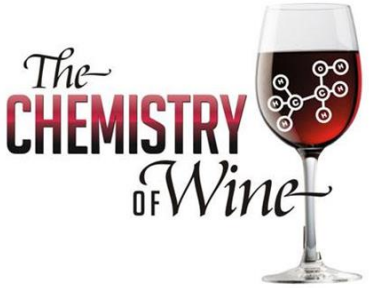
SHIMADZU
Excellence in Science

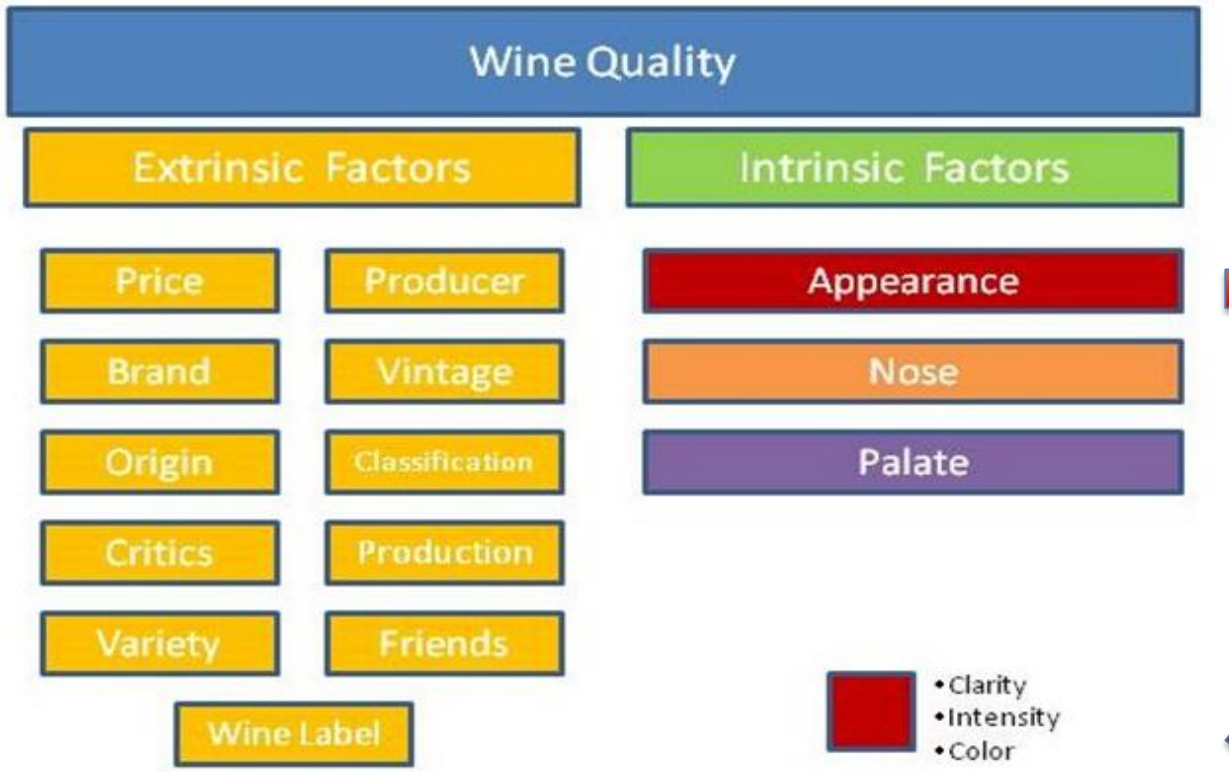


Determination of wine colour by UV-VIS Spectroscopy following Sudraud method

Johan Leinders, Product Manager Spectroscopy

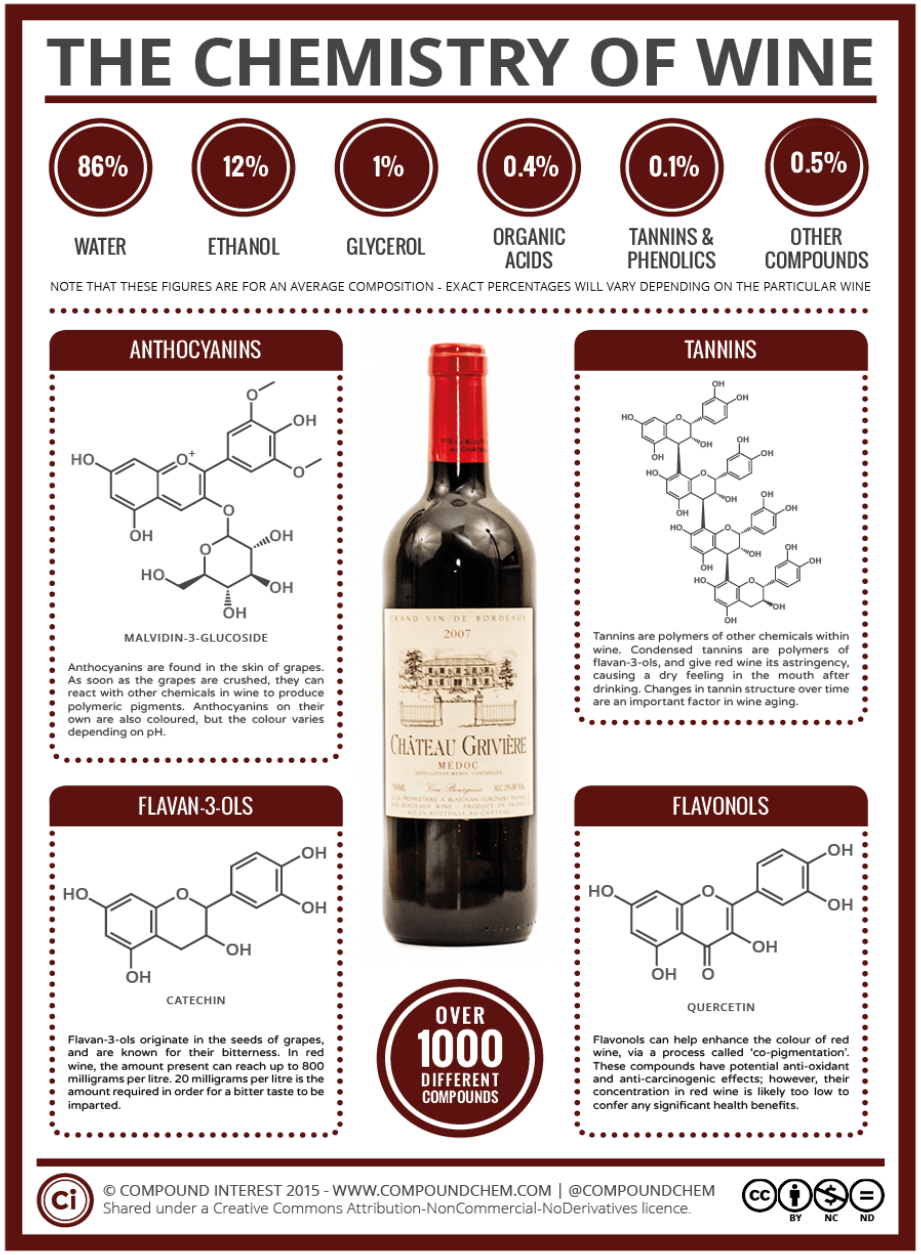
1. A bit of background

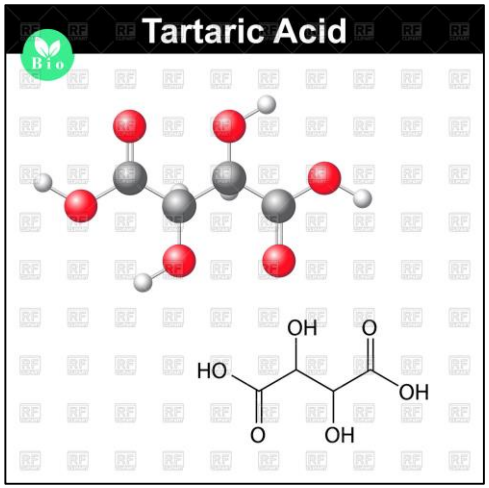
Why measure the colour of wine?

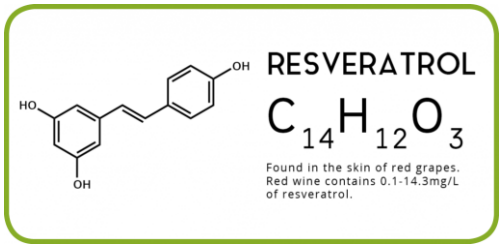


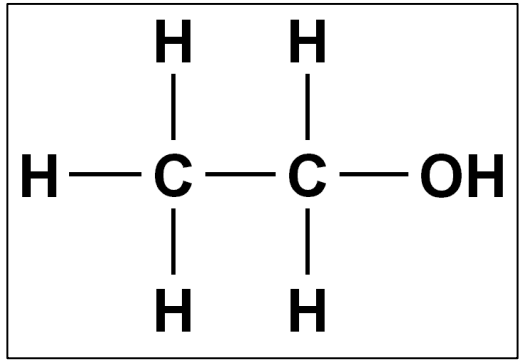
- Verification of lot-to-lot consistency in wine color, particularly with varietal blends.
- Monitoring color consistency over a season and from season-to-season.
- Monitoring the change in wine color over time.

