XIV. THE ESTIMATION OF REDUCING SUGARS BY KENDALL'S SOLUTION AND THE CON- STRUCTION OF A TABLE INDICATING THE REDUCING POWER OF LAEVULOSE.

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While engaged in the estimation of sugars in plant tissues, we had occasion to employ Kendall's [1912] solution, consisting of copper sulphate, potassium carbonate, and salicylic acid. This was found by us to be extremely satisfactory and free from the possibility of error inherent in Fehling's solution, namely the solvent action of the hot sodium hydroxide upon the cuprous oxide. In all determinations Kendall's directions were carefully followed, but instead of estimating the copper reduced by this author's [1911] modification of the iodide method, or by the simpler routine adopted by Peters [1912], we proceeded in the following manner. The cuprous oxide was collected on asbestos in a Gooch crucible, washed with 360 cc. of hot distilled water (which quantity was found sufficient completely to remove the alkaline solution), then with a little alcohol and dried in a water oven. The crucible was placed inside a larger one and heated over a Teclu burner, as recommended by Davis and Daish [1913]. By heating in this manner for half an hour, all the cuprous oxide was converted into cupric oxide, and after allowing to cool in a desiccator for at least an hour, the crucible was weighed. The blue amphibole asbestos employed was treated with sodium hydroxide according to the well-known method of Munson and Walker.

By this method results were obtained for the reducing power of glucose, which were in close agreement with those in Kendall's table. Thus, 0.1345 g. of a sample of glucose, which had been recrystallised three times from 80 % alcohol and dried at 89°, gave 0.4745 g. of cupric oxide, the amount given in the table being 0.4743 g. This, it is worthy of note, was the first determination made by the authors by means of Kendall's solution.

Having thus satisfied ourselves that the method was reliable where pure sugars were concerned, it became necessary to ascertain whether the presence of citrates had any disturbing effect, since Davis and Daish [1913] have shown that citric acid inverts sucrose completely under conditions in which it does not hydrolyse maltose appreciably. Kendall himself tried to adapt Benedict's volumetric method to gravimetric sugar estimations, but came to the conclusion that the citrate in the solution employed, while dissolving but little cuprous oxide, had a depressing effect on the reducing power of the sugar. The following experiments show that it is not permissible to employ 2 % citric acid for the inversion of sucrose, when it is intended to estimate the products of hydrolysis by Kendall's method. Kahlbaum's sucrose from Indian cane was taken, the weight being corrected for the moisture present. The latter was determined by heating the finely powdered sugar in a closed vessel containing phosphoric oxide, the temperature being approximately 100°, till a constant weight was obtained.

A quantity of sucrose, 0.518 g., was made up to 100 cc. (solution *A*) with 2 g. of citric acid, and 20 cc. portions were heated for various times in a boiling water bath. Polarimetric readings showed that the change was not complete in fifteen minutes but was in thirty minutes.

For these observations and for the reductions the aliquot portions were made up to 25 cc. (solution *B*).

Solution <i>B</i>	CuO	
	20 min. heating	60 min. heating
20	0.3051 g.	0.3057 g.

From Kendall's table these values correspond to 0.495 g. sucrose per 100 cc. original solution. Thus it is clear that the citrate interferes seriously with the accuracy of the method. Accordingly it is advisable to employ invertase, free from maltase, for the inversion of sucrose in plant extracts since hydrochloric acid attacks laevulose and dextrose also to some extent.

Kendall's tables are constructed for glucose, invert sugar, lactose and maltose. As it was necessary for the purposes of our research to estimate laevulose with accuracy, a series of direct determinations of the reducing power of this sugar were made. In these experiments Kahlbaum's "laevulose from inulin I" was weighed out after prolonged drying in a desiccator. A portion of this very finely powdered was heated to the temperature of the water oven in a desiccating bottle connected with a tube containing phosphoric oxide, this tube being attached to an air-pump. After an hour of heating no loss of weight, whatever, was found to have occurred and the sugar was

therefore taken to be dry. The powder remained snow white throughout. Taking $[\alpha]_D^{20} = -92.5^\circ$ for laevulose, a solution was found to contain 2.4760 g. by calculation from the rotation measured in a 20 cm. tube at 16.6° and corrected to 20° . The polarimeter used gave readings to a hundredth of a degree. As 2.4851 g. of laevulose had been weighed out the agreement was considered satisfactory and the laevulose was shown to be of a high degree of purity. The weights of the laevulose and of the cupric oxide thus obtained in the reductions were employed in the calculation of an equation of the form $y = a + bx + cx^2$, by the method of least squares. In this equation y denotes the weight of oxide and x represents that of the laevulose, both in grams. It should be noted here that the constants in Kendall's equation are intended for calculations in milligrams.

