Determination of Saponin in Granulated Sugar: Method of H. G. Walker Adapted to Routine Control

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The analytical determination in commercial granulated sugar of trace quantities of substances loosely classified as "floc" which precipitate in acidified aqueous solution has become increasingly important the last three or four years. Before that time, spot checks were made in most sugar laboratories on the turbidity produced in sugar solutions by the addition of citric or phosphoric acid to a specified pH. Many adaptations to this general method have been tried. Various concentrations of sugar were used as were most of the common food and mineral acids at various pH's. Heating to different temperatures for varying periods of time as well as allowing the test to stand for periods ranging from one hour to ten days was specified. None of these modifications resulted in solutions from which quantitative measurements on floc-producing substances could be obtained.

As a result, it was impossible to predict in more than about 50 percent of the tests which sugars would tend to floc in susceptible beverages. Because the criterion on which the sugar is judged is the presence or absence of floc in these beverages, it seemed logical to base our search for a routine method of analysis on the behavior of sugar containing various concentrations of floc (as measured by the various methods of analysis) in one of the more susceptible carbonated beverages. The beverage chosen was 7-Up, not only because it is one of the most susceptible, but because it forms a clear water-white solution. We are indebted to Dr. B. C. Cole of the 7-Up product Department for furnishing the formula and flavoring ingredients for making 7-Up in the laboratory.

Cases of capped 7-Up bottles containing only carbonated water were obtained from the local 7-Up bottler. These were refrigerated to 0° C. The 7-Up syrup containing exact amounts of the various components including the sugar in question is prepared. The bottles of carbonated water are removed from the refrigerator and uncapped. Exactly 1 ounce of the water is poured off, exactly 1 ounce of the above syrup is added, and the bottles immediately recapped. At 0° C. practically no CO_2 is lost. Gas volumes on the recapped beverage varied from 3.7 to 4.1; 7-Up Company specifications are 3.5 to 4.0. After mixing, the bottles were allowed to stand at room temperature and observations for floc were made at intervals for periods of up to 8 weeks.

In 1952, F. G. Eis, et. al, published a method (1)[2] in which the floc from five pounds of sugar dissolved in five liters of water is precipitated in hot solution brought to pH 2.0 with HCl, filtered through a 600 ml. F-fritted glass funnel, extracted with hot methanol, the methanol evaporated and the triterpenes weighed. This method, while possessing a suitable degree

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[2] Numbers in parentheses refer to literature cited.

of quantitativeness, was too cumbersome for our purpose. The difficulties in filtering 5 pounds of sugar in 5 liters of water through a F-frit coupled with the small amount (6 to 10 mg. in the critical range for susceptible beverages) of recovered triterpenes rendered this method unsuitable for routine mass analysis.

Howard G. Walker, Jr., and Harry S. Owens, working on the acid-insoluble constituents in selected samples of sugar (2), found a fairly large percentage to be saponin and Mr. Walker devised a method for the determination of this component (3).

In the Walker method, the sugar is dissolved in water and the saponin precipitated by the addition of HCl. The solution is filtered through analytical Celite and the contents of the funnel dried. The saponin is then eluted with hot glacial acetic acid. To an aliquot of this eluate is added antimony pentachloride-chloroform reagent and the developed color measured by absorption at 535 mu.

We found that Walker's method could be readily adapted to routine mass testing of sugar and when evaluated by comparison of floc produced in 7-Up, gave consistent and reproducible results. This general method was, therefore, adopted and all values for saponin shown in this paper were obtained by our adaptation of this method. Table I shows typical results in 7-Up made with different sugars and mixtures of sugars from the seven Utah-Idaho factories.

Table I.—Floc Production in 7-Up Made With Sugar Containing Various Concentrations of Saponin.

Appearance of Floc	Beverage Sample No.	Sugar—p.p.m. Saponin	Elapsed Time Days
Heavy, fine, dispersed	1	14.6	10
Large flakes	2	11.0	13
Heavy ring, deposit on bottle	3	8.0	17
Very fine, dispersed	4	7.5	13
Very fine, slight ring	5	6.0	20
Light film on surface	6	4.4	28
No floc	7	2.6	56
No floc	8	1.6	56

Three major conclusions were drawn from our work with the 7-Up.

