

A PRELIMINARY STUDY OF THE CLARIFICATION OF EXTRACTS FOR ESTIMATING SUGARS IN PLANT MATERIALS.

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The accuracy of estimating sugars in plant extracts and juices is affected by a number of associated materials such as colouring matter, proteins, tannins and organic acids. These interfering constituents are removed by different clarifying agents, such as lead acetates, alumina cream and dialysed iron.* Among the clarifiers, basic and neutral lead acetates have been widely employed. They have also been subjected to some critical studies with particular reference to sugar solutions.

Bryan (*U. S. Dept. Agric. Bur. Chem. Bull.*, 1911, No. 146) first pointed out the disadvantages arising from the use of basic and neutral lead acetates interchangeably. These reagents are generally added as solutions, but Horne (*J.A.C.S.*, 1904, 26, 186) first recommended dry defecation with solid basic lead acetate. Lead salts suffer from the singular disadvantage in that they introduce volume errors through the formation of bulky precipitates. Thomas and Dutcher (*J.A.C.S.*, 1924, 46, 1662) recommended the use of mercuric nitrate, but excess of that reagent cannot be completely removed. Moreover, mercury salts possess a reducing action on sugars according to Knapp (*Z. Analyt. Chem.*, 1870, 9, 395). Rumsey (*Ind. Eng. Chem.*, 1923, 15, 270) employed sodium tungstate as a convenient clarifying agent. The use of zinc salts has been studied by Somogyi (*Proc. Soc. Expt. Biol. Med.*, 1928-29, 26, 353) and Evans (*Ann. Bot.*, 1928, 42, 1) while the present author adopted precipitations with colloidal iron. Whether by the employment of these clarifiers, all the interfering constituents such as tannins, etc., are completely removed or not, evidence is lacking. The problem is thus one which deserves close examination.

In practice, lead salts have been found to be the most efficient clarifiers. With a view, however, to overcoming the defects noted above, Davis suggested deleading the solution immediately (*Jour. Agric. Sci.*,

* In a few cases, such as that of the apple extract, there seems to be no need for any treatment (Heynes and Archbold, *Ann. Bot.*, 1928, 42, 965; Archbold and Widdowson, *Biochem. J.*, 1931, 25, 101).

1916, 8, 7) but Harris (*Ind. Eng. Chem.*, 1921, 13, 925) preferred to add the deleading agent without separating the first precipitate and before making up the volume. Apart from this, the real difficulty lies in the choice of a suitable deleading agent. Sodium phosphate has long been in vogue, but Eynon and Lane (*Jour. Soc. Chem. Ind.*, 1923, 42, 143T) found it unsatisfactory as it does not remove entirely the alkaline earth metals. Sawyer (*J.A.C.S.*, 1904, 26, 1631) recommended the use of potassium oxalate which has since been adopted by several workers including Willaman and Davison (*Jour. Agric. Res.*, 1924, 28, 479), Loomis (*Plant Physiol.*, 1927, 1, 179) and recently by Saywell and Phillips (*Ind. Eng. Chem. Anal. Edn.*, 1934, 6, 116) in their studies with pure sugar solutions. In spite of these observations, a satisfactory deleading agent is yet to be selected. A preliminary study made in this direction is presented in this communication.

EXPERIMENTAL.

Extracts from sandal and cotton leaves were used in the following experiments. Mature leaves (about 1 kg.) were first treated by the method of Davis and Daish (*Jour. Agric. Sci.*, 1912-13, 5, 437) to extract the sugars. The combined alcoholic extracts were concentrated *in vacuo*. The residue was next taken up in warm water and made up to one litre after filtering through cotton wool to remove mechanical impurities. These filtrates were slightly turbid and generally coloured.

