Green with Envy: On Citation of the Chlorophyll Assay—Commentary

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15

This article by Daniel I. Arnon has been cited more than 10,000 times and is still cited today, more than 60 years after its original publication, for what is now a "routine" laboratory method. However, chlorophyll measurement was only an incidental procedure in the study of a redox enzyme, polyphenoloxidase, in chloroplasts. The chloroplast had only just been recognized as the site of oxygen evolution and the "Hill reaction," and an obvious research objective was the resolution of individual enzymes and components of photosynthesis. Therefore, chloroplast-localized redox enzymes were prime targets of study. Since copper was appreciated for its catalysis of redox reactions and oxygen chemistry and because copper "poisons" inhibited photosynthesis, copper-containing polyphenoloxidase (at the time indistinguishable from copper enzymes of fungi and animals) was of considerable interest. Arnon appreciated even then that the protein was not likely to be an essential component in photosynthesis because it is not conserved in all chloroplast-containing organisms. Today, we know that the probable targets of copper poisons in photosynthesis and respiration in *Chlorella* are plastocyanin and cytochrome oxidase, respectively. Nevertheless, the methods for chloroplast isolation were instrumental in Arnon's measurement of photosynthesis in isolated chloroplasts, the description of chloroplasts as a complete photosynthetic unit, and his discovery of photophosphorylation (for review, see Arnon, 1984). A few months before his *Plant Physiology* article, Arnon had published a preliminary article on the localization of polyphenoloxidase in the journal *Nature* (Arnon, 1948). That article was cited only 14 times (in the early 1950s). Undoubtedly, the abbreviated format of the journal precluded detailed methodologies, which turned out to be the most valuable components of the publication.

Students of the history of photosynthesis and chloroplast biology will find detailed description and analysis of chloroplast isolation (would an editor allow an author to use the word "mash" today?) and the development of a single-step pigment extraction protocol together with simultaneous equations for using the extinction coefficients of chlorophyll *a* and *b* at 663 and 645 nm to calculate total chlorophyll concentration. Arnon also describes the simpler method involving the extinction at 652 nm (isosbestic point for the two chlorophylls), but he points out that the two-wavelength method is more "trustworthy." I confess that I learned the simpler method as a student and used it for nearly a decade. Today, my laboratory prefers the more accurate extinction coefficients described by Porra and coworkers in 1989 (published 40 years later and cited over 1,800 times since then; Porra et al., 1989). Porra used chromatographically pure chlorophylls for determination of the coefficients and further verified them by using atomic absorption spectrometry for measuring the central magnesium ion. He despairs the continued use of the Arnon equations (Porra, 2002); I suspect that this is a result of "hand-me-down" protocols rather than obstinate refusal to accept the improved extinctions and extraction procedures.

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www.plantphysiol.org/cgi/doi/10.1104/pp.110.159848