1. Sugar saponin concentrations greater than 4 p.p.m. always resulted in detectable floc in the beverage if allowed to stand a suitable time.
2. The time required for the floc to become detectable is a function not only of the saponin concentration in the sugar but also of the source of the sugar.
3. The physical appearance of the floc varies rather widely.

In adapting Walker's method to the routine testing of sugar, we discovered very early that reagent grade antimony pentachloride contains free chlorine and must be purified before use. We attempted to remove this contaminant by adding crystalline antimony trichloride and by bubbling dry air through the reagent. Neither of these procedures was completely

effective on large batches (3 pounds) so vacuum distillation was necessary. As finally worked out, the distillation set-up consisted of all Pyrex glass assembly with standard taper connections throughout, a 2-liter distillation flask and a 1-liter receiving flask. The reagent is highly corrosive; also it decomposes in the presence of moisture. To exclude moisture and protect the laboratory vacuum pump we use a rather elaborate absorption train. Dr. Walker recommends using an all-glass aspirator with a freshly prepared $CaCl_2$ drying tube between the receiver and water pump.

Three pounds of reagent-grade $SbCl_5$ are placed in the 2-liter distillation flask along with approximately 10 gm. of Hengar boiling granules. The condenser should be full of water but the water should not be circulating. Pumping down time to an absolute pressure of 1 inch or less of mercury is approximately 2 hours at our laboratory because the rate at which the absorption train will take the gas evolved is rather low. As the vacuum reaches about 10 inches, evolution of gas (mostly chlorine) begins and continues without the application of heat for considerable time. When the absolute pressure reaches 1 inch Hg, heat is applied and the first vapors of $SbCl_5$ allowed to sweep the system. At this point water is immediately started through the condenser. The distillation rate is adjusted to give maximum recovery of reagent with minimum loss to the absorption train. This rate is such that about 1.25 hours are required and about 510 ml. of distillate recovered. By the time this much material has distilled over, the residue in the distillation flask is very dark and has developed a tendency toward unstable boiling. Another indication that distillation should be stopped is that the condensate in the connecting tube changes from a film to oily droplets.

The purified $SbCl_5$ is not stable under ordinary storage conditions and begins to darken in a matter of days. Therefore, the freshly distilled compound should be diluted immediately with chloroform. (12.5 percent $SbCl_5$ in $CHCl_3$, vol./vol.) This diluted reagent has been kept for periods up to six months with no significant deterioration when normal precautions were taken to exclude atmospheric moisture. In no case did the variation in absorbance during this period exceed 5 percent of the value of the 5 ppm. standard. As a result, the slope of the calibration curves remained nearly constant over the linear range (0-10 p.p.m. saponin) for a given batch of reagent. Successive batches of reagent prepared by the same technique gave deviations in absorbance values for saponin standards of 10 percent or less.

Saponin Standards

Saponin standards used in this work were obtained from different sources but were both extracted from diffusion juice by the method recommended by Walker (1). Sample "A" was extracted from concentrated diffusion juice (source unknown) and sample "B" was extracted from concentrated diffusion juice from one of the Utah-Idaho factories. Absorbance values for these two saponins made up in glacial acetic acid solution and read in an 18 mm. round tube at 535 mu are shown in Table 2.

Table 2.—Corrected Absorbancies on Saponin Standards.

Concentration	Sample A	Sample B
.05 mg./ml.	.250	.265
.10 mg./ml.	.500	.540
.15 mg./ml.	.610	.660

It may be seen that there is a slight difference in absorbance at the same concentration between these samples. It should be remembered, however, that the so-called "saponin" in each case is actually the alcohol-soluble fraction of the acid precipitate from diffusion juice. This fraction undoubtedly contains traces of other compounds which affect color development. We do know that the degree of purification of the saponin has a bearing on the color development. Saponin to be used for standards should be dissolved in alcohol and reprecipitated sufficient times to give a very light-tan-colored solid.

Analytical Method

To precipitate the floc producing substances, Walker (3) recommends the addition of a measured amount of concentrated HCl to the solution of the sugar under test. Eis, et. al. (1) acidify to pH 2.0, heat to boiling, and simmer for 10 minutes; Walker and Owens (2) recommend pH 1.0 to 2.5 for fairly rapid formation. We have found in working with granulated sugar that at room temperature and at a concentration of 50 gm. sugar in 150 ml. water, maximum recovery of saponin is not realized above pH 1.0 (see Table 3).