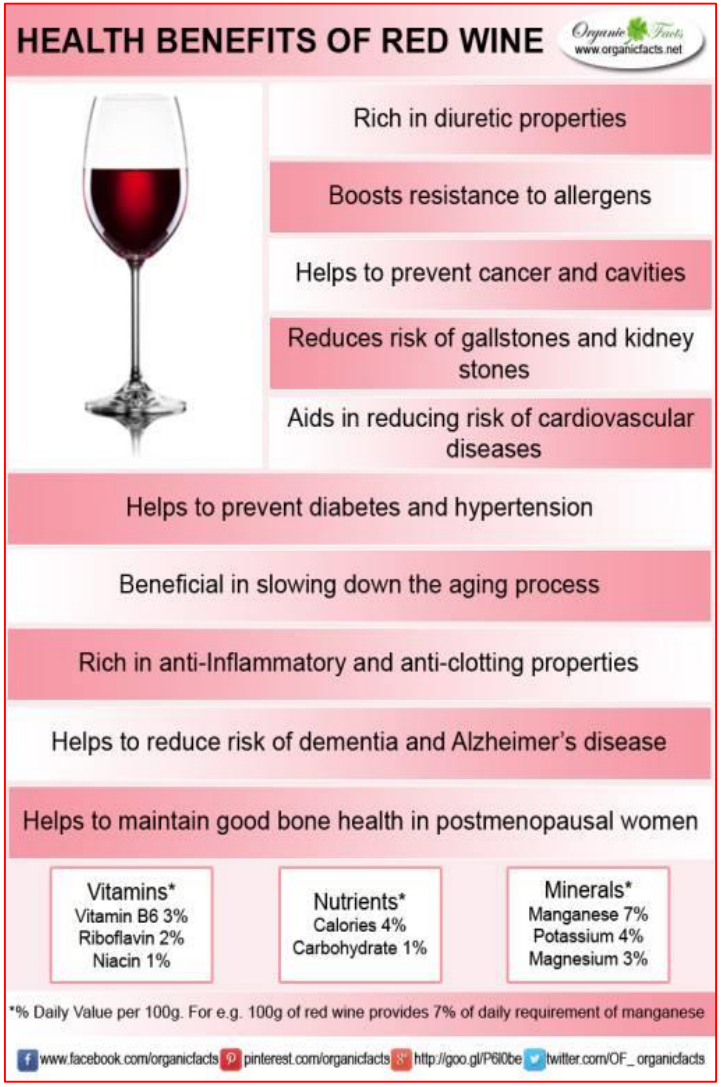
1. A bit of background





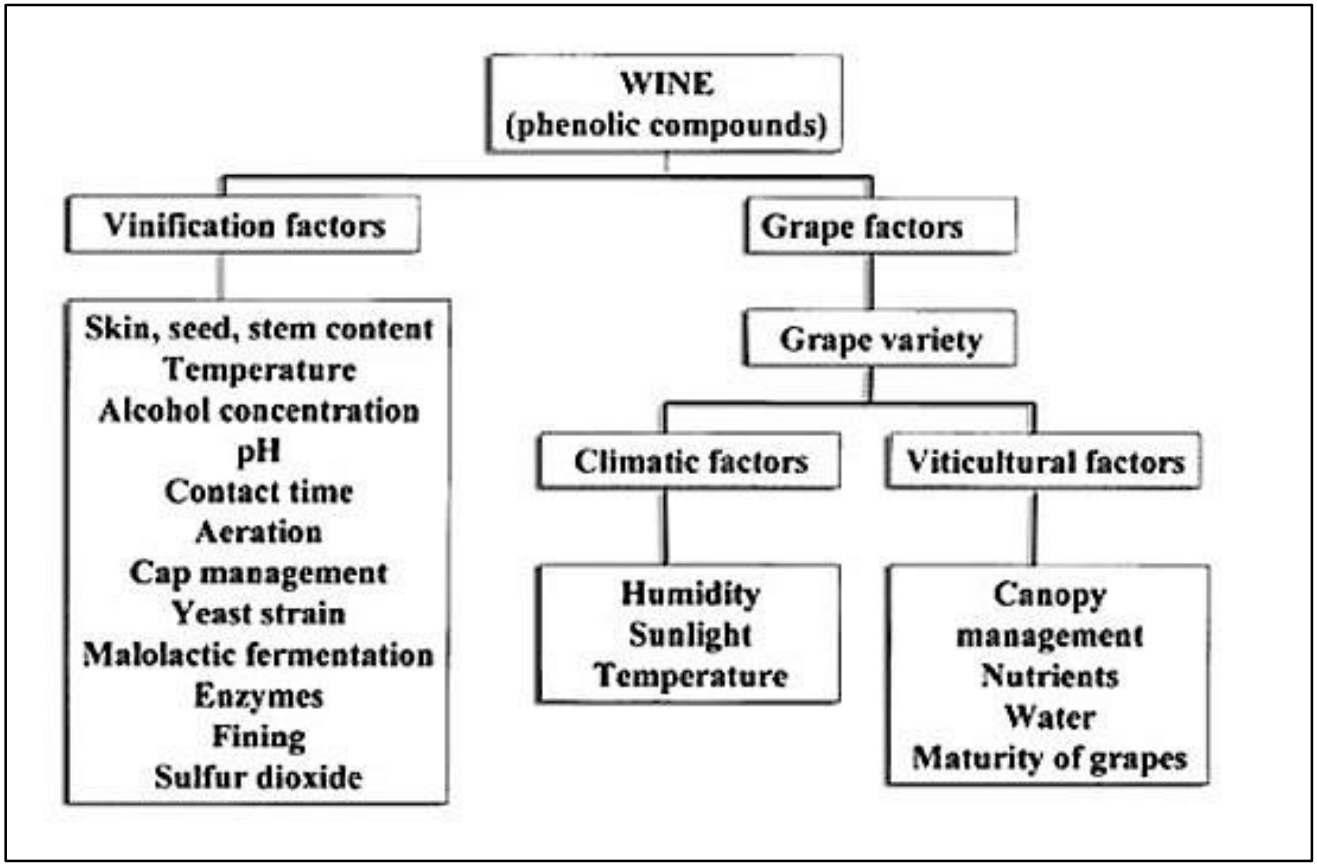






1. A bit of background

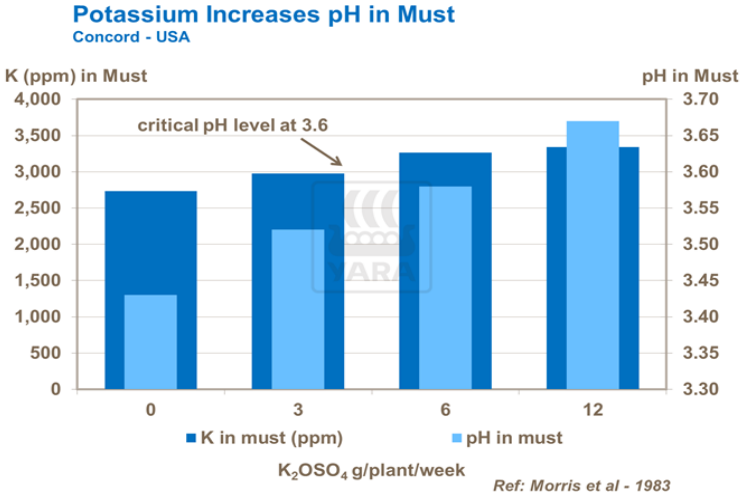
- **Soil composition (available trace elements) will have an impact on the final properties.**



- **Elements like potassium in combination with grape properties will influence the final colour.**

1. A bit of background

- **High K concentrations are typical for red wines; equilibrium between K, tartaric acid and anthocyanin pigments complex, which is responsible for the red colour.**



Wine pH and Color
Muscadine, USA

Wine pH	Anthocyanin (mg/l)
2.9	788
3.2	692
3.8	603

Ref: Sims & Morris - 1984

- **Anthocyanin is a natural colorant which is found in leaves and grapes. About 250 different variations of the anthocyanin complex are known. They all contain the structure of the common "Flavin" molecule which will be substituted at 7 possible positions from simple -OH to complex sugar molecules.**

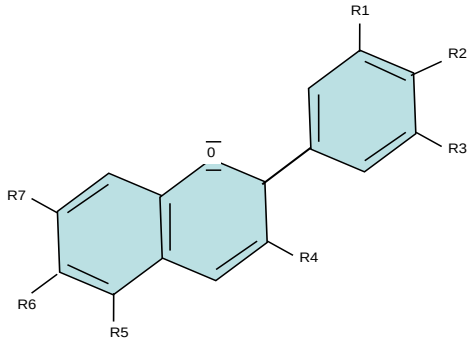
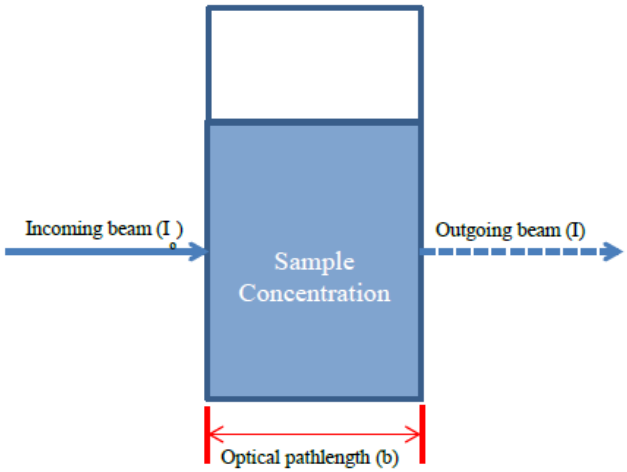


Fig. 2: Flavene is the base of the anthocyan structure, R1 to R7 represent organic groups which will generate the difference among the anthocyanes

2. Wine Colour Determination

- **The definition of wine colour: analysis of absorption spectra of wine samples are required.**
- **Physically, the colour is a light characteristic, measurable in terms of intensity and wavelength.**
- **Anthocyanin pigments are absorbing in the GREEN portion of the visible spectra, giving to human eye the sensation of colour RED.**