Evaluation of the constants gave the following figures:

$$y = 0.0006 + 3.910x - 2.601x^2.$$

The observed and calculated values of y are recorded below.

x	y calculated	y found	Difference
0.0321	0.1234	0.1230	+0.0004
0.0400	0.1528	0.1533	-0.0005
0.0493	0.1871	0.1856	+0.0015
0.0777	0.2887	0.2914	-0.0027
0.0926	0.3404	0.3406	-0.0002
0.1285	0.4601	0.4611	-0.0010

From these figures it is clear that the equation yields values which agree fairly well with those found by direct experiment.

The accuracy is increased by the large amount of oxide obtainable by Kendall's method relatively to the amount of sugar employed. Thus the ratio CuO/laevulose varies from 3.83 to 3.58 in the above table.

Accordingly an error of 0.001 g. cupric oxide corresponds to less than 0.0003 g. laevulose. In the table which follows, the weights of oxide were calculated for every two milligrams of laevulose and the intermediate values interpolated. It may be added that for some as yet obscure reason it is not advisable to estimate laevulose by this method beyond the limits of concentration recorded in our table. Very numerous determinations were carried out at higher concentrations but the results obtained were not of such a degree of accuracy as to justify their use in constructing a table. We hope to study the point further.

In conclusion we wish to acknowledge our indebtedness to Prof. H. H. Dixon for all facilities for carrying out this work in the School of Botany, Trinity College, Dublin; the expenses were defrayed by the funds of the laboratory.

Laevulose mg.	Cupric oxide mg.	Laevulose mg.	Cupric oxide mg.	Laevulose mg.	Cupric oxide mg.
10.0	39.4	31.6	121.5	53.2	201.2
10.4	40.9	32.0	123.0	53.6	202.7
10.8	42.5	32.4	124.5	54.0	204.2
11.2	44.0	32.8	126.0	54.4	205.6
11.6	45.6	33.2	127.5	54.8	207.1
12.0	47.1	33.6	129.0	55.2	208.5
12.4	48.6	34.0	130.5	55.6	210.0
12.8	50.2	34.4	132.0	56.0	211.4
13.2	51.7	34.8	133.5	56.4	212.8
13.6	53.3	35.2	135.0	56.8	214.3
14.0	54.8	35.6	136.5	57.2	215.7
14.4	56.3	36.0	138.0	57.6	217.2
14.8	57.9	36.4	139.5	58.0	218.7
15.2	59.4	36.8	140.9	58.4	220.1
15.6	61.0	37.2	142.4	58.8	221.5
16.0	62.5	37.6	143.9	59.2	222.9
16.4	64.0	38.0	145.4	59.6	224.4
16.8	65.6	38.4	146.9	60.0	225.8
17.2	67.1	38.8	148.3	60.4	227.2
17.6	68.7	39.2	149.8	60.8	228.7
18.0	70.2	39.6	151.3	61.2	230.1
18.4	71.7	40.0	152.8	61.6	231.6
18.8	73.2	40.4	154.3	62.0	233.0
19.2	74.8	40.8	155.8	62.4	234.4
19.6	76.3	41.2	157.3	62.8	235.9
20.0	77.8	41.6	158.8	63.2	237.3
20.4	79.3	42.0	160.3	63.6	238.8
20.8	80.8	42.4	161.8	64.0	240.2
21.2	82.4	42.8	163.2	64.4	241.6
21.6	83.9	43.2	164.7	64.8	243.0
22.0	85.4	43.6	166.2	65.2	244.4
22.4	86.9	44.0	167.7	65.6	245.9
22.8	88.4	44.4	169.1	66.0	247.3
23.2	89.9	44.8	170.6	66.4	248.7
23.6	91.4	45.2	172.0	66.8	250.2
24.0	92.9	45.6	173.5	67.2	251.6
24.4	94.4	46.0	175.0	67.6	253.1
24.8	95.9	46.4	176.4	68.0	254.5
25.2	97.4	46.8	177.9	68.4	255.9
25.6	98.9	47.2	179.3	68.8	257.3
26.0	100.4	47.6	180.8	69.2	258.7
26.4	101.9	48.0	182.3	69.6	260.2
26.8	103.5	48.4	183.7	70.0	261.6
27.2	105.0	48.8	185.2	70.4	263.0
27.6	106.6	49.2	186.6	70.8	264.4
28.0	108.1	49.6	188.1	71.2	265.8
28.4	109.6	50.0	189.6	71.6	267.2
28.8	111.1	50.4	191.0	72.0	268.6
29.2	112.6	50.8	192.5	72.4	270.0
29.6	114.1	51.2	193.9	72.8	271.5
30.0	115.6	51.6	195.4	73.2	272.9
30.4	117.1	52.0	196.9	73.6	274.4
30.8	118.5	52.4	198.3	74.0	275.8
31.2	120.0	52.8	199.8	74.4	277.2

