Symposium on New Methods, Procedures and Instruments for Research and Control Laboratories*

Simplicity in Analytical Methods—W. A. HARRIS AND L. W. NORMAN[1] The importance of analytical methods in the sugar beet industry needs no emphasizing. Obviously it is only through analytical procedures that the complex nature of the sugar beet is revealed, and some understanding is obtained of the effects of the various chemical constituents on the growth of the beet and the problems they present in the extraction of pure and marketable sugar.

Through continued development of new techniques, our understanding of the agronomic and processing problems can be broadened, and improved guidance and controls can be instituted in the agricultural and processing phases of sugar production.

Any method of analysis must give reproducible results and an accuracy that is suitable to the problem at hand. But simplicity and speed must be the keynote. This is necessary for routine factory control, or for the handling of the many samples necessary in procuring data in the study of a particular problem.

The fact that a method of analysis has been accepted as standard should not preclude an appraisal of other possible approaches or other techniques. For example, is the calculation of raffinose—from direct and invert polarizations—more satisfactory than its evaluation from a paper chromatogram? Certainly for a large number of determinations the chromatographic approach offers speed and simplicity—along with reasonable accuracy. Again, are the long-used gravimetric and titrimetric methods for invert sugars more preferable than simpler chromatographic or colorimetric techniques? Certainly those methods are subject to inaccuracies if other reducing substances are present. The chromatographic evaluation has even more possibilities now that the Eli Lilly Company has introduced a new reagent that seems to be absolutely specific for glucose.

We know there are materials in the beet that we should be more cognizant of in our efforts to pinpoint individual factors in making beet selections in our breeding programs. We are aware that some compounds or groups of compounds need further study

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* Conducted as a scheduled symposium under the Chemistry and Factory Operation Section, American Society of Sugar Beet Technologists, February 6, 1962.

for their effects on juice behavior in processing. For lack of simple techniques, we are inclined to omit analyses of this kind in our studies.

Betaine, for instance, is an abundant nitrogenous component that we really don't know too much about. Techniques for its determination have been somewhat unwieldy for routine analyses. True, the development of the colorimetric determination of betaine reineckate has helped considerably (1)[2], but the determination does require considerable time and manipulation. A simpler technique would certainly be an invitation to include more betaine determinations in our studies.

We think there is a fair possibility that a chromatographic procedure can be developed for betaine. Preliminary work that we have done along this line may offer a clue to something that could lead to such a development.

We have tried, using a variety of solvents, to move betaine reineckate on paper, but instead of moving as a unit, the complex dissociates and only the reinecke is revealed with a ferric chloride spray—and even this seems to fragment into two or three spots. Since some alkaloid reineckates have been separated on aluminum oxide columns (2), one wonders if some carrier other than paper might allow the betaine reineckate to move intact. Here the new technique of thin layer chromatography would have application.

At the moment it appears that if paper chromatography could be used, a reagent must be found that will reveal the betaine spot with adequate sensitivity. So far as we have pursued the matter, a solution of about 1% iodine in a water-free solvent—such as absolute ethanol or ethyl ether—has been the most effective reagent. We have been able to detect known betaine spots in the range of 15 micrograms per 15 microliter spot. However, spot intensities have not been uniform. Further, short runs in an isopropanol-benzene-butanol-water solvent failed to separate betaine from interferences. It is hoped that further efforts, by ourselves or one of you, will be fruitful.

An ever-present problem in the industry is that of the tendency of some sugars to form floc in carbonated beverages. Testing for floc is imperative for the proper marketing of our sugars.

Probably the most used and reliable measurements is by the well known "Spreckels Test"—or some variation of it. Yet this test has obvious disadvantages. Precipitation of floc with quaternary amines (3) has not been entirely acceptable.

It has long been known that traces of saponin carrying through to the final product may be held responsible for floc formation (4)—at least to some extent. Consequently, methods have been

devised for the colorimetric measurement of saponin. These involve the precipitation of saponin from acidic sugar solution, removal of the precipitate on a fine-fritted glass funnel by suction filtration, extraction from the funnel with a suitable solvent, and color development. Antimony pentachloride has been used after extraction with glacial acetic acid (5), and concentrated H_2SO_4 heated with a methanol extract gives a color reaction with saponin (6).

