Rapid Digestion Procedures for Determination of Metallic Ions and Total Nitrogen in Sugarbeet Samples[1]

GRACE W. MAAG[2]

Received for publication May 7, 1968

Introduction

Chemical digestion of sugarbeet material is always a problem because of the high sugar content involved. The sugar causes excessive foaming and charring and, consequently, the digestion is usually a long, tedious process. When there are many samples to be analyzed, digestion time can become an important factor.

This discussion is limited to digestion of sugarbeet samples for atomic absorption spectrophotometric analysis and for total nitrogen determinations. It should be noted, however, that the digested samples which result from the procedures can be used sometimes for other analyses. Atomic absorption spectrophotometry is an accurate and highly sensitive method for determination of many metallic ions. For this analysis, samples must be in a solution that can be easily aspirated through the sample tube of the burner-atomizer and that has the fewest interfering ions possible. Total nitrogen determinations are important in purity, quality and genetic studies; therefore, a reliable rapid digestion procedure for analysis of micro-samples is desirable.

Digestion for Atomic Absorption Analyses

Most atomic absorption spectrophotometric procedures suggest digestion of samples with nitric acid (10)[3] or a mixture of nitric and perchloric acids (1,6). Sometimes a mixture of nitric, perchloric, and sulfuric acids is used (5). We have found that nitric acid, if used alone, is unsatisfactory for sugarbeet samples because of excessive foaming and incomplete digestion. The nitric-perchloric acid mixture will eventually give complete digestion; but several additions of the acid mixture to the digesting sample must be made before complete digestion takes place. Both acids are quite volatile and decompose upon heating. This

[1] Contribution by the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, in cooperation with the Colorado Agricultural Experiment Station and the Beet Sugar Development Foundation. Publication approved by the Director, Colorado Agricultural Experiment Station, as Scientific Series Paper No. 1290.

[2] Research Chemist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Fort Collins, Colorado.

[3] Numbers in parentheses refer to literature cited.

procedure is also dangerous. If the mixture is allowed to boil down too far, a violent explosion can result through dehydration and decomposition of some of the components.

A mixture of nitric, perchloric and sulfuric acids is much safer to use. Since sulfuric acid is more stable at higher temperatures, its presence helps prevent dehydration which may cause the formation of the explosive compounds during the digestion. Again, this mixture requires at least 1 hour for complete digestion, and usually requires addition of more acid during the process.

Bolin and Stamberg (3) suggested the use of a digestion mixture of perchloric and sulfuric acids with some molybdenum added as a catalyst for determination of phosphorus. The presence of the molybdenum markedly increases the rate of oxidation of organic matter. This mixture is satisfactory for digestion of many samples, but in atomic absorption analyses, the high sulfate ion content that results sometimes causes interference. Bolin and Stamberg suggested the following proportions:

30 g sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 150 ml H_2O
150 ml concentrated sulfuric acid (H_2SO_4)
200 ml of 70 - 72% perchloric acid ($HClO_4$)

This procedure was modified for atomic absorption sample preparation to decrease the sulfate ion content (8). We use the following combination:

350 ml 70 - 72% perchloric acid ($HClO_4$)
100 ml Conc. sulfuric acid (H_2SO_4)
1500 ml Conc. nitric acid (HNO_3)
2 g sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 100 ml H_2O

The amount of sodium molybdate suggested by Bolin and Stamberg was reduced to decrease the light scattering that results from an intense sodium flame. Excess scattering may decrease the sensitivity in atomic absorption analysis for other cations.

