# APPLICATION OF POTASSIUM FERRICYANIDE METHOD FOR THE ESTIMATION OF CARBOHYDRATE IN CANE LEAVES

By K. L. KHANNA AND S. C. SEN

*(Sugarcane Research Scheme, Bihar)*

Received January 2, 1942

## *1. Introduction*

THE permanganate method of Bertrand (1923) and the iodine sulphate method of Schaffer and Hartmann (1921) for the estimation of carbohydrate in leaves are widely accepted. The principle underlying both these methods consists in the reduction of copper sulphate solution in the presence of weak alkalis the reduced copper being measured from the titration values of N/20 $KMnO_4$ or N/10 $Na_2S_2O_3$ solution. The permanganate method claims superiority due to its applicability to highly coloured solutions which often interfere with the end-point of the iodine titration although the authors during the course of their work experienced considerable difficulty in accurately defining the neutral point even in this case. Neither of the two methods, however, gives direct values for glucose, they being read from the graphs drawn with N/20 $KMnO_4$ or N/10 $Na_2S_2O_3$ solutions against mgms. of glucose, so that results obtained therefrom cannot always represent the true values. In addition the methods are time consuming, laborious and costly. Thus a method that would enable the direct estimation of glucose besides being simpler, less laborious and more economical is a desideratum.

Attempts were, therefore, made to see if the potassium ferricyanide method so succesfully used by the authors (1938) in cane juice analysis could be equally successfully employed in the estimation of glucose in cane leaves and the results obtained from experiments during the last crushing season have amply shown that this latter method besides being simpler, quicker and more economical gives excellent comparable results with the standard methods while as regards precision it is decidedly superior.

## *2. Experimental Procedure*

Method of extraction of carbohydrates in leaves is similar in all the three methods and largely follows the standard procedure namely that 10·0 gm. of sampled cane leaves are treated with 150 c.c. of boiling alcohol, the solution decanted and the plant materials are washed out with 85% alcohol into a soxhlet extractor fitted into a 250 c.c. flask three-fourth full of alcohol (with a spatula tip full of $CaCO_3$ in it). The extracter is then fitted to a condenser and the whole apparatus heated on a water-bath

for 5–6 hours. The alcohol is poured off through filter-paper into a 500 c.c. flask, the filter-paper being washed with 85% alcohol and to this is added the original decanted extract. This filtrate is cooled, made up to volume, shaken well and 50 c.c. taken out for the estimation of alcoholic soluble matter. This alcoholic extract (450 c.c. or 500 c.c. in case no solution is taken for the estimation of alcoholic soluble matter) is now heated in a vaccum flask on a water-bath at 50–55° C. and the distillation is continued until only 15 c.c. approximately of the extract remains. This residue is then washed into a beaker with hot water and 20 c.c. of saturated solution of neutral lead-acetate added and the beaker allowed to stand for an hour when it is filtered into another 500 c.c. beaker, the bottom of which is covered with potassium oxalate crystals. Lead oxalate precipitates out and the washings are continued till about 400 c.c. of the filtrate is collected in the beaker. This is allowed to stand overnight, when lead oxalate is filtered off, the clear filtrate being collected in a 500 c.c. flask, washed with cooled distilled water and made up to mark. At this stage the filtrate (A) is ready for the estimation of glucose in cane leaves.

For the estimation of disaccharides in leaves 100 c.c. of the filtrate (A) is treated with 10 gm. of citric acid and kept for 18 to 24 hours for complete hydrolysis to glucose. The solution is then treated with fairly strong caustic soda until it is slightly alkaline (using litmus paper as an indicator) and then completely neutralised with a drop or two of dilute hydrochloric acid. This solution is then made up to 200 c.c. (referred subsequently as B) and total sugars are estimated as glusose.

In both permanganate and the iodine sulphate methods 50 c.c. of the extracted solution (A or B) are boiled with 50 c.c. of copper sulphate solution spcially made for these reactions and the reduced copper thus formed is dissolved and titrated against N/20 $KMnO_4$ or N/10 $Na_2S_2O_3$, the values for glucose being obtained from appropriate graphs.