Hibbert and associates (7) recently pointed out the desirability of a more general method of surface active impurities, and have adopted the "polarographic purity" method on Vavruch (8). This method employs the fact that minute quantities of surface active materials strongly suppress the so-called oxygen maxima that are encountered in polargraphic current-voltage curves. On evaluating sugar solutions, the amount of suppression of the peak height indicates the amount of surface active materials present.

The method is rapid—certainly a great advantage for determining immediately whether a strike is suitable for bottlers' trade. It would appear likely that this may be the most suitable and accurate of the objective methods now available.

However, it may be difficult to justify the expense of polarographic equipment, for control purposes, at each factory producing bottlers' sugar, if other means of evaluating floc can keep us out of trouble.

Recently we have started to investigate possibilities of simplifying chemical methods. Two or three things have come to light which appear to offer potential.

First, the filtered floc from an acidified sugar solution may be extracted with H_2SO_4 of 80 to 85% concentration. Heating the extract gave color gradations according to the amount of saponin present—very much like that obtained with heating a methanol extract with an equal volume of concentrated H_2SO_4 as described by Bauserman and Hanzas (6). This would eliminate the ticklish procedure of adding H_2SO_4 to methanol, cooling and making to volume. Possibly, the drying step would be unnecessary.

Secondly, a much more intense color was obtained when this acid extract was heated for 10 minutes, a few drops of potassium chromate or dichromate added and heating continued for 10 minutes, then chromotropic acid added. Here, only 10 grams of sugar in solution was required to show good differentiation between samples of different saponin content.

Another approach is based on the observation that saponin has reducing properties that might be utilized. The ferric ion, for instance, is reduced to the ferrous ion—which responds to the very sensitive reagent ortho-phenanthroline. The reaction was found to occur in aqueous solutions that were neutral or slightly alkaline, in alcoholic solution, or in methyl cellosolve solutions. The orange-red color developed with about 5 minutes of heating in a boiling water bath and was proportional to the amount of saponin present.

Thus the precipitated and filtered floc could be extracted with methyl cellosolve, a few drops of 1 to 2% solution of o-phenanthroline containing a small amount of ferric chloride or ferric ammonium sulfate added, and the color developed with a few minutes heating. We don't know yet if the reaction is sensitive enough that smaller amounts of sugar solution can be used to cut down on filtration time but obviously the procedure would eliminate some steps in saponin determinations, and requires no unpleasant chemicals.

The most desirable situation, of course, would be to carry out this reaction directly in the sugar solution. Indeed, we did find that 5 ml. aliquots of 40% sugar solutions having different saponin content gave color intensities according to the amount of saponin present. However, all colors were darker than those produced with the isolated saponins. It is probable that reducing sugars would interfere in such a simple scheme.

These are all very preliminary visual observations. As yet we have made no colorimetric measurements to check reproducibility. So we do not offer a new method for floc determinations, but rather, the hope that a simple system can be devised that will lend itself more readily to rapid routine evaluations.

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Dual Laboratory Continuous Dorr System First Carbonation Apparatus—F. G. EIS[1] A laboratory continuous Dorr system first carbonation apparatus developed by Dr. R. A. McGinnis has been described in previous publications (2) (3) (4)[2]. Results of unquestionable significance obtained with the use of such apparatuses have been reported (1) (2) (4).

Various European investigators have preferred using dual carbonators. The determination of the effects of carbonation variables on processing using a single unit requires special procedures for assurance of uniformity of the raw materials being treated. Raw juice is known to be susceptible to changes during retention which have an influence on processing. Increased numbers of tests are often required to compensate for the variability of raw juice when using a single carbonation unit.

Even though the fundamental effects of carbonation variables are well known, at times it is desirable to check the effects of these variables as the processing characteristics of beets are subject to changes. Beets with abnormal processing characteristics sometimes cause operating difficulties and speed in obtaining data is essential for checking the effect of variables to assure factory operation at optimum conditions.

