$SbCl_5$ Quantitative Method:

Originally it was not possible to quantitate directly the saponin content of beet sugar on the Millipore filter. The pink color of the saponin from beet sugar was evenly distributed over the area of the spot on the Millipore filter surface when antimony pentachloride reagent was applied. This is not the case for purified saponin obtained from limecake or from sugarbeet skin. Such saponin is washed out to the periphery of the spot on treatment with antimony pentachloride, thus making it impossible to quantitate. More recently it was found that saponin obtained from beet sugar molasses acts similarly to the saponin found in beet sugar, thus allowing for direct quantitation on the Millipore filter.

Table 3.—Floc test versus Millipore-$SbCl_5$ control method at research laboratory.

Factory	No. of samples	Bottlers sugar	
		Floc test	Millipore-$SbCl_5$
A	3	1	1
B	13	13	13
C	15	7	5
D	16	0	0
E	16	16	13
F	17	16	13
G	16	0	0
H	15	13	11
Total	111	66	56
% Acceptable		59.5	50.5

Beet sugar free of saponin is fortified with various levels of saponin from molasses. Usually .1000 grams saponin are dissolved in 1 liter of methanol. Thus 1 ml of the methanol solution of saponin is added to 100 grams of saponin-free beet sugar to produce a 1 ppm saponin standard. It is very difficult, if not impossible, to find refined beet sugar free of any saponin. Originally it was thought that cane sugar could be used for preparation of the saponin standards. It was found that the saponin added to cane sugar was partially or completely masked on color development with antimony pentachloride.

We were able to locate a strike of sugar that had no more than, and possibly much less than, .1 ppm saponin. This lot of sugar is being used for preparation of saponin standards. Previous to finding this lot of sugar, we simply fortified with saponin the filtrate of an acidified solution of 100 grams of refined beet sugar of low saponin content which had been filtered through a 1.2 micron porosity Millipore filter.

Table 4 compares the actual floc grade with the amount of saponin present in the sugar.

The control sugar which we had been using to determine Bottlers sugar contained .8 ppm saponin.

Saponin Schleicher & Schuell - $SbCl_5$ Quantitative Method:

It was found that filter media other than the Millipore filters work well with the Millipore Filter Apparatus. Schleicher & Schuell Red Ribbon 589 Filter Paper is fairly retentive. Where it takes less than 2 minutes for a 1.2 micron porosity Millipore to filter an acidified solution of 100 grams of sugar, it takes less than 10 minutes for S & S RR 589. Schleicher & Schuell 507 filter paper is extremely retentive, requiring at least 20 minutes for comparable filtration. The saponin on Red Ribbon 589 paper is best visualized by dipping in antimony pentachloride reagent. The whole filtration area develops a uniform pink color. 507 paper may be visualized either by dipping or by spotting.

Table 4.—Quantitative analysis of saponin by Millipore-$SbCl_5$.

Sugar	Floc grade	Millipore-$SbCl_5$ saponin, ppm
A	1	.3
B	2	1.3
C	2+	1.3
D	3+	1.5
E	2+	1.5
F	4	3.5
Control Sugar	2	.8

Standards: .00, .25, .5, 1.0, 1.5, 2.0, 3.0 and 4.0 ppm Saponin

Half a circle of 11 cm diameter paper is conveniently used for filtration. 507 paper was used prior to the Millipore filter for quantitation with limecake saponin.

Table 5 compares the floc grade, the saponin rank by Millipore-$SbCl_5$, and the ppm saponin with Schleicher & Schuell 507 paper.

Table 5.—Quantitative analysis of saponin with Scheicher & Schuell 507

Sugar	Floc grade	Millipore-$SbCl_5$ Saponin rank	SS 507-$SbCl_5$ Saponin, ppm
A	1	1	.2
B	1	2	.4
C	1+	3	.5
D	1+	4	.7
E	2+	7	1.3
F	2+	6	1.3
G	4	8	4.0
Control	2	4	.8

Standards: .25, .5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 ppm saponin

All of the sugars evaluated with the 507 paper, be they unknown saponin content or the standards, were first made up as basic solutions and filtered through a 1.2 micron porosity Millipore. This was done to remove fine sediment which would impede filtration through the very fine porosity 507 paper. The basic sugar filtrates and washings were then adjusted to pH 2.0 with concentrated HCl. The acidified sugar solutions were filtered through the 507 paper. The Millipore filters of the basic sugar solutions were tested for saponin content and found to contain none.

Conclusion

The authors recommend the use of the 1.2 micron Millipore filter for routine control work and especially where quantitation is desired. By this means the analyst should be able to complete a test easily in less than 15 minutes.

Where cost of the filter media is of consideration and the time element not critical, regular fine grade filter papers may be used.

In the event of background interference from the presence of fine sediment on the filter, it is recommended that this material be removed by filtration from an alkaline solution before proceeding with the test.

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Literature Cited

(1) BAUSERMAN, H. M. and P. C. HANZAS. 1957. Colorimetric determination of saponin as found in beet sugars. J. Agr. Food Chem. 5: 773-776.

(2) BAUSERMAN, H. M. and P. C. HANZAS. 1957. Paper chromatography of sugarbeet compounds. J. Am. Soc. Sugar Beet Technol. 9: 295-299.

(3) EIS, F. G., L. W. CLARK, R. A. MCGINNIS and P. W. ALSTON. 1952. Floc in carbonated beverages. Ind. Eng. Chem. 44: 2844-2848.

(4) GADDIE, R. S. and R. R. WEST. 1958. Relationship between white pan pH and saponin content of the sugar. J. Am. Soc. Sugar Beet Technol. 10: 171-176.

(5) HANZAS, P. C. and R. W. KOHN. 1961. Saponin survey of various factories of the 1958 campaign. J. Am. Soc. Sugar Beet Technol. 11: 519-527.

(6) JOHNSON, R., JR. 1960. The occurrence and elimination of saponin in process juice. J. Am. Soc. Sugar Beet Technol. 11: 201-205.

(7) JOHNSON, J. R. and N. R. DIEHL. 1956. Determination of floc in refined sucrose by coagulation with quaternary amines. J. Am. Soc. Sugar Beet Technol. 10: 171-176.

(8) MADRU, J. E. 1952. "Floc" in white sugar. Proc. Amer. Soc. Sugar Beet Tech. 764.

(9) MILLIPORE CORPORATION. Catalog MF-67.

(10) SABINE, F. M. 1952. The "floc" problem in beet sugar. Proc. Amer. Soc. Sugar Beet Tech. 760-763.

(11) STANSBURY, M. F. and C. L. HOFFPAUIR. 1959. Composition of "floc" formed in acidified sirups from refined granulated cane sugars. J. Agr. Food Chem. 7: 353-358.

(12) WALKER, H. G., JR. 1956. Determination of saponins in refined beet sugars. J. Am. Soc. Sugar Beet Technol. 9: 233-237.

(13) WALKER, H. G., JR. and H. S. OWENS. 1953. Beet sugars, acid insoluble constituents in selected samples. J. Agr. Food Chem. 1: 450-453.

(14) WEST, R. R. and R. S. GADDIE. 1956. Determination of saponin in granulated sugar. Method of H. G. Walker adapted to routine control. J. Am. Soc. Sugar Beet Technol. 9: 156-152.