Bouguer-Lambert-Beer's Law



Light incident on a sample (I_o) can be reflected, absorbed, or transmitted. The ratio of light transmitted through the sample to the light incident on the sample, I/I_o, is defined as the transmittance through the sample (T). Absorbance (A) can be calculated from transmittance using the following relationships:

$$T = \frac{I}{I_o} = 10^{-kcb} \; and \; \%T = \frac{I}{I_o} * 100 \qquad A = -\log \frac{I}{I_o} = kcb$$

Samples are routinely measured in absorbance because absorbance is proportional to the concentration of the sample (Beer's law) and is proportional to the optical path length (Bouguer's law). The proportionality constant (k) is unique for every species. When the optical path length is 1 cm and the sample concentration is 1 mole/L, the proportionality constant (k) for a given species becomes the molar absorptivity (ε) yielding the more commonly seen equation relating absorbance and concentration:

$$A = \varepsilon bc$$

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2. Wine Colour Determination

- **The colour of wine can be defined by three terms as follows:**

1. Intensity **or luminosity: related to the "Intensity" of colour**
2. Chromaticity**: representing the colour in a 3 dimensional space**
3. Brightness **or purity: related to the clearness**

- **The methods to give results of chromatic characteristics of wine are:**

Glories Method (1984, Intensity):

Very similar to Sudraud, correcting for different % contribution of pigment categories. Absorbance is measurements at 420nm (yellow), 520 nm (red) and 620 nm (blue).