In order to obtain data in as short an elapsed time as possible and to bypass the effects of changes in composition of raw materials, a dual laboratory continuous first carbonation apparatus was constructed. Each unit was built to the design of the original tested apparatus and the units constructed to operate in parallel with separate control of any desired operating variable.

Parallel operations allow a direct comparison of carbonation effluents and a direct measure of the effect of the variable under investigation. Possible inherent differences between the two units, even though constructed as nearly alike as possible, can be compensated for by alternating the test variable by units.

The time required to reach steady state conditions is normally an appreciable part of the total time required for a test. In determining the effect of a variable, a dual unit allows an apprec-

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[2] Numbers in parentheses refer to literature cited.

iable savings in elapsed time as well as man-hours since one man is required for operation of a single unit but can operate the dual unit without difficulty.

The results of a test on the effect of the point of lime addition to first carbonation can be used to illustrate the performance of the dual carbonation unit.

The apparatus was set up in the factory with raw juice, saccharate milk, and carbon dioxide supplies common to both units. The units were adjusted to operate at a recirculation ratio of 8 to 1 with an equivalent of 2% CaO in first carbonation effluent. Carbonation at 80°C was controlled at an alkalinity of 0.085% CaO. Saccharate milk was fed into the secondary carbonation tank, the gassing tank, of one unit in the normal manner and into the primary tank of the other unit. Samples were taken for analysis after reaching steady state conditions. Settling tests were made on the first carbonation effluent by the Dorr-Kynch method as described by Talmage and Fitch (5). The lime salts and color of thin juice were determined after a batch second carbonation with gassing at the boiling point for three minutes followed by five minutes of boiling. The point of lime addition was reversed between units after the first two samples were taken.

The data indicate that the point of lime addition has a major effect on the results of first carbonation. Addition of lime to the primary carbonation tank rather than the secondary, decreases the settling rate of the first carbonation sludge, and causes an increase in the lime salts and a decrease in the color of thin juice at equal Dorr retention periods.

It is interesting to note that the data on lime salts would not have been considered statistically different at the 95% level of

Effect of point of lime addition in first carbonation

Sample No.	Point of lime addition	Settling capacity lbs. solids/sq. ft/hr	Thin juice	
			Lime salts CaO/100 rds	Color 100 (-log T_D) 10 rds, 5 cm cell
1	Primary	13	.221	40
	Secondary	40	.215	47
2	Primary	14	.242	37
	Secondary	56	.178	40
3	Primary	15	.192	37
	Secondary	40	.178	44
4	Primary	15	.254	32
	Secondary	38	.228	50
5	Primary	16	.290	38
	Secondary	40	.237	46
Average	Primary	15	.240	37
	Secondary	43	.207	45

confidence if the samples could not have been paired for statistical analysis. The difference between averages is 0.033 while the LSD_{95} is 0.038 without inherent pairing.

The results of the test reported illustrate the reason for the Dorr Company's choice of the point of lime addition to carbonation. Data were not recorded for rates of filtration but it was observed that filtration of both first and second carbonation juices was decreased by lime addition to the primary rather than the secondary carbonation tank.

The dual laboratory carbonation unit has been found to be highly satisfactory. Its use has allowed a significant savings in elapsed time and man-hours required for testing. Statistically significant results are more readily obtained on factory feed materials since the effects of changes in composition of the feed are minimized.

Ratio of values: lime addition to primary tank/secondary tank

Sample	Settling capacity	Lime salts	Color
1	.33	1.03	.85
2	.25	1.36	.93
3	.38	1.08	.84
4	.39	1.11	.64
5	.40	1.22	.83
Ratio Average	.35	1.16	.82
Difference of ratio from 1.0	.65	0.16	.18
LDS_{95} from ratio of 1.0	.06	0.13	0.10

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Wet Screening of Sugar Crystals from Low Purity Massecuites and Sugars—ROBERT R. WEST AND ROBERT S. GADDIE[1]

In any program involving raw sugar boiling improvement (1)[2], it is of great advantage to be able to make routine determination of size and degree of uniformity of sugar crystals in samples of

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[2] Numbers in parentheses refer to literature cited.

sugars and massecuites. Saint and Trott (3), working with raw cane sugar, developed a wet screening method which, with suitable modifications, can be applied to low purity beet house products containing very small crystals.