Table 3.—Recovery of Saponin at Various pH.

pH	Saponin, p.p.m.	Recovery, Percent of Maximum
1.0	11.2	Maximum (100%)
1.5	9.6	85
2.0	4.0	36
2.5	2.7	24
3.0	1.6	14

Reproducibility of Method

During the 1954-55 campaign, numerous samples of the same sugar from each factory were run on successive days. Sugars were chosen with floc contents ranging from 1 to 12 p.p.m. A summary of results is shown in Table 4.

Table 4.—Reproducibility of Method.

Factory	Sample No.	Average Floc p.p.m.	Maximum Deviation p.p.m.	Number of Analyses
A	1	10.0	± 0.3	8
A	2	1.6	± 0.1	12
B	1	1.8	± 0.1	12
C	1	1.4	± 0.1	8
D	1	2.0	± 0.2	8
E	1	8.0	± 0.3	12
E	2	12.0	± 0.4	12
F	1	11.6	± 0.5	16

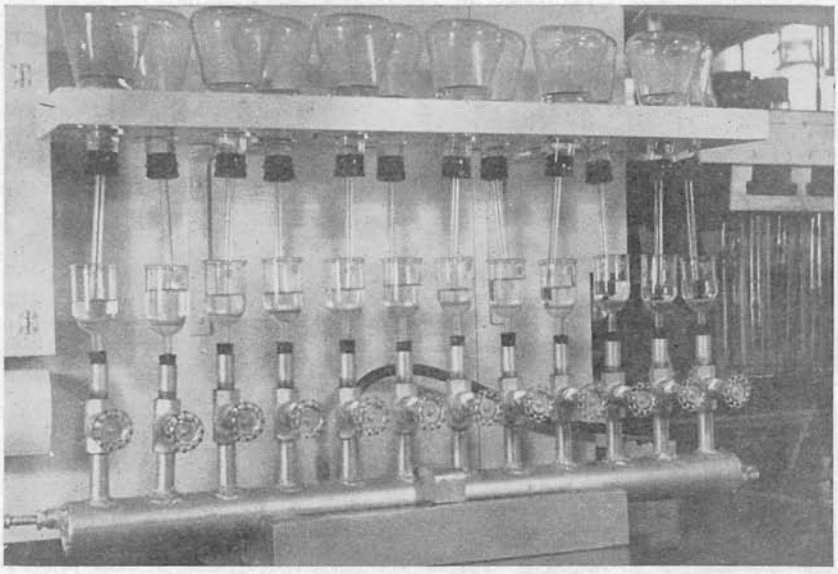
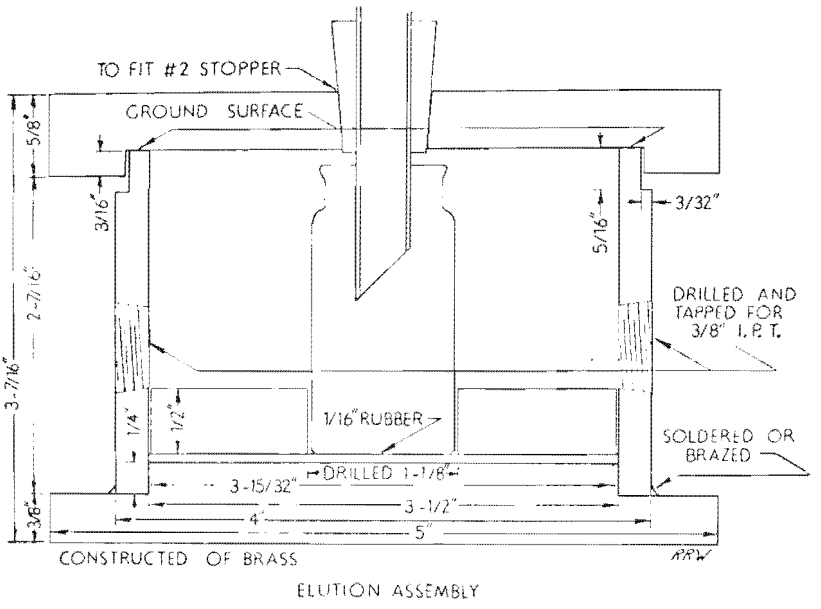


Figure 1.—Multiple filtration apparatus.

