

This Week's Citation Classic[®]

Clegg K M. The application of the anthrone reagent to the estimation of starch in cereals. *J. Sci. Food Agr.* 7:40-4, 1956.
[Rowett Research Institute, Aberdeen, Scotland]

Thirty years ago, the analysis of starch and sugars depended on tedious volumetric and polarimetric methods. The discovery of the anthrone/sugar chromophore meant that conditions for the reaction could be standardised to provide a simple, quantitative method of analysis for starch. [The *SCI*[®] indicates that this paper has been cited in over 130 publications.]

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The development of this analytical method was an offshoot from the main topic of research for my doctoral thesis to be submitted to Aberdeen University; the practical work was carried out at the nearby Rowett Research Institute for Animal Nutrition.

I was interested primarily in the determination of the metabolisable energy content of poultry rations. The direct method of energy assessment was by feeding trials in which the energy contents of the hens' diet and of their excreta were established by bomb calorimetry; the difference between the two results is the metabolisable energy value for the quantity of diet eaten. I thought that a simpler and quicker way would be to use the chemical composition of the ration or cereal to predict the metabolisable energy from its totally assimila-

ble components, viz., the protein, lipid, and available carbohydrate components. The quantitative determination of the first two constituents presented no problem, but a quick, simple chemical method for starch determination was not available 30 years ago. However, some research workers were reporting on the colour reaction between anthrone and sugars.

Kenneth Carpenter (now professor of nutritional sciences, University of California, Berkeley) was my guide and mentor and encouraged me to devise the anthrone method, standardise the conditions, establish the accuracy, confirm the recovery of added starch, and overcome any problems from interference from refractory compounds that might be present in poultry rations.

Previously, I had been initiated in the importance of an internal standard in the microbiological analysis of B vitamins in biological materials when working at the Dunn Nutritional Laboratory, Cambridge; a similar situation existed in the anthrone method where slight variations in the environmental conditions for the reaction could affect the colour intensity, but were nullified by the use of an internal standard. The final form of the anthrone method for the estimation of starch gave a standard deviation of 2.2 percent when applied to a range of cereals and cereal by-products; it is not a precise technique but is sufficiently accurate for most applications in applied research.

Nowadays, with the introduction of enzymic methods for the analysis of starch,¹ accuracies of 0.5 percent can be obtained. However, the use of the new techniques is restricted mainly to pure research, whereas the workaday food and animal feedstuff laboratories are looking for a close approximation of starch content or for significantly large differences in their products; a simple colorimetric method suffices. It is for this reason that the anthrone method has stood the test of time. Incidentally, the combination of the development of the anthrone method and the practical work with poultry was successful for the award of my doctorate!

1. Karkalas J. An improved enzymic method for the determination of native and modified starch. *J. Sci. Food Agr.* 36:1019-27, 1985.