Sudraud method (1958, Intensity):

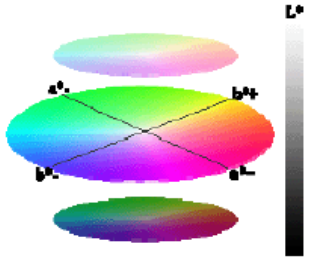
Defining the Intensity I as the sum of absorbances measured at 420nm and 520nm and the Tone T as the ratio of above mentioned absorbances on a 1cm cell.

Tristimulus method (Chromaticity):

Based on CIE x, y, z space coordinates, showing the red, green and blue content to define colour in a 3 dimensional space. Transmittance values at 4 wave lengths.

Cielab Space L* a* b* values (1988, Chromaticity):

Advanced version of Tristimulus, complex algorithms for continuous Cartesian representation on 3 orthogonal axis.



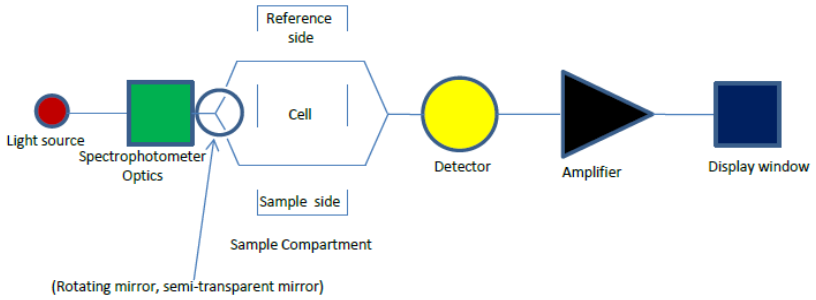
3. Sudraud Method for colour intensity

- An absorption spectrum (350nm – 700nm) of wine is collected.
- 10mm Quartz cuvettes
- Absorbance value determined at 420nm (yellow) and 520nm (red = max Anthocyanin)

- Calculations:
- Intensity I (Density) = Absorbance at 420nm + Absorbance at 520nm
- Hue T (Tone) = Absorbance at 420nm / Absorbance at 520nm

3. Required Hardware

- **Double Beam Spectrophotometer: UV-1800**
- **2 x 10mm quartz cuvettes**

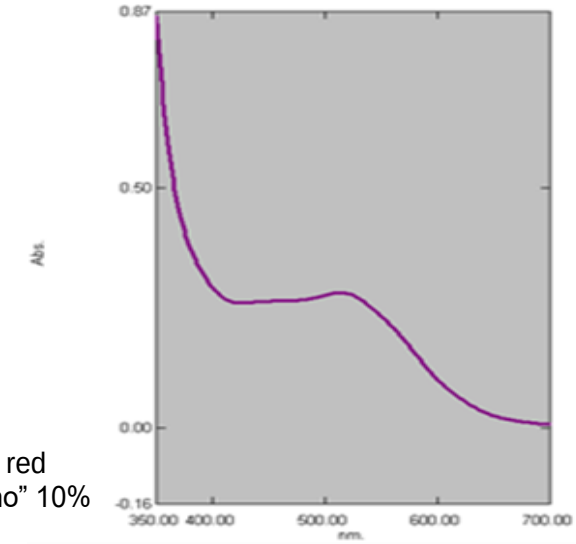


- **UV-Probe software allowing direct calculation of I & T in software**

Sample Table - (Active)

	Sample ID	WL420.0	WL520.0	I	T
1	Red wine original 1cm	2.59143	2.91382	5.50525	0.88936
2	Red wine original 2mm	0.53064	0.57776	1.10840	0.91844
3	Redwine 1:1 diluted water 1cm	1.28052	1.37573	2.65625	0.93079
4	Redwine 1:1 diluted water 1cm_1	1.28259	1.37573	2.65833	0.93230
5	Redwine 1:1 diluted water 1cm_2	1.28357	1.37573	2.65930	0.93301
6	Redwine 1:1 diluted water 1cm_3	1.28357	1.37573	2.65930	0.93301
7	Redwine 1:1 diluted water 2mm_1	0.25916	0.27405	0.53320	0.94566
8	Redwine 1:1 diluted water 2mm_2	0.25940	0.27417	0.53357	0.94613
9	Redwine 1:1 diluted water 2mm_3	0.26086	0.27600	0.53687	0.94516
10					

Result of Sudraud method in a UVProbe software table,



This is the absorption spectrum of a red Italian table wine “colore rosso rubino” 10% Volume measured with UV-1700.

Application Note Available

Application Note

Determination of wine colour with UV-VIS Spectroscopy following Sudraud method

IN VINO VERITAS. A very famous Latin phrase becomes reality in case you prove the colour of the wine.
Wine is more or less natural product having all substances inside like sugars, wine acids and alcohol. Depending on the earth where it was grown trace elements have their influence. Other elements like potassium (K) in combination with the graves will have influences on the colour. Colour can be white and red or different depending on theoretical classification and practical subjective identification of human eyes. High K concentration is combined with the red wine because it exists an equilibrium between K, tartaric acid and the anthocyan pigments complex, which is responsible for the red colour.

