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THE DETERMINATION OF OXALIC ACID IN PLANTS.

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SUMMARY.

A titrimetric adaptation of Myers' modification of Bau's gravimetric method for determining water and acid extractable oxalate in plant material is described.

INTRODUCTION.

A gravimetric method for determining water and acid extractable oxalate in plant material was described by Bau (1919) and modified by Myers (1947). Myers specified a temperature of 70°C. for the extraction of water and acid extractable oxalate, and the precipitation of calcium oxalate from the boiling extract at a pH of approximately 5.2. In the original method of Bau, calcium oxalate is precipitated from a cold extract at a pH of approximately 4.0.

A titrimetric method involving the double precipitation of oxalate as calcium oxalate was described by Rimington and Steyn (1933). In this method, the plant is extracted with 0.25N. HCl at 100°C. for one hour. The oxalate is precipitated from the boiling extract at an indefinite pH on the acid side of neutrality. This method is inapplicable to some plant species, due to the presence of organic matter, which is reprecipitated with the calcium Queensland pasture plants (Mathams and Sutherland, 1952).

The method described in this paper was designed for ease in handling large numbers of samples in a survey of the oxalate content of some Queensland pasture plants (Mathams and Sutherland, 1952).

It is a titrimetric method adapted from Myers' modification of the gravimetric method of Bau. The extraction at 70°C. is combined with the precipitation of calcium oxalate from a cold solution at a pH of 4.0. Temperature control at 65°C. to 75°C. is important for the quantitative extraction of oxalate with a minimum extraction of interfering organic matter. The precipitation of the calcium oxalate from a cold aliquot of this extract at a pH of 4.0 gives consistently a relatively pure, crystalline precipitate of calcium oxalate. The nature of the calcium oxalate precipitated under these conditions favours its separation by centrifuging.

Bau allows for the solubility of calcium oxalate in water used for washing the precipitate by noting the volume of wash water used. Myers found that the solubility of calcium oxalate could be neglected when the oxalate was determined by his modified method. The greater solubility of calcium oxalate in Bau's method could be due to the fine nature of the calcium oxalate crystals when oxalate is precipitated from a cold solution.

In the method described below, an alcohol ammonia mixture, in which calcium oxalate is for practical purposes completely insoluble, is used for washing the precipitate. The use of this reagent has the further advantage that its removal by heating prior to the titration by permanganate renders traces of impurity insoluble in the acid used for the titration.

REAGENTS.

(1) Approximately 0.25N. HCl made by diluting concentrated acid (S.G. 1.16) 40 times.

(2) Precipitating reagent. (To facilitate weighing, calcium acetate is substituted for calcium chloride in the "Kalkessig" reagent described by Bau).

Solution A. 96.5 g. anhydrous sodium acetate is dissolved by warming in distilled water and made to 250 ml.

Solution B. 18 g. anhydrous calcium acetate is dissolved in 250 ml. of 50 per cent. aqueous acetic acid.

Solution A is mixed with Solution B and filtered after standing in a refrigerator for 48 hours. The reagent is filtered if necessary before use. It keeps indefinitely when stored in a refrigerator.

(3) Calcium oxalate washing reagent: 240 ml. of 96 per cent. alcohol and 125 ml. of concentrated ammonia solution (S.G. 0.92) are made to 500 ml. with distilled water.

(4) Approximately 2N. H_2SO_4 made by diluting concentrated acid (S.G. 1.84) 18 times.

(5) 0.02N. potassium permanganate prepared before use from 0.1N. stock solution.

METHOD.

1. Total Oxalate.

The plant is dried sufficiently at 100°C. to permit its being finely ground in a laboratory mill. A moisture determination is made on the milled material.

The sample is weighed out to 2.50 g. and transferred to a 250 ml. volumetric flask. Approximately 220 ml. of 0.25N. HCl is added and the flask heated in a water bath at 70°C. for one hour. When cool, 0.25N. HCl is added to make 250 ml. and the contents thoroughly mixed. Approximately 10 ml. is filtered through an 11 cm. Whatman No. 2 filter paper and this

portion is rejected. A further 10 ml. is filtered through the same filter paper and 5 ml. is pipetted into a 10 ml. centrifuge tube. This is cooled to about 10°C. and 1 ml. of the precipitating reagent added with stirring. The stirring rod is washed with a few drops of water. The oxalate is precipitated overnight in a refrigerator and is then centrifuged for 5 minutes at an R.C.F. value of 1,800 (approx. 2,700 r.p.m.). The supernatant liquid is carefully poured off by inclining the tube and the drop collecting on the rim is removed with filter paper. The precipitate is dissolved in 5 ml. of 0.25N. HCl and precipitated and separated as before. To this, 5 ml. of washing reagent is added down the sides of the tube, stirred and the stirring rod washed with water. After centrifuging and separating, the tube is heated in an oven at 100°C. for 30 minutes. The dried precipitate is dissolved in 5 ml. of 2N. H₂SO₄, heated in a beaker of boiling water and titrated with 0.02N. KMnO₄ from a burette graduated to 0.02 ml. divisions. The burette should be read to the nearest 0.01 ml.

A glass rod bent at right angles, to serve as a stirring rod, facilitates the titration. If the titration is likely to exceed 3 ml. the contents should be washed into a small flask.

Calculation: Corrected for moisture, the result is expressed as per cent. anhydrous oxalic acid.

Titration in ml. \times 1.801 = per cent. anhydrous oxalic acid.

2. Water Soluble Oxalate.

In a 250 ml. volumetric flask 2.50 g. of the milled plant material is extracted with 220 ml. of distilled water at 70°C. for one hour, made to 250 ml., and 5 ml. of the filtered extract pipetted into a 10 ml. centrifuge tube. Then 0.12 ml. of concentrated HCl is added and the contents mixed, then heated at 70°C. for 20 minutes in a water bath. If a precipitate forms, this is removed by centrifuging and the supernatant liquid poured into another centrifuge tube. By centrifuging again for a few seconds with about 0.2 ml. of water, the remaining drop may be removed almost completely. The oxalate is precipitated and titrated as for total oxalate and similarly calculated.

DISCUSSION.

The accuracy of the above methods has been tested with standard oxalate solutions and with recoveries on plants containing oxalate and on plants containing no oxalate. The analyses agreed with the theoretical value within \pm 0.01 ml. titration, equivalent to 0.02 per cent. oxalic acid in the plant as analysed. This applies to concentrations of oxalic acid up to 5 per cent. The percentage error does not exceed 1 per cent. for concentrations higher than 5 per cent.

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