THE DETERMINATION OF TOTAL CHLOROPHYLL IN METHANOL EXTRACTS

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ABSTRACT — The colorimetric determination of chlorophyll is dependent upon the efficiency of the solvent used for extraction. Using reported absorption coefficients for chlorophylls a and b in methanol, we have derived the following formula for chlorophyll in mg per liter of extract:

$$C = C_a + C_b = 25.5\ D_{665} + 4.0\ D_{650}.$$

A critical step in procedures for the determination of chlorophyll in plant tissues is that of extraction. In work with apple leaves, methanol has been found to be a much more efficient chlorophyll solvent than is acetone. Compton and Royston (1945) developed a method for determining chlorophyll in methanol extracts of apple leaves. MacKinney (1941) and Harris and Zscheile (1943) have determined the absorption coefficients of chlorophylls a and b in several solvents including methanol and acetone. Arnon (1949) used the absorption coefficients (K values) of chlorophyll in acetone as reported by MacKinney, to derive the following formula for the estimation of total chlorophyll in acetone extracts in plant tissues.

(1) $C = C_a + C_b = 20.2\ D_{645} + 8.02\ D_{663}$

where C_a and C_b are chlorophylls a and b in mg/liter, and D is the optical density of extracts at wavelengths 645 and 663 millimicrons.

Arnon's formula, although it has been widely used for chlorophyll studies, is applicable only for acetone solutions because of the solvent effect on absorption coefficients, MacKinney (1941). The use of methanol for the extraction of chlorophyll from apple leaves necessitates a change in Arnon's formula for the estimation of total chlorophyll. Using MacKinney's absorption maxima of 664 and 651 millimicrons with K values of 74.5 and 36.4 for chlorophylls a and b, we have modified Arnon's formula for chlorophyll extracted in methanol.

The following simultaneous equations of Arnon's with appropriate values for methanol as a solvent are presented.

(2) $D_{651} = 74.5\ C_a + 18.3\ C_b$.

(3) $D_{664} = 27.6\ C_a + 36.4\ C_b$.

From equation (3):

$$(4)\quad C_a = \frac{D_{664} - 36.4\ C_b}{27.6}$$

Substituting into equation (2) and solving for C_b we obtain:

(5) $C_b = 0.0338\ D_{664} - 0.0125\ D_{651}$

Substituting this equation for C_b in equation (3) and solving for C_a:

(6) $C_a = 0.0164\ D_{664} - 0.0683\ D_{651}$

Therefore, total chlorophyll in g/liter is:

(7) $C = C_a + C_b$

$C = 0.0256\ D_{664} + 0.0040\ D_{651}$

or:

(8) $C = 25.5\ D_{664} + 4.0\ D_{651}$ mg/liter of chlorophyll in methanol.

The procedure used is as follows. Sampling and preparation are carried out under red light. Ten to 30 discs of 11-12 mm diameter are punched from fresh leaves and their total fresh weight is recorded. The discs, covered with watch glasses, are left for 15 to 18 hr in 50 ml of 99.8% methanol. At the end of this time the chlorophyll is leached into the methanol, leaving the discs practically colorless. Five ml of the 50 ml methanol extracts are measured at 651 and 664 millimicrons in a Beckman model DU spectrophotometer. Substituting the density values (D) of these two readings in equation (8) one can estimate the methanol soluble chlorophyll content of apple leaves. Results are expressed as mg/disc, mg/g of fresh wt, or mg/cm² of leaf surface.

Ozerol (1964) has found that total chlorophyll values, as described above

correlate well with both leaf nitrogen and spectral reflectance curves of intact leaves.

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