In the potassium ferricyanide method the procedure is similar to that described by the authors (*loc. cit.*) earlier and it consists in taking 5 c.c. of 1% potassium ferricyanide solution (previously standardised against extra-pure glucose solution) from burette in a 150 c.c. Erl nmeyer flask and adding to it 2 c.c. of 5% caustic potash and a drop of 1% methylene blue as an internal indicator shortly before the end-point is reached. Into this cold solution is run from the burette containing extracted solution (A or B ready for the glucose estimation) roughly half the total quantity of sugar solution required for complete titration and the flask is heated to boilng on wire-gauze over a flame. After each boiling the flame is lowered to permit of more solution being added from the burette and this process

is repeated till the entire fluid in the flask is decolourised when reading for the quantity of sugar solution used is taken. The glucose percentage can then be directly estimated as 1 c.c. of 1% potassiun ferricyanide solution is equivalent to 1 mgm. of glucose.

*3. Results Obtained*

In the standard methods glucose is estimated indirectly as pointed out above from the graphs (*loc. cit.*) which were prepared (Fig. 1) from the data

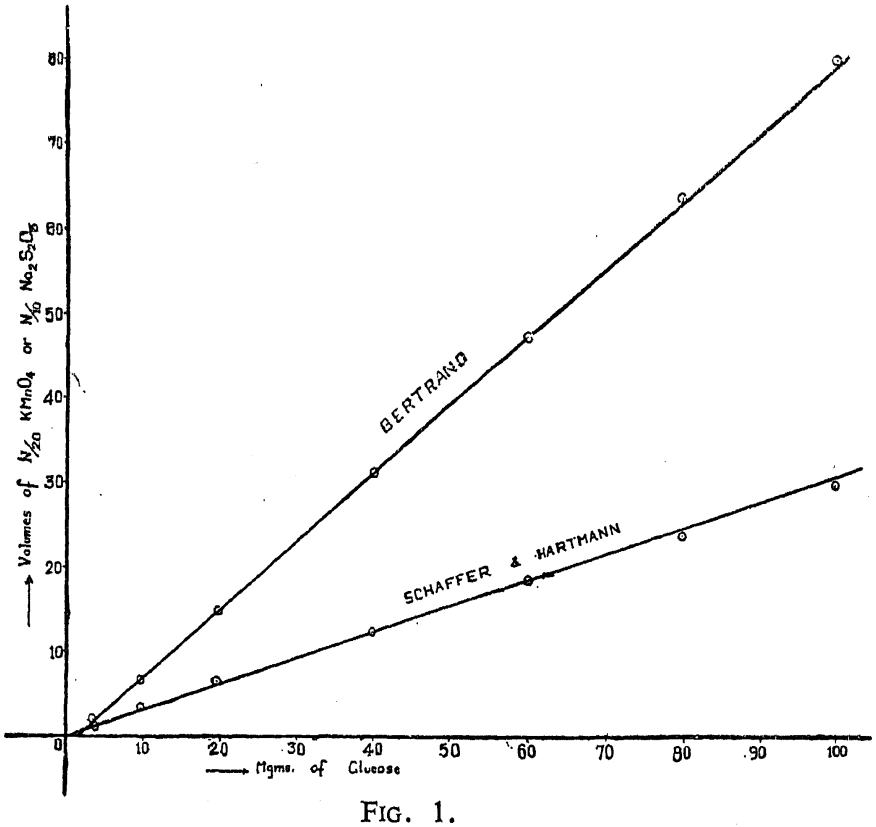


FIG. 1.

(Table I) obtained during these experiments for the estimation of glucose from the titration values.

TABLE I

| Quantities of glucose in mgm. taken for the experiment | Volume of N/20 $KMnO_4$ required for neutralisation | Volume of N/10 $Na_2S_2O_3$ required for neutralisation | Remarks |
|---|---|---|---|
| 4·0 | 1·65 | 1·10 | The mean results of 4 readings tabulated |
| 10·0 | 6·50 | 3·20 | |
| 20·0 | 14·70 | 6·30 | |
| 40·0 | 31·00 | 12·50 | |
| 60·0 | 47·00 | 18·40 | |
| 80·0 | 63·40 | 23·40 | |
| 100·0 | 80·20 | 29·40 | |

The comparative results of glucose estimation by the three methods are given in Table II. Duplicate estimations were made by each method for each of the twenty-five varieties of leaves under study.

