

# ETS Red Wine Phenolic Analysis

## ETS Red Wine Phenolic Analysis measures twelve different phenolic compounds

Our phenolic analysis includes compounds that are indicators of important phenolic reactions as well as compounds that directly affect wine flavor and color.

Phenolic results are presented in a report format that is an excellent tool for monitoring the processes that influence phenolic accumulation, extraction, and development in wine. This publication describes the report format and briefly reviews the origins and importance of the measured compounds.

### An Example Report

*This example shows the effects of a maceration enzyme on the phenolic profile of a Pinot Noir wine. The reference is a control wine made without enzymes. Many winemaking treatments have global effects on phenolics and can modify individual phenolic compounds in different ways.*

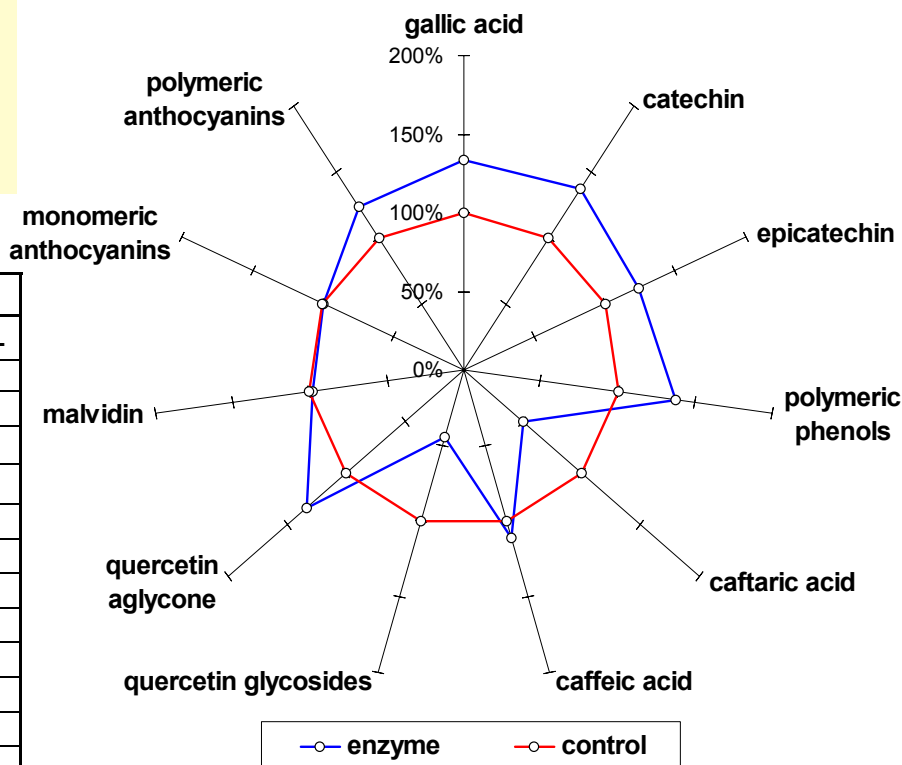
Quantitation Table	
Phenolic Compound	mg/L
gallic acid	20
catechin	110
epicatechin	31
tannin	524
caftaric acid	1
caffeic acid	20
total anthocyanins	165
malvidin glucoside	111
monomeric anthocyanins	144
polymeric anthocyanins	21
quercetin glycosides	11
quercetin aglycone	8

## Report Format

The data is presented in tabular form as concentration in mg/L, and graphically, as a radar plot.

The radar plot is a tool for quickly viewing all the measured compounds in the same scale. The sample data is presented relative to a reference. The reference values are defined as 100% (the red graph) and the sample data as a percentage of the reference (blue graph).

There are several options for choosing a reference value. The reference could be an average of all the samples in a group, the control in an experiment, a target wine, or a reference supplied by ETS. The reference influences the appearance of the radar plot, but has no impact on the sample quantitation table.



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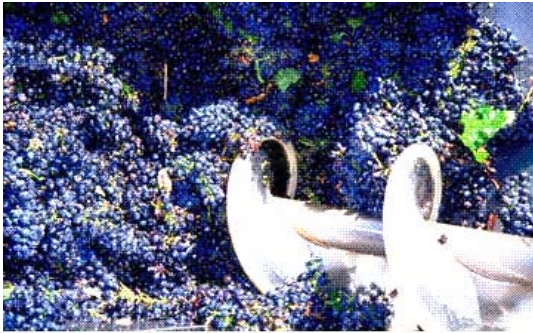


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## Gallic Acid

The main sources of gallic acid in wine are grape seeds and oak cooperage. Gallic acid is present in seeds as free gallic acid and as an ester attached to procyanidin polymers. Gallic acid is also present in grape stems and may be increased by whole cluster fermentations.