They were then treated (in 400 c.c. lots) with basic lead acetate (prepared according to the A.O.A.C.) and neutral lead acetate (saturated solution) till no more precipitate was formed and immediately made up to volume. These were centrifuged and the clear supernatant liquids transferred to conical flasks. Aliquots of these (75 c.c.) were delead with potassium sulphate, sodium phosphate, sodium oxalate and potassium oxalate respectively. The deleaders were added as dry powders. They were next filtered through dry filters, the earlier portions of the filtrate being rejected. In the final portions, sugars were estimated, in triplicates, by the method of Bertrand and computed as glucose. Since the volume factors were identical in all cases, the divergences in result are traceable to the chemicals added. In the case of basic lead acetate, pure glucose was added to ascertain whether any of the sugar was retained by the precipitate.

TABLE I.

Comparative clarification of leaf extracts with lead acetates.

(Reducing sugars expressed as grams of glucose in 100 c.c.)

Clarified extract delead- ed with	Sandal extract clarified with		Cotton extract clarified with	
	Basic lead acetate	Neutral lead acetate	Basic lead acetate	Neutral lead acetate
Total reducing sugars as estimated				
Potassium oxalate ..	0.201	0.501	0.222	0.298
Sodium oxalate ..	0.213	0.499	0.222	0.306
Disodium phosphate	0.222	0.498	0.226	0.279
Potassium sulphate ..	0.213	0.525	0.222	0.303

TABLE II.

Clarification of cotton and sandal leaf extracts with basic lead acetate.

Clarified extract delead- ed with	Sandal			Cotton		
	Reducing sugar (in gms.)		Glucose recovered in per cent.	Reducing sugar (in gms.)		Glucose recovered in per cent.
	Originally present	After glucose addition		Originally present	After glucose addition	
	Glucose added 0.355 g.			Glucose added 0.286 g.		
Potassium oxalate ..	0.201	0.551	98.7	0.222	0.484	91.9
Sodium oxalate ..	0.213	0.545	93.5	0.221	0.468	86.0
Disodium phosphate ..	0.222	0.554	93.8	0.226	0.459	81.3
Potassium sulphate ..	0.213	0.554	96.1	0.222	0.468	86.0

The abnormally high glucose values obtained with normal lead acetate may be traceable to the fact that the clarified extracts were still coloured, while with the basic lead acetate treatments, the resulting solutions were practically colourless. It is probable that in the former case the acidity of the final extracts were responsible for the incomplete removal of the interfering constituents. When the basic

salt is employed, potassium oxalate was found to be the best deleading agent, while with the neutral one, sodium phosphate is to be preferred. Considerable differences have been noticed with these reagents with added glucose in both sandal and cotton extracts and these emphasise the necessity for a more detailed examination.

The action of different clarifying agents on the sugar content of plant extracts.—The extracts were prepared as before from cotton and sandal leaves and made up to two litres. Aliquots of these were treated with the following clarifiers, excess being suitably removed wherever necessary: (a) basic lead acetate solution (excess deleading with potassium oxalate); (b) neutral lead acetate (saturated solution, excess deleading with disodium phosphate); (c) alumina cream; (d) dialysed iron (excess iron removed through potassium sulphate); (e) mercuric nitrate in nitric acid (excess removed by sodium carbonate); (f) basic lead nitrate; and (g) control (untreated). The following determinations were carried out on the final extracts:— (1) total nitrogen by Kjeldahl-Gunning method to include nitrates following concentration of the neutralised extract, (2) free amino nitrogen by Van Slyke's method, (3) free reducing sugars reckoned as glucose, and (4) aliquots hydrolysed with dilute HCl for 5 minutes at 70° C. and sucrose determined as invert sugar after neutralisation. Bertrand's method of estimating sugars was adopted.

TABLE III.

Effect of clarifiers on the sugar present in cotton leaf extract.

(Results reported as in 100 c.c. of extract in each case.)