ESTIMATION OF SUGARS BY KENDALL'S SOLUTION 141

Laevulose mg.	Cupric oxide mg.	Laevulose mg.	Cupric oxide mg.	Laevulose mg.	Cupric oxide mg.
74.8	278.6	93.6	343.8	112.0	405.9
75.2	280.0	94.0	345.2	112.4	407.2
75.6	281.4	94.4	346.6	112.8	408.5
76.0	282.8	94.8	347.9	113.2	409.9
76.4	284.2	95.2	349.3	113.6	411.2
76.8	285.6	95.6	350.7	114.0	412.6
77.2	287.0	96.0	352.0	114.4	413.9
77.6	288.4	96.4	353.4	114.8	415.2
78.0	289.8	96.8	354.7	115.2	416.5
78.4	291.2	97.2	356.1	115.6	417.9
78.8	292.6	97.6	357.5	116.0	419.2
79.2	294.0	98.0	358.8	116.4	420.5
79.6	295.4	98.4	360.2	116.8	421.8
80.0	296.8	98.8	361.5	117.2	423.1
80.4	298.2	99.2	362.9	117.6	424.5
80.8	299.5	99.6	364.3	118.0	425.8
81.2	300.9	100.0	365.6	118.4	427.1
81.6	302.3	100.4	366.9	118.8	428.4
82.0	303.7	100.8	368.3	119.2	429.7
82.4	305.1	101.2	369.6	119.6	431.0
82.8	306.5	101.6	370.9	120.0	432.3
83.2	307.9	102.0	372.3	120.4	433.6
83.6	309.4	102.4	373.7	120.8	434.9
84.0	310.8	102.8	375.0	121.2	436.3
84.4	312.2	103.2	376.4	121.6	437.6
84.8	313.5	103.6	377.8	122.0	439.0
85.2	314.9	104.0	379.1	122.4	440.3
85.6	316.3	104.4	380.5	122.8	441.6
86.0	317.6	104.8	381.8	123.2	442.9
86.4	319.0	105.2	383.2	123.6	444.2
86.8	320.4	105.6	384.6	124.0	445.5
87.2	321.8	106.0	385.9	124.4	446.8
87.6	323.3	106.4	387.2	124.8	448.1
88.0	324.7	106.8	388.5	125.2	449.4
88.4	326.0	107.2	389.9	125.6	450.7
88.8	327.4	107.6	391.2	126.0	452.0
89.2	328.7	108.0	392.6	126.4	453.3
89.6	330.1	108.4	393.9	126.8	454.6
90.0	331.4	108.8	395.2	127.2	455.9
90.4	332.8	109.2	396.5	127.6	457.2
90.8	334.1	109.6	397.9	128.0	458.5
91.2	335.5	110.0	399.2	128.4	459.8
91.6	336.9	110.4	400.5	128.8	461.1
92.0	338.3	110.8	401.8	129.2	462.4
92.4	339.7	111.2	403.2	129.6	463.7
92.8	341.0	111.6	404.5	130.0	465.0
93.2	342.4				

REFERENCES.

- Davis, W. A., and Daish, A. J. (1913), *J. Agric. Sci.* 5, 437.
 Kendall, E. C. (1911), *J. Amer. Chem. Soc.* 33, 1947.
 — (1912), *J. Amer. Chem. Soc.* 34, 319.
 Peters, A. W. (1912), *J. Amer. Chem. Soc.* 34, 422.