Gallic acid concentrations range from 10 to more than 100 mg/L. Concentrations in the upper end of this range are usually associated with new oak cooperage.

## Catechin and Epicatechin

Catechin and epicatechin are known as flavonols, flavon-3-ols, or procyanidins. They are present as monomers in grapes and wine and are the primary components of tannin. Both compounds are found in high concentrations in seeds and stems and may be found in the skins of immature grapes. Pinot noir has much higher concentrations of both compounds than Cabernet, Merlot, or Zinfandel.

As grapes mature, the phenolics on the seed surface combine with other compounds to form the seed coat. As a result, seed phenolics become increasingly difficult to extract as seeds mature. Catechin and epicatechin are generally lower in wines made from grapes with riper, more developed seeds.

Catechin is a useful indicator of seed extraction in wine. Fermentation practices that increase seed extraction such as extended macerations, higher temperatures, or aggressive punch downs usually result in an increase in catechin.

Catechin and epicatechin concentrations tend to be low in Syrah/Shiraz. Catechin in Cabernet Sauvignon and Merlot can range from less than 10 to more than 100 mg/L depending on seed ripeness and winery production practices. Pinot Noir wines range from 30 to more than 400 mg/L reflecting the wide range of seed maturity in Pinot Noir at harvest.

## Tannin

Wine tannins are complex polymers that strongly affect wine flavor and color. Tannin includes phenolic polymers from grape seeds, skins, and stems as well as compounds formed or modified in wine.

Tannin polymers primarily consist of linked proanthocyanidin units such as catechin, epicatechin, epigallocatechin and epicatechin gallate. After crushing, polymeric phenols can be modified by additions of anthocyanins (see polymeric anthocyanins below), other phenolic compounds, proteins, polysaccharides and metal ions.

Tannin may account for 50% of the phenolics in young wine with the percentage increasing as wines age. The concentration and structure of tannin along with tannin interactions with other wine components are responsible for much of the mouth-feel of red wine. Tannin is the strongest antioxidant in red wine and acts as an oxidative buffer to protect wine from oxidative spoilage.

The concentration of tannin in wine is the result of a balance between the amount of extractable tannin in grapes, extraction of grape tannin during fermentation/maceration, tannin additions, and tannin precipitation.

Extraction is driven by temperature, time, alcohol content and physical manipulations such as pump-overs.

Tannins are often added to wine. These compounds vary greatly due to the source material and the manufacturing process used. When added during crush, enological tannin does not always increase tannin in the finished wine and in some cases can reduce total wine tannin content.

Loss of wine tannin by precipitation is caused by tannin binding to proteins from grapes and yeast. The protein reaction does not remove tannin

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uniformly. More likely it removes more reactive tannin molecules (larger tannins and tannins with epicatechin gallate units are more protein reactive). natural fining event is often overlooked; however it is the probable cause of wine “softening” often observed at the end of an extended maceration.

Tannin can be measured many ways and the measurement method is important to understand when evaluating results. The tannin analysis method used at ETS Laboratories is primarily measuring tannin based on molecular size. Phenolic polymers with at least four linked proanthocyanidin units are included in the quantitation. Tannin results are expressed as mg/L of catechin equivalents. Unlike protein reactivity assays, this measure of tannin does not change substantially as wines age.

Tannin concentrations in red wines range from 100 mg/L in very light wines to more than 1500 mg/L in very tannic wines.

## Caftaric and Caffeic Acid

Caftaric acid is an ester formed from caffeic and tartaric acids. It is found in grape skins, pulp, and stems. It is not present in seeds. Caffeic acid is a free cinnamic acid. Both the ester and free cinnamate are found in wine. Other cinnamate esters in grapes include fertaric and coutaric acids. The relative proportions of the different cinnamic acid esters varies by variety.

Caftaric acid is readily oxidized during processing and fermentation. In the presence of polyphenol oxidase enzyme, caftaric acid forms a highly reactive quinone that can oxidize other compounds in coupled oxidations. The quinone can also combine with other compounds to form adducts.

Glutathione and caftaric acid react to form a compound known as grape reaction product.

In the presence of cinnamic esterase enzymes, tartaric acid is cleaved leaving free caffeic acid. Some extraction enzyme preparations have significant esterase activity. Free cinnamates are precursors for several aromatic phenols in wine including 4-ethylphenol and 4-ethylguaicol.

