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Estimation of protein in potato tissue by dye binding

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A rapid, low-cost, dye-binding procedure for the determination of 'true' protein in freeze-dried potato tissue is described. The method employs CI Acid Orange 12, and it may be readily adapted for the analysis of other vegetative tissues. Good agreement with conventional analysis based on the determination of elemental protein nitrogen was obtained (r = 0·97; standard error of estimate = 0·022).

INTRODUCTION

Potato cultivars that are employed for food use typically contain 6–8% protein on a dry matter basis; and, because of the high yields of dry matter associated with this crop, it produces as much protein per unit area of land as wheat and other seed crops (Desborough & Weiser, 1974). This protein is well balanced nutritionally and it makes a significant contribution to protein intake of many populations (Woolfe, 1987). As yet, attempts by plant breeders to improve the protein content of the potatoes have been relatively ineffective, even though genotypes are available that are potentially capable of producing 16–18% protein on a dry matter basis (Desborough & Weiser, 1974).

A key reason for the lack of improvement of the protein status of the potato crop is the absence of an analytical method that is capable of accurately measuring the 'true' protein content, and yet will ensure a rapid throughput of samples at relatively low cost (Mohyuddin & Mazza, 1978; Snyder & Desborough, 1978). This is because, in common with many vegetative tissues, potatoes contain substantial amounts of non-protein nitrogenous compounds (e.g. 37–64% of the total nitrogen is present as non-protein nitrogen; Neuberger & Sanger, 1942). Hence the usual approximation of equating total nitrogen content with protein nitrogen content (as used for many seed tissues) is not valid for the analysis of potato tissue. In attempting to resolve this problem, several workers have undertaken comparisons of a variety of analytical procedures for the measurement of 'true' potato protein (Desborough, 1975; Hoff, 1975; Li & Sayre, 1975; Vigue & Li, 1975; Mohyuddin & Mazza, 1978; Snyder & Desborough, 1978; Fomicheva & Roenko, 1984). Although the conclusions from these studies are somewhat conflicting, the general consensus is that such determinations are fraught with difficulty, and that the most accurate approach is to determine the nitrogen content of the residue that is insoluble in 80% aqueous ethanol (e.g. Li & Sayre, 1975). However, Snyder and Desborough (1978) considered that, while this was a satisfactory solution, it was both expensive and time consuming. As an alternative, they proposed the use of a procedure based on that of Bradford (1976), which involves the adsorption of Coomassie Brilliant Blue G-250.

Unfortunately, Bradford's procedure is dependent upon the formation of a soluble, coloured dye–protein complex produced by the adsorption of Coomassie Brilliant Blue G-250, and so it is not ideal for the analysis of solids because it does not respond to insoluble proteins. Thus, before using this procedure for the analysis of potato powder, Snyder & Desborough (1978) found it necessary to pre-extract the protein by soaking samples in 0·5 M sodium hydroxide for 2·5 h.

Although, compared with Bradford's procedure, those dye-binding procedures that involve the formation of insoluble dye–protein complexes are less sensitive for protein estimation, they have been routinely used for the analysis of a wide range of food tissues, many of which contain insoluble proteins (Lakin, 1978). One such method, employing Orange G (CI Acid Orange 10) was introduced by Kaldy *et al.*

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(1972) for the determination of protein in fresh potato tissue, but this was found to be unsatisfactory for the analysis of potato powders (Desborough, 1975; Mohyuddin & Mazza, 1978). From our experience with these procedures, it was postulated that this difficulty was probably because of a failure to establish the correct experimental parameters before embarking on the analysis of samples. It was therefore decided to investigate this aspect. Also, in order to improve sensitivity to the amount of protein present, CI Acid Orange 12, rather than Orange G, was employed (Udy, 1971).

The strategy adopted for the experimentation (i.e. the construction of an adsorption isotherm followed by the investigation of reaction time) has already been successfully employed for several food systems, and may be readily adapted for other vegetative tissues.

MATERIALS AND METHODS

Potato sample preparation

Potato tubers were peeled, cut into 0·5 cm slices and freeze-dried. The samples were then milled (1·25 mm screen) and stored at −20°C.

Determination of protein nitrogen

In order to separate proteins from non-protein nitrogenous compounds, freeze-dried potato powder (2 g) was dispersed in 40 ml tungstic acid solution (0·5%, m/v, sodium tungstate in 0·2%, v/v, sulphuric acid) and allowed to stand for 30 min (Kent-Jones & Amos, 1967). The protein-containing residue was separated by filtration, washed on the filter with three successive 40 ml aliquots of the tungstic acid solution, and dried *in vacuo* at 75°C. Separation by means of 80% aqueous ethanol, as described by Li & Sayre (1975) and Snyder & Desborough (1978), was not employed because Vigue & Li (1975) had shown that some native potato protein fractions were soluble in this reagent.