Sugarbeet thin juice samples, prepared according to Carruthers and Oldfield (4), may be read spectrophotometrically without digestion. Pressed juice and dried leaf and petiole samples must be digested. The ratio of the digestion mixture to the amount of sample may be adjusted as required. The procedure we use is as follows:

To 0.5 g of finely ground dried leaf or petiole sample in a pyrex digestion tube, add 5 ml of the acid digestion mixture. If this mixture is allowed to stand overnight, foaming is reduced considerably when first heated. Heat at medium temperature on a digestion rack. We use an electric rotary digestion rack. The sample will boil with some foaming and charring. Then a rapid stage of oxidation takes place, during which the solid material disappears and the sample becomes colorless. After this

reaction, continue heating at a high temperature until the sulfuric acid begins to reflux up the tube. This refluxing action digests any sample particles remaining on the sides of the tube. A total of about 25 minutes is required for the complete digestion. Cool. Dilute the sample to 25 ml. If necessary, further dilution of the sample is made if the concentration of the metallic ions is not within the sensitivity range of the atomic absorption spectrophotometer. In some determinations, additions of other reagents may be necessary as indicated in methods outlined in the procedure for atomic absorption analysis for a particular cation. For example, in calcium determinations (7), lanthanum chloride is added to mask interference by phosphorus and aluminum ions which may be present. Also, the sulfate ion concentration in the diluted sample and in the standard solution should be about 1%. All samples are read in comparison with standard solutions on an atomic absorption spectrophotometer.

This digestion procedure is satisfactory for atomic absorption analysis for various cations, except sodium and molybdenum. The presence of molybdenum causes no interference in any of the analyses we have made.

Digestion for Total Nitrogen

Total nitrogen determinations are time-consuming and difficult, especially when it is desirable to determine the nitrate nitrogen along with other nitrogen present.

Many variations of the Kjeldahl procedure have been used. The procedure which we have found to be the most satisfactory, and which will also determine nitrate nitrogen, is described in the Association of Official Agricultural Chemist's "Methods of Analysis" (2). This procedure uses salicylic acid in concentrated sulfuric acid along with sodium thiosulfate. Copper and potassium sulfate (Kel Pak) are added as catalysts and to raise the boiling point. The total digestion time required for this digestion of dried plant or juice samples is about 4 to 5 hours. The nitrate reacts with the salicylic acid in the presence of strong acids and, thus, is eventually converted to the ammonium ion in the digestion process.

The proposed digestion mixture given here still maintains the use of salicylic acid along with concentrated sulfuric acid. In addition, perchloric acid is added as a strong oxidant and sodium molybdate as a catalyst. The reagents are made up as follows (Reagent I should be made fresh just before use.):

Reagent I
150 ml conc. H_2SO_4
25 g salicylic acid
Mix well
Add 200 ml 70 - 72% $HClO_4$

Reagent II
10 g $Na_2MoO_4 \cdot 2\ H_2O$ in 150 ml H_2O

In digesting thin juice samples, place 0.5 ml thin juice in a 50 ml-calibrated pyrex digestion tube or micro-Kjeldahl flask. Boiling stones are added to prevent bumping. Add 1.5 ml of Reagent I and allow to stand overnight to let the nitrate react with the salicylic acid. Just before digestion, add 1.0 ml of Reagent II (sodium molybdate solution). Place on a digestion rack and heat at medium temperature. After initial foaming and charring, the samples go through a rapid stage of oxidation during which the solid matter disappears and the mixture becomes colorless. Turn to high heat until the sulfuric acid refluxes up the sides of the tube. This will digest any remaining sample particles on the sides of the tube, and drive off excess hydrogen chloride and other volatile components which may interfere with the color formation if direct nesslerization is used later. After digestion, the samples may be read by direct nesslerization, by nesslerization of an aliquot of the digested sample, or by a steam distillation method. If nesslerization is used on aliquots of the digested sample, or if a steam distillation method is utilized in reading the sample, larger amounts of sample and digestion mixture can be used.