## TABLE II

*Comparative results of Glucose Estimations by the three methods*

| Sl. No. | Leaves of the varieties | Age of the plant | Alcoholic soluble matter | Bertrand's | | | | Schaffer and Hartmann | | | | Potassium Ferricyanide | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Volume of N/20 $KMnO_4$ | | % Glucose | | Volume of N/10 $Na_2S_2O_3$ | | % Glucose | | Volume of 'A' or 'B' | | % Glucose | |
| | | | | I | II | I | II | I | II | I | II | I | II | I | II |
| | | | | | | *Glucose alone—Solution 'A'* | | | | | | | | | |
| | | days | | | | | | | | | | | | | |
| 1 | Co 213 | 360 | 9·84 | 3·5 | 3·5 | 0·787 | 0·787 | 2·1 | 2·1 | 0·738 | 0·738 | 36·3 | 37·5 | 0·759 | 0·733 |
| 2 | ,, | ,, | 9·70 | 4·5 | 4·7 | 0·845 | 0·886 | 2·2 | 2·6 | 0·833 | 0·920 | 29·8 | 30·0 | 0·926 | 0·921 |
| 3 | ,, | 120 | 8·29 | 6·4 | 6·6 | 1·111 | 1·128 | 3·2 | 3·4 | 1·111 | 1·193 | 21·8 | 21·8 | 1·261 | 1·261 |
| 4 | ,, | ,, | 8·15 | 7·9 | 8·2 | 1·300 | 1·344 | 3·9 | 4·2 | 1·383 | 1·468 | 19·75 | 19·45 | 1·395 | 1·379 |
| 5 | ,, | 50 | 6·65 | 2·9 | 3·5 | 0·616 | 0·700 | 1·9 | 2·1 | 0·667 | 0·744 | 40·0 | 40·2 | 0·687 | 0·686 |
| 6 | ,, | ,, | 7·27 | 2·5 | 3·0 | 0·561 | 0·633 | 1·7 | 1·9 | 0·633 | 0·694 | 45·0 | 44·8 | 0·611 | 0·614 |
| 7 | Co 299 | 120 | 7·83 | 10·5 | 11·0 | 1·655 | 1·722 | 4·5 | 4·7 | 1·589 | 1·667 | 17·8 | 17·4 | 1·545 | 1·581 |
| 8 | ,, | ,, | .. | 10·8 | 11·0 | 1·525 | 1·550 | 4·7 | 5·0 | 1·500 | 1·590 | 17·4 | 17·8 | 1·581 | 1·546 |
| 9 | Co 313 | 360 | 6·37 | 6·6 | 6·9 | 1·122 | 1·167 | 3·5 | 3·8 | 1·233 | 1·355 | 20·3 | 20·0 | 1·358 | 1·375 |
| 10 | ,, | ,, | 5·78 | 7·5 | 7·9 | 1·244 | 1·300 | 4·0 | 3·8 | 1·422 | 1·333 | 19·6 | 19·8 | 1·403 | 1·390 |
| 11 | ,, | 120 | 6·83 | 4·8 | 5·0 | 0·878 | 0·905 | 2·3 | 2·5 | 0·817 | 0·889 | 30·0 | 30·4 | 0·915 | 0·909 |
| 12 | ,, | ,, | .. | 4·9 | 5·0 | 0·805 | 0·815 | 2·6 | 2·7 | 0·826 | 0·864 | 29·0 | 29·2 | 0·854 | 0·848 |
| 13 | Co 356 | ,, | 6·62 | 3·4 | 3·3 | 0·683 | 0·672 | 1·8 | 2·0 | 0·639 | 0·694 | 40·8 | 40·9 | 0·674 | 0·672 |
| 14 | ,, | ,, | .. | 4·0 | 3·3 | 0·690 | 0·600 | 2·3 | 1·9 | 0·680 | 0·600 | 35·1 | 35·2 | 0·705 | 0·703 |
| 15 | Co 419 | ,, | .. | 5·3 | 5·5 | 0·852 | 0·875 | 2·6 | 2·4 | 0·830 | 0·765 | 27·0 | 28·0 | 0·900 | 0·884 |
| 16 | ,, | ,, | 6·65 | 5·2 | 4·6 | 0·933 | 0·850 | 2·35 | 2·6 | 0·822 | 0·911 | 32·2 | 31·2 | 0·852 | 0·863 |
| | | | | | | *After inversion—Solution 'B'* | | | | | | | | | |
| 17 | Co 299 | 120 | 7·83 | 7·7 | 7·4 | 2·546 | 2·467 | 3·6 | 3·3 | 2·560 | 2·330 | 21·8 | 21·3 | 2·524 | 2·584 |
| 18 | ,, | ,, | .. | 7·8 | 7·3 | 2·320 | 2·191 | 3·5 | 3·7 | 2·230 | 2·340 | 21·1 | 21·5 | 2·346 | 2·305 |
| 19 | Co 313 | 360 | 6·37 | 10·75 | 10·8 | 3·626 | 3·655 | 4·75 | 4·90 | 3·356 | 3·466 | 16·0 | 15·7 | 3·438 | 3·514 |
| 20 | ,, | ,, | 5·78 | 10·0 | 10·5 | 3·178 | 3·326 | 4·75 | 4·75 | 3·356 | 3·356 | 16·0 | 16·3 | 3·438 | 3·377 |
| 21 | ,, | 120 | 6·83 | 4·4 | 4·0 | 1·640 | 1·533 | 2·2 | 2·4 | 1·560 | 1·711 | 32·6 | 33·0 | 1·689 | 1·668 |
| 22 | ,, | ,, | .. | 2·7 | 3·0 | 1·0600 | 1·130 | 1·8 | 1·9 | 1·150 | 1·200 | 41·6 | 42·1 | 1·190 | 1·176 |
| 23 | Co 356 | ,, | 6·62 | 4·8 | 5·0 | 1·755 | 1·811 | 2·6 | 2·5 | 1·844 | 1·778 | 30·3 | 30·1 | 1·816 | 1·827 |
| 24 | Co 419 | ,, | 6·65 | 5·6 | 5·3 | 1·990 | 1·890 | 2·8 | 2·5 | 1·990 | 1·770 | 28·4 | 27·7 | 1·940 | 1·984 |
| 25 | ,, | ,, | .. | 6·3 | 6·2 | 1·950 | 1·930 | 3·2 | 3·1 | 2·040 | 1·980 | 24·6 | 24·7 | 2·013 | 2·004 |

