Summary 45

VITICULTURE & ENOLOGY
UNIVERSITY OF CALIFORNIA DAVIS

Title: **"Measuring phenolics in the winery"**

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In: Am. J. Enol. Vitic. 57(3):280-288. 2006

If the previous summary dealt with the various methods to measure phenols *in the research lab*, where sophisticated equipment is common, this one reviews the methods available to measure phenols (color, total phenols and tannins) *in the winery*, where the most exotic equipment is probably a visible/UV spectrophotometer. Still, this article is not for the chemically faint of heart.

Measurements of Color:

- It is rather remarkable that 99.5 % of the components in wine are transparent, and only 0.5% of components are responsible for the coloration of red and white wines.

- Anthocyanins are the main source of pigmentation in red wines. Copigmentation, the enhancement of visible color due to complexes between anthocyanins and colorless cofactors, also contributes to red wine color. Finally, some cofactors cause the wine to take a bluish appearance, known as a "blue shift" (or bathochromic shift).

- When grapes are crushed, anthocyanins are able to react with many other compounds – acetaldehyde, keto-acids, tannins, and cinnamates-, forming what we know as *polymeric pigments.* Polymeric pigments are the "stable form of color", resistant to bisulfite bleaching and to changes in pH. But as the authors point out, the term "polymeric pigments" is a misnomer. This is partly because it does not refer to all the *pigments* that contain anthocyanins; and partly because there are compounds that are *not polymeric* that anthocyanins can react with. Even if the name is less than perfect, the practice of measuring polymeric pigments - the color fraction resistant to bleaching- continues to be very useful.

- The authors review 5 methods for measuring color:

1) **Tri-stimulus values**. In this method, any wine color is matched by a combination of three primary colors, represented by 3 tri-stimulus values (X,Y,Z). To do that, the absorbance of the wine across the visible spectrum needs to be measured. Then, the information is mathematically transformed and mapped into a two-dimentional color spectrum (called sometimes the "color tongue" because of its shape). One of the drawbacks of this method is having to measure absorbances across the entire visible spectrum.

- 2) **CIELAB coordinates.** In this method the tri-stimulus values are converted into CIELAB values (L*, a*, b*), and then the wine color is characterized by plotting those values in a 3-dimensional space. In a CIELAB space, L* measures *luminosity* (amount of light), a* measures *chroma* (hue), and b* measures *intensity* (for that particular hue). Even though each color is precisely characterized, it is difficult to visualize with these two methods exactly what color is it that is in the wine in the glass.

• 3) **Somers assay**. Basically, this method measures 3 components: *total anthocyanins* (pigments resistant to SO2 bleaching, after shifting the wine to an acidic pH), *ionized anthocyanins* (or anthocyanin levels at the natural wine pH), and *polymeric pigment* (pigments bleachable with SO2). Please refer to the original text for how to measure each of these components.

• 4) **Copigmentation assay**. The main difference between this assay and the Somers' is the inclusion here of a "copigmentation" fraction. This is achieved by comparing diluted and undiluted wine samples (dilution renders anthocyanins inaccessible for copigmentation). Thus, this method measures: *anthocyanin color, polymeric pigment color,* and *copigmentation color*. The main drawback of this method is that it does not measure color at the wine's natural pH (colors need to be standardized to pH 3.6 first). But, as the authors point out, it is the **only method available for measuring <u>all</u> main wine color components** with a spectrophotometer.

• 5) **Color intensity and hue.** *Color intensity* is traditionally the sum A420+A520+A620 (or sometimes A420+A520). *Color hue* is calculated as the ratio A420/A520. *Color intensity* gives an idea of how much color there is, whereas *color hue* is normally used to monitor wine aging (hue values increase with aging). The problem with these measurements is that their **utility for comparing multiple wines is very limited**. This is because wine color is pH dependent, and different wines have different pHs. However, the method is so simple that it is an attractive alternative to measuring actual color components.