The direct nesslerization procedure involves, first, adding about 15 ml of distilled water to the cooled, digested sample in the calibrated digestion tube. Some of the excess acid is neutralized by the addition of 2 to 3 ml of 10 percent sodium hydroxide. Allow this mixture to cool. Add 1 ml of 2% gum ghatti solution to aid in stabilization of the colloidal solution when it is formed later. Using a pipet, blow in 12 to 14 ml Nessler's solution. Make up to 50 ml with distilled water. Mix well and read at 490 $m\mu$ on a spectrophotometer. The amount of sodium hydroxide and Nessler's solution should be adjusted to the samples being analyzed. Some types of samples may require more acid in digestion than others. The Nessler's solution must be sufficient for the colored colloidal compound of dimercuric ammonium iodide to form in ratio to the ammonium radical ion present. The pH of the resulting solution should be about 12. If the pH is too low, a red precipitate will form. If the pH is too high, the resulting solution may become cloudy. Cloudiness may also result if the Nessler's solution is not mixed thoroughly as it is added to the sample.

High concentration of contaminates, such as silicates or soluble salts, may also cause precipitation of the colloidal compound. For this reason, it is difficult to read digested leaf or petiole samples by direct nesslerization. However, these samples

may be read by the nesslerization method if an aliquot of the sample is used. To do this, dilute the digested sample to 50 ml with water. After thorough mixing, measure a 2 ml-aliquot into a second 50 ml-calibrated tube. Add about 15 ml of water. With a pipet, blow in 5 ml of Nessler's solution. Dilute to 50 ml, mix well, and read at 490 mμ as above. The dilution factor must be included in calculating the amount of nitrogen in the original sample. The colloidal solution resulting from nesslerization, if properly prepared, should be golden to reddish-brown in color and sparkling clear.

A steam distillation method may be used, if preferred, when a high concentration of interfering ions are present in the digested sample. It is then preferable to digest the samples in micro-Kjeldahl flasks which can be used later with the steam distillation apparatus. After digestion, the sides of the cooled sample flask are washed down with about 10 ml of distilled water. Connect the flask to the steam distillation apparatus and then add sufficient 40 percent sodium hydroxide to more than neutralize the acid present. The ammonia, which is driven off by steam distillation, can be collected in Nessler's solution and read as above. We prefer collecting the ammonia in a 2 percent boric acid solution which contains bromcresol green indicator, and titrating the resulting solution with 0.0143 N sulfuric acid (9). Each ml of the 0.0143 N sulfuric acid used is equivalent to 0.2 mg nitrogen in the sample.

Table 1 shows the average amount of total nitrogen, in mg per 100 ml thin juice, obtained on three thin juice samples. Each thin juice sample was analyzed three ways as follows: 1) Duplicate 0.5 ml portions of each sample were digested with 1.5 ml of the proposed digestion mixture (H_2SO_4, $HClO_4$, salicylic acid and Na_2MoO_4) and read by direct nesslerization. The amount of sample and reagents added is kept to a minimum here because direct nesslerization is used for reading after digestion. 2) Duplicate 1.0 ml portions were digested with 5.0 ml of the proposed digestion mixture and read by the steam distillation-boric acid-sulfuric acid method. 3) Duplicate 1.0 ml portions were digested with 5.0 ml of the standard AOAC digestion mixture (H_2SO_4, salicylic acid, $Na_2S_2O_3$, and Kel Pak) and read by the steam distillation-boric acid-sulfuric acid method.

Thin juice samples were also run with known amounts of nitrate nitrogen added to the samples. The check results on these tests samples were satisfactory.

When analyzing pressed juice samples, best results are obtained when at least 5.0 ml of the proposed digestion mixture are used for each ml of pressed juice. Again, three pressed juice samples were analyzed using the proposed digestion mixture.

Table 1.—Mg of nitrogen per 100 ml thin juice obtained on three samples analyzed by three methods.

Sample	Method I	Method II	Method III
	Proposed digestion mixture		AOAC digestion mixture
	Direct nesslerization	Steam distillation	Steam distillation
1	18.38 mg N/100 ml	19.00 mg N/100 ml	18.88 mg N/100 ml
2	14.15	14.00	13.95
3	61.75	61.90	61.80

The results were checked against results obtained on the same three samples digested with the AOAC digestion mixture. The steam distillation-boric acid-sulfuric acid method was used in each case to read the sample after digestion. Table 2 shows results of these samples with the total nitrogen given in mg nitrogen per 100 ml pressed juice.