After further drying to constant weight by storing in a desiccator over silica gel, the residue was weighed and ground to a powder with a pestle and mortar. Samples (150 mg, duplicates) of the residue were taken for nitrogen determination by means of an automated Dumas procedure (LECO Nitrogen Analyzer, LECO Corporation, St Joseph, USA), and the content of protein nitrogen in the potato powder was calculated from the analytical data.

The automated Dumas procedure was found to be more convenient and much quicker than the determination of nitrogen by the Kjeldahl procedure; and preliminary experimentation had shown good agreement between the two procedures for the determination of the total nitrogen contents of potato powders (r = 1·00 for eight samples; standard error of estimate = 0·019).

Dye-binding procedure

Dye reagent

CI Acid Orange 12 was re-crystallised from aqueous ethanol (as described by Udy, 1971) and the specified weight quantitatively dissolved in 0·2 M citric acid solution. Normally, the dye concentration was maintained at 1·6 g litre^{-1}, but a stronger solution (2 g litre^{-1}) was used in the preliminary experiment to ascertain the most appropriate working strength. (This reagent is stable for long periods when stored in a closed cupboard.)

Standard dye solution

Re-crystallised CI Acid Orange 12 (0·25 g) was dried *in vacuo* at 110°C for 24 h, quantitatively dissolved in water (100 ml), and stored in a closed cupboard. (In order to provide a check on measurements, two such solutions were employed in each series of readings.)

Basic procedure

Ground, freeze-dried potato tissue (1 g, duplicates) was weighed into a 100 ml polythene bottle and 50 ml Dye Reagent added, together with a glass bead (to facilitate agitation). After shaking the sealed bottle at 25°C for the specified period (normally 2 h), an aliquot of the suspension was clarified by centrifugation (15 min at 2000 rev/min) and the supernatant quantitatively diluted to give an absorbance reading in the range 0·5–0·8.

The concentrations of dye in the supernatant and in the Dye Reagent were determined spectrophotometrically by reference to the Standard Dye Solution (1 cm cells at 482 nm). From these data, the amount of dye bound by the sample (mg g^{-1}) was calculated.

Effect of residual dye concentration on amount of dye bound

Sub-samples taken from the same lot of ground, freeze-dried potato tissue (1 g, duplicates) were weighed into 100 ml polythene bottles. In order to give a range of residual dye concentrations in the supernatants, varying volumes (7–50 ml) of Dye Reagent (CI Acid Orange 12, 2 g litre^{-1}, in 0·2 M citric acid) were added to the bottles, and the total volumes were made up to 50 ml by the addition of 0·2 M citric acid, as necessary. The Basic Procedure was then followed, using a shaking period of 4 h (i.e. a longer time than normally required).

Effect of shaking period on amount of dye bound

The Basic Procedure was followed, using the dye concentration deduced from the results of the above experiment (i.e. 1·6 g litre^{-1}, normal strength Dye Reagent). Shaking times ranged from 0·5 to 24 h. At the end of each reaction period, rapid separation of the dye–protein coagulum was effected by immediate filtration through glass fibre papers.

Evaluation of the Basic Procedure

Having established the required experimental parameters (i.e. residual dye concentration in the supernatant and the reaction time), the Basic Procedure was evaluated by comparing the amounts of dye bound with the protein contents of powders prepared from 73 different lots of potatoes (nine samples of mature tubers of different commercial cultivars, four samples of sprouted 'seed' potatoes, and 60 samples of potatoes freshly taken from trial plots).

RESULTS AND DISCUSSION

Effect of residual dye concentration on amount of dye bound

Figure 1 shows the adsorption isotherm obtained by plotting the amounts of dye bound against the residual dye concentrations of the corresponding supernatants. It can be seen that effective saturation of the potato powder with CI Acid Orange 12 occurs at dye concentrations exceeding 0·8 g litre^{-1}, when the amount of dye bound is approximately 20 mg g^{-1}.