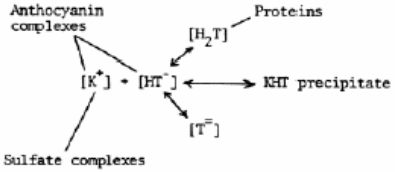


Fig. 1: Equilibrium between Anthocyan color and tartaric acid, K^+ = Potassium, H_2T = Tartaric acid, $T^=$ and HT^- = tartaric acid salts [1]
The anthocyane is a natural colour which is found in plants like in leaves or in fruits like the grapes. From these anthocyan complexes are about 250 different structures known. They have partly in common the structure (Fig. 2) of the flaven molecule which will be substituded at 7 possible positions from simple -OH to complex sugar molecules.

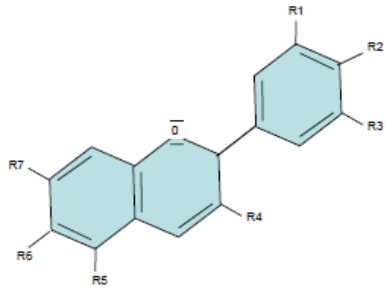


Fig. 2: Flavene is the base of the anthocyan structure, R1 to R7 represent organic groups which will generate the difference among the anthocyanes

Wine Colour Determination [2]
The definition of Wine Colour allows us to run analysis of absorption spectra of wine samples.
Physically, the colour is a light characteristic, measurable in terms of intensity and wavelength.
The human eye can reveal a substance as coloured due to two reasons:

- absorption of all of radiation of the visible spectra and non-absorption of the colour under analysis;
- absorption of the complementary of the colour under test.

In the case of grapes and wine, we can approximately say they are RED, because the anthocyan pigments are absorbing in the GREEN portion of the visible spectra, giving to human eye the sensation of colour RED.

The colour of wine can be defined by three terms as follows:

1. **Intensity or luminosity**: related to the "amount" of colour;
2. **Tone or chromaticity**: representing the wine colour shading off;
3. **Brightness or purity**: related to the clearness.

For better understanding the Wine Colour analysis, have a look on one spectrum of it. The methods to give results of chromatic characteristics of wine are:

- tristimolous method: based on X, Y, Z values, showing the RED, GREEN and BLUE content to define colour (according to CIE);
- Sudraud method: defining the Intensity ***I*** as the sum of absorbance measured at 420nm and 520nm and the Tone ***T*** as the ratio of a.m. absorbance, on a 1cm cell.

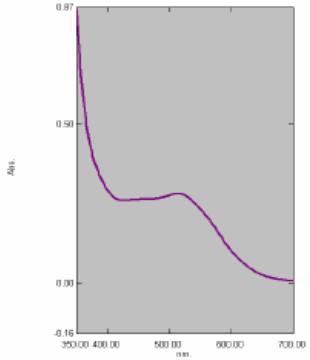


Fig. 3: This is the absorption spectrum of a red Italian table wine "colore rosso rubino" 10% Volume measured with UV-1700.

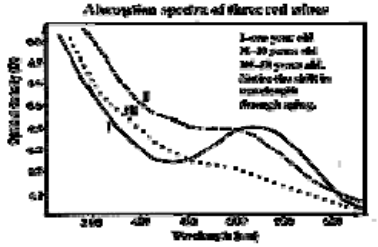


Fig. 4: This is a wine and its variation while aging or oxidising [3].

Sudraud Method
I=A420 + A520
And
T = A420 / A520

We can observe the A520 becoming smaller as much as old the wine is.
The tone is also varying and is below 1 for young wines, and greater than 1 when old; in particular, for red wines the colour is changing from ruby red up to orange red.
T value can give some indication about the age of wine, and if this is knew, on preservation condition and if possible to be aged again.

Sample Table - (Active)

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10					

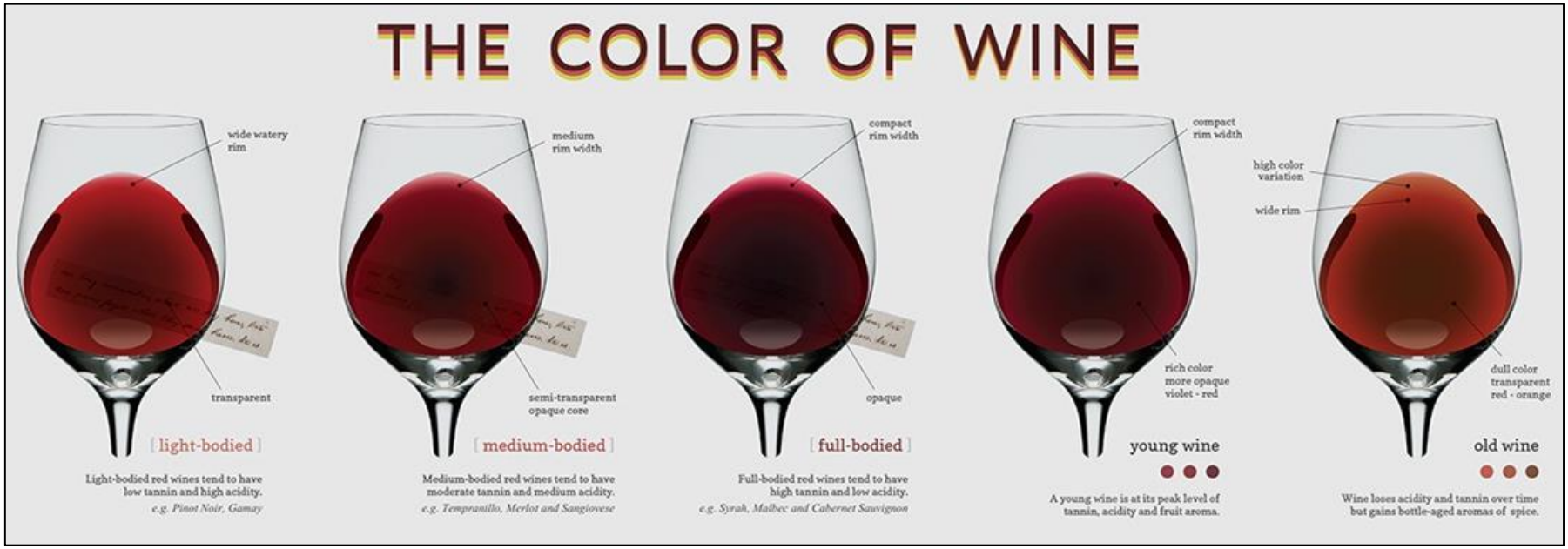
Fig. 5: Result of Sudraud method in a UVProbe software table, sample see fig. 3.