Analytical determinations	Control	Treatment of extract with					
		Dialysed iron	Lead acetate		Alumina cream	Basic lead nitrate	Mercuric nitrate
			Neutral	Basic			
Direct reduction (in gm. glucose)	0.461	0.361	0.311	0.306	0.306	0.334	0.197
Sucrose (in gm. invert sugar) ..	0.585	0.280	0.394	0.459	0.375	0.468	0.517
Total nitrogen in terms of c.c. N/10 acid ..	155.1	139.4	104.5	108.8	126.9	109.2*	108.2*
Free amino nitrogen in c.c. gas ..	49.0	40.5	28.5	34.5	38.9	36.0	30.0

* Corrections have been made for the amount of nitrates added.

TABLE IV.

Sandal leaf extract clarified with different clearing agents.

(Results reported as in 100 c.c. of extract in each case.)

Analytical determinations	Control	Treatment of extract with					
		Lead acetate		Alumina cream	Dialysed iron	Basic lead nitrate	Mercuric nitrate
		Neutral	Basic				
Direct reducing power in gms glucose ..	0.209	0.069	0.048	0.123	0.103	0.067	0.079
Sucrose as gms. invert sugar ..	0.360	0.146	0.128	0.248	0.246	0.189	0.268
Total nitrogen in terms of c.c. 0.1N acid ..	38.5	33.8	30.2	36.8	31.5	34.9*	36.6*
Free amino nitrogen in c.c. gas ..	16.5	4.5	3.0	12.0	9.0	5.4	6.8

* Corrections have been made for the amount of nitrates added.

It may be pointed out that basic lead acetate is the best clarifier for sandal leaf extracts while dialysed iron already in use is to be avoided when absolute values are required. From the viewpoint of removing nitrogenous compounds even basic lead acetate removes only a third of that originally present. With cotton leaves neutral lead acetate is preferable. The nitrogen left in the extract after clarification is still high.

DISCUSSION.

A study of Table I shows that basic lead acetate clarification is better than that with the neutral salt. The high sugar values obtained with the latter cannot be easily accounted for. In the case of sandal, potassium oxalate happens to be the best deleader while the high sugar values obtained following removal of lead through phosphate are difficult to explain. Whether this is due to the retention of calcium salts as suggested by Eynon and Lane (*loc. cit.*) is yet obscure. From the point of view of securing a colourless extract, basic lead acetate is the best. The above conclusions are borne out even with added glucose, the greatest per cent. recovery of which was secured when the solution was deleaded with potassium oxalate (Table II). Why the corresponding sodium salt has failed is still not clear. (In all these cases, the presence of excess of the deleading agent was ensured.) The

relation of acidity of the final extract to the interfering constituents present is under examination.

When a selection has to be made between different clarifiers, the problem is indeed complex. The study reported here relates to the elimination of nitrogenous compounds present in plant extracts. In the case of cotton leaf, where the largest portion of nitrogenous material is removed, the values obtained for sugars are not necessarily the lowest. The low sugar figures for mercuric nitrate are indeed inexplicable (Table III). The nature of the compounds removed by each one of these is probably different. Moreover, less than 50 per cent. of the amino acids is eliminated, while more than 60 per cent. of the total nitrogen appears to be retained. With sandal leaf extract (Table IV) a greater part of the amino nitrogen is removed by lead salts only. Neutral lead acetate appears to react better with cotton extracts than the basic salt. This may probably be correlated with the reaction of the original extract. Further work is in progress on the different factors involved in the elimination of interfering constituents.

SUMMARY.

1. Cotton and sandal leaves were examined with a view to ascertaining the factors affecting the accuracy of the determination of sugars present in them.

2. While sandal leaf extract is clarified best with basic lead acetate, that from cotton reacts best with neutral lead acetate.

3. The removal of excess of lead is best accomplished by potassium oxalate when the basic salt is employed and by sodium phosphate when the neutral salt is used.

4. In cotton leaf extract while a colourless solution is finally obtained with basic lead acetate, the high sugar values recorded would appear to indicate the presence of other reducing substances.

5. In the case of sandal leaf extract, even basic lead acetate does not appear to eliminate all the nitrogenous compounds. Whether the latter affect the accuracy of the sugar estimation is not clear.

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