Caftaric acid can be used to monitor oxidative stresses in wine. Wines exposed to considerable oxidation, such as press wines, have little or no caftaric acid.

## Quercetin

Quercetin is a flavonol present in grape skins and stems as several different glycosides (compounds with attached sugars). Other flavonols in grapes are kaemferol, myricetin, and isorhamnetin.

Quercetin accumulates in grape skins to protect against damage from ultra violet (UV) light. There are high concentrations of quercetin in sun exposed grape skins and in wine made from sun exposed grapes. Quercetin is readily extracted from grape skins during fermentation. Stems may contribute additional flavonols in whole cluster fermentations.

Quercetin glycosides may be hydrolyzed in wine to form quercetin algycone (quercetin without a sugar, usually just called quercetin). This process is similar to the hydrolysis that can occur with anthocyanins. Unlike anthocyanins, flavonol aglycones are stable in wine and can be used to monitor hydrolysis reactions.

Quercetin may cause problems as a precipitate in bottled wines. Sangiovese wines are particularly sensitive to this problem.

Flavonols can interact with anthocyanins, enhancing their red color in a process known as co-pigmentation. This process may also aide anthocyanin color stability.

## Malvidin and Monomeric Anthocyanins

Anthocyanins are red pigments found in grape skins as monomeric glucosides. The primary anthocyanin pigment in most *Vinifera* grapes is malvidin glucoside. It is the phenolic compound found in the greatest concentration in young wine.

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There are five to seventeen monomeric anthocyanins found in wine. The ratios of the different anthocyanins vary by variety. In Pinot noir, malvidin may account for more than 80% of the total monomeric anthocyanins, but in Cabernet Sauvignon, it may account for less than 50%.

High concentrations of anthocyanins in grapes are essential for good color in wine. There are numerous viticultural factors that affect grape anthocyanin content. Light exposure on grape clusters and berry size are two of the most important.

Anthocyanins have several different ionic forms in wine that change based on wine pH. Only one of these forms, the flavillium ion, is a red pigment. At wine pH, somewhere between 10 to 15% of the monomeric anthocyanins present are pigments. The anthocyanin values reported in the ETS Red Wine Phenolic Profile is the total of all the ionic forms, not just the pigmented types.

Anthocyanin extraction and stability are affected by winery production practices. Monomeric anthocyanins are subject to hydrolysis, oxidation, and polymerization in wine. Anthocyanin concentrations are usually highest in wine during the early part of fermentation. They usually are declining during fermentation and continue to decrease during aging. Monomeric anthocyanins are the most labile phenolic compounds in wine, typically decreasing at a rate of about 50% per year.

Wine characteristics such as pH, SO<sub>2</sub>, and acetaldehyde influence these processes and anthocyanin interactions with other phenolic compounds.

## Polymeric Anthocyanins

Anthocyanins can become associated with polymeric molecules in wine through a complex set of reactions. When colored forms of anthocyanins are combined with grape or wine polymers, they become significant contributors to color expression and color stability. These polymers are less effected by pH and SO<sub>2</sub> than monomeric anthocyanin forms and do not usually decrease with wine age.

The degree of anthocyanin polymerization that occurs in wine depends on the types and concentrations of phenolic compounds present, pH, SO<sub>2</sub>, temperature, and the availability of oxygen. Tannin concentrations seem to have more effect than monomeric anthocyanin on the final concentrations of polymeric anthocyanin.

Polymerization of anthocyanins occurs most rapidly during fermentation and maceration, but the process may continue throughout the life of a wine. As wines age, a greater proportion of their anthocyanin content is polymerized. Like the other wine polymers, they may also be removed due to precipitation. As a result fining agents that remove tannin may also remove polymeric anthocyanins and reduce wine color.

## Concentration Data

Many of the phenolic compounds in wine are not available as commercial standards. To quantify these phenolic compounds in wine we compare them to the most closely related available standards.

The concentration data is important as an essential check on the relative information presented in the radar plots and should be incorporated into your own wine information system.

As your phenolic database becomes more richly populated, the history of quantified phenolic levels will become apparent, and valuable interpretations can be made from raw data as well as from comparison to established standards.

## Analytical Details:

<b>Methodology:</b>	High Performance Liquid Chromatography (HPLC)
<b>Sample Size Required:</b>	Representative 60 mL sample
<b>Target Response Time:</b>	5 working days from receipt of samples
<b>Sample Packaging:</b>	Container with minimal headspace

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