Method for Routine Analysis

In a 500 ml. Erlenmeyer flask dissolve 50.0 gm. sugar under test in 150 ml. distilled water at room temperature. Add concentrated HCl to pH 1.0 to 1.4 and mix. Rinse a clean "F" porosity 60 ml. high form fritted glass funnel with 5 ml. hot glacial acetic acid followed by 30 ml. water. Filter the sugar solution through this funnel. Multiple filtrations may be set up by means of an apparatus such as that shown in Figure 1. The unit consists of a vacuum header into which is welded 12 half-inch nipples. Attached to the nipples are half-inch valves to control the vacuum on each. Into each valve is screwed a short length of half-inch pipe squared and reamed at the end. The stem of each funnel is equipped with a No. 1 rubber stopper which just fits into the half-inch pipe. Automatic filtration is provided by a rack to carry the inverted 500 ml. Erlenmeyer flasks which are fitted with 1-hole stoppers into which are inserted short lengths of 10 mm. glass tubing.

When filtration is complete, rinse the flask with 15 ml. of very dilute HCl (1 ml. concentrated HCl + 1 liter of water), pouring rinsings into funnel; wash down sides and fritted surface of funnel using 15 ml. of the same very dilute HCl using gentle suction and several washings of the fritted disc. Dry the funnels for 1 hour at 103° to 105° C. and allow to cool. Greenish spots on the sides or fritted surface of the dry funnels indicate that all the sugar was not washed out. These spots will dissolve during elution causing off-colors and subsequent errors. Place a 5-dram vial in the elution apparatus shown in Figure 2. This elution apparatus is constructed of bronze. The seal between the cover and base is a ground fit, and with the aid of a little silicone stopcock grease, is air tight. There



ELUTION ASSEMBLY

Figure 2.—Elution assembly.

is a hole drilled in the lid which just fits the No. 1 stoppers on the funnels. A recess is drilled in the inside bottom of the base to position the 5-dram vial directly below the hole in the lid. Stopcocks on each side regulate the vacuum.

Place a funnel in the cover and assemble so that the funnel stem extends into the vial. Take up 5.2 ml. hot glacial acetic acid, (a 5 ml. glass hypodermic syringe with a 21-gauge needle is convenient). Elute the saponin by covering the fritted surface and then applying gentle suction. Repeat this several times until the 5.2 ml. of acid is used. Remove the vial and stopper immediately with the plastic stopper. Hold until all samples have been eluted. 5.2 ml. of acid will give very close to 5.0 ml. of eluate.

When a number of samples are ready to read, pipette 1.0 ml. (we use a 3 ml. hypodermic syringe) aliquots of each into clean 5-dram vials. In additional vials pipette 1.0 ml. of the elution acetic acid for a reagent blank and 1.0 ml. of saponin standard. Rinse the syringe twice each time with about 1 ml. of the solution to be pipetted. From a burette equipped with a teflon stopcock, add 7.0 ml. antimony pentachloride-chloroform reagent. Stopper and mix by swirling; avoid splashing on the plastic stopper and do not expose to direct sunlight. At the end of 10 minutes read in the photometer at 535 mu. Read the reagent blank and saponin standard first. On regular graph paper with absorbance (optical density) as ordinates and p.p.m. saponin as abscissas, plot the scale reading of the reagent blank vs. 0 p.p.m. saponin. Plot the scale reading of the saponin standard vs. p.p.m. in the standard. A standard containing 0.05 mg. per ml. (50 p.p.m.) for

samples of low saponin concentration, or one of 0.10 or 0.15 mg. per ml. for samples of high saponin concentration is used. Connect the two points with a straight line. This is the calibration curve for this set of samples. The curve shifts slightly from day to day so it is redetermined each day. The scale reading on the sugar sample is for 10 gm. sugar so the p.p.m. saponin as read must be divided by 10.

The antimony pentachloride-chloroform reagent will react with practically everything we have tried except teflon and glass. Even silicone stopcock grease in a burette stopcock dissolves. We, therefore, use a teflon stoppered burette. Water will decompose the reagent liberating HCl and leaving a deposit of white antimony pentoxide which is insoluble in water. Therefore, all glassware which has come in contact with the reagent must be first rinsed in chloroform, then in concentrated HCl' before finally washing in water. It is good practice to dry the vials and photometer tubes in the oven before using.

After a time the fritted funnels become partially plugged resulting in progressively longer time to effect filtration. By keeping the funnels immersed in dilute chromic acid solution when they are not in use, the life of a fritted funnel is greatly lengthened.

References

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