Measurements of Total phenols:
• The authors review 4 methods for measuring total phenols:
1) **Absorbance at 280 nm.** We love this one because it's so easy! The method is based on the fact that compounds with aromatic rings –like phenols- absorb UV light. Two drawbacks of this method are: it has interferences from non-phenolic compounds that also contain aromatic rings (nucleotides, aromatic aminoacids, peptides, proteins), and it offers no information about the type of phenolics measured.

• 2) **Folin-Ciocalteu assay**. The method is based on the fact that phenols ionize completely under alkaline conditions, and can be readily oxidized by the Folin-Ciocalteu reagent. The oxidation causes a color change from yellow to blue easy to monitor with a spectrophotometer. The main drawback is that the Folin-Ciocalteu reagent is so reactive that it can also oxidize many unintended compounds in wine (like fructose, bisulfite, aminoacids, ascorbic acid). This problem can be partially avoided by adding acetaldehyde (to bind the bisulfite), or multiplying by a correction factor (if the wine is sweet).

• 3) **Iron-chloride method**. This method has been recently re-introduced for the measurement of phenolics in the winery. This method is based on the ability of iron to react with all the phenolics that have more than one hydroxyl group. Thus, it is suitable to measure all phenolics in wine except anthocyanins and monohydroxylated phenols. It is therefore, strictly speaking, a method for measuring "iron-reactive phenols", rather than "total phenols". However, as one of the authors has shown (See *Summary 28* of these series), it can be successfully combined with measurements of anthocyanins, polymeric pigment, and copigmentation, to provide a convenient assay of the main functional types of phenols in wines.

• 4) **Enzymatic method**. This method monitors the conversion of phenolics into colored quinone-imine products in the presence of certain enzyme (horseradish peroxidase). The advantage of the method is that it takes 5 minutes, and the response is linear over a wide range. The method was compared with the Folin-Ciocalteau assay and it correlated well. However, the method is very new and it has still not been demonstrated to work well with the main classes of phenols present in grapes.

Measurements of Tannins

- The authors review 3 methods for measuring tannins, all based on their precipitation by protein.

1) **Glories Gelatin index**. This method, which uses gelatin as the protein, allows for the quantification of both total tannins and protein reactive tannins. The difference is known as the *gelatin index.* The problem with this method is that the precipitation step is very long.

- 2) **Llaudy method**. This new method uses ovalbumin instead of gelatin. Ovalbumin is less sensitive (numbers obtained are lower) than gelatin. But the method is more reproducible and quicker. Unfortunately, it is a bit cumbersome, as it takes 12 samples per wine to run. But it is still quicker than the Glories Gelatin Index, and more reproducible.

- 3) **UC Davis tannin assay**. Developed by Harbertson and Adams, this method uses bovine serum albumine (BSA) to precipitate tannins, which are then measured by adding ferric chloride and measuring absorbance at 510 nm. The advantage of this method is that it can be combined with a measurement of total phenols, as well as a measurement of polymeric pigments. The method has been recently modified so it could handle many samples at a time, as well as provide results on the same day, factors critical for its extended use in the winery (see *Summary 28* of this series).

In summary, the authors review the methods available to measure *color*, *total phenols,* and *tannins* in the winery using a spectrophotometer. Measuring phenols has long been a controversial topic among researchers. The authors make no attempt to recommended one method over the other; the value of their paper is to present and compare what is available.

Methods for measuring:		**Author (or most recent reference)**
Color	Tri-stimulus values Cielab coordinates Somers assay Copigmentation assay Intensity and Hue	Pérez-Caballero 2003 Somers, Evans 1977 Levengood, Boulton 2004
Total phenols	Absorbance at 280 nm Folin-ciocalteau assay Iron-chloride assay Enzymatic method	Singleton 1999 Harbertson 2004 Stevanto 2004
Tannins	Glories Gelatin index Llaudy method UC Davis Tannin assay	Glories 1984 Llaudy 2004 Harbertson, Picciotto, Adams 2003

Author: Bibiana Guerra, Editor: Kay Bogart. This summary series funded by J. Lohr Vineyards and Wines.