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Total Available Carbohydrate in Plant Materials*

by

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Synopsis: A new method worked out at the university of Gottingen by the author for the determination of the Total available Carbohydrate in plant materials by modifying the methods adopted by Lenkeit and Becker with the principles of reducible sugars by Bertrand is discussed in this paper.

Introduction: Various methods of determining the total available carbohydrates by adding together the results of glucose, sucrose and starch are available (Loomis, *et al* 1937; A. O. A. C. 1957). Alternative to this method, estimating the percentage of reducing and non-reducing sugars (Quisumbing and Thomas, 1932; Munson and Walker, 1943) are also adopted in plant analysis. Plant materials are subjected to digestion by taka-diastase under such conditions as are necessary for the breakdown of starch, dextrin and maltose to glucose Weinmann, 1961). Various other methods of determining reducing sugars have been used in conjunction with the TAC extraction. Weinman, (1947) once employed a semi-micro copper reduction method. Many others adopted the iodometric technique of Somogyi, (1945), or Hassid's method or the colorimetric estimation with anthrone (Roe 1955). Except the colorimetric method, all other estimations of total available carbohydrate of plant materials are tedious procedures. In order to condense the method with still more accuracy, a new method was worked out at the University of Gottingen (Pillai 1962) by modifying the methods adopted by Lenkeit and Becker, (1961) with the principles of reducible sugars by Bertrand.

Materials and Methods: In this method plant samples, viz. fresh leaves, roots or grains, after treatment with alcohol, are subjected to HCl digestion of all carbohydrates to fractions of glucose.

After clarification and subsequent purification, the reducing power of the neutralized hydrolysate is determined. The reducing action of sugars is determined by means of Fehling's solution, in which, upon heating a reducing sugar converts cupric hydroxide into cuprous oxide. For exact titration values and correctness of results, the copper salts have been exchanged to ferric salts and volumetric measurements by titrations are made with 1/10-$KMnO_4$ solution.

Procedure: Place a 10 g. sample of fresh plant material or powdered sample of grains in a 250 ml Erlenmeyer flask and add 100 to 150 ml 75% alcohol, just to cover the material, mix thoroughly and attach a reflux condenser with a cork. Boil it on a water bath for 1 to 2 hours by maintaining a temperature of 70° C. Remove the reflux condenser and allow the alcohol to evaporate by maintaining the temperature of the water bath at 80° C. On evaporating the

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alcohol completely add 20 ml H_2O. if necessary. To this extract, along with the remaining materials, add 50 ml of 7·5%HCl and allow it for digestion in the water bath at a temperature of 80°C with the reflux condenser attached for a period of not less than 6 hours. At the end of this period the acid hydrolysable extract is filtered into a 250 ml volumetric flask and the residue should be washed several times with boiled distilled water till free of acid. The filtered acid extract should be neutralised with 40% NaOH and immediately make it to slightly acidic (pH around 6). Add enough (2 to 4 ml) saturated lead acitate solution to produce a flocculent precipitate, shake thoroughly and allow to stand for 15 minutes. Complete leading is to be tested with a drop of dilute potassium oxalate to form a precipitate. Dilute it to the mark with water, mix thoroughly and filter through a dry filter. Add sufficient potassium oxalate or oxalic acid to the filtrate to precipitate all the lead, again filter through a dry filter paper till deleading is completed.

To this method the following solutions are required :—

1. Dissolve 40 g. pure crystalline copper sulphate in one litre of water (Fehling's A).
2. Dissolve 200 g. Rochelle salts (Sodium-Potassium tartarate) and 150 g. Na OH (purified alkali) in water and made up to a litre (Fehling's B).
3. Dissolve 50 g. ferric sulphate in 200 ml pure conc. Sulphuric acid and then made up to one litre, by adding acid to water. This solution should be free from ferrosulphate and other reducing substances.
4. Prepare n/10 Potassium permanganate (3·1606 g. $KMnO_4$ in one litre).

Determination : Pipette out 20 ml of clarified solution (crystal clear) into 150 ml clean Erlenmeyer flask and add 20 ml each of Fehlings A and Fehlings B solutions and keeping them boiling for exactly 3 minutes on an asbestos gauze over a Bunsen burner and by carefully controlling the flame. Use a stop-watch for timings. The solution after boiling should be blue coloured ; otherwise discard and repeat by taking dilute solutions of the known volume of clarified solution.

After 3 minutes boiling, filter the hot solution at-once through a tared porcelain Gooch Crucible provided with asbestos mat or a glass filter crucible 1 G 4. Transfer and wash the precipitate of Cu_2O thoroughly with hot water (60°C). Collect the Cu_2O by dissolving them with ferric sulphate solution (3) in a separate clean Erlenmeyer flask completely, by stirring the crucible with a glass rod, using not more than 30 ml of ferric sulphate acid solution through the filter. Wash the crucible filter with water and the total solution is titrated against N/10 $KMnO_4$ solution, till the change of colour. The end point is the change of green colour of the solution into that of rose.

For calculating the content of the solution, the number of milligram copper from the titrated volume of $KMnO_4$ solution used is to be taken. From the following chemical reactions, it is calculated that 1 ml $KMnO_4$ = 6·3596 mg Cu. (Copper to Sugar table is given as calculated by Bertrand).

$$Cu_2 + Fe_2(SO_4)_3 + H_2SO_4 = 2\,CuSO_4 + H_2O + 2\,FeSO_4$$
$$2\,Fe = 2\,Cu$$
$$10\,FeSO_4 + 2\,KMnO_4 + 8\,H_2SO_4 = 5\,Fe_2(SO_4)3 + K_2SO_4 + 2\,MnSO_4 + 8\,H_2O$$
$$2\,KMnO_4 = (10\,Fe) = 10\,Cu$$
$$2\,(158{\cdot}03) = 316{\cdot}06 = 10\,(63{\cdot}57)$$
$$= 635{\cdot}7\ Cu.$$

1 ccm n/10 $KMnO_4$ = 3·1606 mg $KMnO_4$
= 6·3569 mg Cu.

Table for calculating the Glucose as per Bertrand

Cu mg	D-Glucose mg	Cu mg	D-Glucose mg
20·4	10	59·1	30
22·4	11	77·5	40
24·3	12	95·4	50
26·3	13	112·8	60
28·3	14	129·8	70
30·2	15	146·1	80
32·2	16	162·0	90
34·2	17	117·8	100
36·2	18		
38·1	19		
40·1	20		

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ANNOUNCEMENT

The 47th College day and conference for the year 1964 has been tentatively fixed to be celebrated during the month of November or December 1964 at the Agricultural College and Research Institute, Coimbatore. During the College day, the following prizes will be awarded for which the members are requested to get themselves prepared to participate. The last date for the receipt of papers for the various awards will be announced in due course.

List of Awards: (Open to members only)

1. Ramasastrulu Munagala Prize, 1964. (For the best account of original Research)
2. V. C. Vellingiri Gounder Gold medal. (For the best Research worker)
3. N. Veeraraghavalu Naidu Shield and Medal for Agricultural Botany (including Plant Physiology, Cytogenetics and Plant breeding.)
4. T. Konda Reddy Shield and Medal for Agronomy (including Agricultural Economics, Rural Sociology, Extension, Marketing and Agricultural Meteorology.)
5. O. M. Lakshminarayana Reddy Shield and Medal for plant Pathology, Entomology and Horticulture in Rotation (to be awarded to Horticulture this year.)
6. P. S. G. Ganga Naidu Medal for Sugarcane.
7. Ramakrishna Medal for Cotton.
8. Two Rolling Shields and Medals for Agricultural Extension Workers.