The test consists of successive washings of the sample with an ethyl alcohol-water solution saturated with sugar at the temperature of the test, before transferring the washed crystals to the top sieve of the selected series.

Pre-treatment Before Screening

High Raw Sugar

Sufficient sample to yield 8 to 10 g of final dried crystals is placed in an evaporating dish and 20 to 30 ml of 90% sugar-saturated alcohol is added. A rubber policeman is used to break up all lumps and mingle the sugar thoroughly with the alcohol so that each crystal is separated and washed by the alcohol. The alcohol is carefully decanted and the washing repeated with a second 20 to 30 ml portion of the 90% alcohol. Usually two washings are sufficient, but if the syrup film on the crystals is not completely removed, a third may be used. A final washing is made with sugar-saturated undiluted alcohol.

High Raw Massecuite, Low Raw Massecuite as Spun and Low Raw Sugar

Sufficient sample to yield 8 to 10 g of the final dried crystals is washed as above with successive portions of 20 to 30 ml of 80% sugar-saturated alcohol until no further extraction of color into the alcohol is observed. At least one washing with 90% alcohol is performed with a final washing with undiluted sugar-saturated alcohol.

Low Raw Massecuite as Dropped from the Pan

One washing with 80% alcohol which has been heated to 60°-65°C and saturated with sugar at that temperature is required. To the hot sample (sufficient to yield 6 to 8 g of final dried sugar crystals) direct from the pan is added 20 to 30 ml of the hot 80% alcohol. The mixture is mingled thoroughly with the rubber policeman and decanted as soon is generally sufficient to render the massecuite amenable to further washings with room temperature sugar-saturated 80%, then 90%, and finally undiluted alcohol.

The washed sugar in each case is transferred to the top sieve of the selected series of tared 3-inch Tyler stainless steel sieves immersed in undiluted sugar-saturated alcohol in a 3½ inch cylinder fitted with a gasketed, bolted cover. The cylinder is placed in a Tyler Ro-tap shaker (115 to 120 TPM) and

shaken for 30 minutes. If the washing has been performed properly, this time will be sufficient for all materials. It is important that the alcohol in the retainer is sugar-saturated at the temperature at which the sample will be *shaken*, as the long shaking period results in the retainer and its contents assuming the ambient temperature of the shaker and its surroundings. After shaking, the sieves are removed, separated, and after being allowed to drain, dried with their contents at 105°-110°C for 20 minutes. The screens are cooled, weighed, individual fractions added to obtain a total weight, and the percentage retained on each screen calculated. Generally we express size and uniformity of grain by the Powers method (2)—that is, in terms of "Mean Aperture" (M.A.) and "Coefficient of Variation" (CV).

Notes

1. In making dilutions of alcohol with water, the water normally present in the alcohol is ignored; the 90 + 10 and 80 + 20 dilutions are made volumetrically.

2. The alcohol-water solutions, except in the case of the hot solution used for the first washing of low raw massecuite from the pan, must be saturated with sucrose at the temperature of the area where the washings and other manipulations will be performed.

3. If at any time changing from one alcohol concentration to a higher concentration causes the sugar to ball together and refuse to disperse, it indicates that the preliminary washing has not been sufficient, and it is necessary to rewash with the lower concentration of alcohol.

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Process Liquor Color Determination in the Sugar Factory Control Laboratory—ROBERT R. WEST AND ROBERT S. GADDIE[1]

There are many methods used in control laboratories in the sugar industry for routine determination of color in process juices. We have tried several over the years, but none has been

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wholly satisfactory. Such a test, to be useful, particularly on dark-colored juices, should accomplish the following:

1. The test should give reasonably accurate results and good reproducibility. This involves a photo-colorimeter, preferably a spectrophotometer.

