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This Week's Citation Classic®

Singleton V L & Rossi J A Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents.
Amer. J. Enol. Viticult. **16**:144-58, 1965.
[Department of Viticulture and Enology, University of California, Davis, CA]

Folin-Denis and Folin-Ciocalteu reagents were compared for estimation of plant phenols by color yield, spectrum, time-temperature effects, and interferences. The Folin-Ciocalteu formulation avoided a precipitation problem, gave greater color, less variation, and better recovery. Warming shortened analysis time and gallic acid standards were recommended. [The *SCI*® indicates that this paper has been cited over 145 times, making it the most-cited paper for this journal.]

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It was, in 1965, becoming increasingly clear that the amount and specific mixture of natural phenols are crucial to characteristics, quality, and storage reactions of different wines and other foods and beverages from plants. A predictable method for total phenol analysis was essential; ultraviolet-visible spectral characteristics of different natural phenols were too variable for a general analysis. Folin-Denis colorimetry was considered the best and "official" method[1,2] but was subject to precipitations that interfered with colorimetry. We set out to correct this and to understand the method better.

Joe Rossi was an ideal graduate student, able to help with this project while carrying out master's thesis research that led to two other papers. He was a collegiate wrestler of note, and the only problem between us was that I couldn't open any screw-capped bottle after he closed it! Today he is chief winemaker with Heublein, Inc., at Madera, California.

Part of the reason the paper has been cited frequently is that the improved method is useful for products other than wines and spirits. The molar color yield for a given phenol can be estimated from the number of its oxidizable hydroxyl groups.[3] For example, the flavonoid catechin has three (3) reactive phenolic groups and behaves as an equimolar mixture of phloroglucinol (1) plus protocatechuic or gallic acid (2). By analysis before and after selective removals, the method can be adapted to flavonoid-nonflavonoid[4] and other specialized assays. It has also been automated.[5]

Because these improvements are yet to be incorporated in the "official" methods summary,[2] it remains necessary to cite our paper. Also, the method can have serious interference problems, leading to further work and resultant citation. Readily oxidized substances such as ascorbic acid[3] interfere, as does especially sulfite,[3,6] particularly if free and high relative to the phenol content. High sugar can necessitate large corrections by forming reactive reductones (endiols) in the alkaline final solution, especially if warmed; and aromatic amines react as phenols.[3,5]

Nevertheless, the method remains useful, especially for products such as dry red wines. Also it can provide a reproducible total against which contributions by specific phenols, separately determined, can be compared. High-pressure liquid chromatography and multicomponent spectrophotometry are making colorimetry of this type obsolete for efficient research, but owing to low equipment costs it still has attractions for processors' laboratories.

Selection of this paper as a *Citation Classic* also draws attention to the *American Journal of Enology and Viticulture*, a refereed quarterly scientific journal we believe deserving of wider recognition in the scientific community. On the other hand, the paper itself calls up mixed feelings. It reminds me that there are two further related studies I intend to do and that, like the little girl, when this method is good, it is very, very good and when it is bad, it is horrid.

1. **Folin O & Denis W.** On phosphotungstic-phosphomolybdic compounds as color reagents. *J. Biol. Chem.* **12**:239-43, 1912. (Cited 60 times since 1955.)
2. **Williams S.** ed. *Official methods of analysis of the Association of Official Analytical Chemists.* Arlington, VA: AOAC. 1984. 1,141 p.
3. **Singleton V L.** Analytical fractionation of the phenolic substances of grapes and wine and some practical uses of such analyses. *Advan. Chem. Ser.* **137**:184-211, 1974.
4. **Kramling T E & Singleton V L.** An estimate of the nonflavonoid phenols in wines. *Amer. J. Enol. Viticult.* **20**:86-92, 1969.
5. **Slinkard K & Singleton V L.** Total phenol analysis: automation and comparison with manual methods. *Amer. J. Enol. Viticult.* **28**:49-55, 1977.
6. **Somers T C & Ziemelis G.** Gross interference by sulfur dioxide in standard determinations of wine phenolics. *J. Sci. Food Agr.* **31**:600-10, 1980.