Table 2.—Mg of nitrogen per 100 ml pressed juice obtained on three samples digested by two methods.

Sample	Proposed digestion mixture	AOAC digestion mixture
1	82.25 mg N/100 ml	82.25 mg N/100 ml
2	153.00	153.25
3	61.00	60.60

Finely-ground dried leaf or petiole samples may also be digested with the sulfuric-perchloric-salicylic acid mixture with sodium molybdate added as a catalyst. Again, it is best to allow the samples to stand overnight after addition of the acid digestion mixture to allow the nitrate to react with the salicylic acid and to reduce foaming. Best results were obtained when 6 to 10 ml of the digestion mixture were used with 0.2 gram dried sample. Just before digestion 1.0 ml of the sodium molybdate solution is added. The digestion procedure is carried out the same as with thin and pressed juice samples.

Table 3 gives the mg nitrogen per 100 g sample for a single dried leaf sample. Four 0.2 g samples were digested using 10 ml of the proposed digestion mixture, and four 0.2 g samples were digested using 6 ml of the AOAC digestion mixture. All were read by the steam distillation method in which the ammonia was collected in weak boric acid solution and titrated with 0.0143 N sulfuric acid.

Table 3.—Mg nitrogen per 100 g dried leaf sample obtained in four duplicate analyses by two digestion methods on the same sample.

Sample	Proposed digestion mixture	AOAC digestion mixture
1	4650 mg N/100 g	4670 mg N/100 g
2	4680	4680
3	4620	4700
4	4670	4660

Summary

Good results have been obtained using new, faster digestion procedures for analysis of sugarbeet samples. The digestion mixture for sample digestion for atomic absorption spectrophotometry is made of concentrated nitric, perchloric, and sulfuric acids. A small amount of sodium molybdate is added as a catalyst. For total nitrogen determinations, a mixture of concentrated sulfuric and perchloric acids are used with the addition of some salicylic acid to aid in conversion of the nitrate nitrogen to the ammonium radical ion during digestion. Sodium molybdate again is added as a catalyst. The amount of digestion mixture is adjusted to the kind and amount of sample used.

Literature Cited

(1) ALLEN, J. E. 1961. The determination of zinc in agricultural materials by atomic absorption spectrophotometry. Analyst. 86: 530.

(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS "Methods of Analysis." 1955. 8th Edition, 12p.

(3) BOLIN, DONALD W. *and* OLOF E. STAMBERG. 1955. Rapid digestion method for determination of phosphorus. Industrial and Engineering Chemistry, Analytical Edition 16: 345.

(4) CARRUTHERS, A. and J. F. T. OLDFIELD. 1961. Methods for assessment of beet quality. I. Purity determination using a clarified extract from brei. International Sugar J. 63: 72-74.

(5) DAVID, D. J. 1959. Determination of calcium in plant material by atomic absorption spectrophotometry. Analyst 84: 536-545.

(6) DAVID, D. J. 1962. Atomic absorption spectrochemical analysis of plant materials with particular reference to manganese and iron. Perkin-Elmer Atomic Absorption Newsletter 9:6.

(7) DICKSON, RICHARD E. and C. M. JOHNSON. 1966. Interferences associated with the determination of calcium by atomic absorption. Applied Spectroscopy 20: 214-218.

(8) HARRISON, MERLE and CHARLES ANDRE. 1968. Digestion of sugar beet leaves for atomic absorption spectroscopy. Applied Spectroscopy. In Press.

(9) HAWK, PHILIP B., B. L. OSER and W. H. SUMMERSON. 1954. Practical Physiological Chemistry, 13th Edition. 880p.

(10) PARKER, H. E. 1963. Magnesium, calcium, and zinc in animal nutrition. Perkin-Elmer Atomic Absorption Newsletter 13:2.