These values can be also used for determining the final colour of a red wine, especially when ***I*** is too weak (winery at high latitudes receiving few rays of sun). In fact, wines from southern countries are sometimes used for mixing (technically "cutting") the same of the north. (This is possible when there's no requirement of *origin certification - DOC, DOCG, AOC, etc.* - .) ***I*** has the property to be added to same value of another wine, so it's easy to find the percentages.

Literature:
[1] Bruce Zoecklein, A review of potassium bitartrate stabilization of wine, Publication 463-013, 1988, Virginia Cooperative Extension Service
[2] Emanuele Canu. Shimadzu Italy Milano, Wine Colour analysis, Shimadzu internal document
[3] Sudraud P., Ann. Tech. Agri. 7 (1958) 203
Instrument:
UV-1700 and 1cm liquid cell

4. Chemical Age of Wine

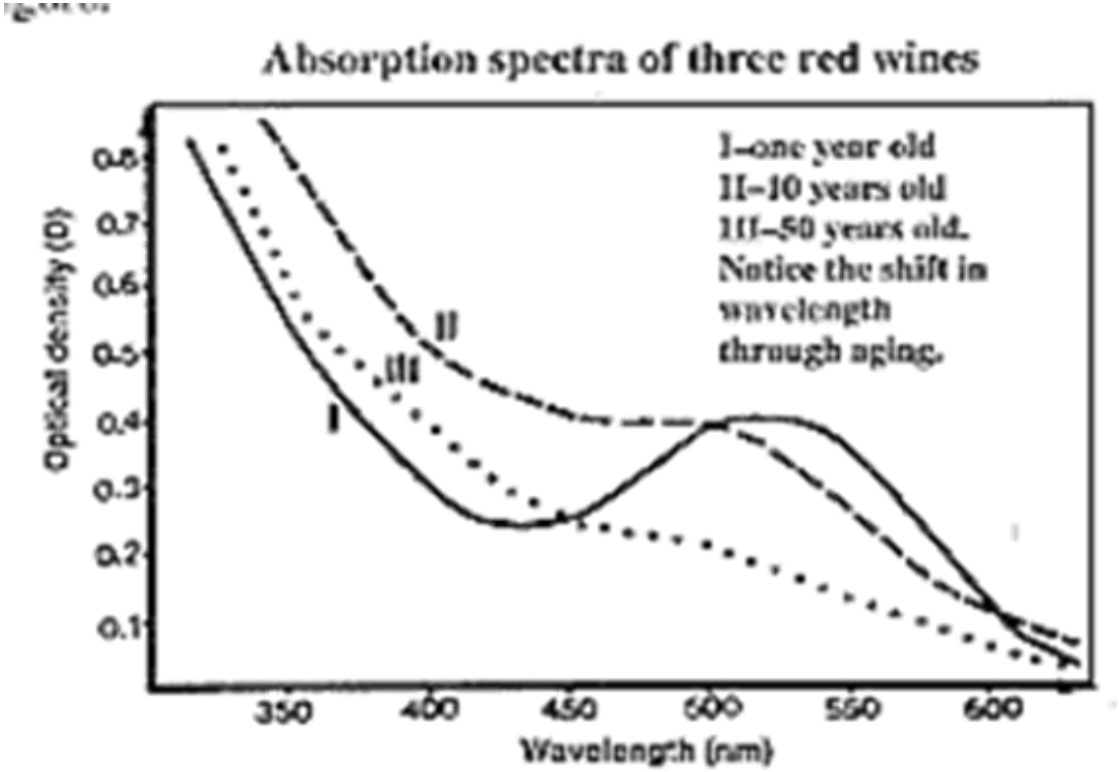
- **As red wine ages, the harsh tannins of its youth gradually give way to a softer mouthfeel. An inky dark color will eventually lose its depth of color and begin to appear orange at the edges, and then later eventually turning brown. These changes occur due to the complex chemical reactions of the phenolic compounds of the wine.**