2. The instrument must be simple to operate, rugged, and not too costly.

3. The test must be quick and simple to perform.

4. If results are to be useful, the color indexes must be equated to constant RDS.

The instrument chosen was the Bausch and Lomb Spectronic "20" spectrophotometer, a grating-dispersion instrument of constant band-width which had been in use for some time in several of the factory laboratories and had proven to be accurate and reliable. To determine the feasibility of such a procedure, we determined first, the absorbance curve of representative standard liquors from 350 to 850 mu; second, the conformity with Beers Law at numerous points on the curve; and third, the reliability of a table which was to be computed to convert observed colors at any RDS to equivalent color values at a constant RDS.

The absorbance curve showed a plateau of high absorbance in the ultraviolet, falling rapidly to a valley between 550 and 675 mu, then rising again to a sharp peak at 800 mu. Wave lengths in the 775 to 825 mu (red) region did not closely follow Beers Law, but at 440 in the blue region a point was found where divergence from Beers Law over a concentration range of 5:1 was in the order of only 2%. The color density was varied by diluting standard liquors with liquid sugars of equivalent RDS. RDS was varied by simple dilution with distilled water on a weight basis (verified by refractometer). As a result of checking 5 different standard liquor colors at three different densities, it was decided that a table correcting for RDS variations could be calculated with adequate accuracy. The table, correcting all observed colors to 70 RDS was then prepared.

If readings were to be made on the absorbance or optical density scale, it would only be necessary to multiply the reading by a factor $\frac{70}{\text{obs RDS}}$ to correct the absorbance to what it would be if that same sample had been at 70 RDS. In view of the fact that the absorbance scale is logarithmic and necessarily has non-uniform divisions and subdivisions, we decided to use the % Transmittance scale which is linear in calibration and therefore much less subject to misreading by an inexperienced operator.

The table actually converts the %T to a color number which represents (absorbance × 100) of the sample corrected to a standard RDS.

The change to refractometer control for purities made available a 1-normal solution of process liquors, and these solutions are used in the analysis. Excess turbidity in the sample, as might be expected, gives color values which are too high, but the error for factory control purposes usually is not serious.

The procedure established for the factory laboratories is as follows:

The determination is made on the 1-normal solution (26 grams of syrup made up to 100 ml.) prepared for purity and RDS determination. The color is determined using the Spectronic "20" equipped with the blue-sensitive photo cell and matched ¾" test tubes. The %T at 440 mu is observed relative to distilled water. Using the %T of the 1-normal syrup and the RDS of the undiluted syrup, the color index is obtained from a table. This color index is directly comparable with any other regardless of the original RDS of the sample. The observation must be made at the time RDS and polarization are being determined, as standing for long periods in the diluted state will cause an appreciable alteration in the readings.

Insecticide Residue in Sugar Beet By-Products—J. R. Johnson and S. E. Bichsel[1] Interest has mounted rapidly in the past few years in what can be termed side effects or long time effects from the increasing use of standard and new or experimental pesticides, fungicides and herbicides that are used on agricultural field crops resulting in a residual carryover into foods for human consumption. The chlorinated hydrocarbon, DDT, has been given a tolerance of essentially zero in milk by the Food and Drug Administration.

Alarmingly high amounts of DDT have been found in milk in some isolated areas. The source of DDT was traced to alfalfa feed which had been exposed to aerial spraying either directly or by wind drift. This finding focused attention on all livestock feed in that particular area. Other incidents of a similar nature have made it expedient to know something about the possible level of DDT in dried beet pulp.

The USDA and The Amalgamated Sugar Company, agronomy section, at Twin Falls undertook a series of tests to determine the level of DDT in beet roots grown in soil treated prior to planting with an exceptionally heavy application of DDT amounting upwards to 400 pounds of 5% dust, or 20 pounds

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active DDT per acre. The roots and foliage were analyzed after harvest for DDT and were found to contain less than 0.2 ppm DDT which was the lower limit of accuracy of the method employed.

DDT is now registered for use as a soil treatment on sugar beets. However, it is often used for other crops and is known to remain active for several years in soil and will build up due to repeated treatment.

Since DDT was not positively found in the roots, it is logical to assume that there would be a small chance at best for any DDT to survive processing and be carried over into pulp or sugar itself. However, unless the product as sold, pulp in this case, has been tested for DDT it is impossible to state that its presence is negative. Consequently, as a precaution, we have adopted the policy of analyzing weekly composites of all dried molasses beet pulp produced at each of our three pulp driers.