It has been possible to eliminate the varietal differences as exhibited in their glucose contents. The following Table III gives the variances in each method due to varieties and those within a variety. The latter shows the variances due to laboratory errors.

TABLE III

| Sl. No. | Sources of error | D.F. | Permanganate method A | Thiosulphate method B | Ferricyanide method C |
|---|---|---|---|---|---|
| 1 | Mean squares between varieties | 24 | 1·3009 | 1·2359 | 1·2789 |
| 2 | Mean squares within a variety | 25 | 0·00236 | 0·00510 | ·00044 |
| 3 | Observational errors | | ± ·0486 | ±0·0714 | ±0·02095 |
| 4 | General mean 50 values of glucose % | | 1·4308 | 1·4433 | 1·4723 |
| 5 | Errors of observation as a percentage of the mean | | 3·401 | 4·949 | 1·423 |

It is apparent from the above table that the three methods do not differ in the mean values but they differ appreciably in their errors of observation. The potassium ferricyanide method gives the least error, *i.e.*, 1·423% whereas the other methods give 3·401% and 4·949% respectively. Thus as regards precision ferricyanide method is much superior to the other two standard methods.

## *4. Summary*

1. A method for the quick estimation of carbohydrates in cane leaves is described and it consists in the direct use of lead oxlate filtrate for the titration of glucose with 1% alkaline potassium ferricyanide solution.

2. This method is shown to be simpler, quicker and more economical than the standard methods of Bertrand and Schaffer and Hartmann.

3. The three methods are shown to give excellent comparable results for glucose in cane leaves while the new method gives the least observational error thus adding to the precision of results.

## *5. Acknowledgements*

The work was carried out at the Sugarcane Research Station, Bihar, financed by the Imperial Council of Agricultrual Research. The assistance rendered in the analytical work by M. Farooque is appreciated.

## REFERENCES

Bertrand, G. .. *Compt. Rend. Acad. Sci.*, 1923, **176**, 1583-87.

Khanna, K. L., and Sen, S. C. *Ind. Jour. Agric. Sci.*, 1938, **8**, 441-46.

Schaffer, P. A., and Hartmann, A. F. *Jour. Biol. Chem.*, 1921, **45**, 349-90.