Since previous experience has shown that, when estimating protein by dye binding, linear relationships are not obtained unless residual dye concentrations are greater than that causing effective saturation of the proteins in the sample, 0·8 g litre^{-1} corresponds to the least residual concentration permissible for this procedure. Therefore, in order to allow for variations in the protein contents of samples, a safety margin must be employed (e.g. 1·5 × the permitted minimum), and so the required residual dye concentration was set at 1·2 g litre^{-1}. This meant that the concentration of CI Acid Orange 12 in the Dye Reagent to be used in the Basic Procedure would be 1·6 g litre^{-1}.

Effect of shaking period on amount of dye bound

Figure 2 shows the effect of shaking time on the amount of dye bound by the potato powder. It can be seen that the adsorption of the dye is complete after 1 h, which was taken to be the minimal reaction period. For convenience, a shaking time of 2 h was employed in the present work.

Evaluation of the Basic Procedure

In a preliminary experiment, the Basic Procedure was applied to 10 sub-samples taken from the same sample of potato powder. The standard deviation for the amount of CI Acid Orange 12 bound by the potato powder was found to be 0·25 mg g^{-1} (coefficient of variation = 1·2%).

Figure 3 shows the scatter diagram obtained by plotting values for the protein nitrogen contents against the amounts of dye bound by 73 samples of potato powder (r = 0·97; standard error of estimate = 0·022), the regression equation for these data being:

$$y \text{ (protein-N, mg g}^{-1}) = 0{\cdot}766 + 0{\cdot}343x \text{ (dye bound, mg g}^{-1})$$

Under the conditions employed in the Basic Procedure, therefore, 20·0 mg CI Acid Orange 12 was bound by 7·63 mg potato protein nitrogen (or 47·7 mg total potato protein, conversion factor = 6·25). It should be noted, however, that since the amount of dye bound by any given sample is dependent on the residual dye concentration (see Fig. 1), this value should be regarded as a characteristic of the experimental procedure only, and should not be employed for the purpose of analysis. Furthermore, although the regression equation obtained in this study may be used elsewhere with a reasonable degree of accuracy, the protocol for the Basic Procedure must

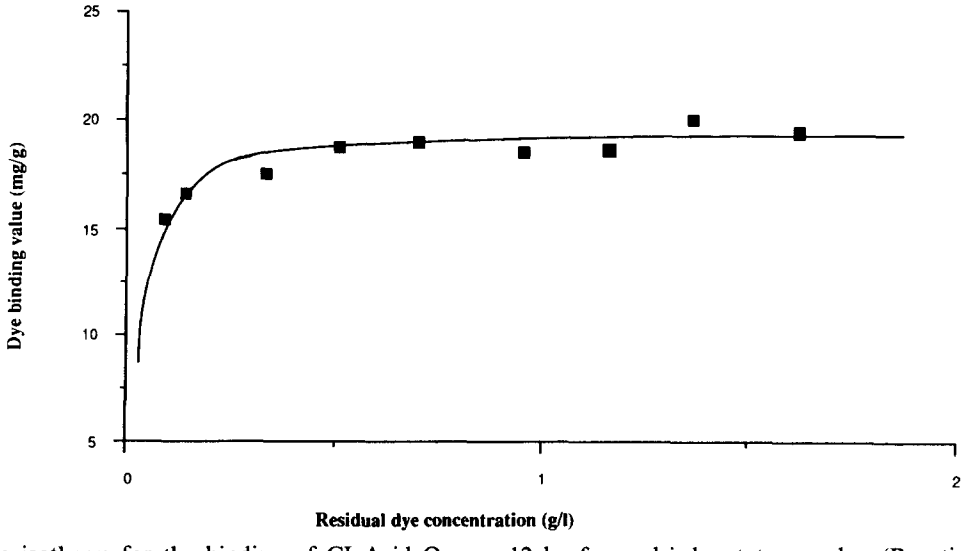

Fig. 1. Adsorption isotherm for the binding of CI Acid Orange 12 by freeze-dried potato powder. (Reaction medium: 0·2 M citric acid; reaction period: 4 h; temperature: 25°C.)