There is no simple analytical method available for the positive quantitative estimation of DDT in ranges of less than 1.0 ppm. We will not attempt to prescribe any particular method at this time. Instead, an attempt will be made to point out a few of the aspects of several methods which may be used to estimate the level of DDT contamination in dried pulp if any is present.

Extraction, cleanup and concentration procedures are common to any method chosen. In the case of dried molasses beet pulp we have adopted the procedure of extracting 50 grams of pulp with 400 mls of U.S.P. chloroform using a Waring blender. The blender is operated through a Power-stat in order to control the apparatus to slow speeds. Mixing is started and stopped on 30 second intervals for a total mixing time of two minutes.

Chloroform is a satisfactory extracting solvent for DDT, hexane, benzene and benzene-acetone or alcohol mixtures are also recommended in the literature.

After extracting, filtering and washing the pulp, the solvent volume is quite large and must be evaporated to a volume of approximately 25 mls. The solvent is then transferred to a 50 ml glass stoppered flask and made to volume with n-hexane.

The cleanup procedure is designed to remove fats, waxes, moisture and any other material soluble in the solvent which may interfere with the subsequent determination. A florisil column topped with anhydrous sodium sulfate is recommended for DDT. The column is first pre-wetted with n-hexane. An aliquot of the DDT suspected solution is added to the column. The column is then eluted with n-hexane at a rate of 8 to 10 ml per minute. 250 mls of n-hexane is sufficient to elute the column thoroughly.

If the colorimetric method of Stiff and Castillo (1) is to be used, treatment of the eluate consists only of evaporation to 1 to 2 mls, transferring to a graduated test tube and further evaporation to dryness with an air stream at room temperature prior to further handling.

If the DDT is to be determined by paper chromatographic procedures, additional cleanup with an acetonitrile extraction is required. This step is necessary in order to yield an essentially pure compound for the chromatogram.

In general the cleanup procedures required for any type of chlorinated hydrocarbon assay for trace quantities is the most important part of the analysis. Plant materials contain a large number of organic compounds all more or less soluble in the solvents used for extraction. In many cases these compounds are present in larger amounts than the pesticide residue sought. For this reason, it is extremely difficult to develop specific methods that are accurate to less than 1 ppm.

After extraction, cleanup and concentration there are several courses open for the analysis of DDT or other chlorinated hydrocarbons. Three will be briefly mentioned in this report.

1. Colorimetric Method.

The only colorimetric method we have used is a modification of the Stiff and Castillo (4)[2] method which is specific for DDT and one of the analogs of DDT. The p,p'-DDT yields slightly more color than o,p'-DDT. Color development depends upon the reaction between DDT and the xanthydrolpyridine - KOH reagent. This reaction is negative for DDT. The lower limit of accuracy is about 0.2 ppm.

This method is satisfactory for control purposes where only four or five determinations a week are required. It will take one technician approximately two days a week for the analysis.

2. Paper Chromatographic Method (2) (3).

In order to prepare a concentrated extract for paper chromatography, the cleanup procedures are somewhat more elaborate. The chlorinated hydrocarbon must be essentially free of other organic materials in order to evaluate the developed chromatogram.

Chromatographic procedures enable us to determine quantitatively some thirteen pesticides if required. Modifications and the proper technique can be extended to the identification of some 114 chlorinated organic pesticides (3). Here again the lower limit of accuracy is in the range of 0.2 ppm.

[2] Numbers in parentheses refer to references.

3. Gas Chromatography.

Gas Chromatography is being developed rapidly for the determination of pesticides. Recent equipment modifications and the development of improved sensing components has made it possible to screen a large number of pesticide residues. The extraction procedure is designed for a catch-all type reaction and cleanup procedures if necessary at all can be rather crude.

Gas chromatographic equipment however is expensive and may not be used extensively by sugar factory laboratories for some time to come.

The following is a list of references which point to the interest and mass of work being done in the pesticide residue field. Many others can be found in 1960 and 1961 issues of Ag. and Food Chem.

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