- **Visual: For red wines the colour is shifting from ruby red to orange red.**

4. Chemical Age of Wine

- **Typical the absorbance A at 520nm will decrease and absorbance at 420nm will increase as the wine is aging**
- **Reason: transition of monomeric Anthocyanins into polymeric Anthocyanins**
- **As a result, typically the Hue T (tone) is <1 for young wines (0,5 – 0,7) and >1 when older (max 1,2 – 1,3) (Development towards orange)**



4. Chemical Age of Wine

- **Artificial ageing: micro-oxygenation, shaking the wine, exposing it to radiation, magnetism or ultra-sonic waves, high-voltage electricity, metallic object that is dipped into wine.**

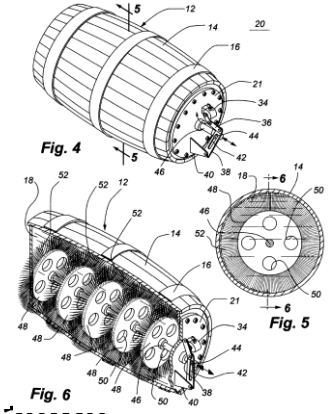


Fig. 4

Fig. 5

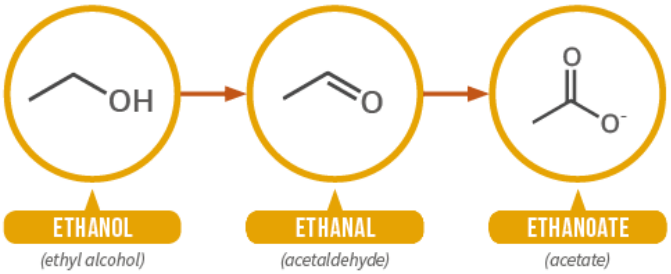
Fig. 6


FIG. 9

- **In general, wines with a low pH have a greater capability of aging.**
- **With red wines, a high level of phenolics (most notably tannins), will increase the likelihood that a wine will be able to age.**
- **The ratio of sugars, acids and phenolics to water is a key determination of how well a wine can age**
- **Pigmented tannins, anthocyanins, colloids, tannin-polysaccharides and tannin-proteins not only influence a wine's resulting color but also act as preservatives**

THE CHEMISTRY OF A HANGOVER

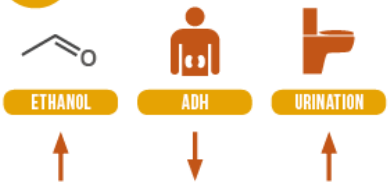
For most of us, a hangover is the price to pay for a night of drinking. However, we still don't know what exactly it is that causes them. In this graphic, we look at what happens to alcohol in your body, and some of the prime suspects for causing your hangover.



WHAT HAPPENS TO ALCOHOL IN YOUR BODY?

In the liver, ethanol is converted to acetaldehyde by the alcohol dehydrogenase enzyme, and then subsequently converted into acetate by the aldehyde dehydrogenase enzyme. Acetate can be broken down into carbon dioxide and water, then eliminated from the body. On average, the liver can break down alcohol at the rate of one unit (8 grams or 10 millilitres of pure alcohol) every hour.

1 DEHYDRATION

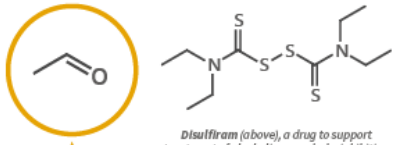


During alcohol intoxication, release of the anti-diuretic hormone (ADH) vasopressin is decreased, resulting in increased urination.

Alcohol has a diuretic effect on the body, increasing urine production. Alcohol-induced dehydration has been suggested as a cause for some hangover symptoms, but research suggests it isn't a major factor.

2 ACETALDEHYDE

Acetaldehyde is rapidly converted into acetate in the liver

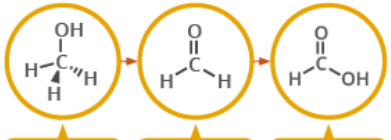


***Disulfiram** (above), a drug to support treatment of alcoholism, works by inhibiting the breakdown of acetaldehyde, producing unpleasant, hangover-like symptoms.*

Acetaldehyde, produced by the breakdown of alcohol, has toxic effects that could cause hangover symptoms. However, acetaldehyde concentration doesn't significantly correlate with hangover severity.

3 CONGENERS

Methanol is found in very small amounts in many alcoholic drinks



Congeners are compounds other than ethanol in drinks. These include alcohols such as methanol, which breaks down into toxic formaldehyde and formic acid. Congeners can increase hangover severity.

4 IMMUNE SYSTEM

Cytokines are small proteins released by cells which affect other cells. They play an important role in the immune system.

CYTOKINES INCREASED BY ALCOHOL CONSUMPTION

In particular, IF-12 & IFN-γ concentration changes show significant correlations with hangover severity

Alcohol causes changes in cytokine concentrations in the immune system. Studies have shown the effects caused by some cytokines are very similar to those of a hangover, strongly supporting their role.

5. Summary

- Anthocyanins are mainly responsible for the color of wine
- Sudraud method has been around for 60 years, easy to implement
- Absorption measurement at 420nm and 520nm to determine I and T
- Double beam UV-Vis, 10 mm cuvettes, software
- I and T will shift during aging of wine, color will shift from rube red to orange red

- **QUESTIONS?**