

Vernon L. P. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. *Anal. Chem.* 32:1144-50, 1960.  
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This paper describes a spectrophotometric procedure for the determination of chlorophyll a (Chl a), chlorophyll b (Chl b), pheophytin a, pheophytin b, total chlorophyll, and total pheophytins in plant extracts. The method utilizes equations derived from determined specific absorptivities at absorption maxima and changes in specific absorptivities for the four components at appropriate wavelengths in 80-percent acetone. [The SCI® indicates that this paper has been cited in over 330 publications since 1960.]

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After joining the faculty of Brigham Young University in 1954, I was able to attract a few graduate students who chose to work on the photochemical reactions of plant chloroplasts and bacterial chromatophores. I was contacted by the US Department of Agriculture to see if I would be interested in developing a method that could be used to determine the extent of conversion of chlorophylls into pheophytins in cooked vegetables. I had previous experience in chlorophyll isolation and chemistry as a graduate student under Sam Aronoff at Iowa State University, so I was interested in pursuing this project. Since my graduate students were all involved in other projects, it became apparent that if this work was to be done, I would need to do it myself.

The approach to the problem was straightforward. It required the simultaneous determination of the amounts of Chl a, Chl b, and the corresponding pheophytins in plant material. The method of choice was obviously spectrophotometric since each of these components has a unique absorption spectrum. Accordingly, it became necessary to prepare the pure chlorophylls and the pure

pheophytins and to determine their specific absorptivities at their absorption maxima and at those wavelengths exhibiting the greatest change in absorptivity upon conversion of the chlorophylls to pheophytins. Therefore, the limitations of the method would be the purity of the chlorophylls and pheophytins and the accuracy of the spectrophotometer used to determine the absorptivities. The method used for separation of the chlorophylls was chromatography on powdered-sugar columns. I had done this many times in the laboratory of Aronoff and had learned how to uniformly pack the columns so that the developing bands eluted cleanly.

Because some of the measurements made on the pigments would be at wavelengths on the steep part of the absorption curve, it was necessary that the spectrophotometer be accurate. The only instrument available was the workhorse of the biochemical laboratory in those days, a Beckman DU spectrophotometer. Considerable time was spent calibrating the wavelength scale. The accuracy of the spectrophotometric measurements was confirmed by determining the magnesium concentration of the isolated chlorophyll.

Biochemists working with plant material need to determine chlorophyll concentrations. The method commonly used in many laboratories is that reported by Arnon,<sup>1</sup> which uses the specific absorptivities of Chl a and Chl b determined earlier by MacKinney.<sup>2</sup> The attraction of this procedure is that the measurements need only be made at one wavelength where the specific absorptivities of Chl a and Chl b coincide. Unfortunately, this is on a steep part of the absorption curve for Chl a, which introduces a significant error if the wavelength is not correct. Inskeep and Bloom<sup>3</sup> have redetermined the extinction coefficients of Chl a and Chl b in 80-percent acetone and compared them with earlier results presented in several published papers. It is comforting to find that their data agreed closely with those that I published earlier. Their data also indicate that the extinction coefficients reported by MacKinney<sup>2</sup> may be low, which would indicate that the procedure utilized by Arnon<sup>1</sup> may be in error.

- 1 Arnon D I. Copper enzymes in isolated chloroplasts Polyphenoloxidase in *Beta vulgaris* *Plant Physiol* 24 1-15, 1949 (Cited 4,725 times since 1955)
- 2 MacKinney G. Absorption of light by chlorophyll solutions *J Biol Chem* 140 315-22, 1941 (Cited 105 times since 1955)
- 3 Inskeep W P & Bloom P R. Extinction coefficients of chlorophyll A and B in N,N-dimethylformamide and 80% acetone *Plant Physiol* In press