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SHORT COMMUNICATION

EVIDENCE OF SUBSTANCES INTERFERING WITH THE LOWRY TEST FOR PROTEIN IN PLANT LEAF TISSUE[1]

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Abstract—Buffer soluble plant leaf proteins can be estimated by a modified Lowry technique provided they are first precipitated with trichloroacetic acid. If the proteins are not precipitated shortly after extraction, interfering substances contribute to large errors in the determinations. Precipitated proteins can be redissolved in buffer and treated with Lowry reagents as long as 48 hr later with little loss in precision.

INTRODUCTION

THE MICRO-Kjeldahal[2] and micro-biuret[5] methods are useful for determining total protein content but lack sufficient sensitivity whenever plant leaf proteins are fractionated. The Lowry technique[3] as modified by Eggstein and Kreutz[4] possesses sufficient sensitivity provided the proteins first are precipitated with trichloroacetic acid (TCA) prior to the addition of the Lowry reagents. Early investigations in our laboratory with this method, however, gave erratic results. If aliquots from the same extract are precipitated with TCA and the proteins estimated on successive days, the protein content as determined colorimetrically increases, suggesting the presence of other interfering substances.

These studies indicate that TCA-precipitatable substances interfering with the Lowry reagents are formed from precursors in borate buffer and their concentration increases with time. They also show that this interference can be eliminated if the proteins are precipitated within a few hours after buffer extraction and kept separately.

RESULTS AND DISCUSSION

Apple leaf tissue was extracted with borate buffer, pH 8·3,[6] and divided into two portions. Triplicate aliquots from one-half of the extracts were taken on successive days, the proteins precipitated with 10 per cent TCA, redissolved in buffer and analyzed for protein content. Prior to the addition of the Lowry reagents absorbance spectra were obtained and the superimposed curves are shown in Fig. 1. The other half was divided into 5 series of triplicated

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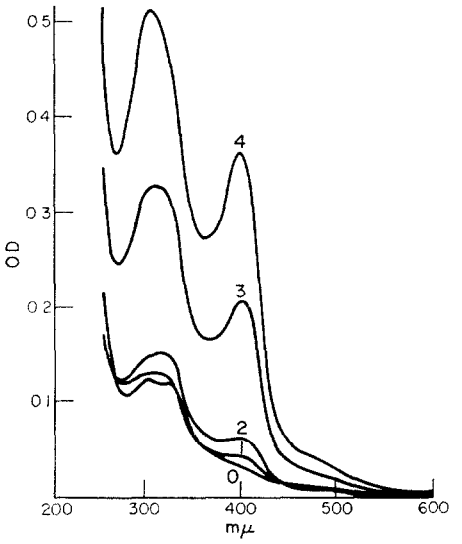

FIG. 1. CHANGE IN SPECTRA ASSOCIATED WITH DURATION OF TIME BETWEEN BUFFER EXTRACTION AND TCA EXTRACTION FOLLOWED BY ESTIMATION OF PROTEIN IN LOWRY TEST.

0—TCA precipitation and estimation of protein immediately following buffer extraction; 1—one day later; 2—two days later; 3—three days later; and 4—four days later.

aliquots and all precipitated with 10 per cent TCA the day of extraction. Precipitates of one triplicated series was immediately redissolved in 0·1 N sodium hydroxide, treated with Lowry reagents and the proteins estimated the same day of extraction. This procedure was repeated for each triplicated series on subsequent days and these data listed in Table 1.

TABLE 1. BORATE BUFFER SOLUBLE PROTEINS OF LYOPHILIZED APPLE LEAF TISSUE AS ESTIMATED WITH THE LOWRY TECHNIQUE

Days after extraction with borate buffer			
TCA precipitation	Treatment with Lowry reagents	Protein μg/ml	% increase over control
0	0	142 8	
1	1	178 5	26
2	2	191·0	29
3	3	342·7	140
4	4	517·5	262
0	0	141·0	
0	1	143·7	1 9
0	2	159·8	13·0
0	3	182·1	29·0
0	4	189·0	34

These data indicate that substances reacting with the Lowry reagents increase as the interval between extraction and TCA precipitation increases. This increase ranges from 25 to 30 per cent for the first 2 days and amounts to as much as 140 per cent on the 3rd day.

Increasing the interval to 4 days results in increases of over 260 per cent. On the other hand, if the proteins are precipitated on the day of extraction and then treated with the Lowry reagents on subsequent days, there is no appreciable increase during the first 2 days. Thereafter increases in excess of 15 per cent occur, possibly due to breakdown of proteins.

Figure 1 shows increases in absorbancies, the curves demonstrating quantitative changes and indicating the presence of interfering substances. The exact identity of these interfering substances are not known, but they may be phenolic compounds. Phenolics are ubiquitous in plant leaf tissues and can interfere with the Lowry test.[3]

EXPERIMENTAL

Leaf tissue was collected from apple and lyophilized. When fully dried the tissue was pulverized in a Spex mixer mill and stored in stoppered containers under refrigeration. Appropriate aliquots were weighed and borate buffer added at a ratio of 1 ml for 10 mg of powder. These suspensions were agitated for two hours at 4° and then centrifuged at 5000 *g* for 20 min to remove the extraneous material. Pellets were washed once and the supernatants combined and made to volume.

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