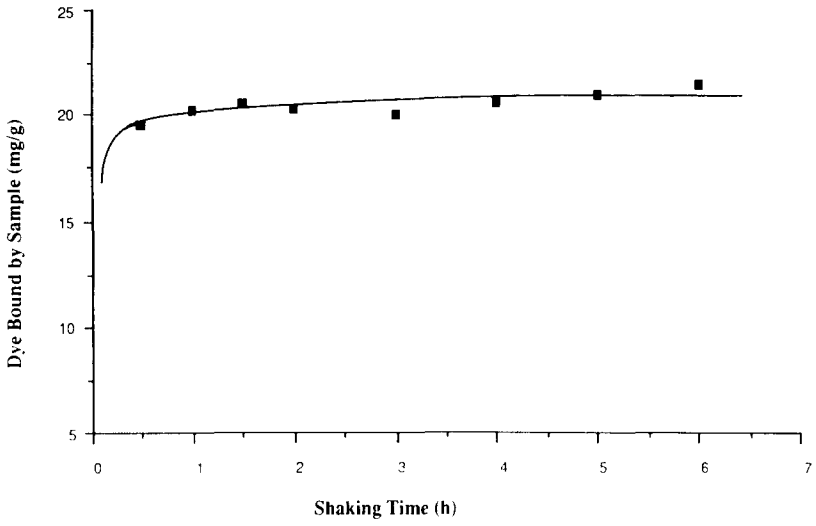

Fig. 2. Effect of reaction time on the binding of CI Acid Orange 12 by freeze-dried potato powder. (Dye Reagent: 1·6 g litre^{-1} dye in 0·2 M citric acid; temperature: 25°C.)

be strictly followed. This is because the chemical mechanisms of dye-binding procedures are somewhat complex, the amounts of dye bound being influenced by the experimental conditions (Lakin, 1978). The most satisfactory approach, therefore, is to calibrate every dye-binding procedure, as used, with the preferred procedure for the determination of protein nitrogen.

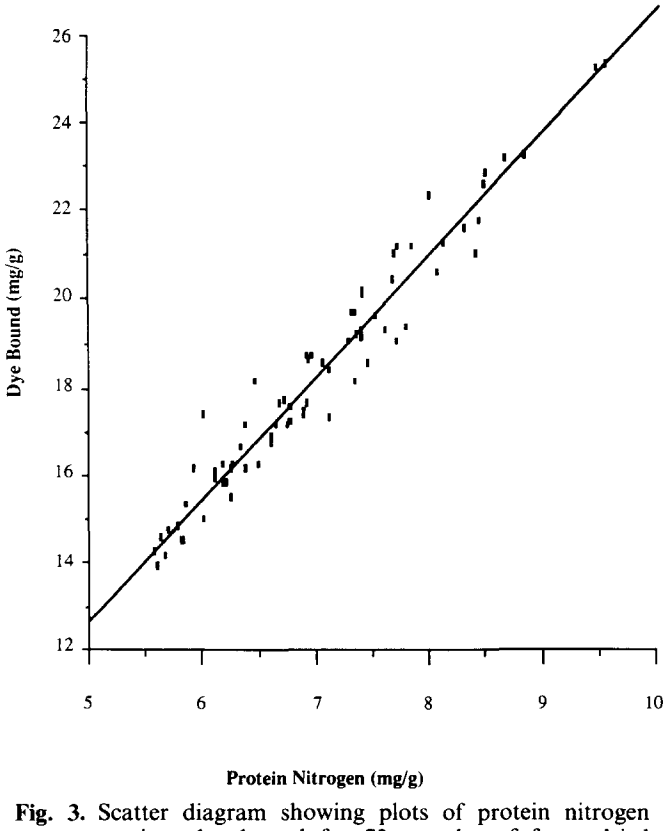

Fig. 3. Scatter diagram showing plots of protein nitrogen content against dye bound for 73 samples of freeze-dried potato powders of different types. (Dye Reagent: 1·6 g litre^{-1} dye in 0·2 M citric acid; reaction period: 4 h; temperature: 25°C.)

CONCLUSIONS

This study has shown that, when using the correct experimental parameters, measurement of the binding of CI Acid Orange 12 can be successfully employed for the estimation of 'true' protein in potato powder. Also, in keeping with other dye-binding procedures that have been used for plant breeding purposes (Mossberg, 1969), the procedure is rapid, reproducible and relatively inexpensive. Even without any special facilities, a throughput of 50 analyses per person per day is readily attainable, and this can be more than doubled by the use of liquid dispensing systems and an automated system for the spectrophotometric measurements. (As always when analysing solid materials, the limiting factor on throughput is the weighing of samples.)

With regard to the difficulty experienced by Desborough (1975) and by Mohyuddin & Mazza (1978), when attempting to use the procedure of Kaldy *et al.* (1972) for the analysis of potato powders, it is suggested that this was because the correct experimental conditions were not employed. Kaldy's procedure was developed for the estimation of protein in fresh tuber tissue, and so it would have been necessary to have changed both the concentration of dye and the reaction time in order to apply it to dried material.

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