APPLICATION NOTE

UV/Visible Spectroscopy

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Wine Analysis Using the LAMBDA Series Spectrophotometers

Introduction

Wine has been made for the past 8,000 years using traditional methods. In recent years, there has been an worldwide increase in production – much of it coming from "New-World" wine producers such as the USA (California, in particular), Australia, New Zealand, South Africa, and some South American countries – notably Argentina and Chile. Even unlikely candidates as wine producers, such as the UK and Canada, are producing quality wines in relatively small quantities. This broadening of geographic locations has also led to an increase in the technology used in both the manufacturing and testing of the product to ensure consistency of flavor and product safety. Modern analytical techniques, such as Gas and Liquid Chromatography (GC and HPLC), are used by big producers. Another technique is UV/Visible spectroscopy.

One indicator of wine quality is its color. In previous times, this would have been done by eye but this is semi-quantitative at best. It is useful to be able to assign numbers to wine color using instrumentation. Although color is mainly a quality consideration, it does also help with safety issues as a change in color may indicate a fault in the brewing process or indicate bacterial or other contamination.

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Wine Color Intensity and Hue

One of the simplest approaches is to measure the absorbance at three wavelengths in the visible region. Normally, these are at 420 nm, 520 nm, and 620 nm. These can be summed (the Wine Color Intensity) or a ratio of A_{420} / A_{520} can be measured (Wine Hue). The calculations are based on the absorption of wine in a 1cm (10 mm) pathlength cuvette. In the case of red wines, it is necessary to use shorter pathlengths (e.g. 1 mm) in order to obtain spectra within the range of the instrument. The readings can then be recalculated for a 1 cm cuvette.

This simple approach has been adopted as it allows measurement in relatively simple instrumentation without wavelength scanning ability.

CIE Color Based Analysis

A more accurate approach is to utilize the entire visible region from 380 nm to 780 nm, and apply standard color methodology as developed by CIE (Commission Internationale D'Eclairage, based in Vienna). This is an international body responsible for the whole area of representing colors numerically. Its first specification was issued in 1931 and has been developed steadily over the years. The color calculation is a weighted transmittance value that takes into account the illumination conditions and the spectral responses of the eye to the three primary colors of light – red, green, and blue. It also takes into account the two types of receptor in the retina – rods (for monochromatic vision in low-light conditions) and cones (color receptors which work in relatively well illuminated conditions).

Wine analysis is normally performed using the 1964 calculation. This uses a 10° observer angle (this takes into account the contribution of both rods and cones whereas the earlier 1931/ 2 degree standard was biased towards cones only) and Illuminant D65 (daylight with a color temperature of 6500 K). This calculation produces three values for the amount of each of the three primary colors – red, green, and blue. These are the tristimulus values and are X, Y, and Z for red, green, and blue, respectively. The calculation involves adjusting the absorbance scan to the equivalent of a 1 cm pathlength and then converting it to transmittance (%T). CIE publish weighting tables for the illuminant and for red, green, and blue responses in the eye. The calculation is as follows:

$$X_{10} = K\int_{380}^{780} S(\lambda)\cdot \bar{x}(\lambda)\cdot R(\lambda)d\lambda \qquad (1)$$

$$Y_{10} = K\int_{380}^{780} S(\lambda)\cdot \bar{y}(\lambda)\cdot R(\lambda)d\lambda \qquad (2)$$

$$Z_{10} = K\int_{380}^{780} S(\lambda)\cdot \bar{z}(\lambda)\cdot R(\lambda)d\lambda \qquad (3)$$

$$K = \frac{100}{\int_{380}^{780} S(\lambda)\cdot y_{10};(\lambda)\cdot \bar{R}(\lambda)d\lambda} \qquad (4)$$

Where S(λ) = relative spectral power distribution of the illuminant (D65) as supplied by CIE

x_{10}, y_{10} and z_{10} = the color matching functions for a 10 degree observer (as supplied by CIE 1964)

R(λ) = Spectral reflectance of sample

These values can then be normalized so that x + y + z =1. This means that the value of z is no longer necessary as it is implied and so a color can be described using the x and y values.

$$x = \frac{X}{X+Y+Z} \qquad (5) \qquad y = \frac{Y}{X+Y+Z} \qquad (6) \qquad z = \frac{Z}{X+Y+Z} \qquad (7)$$

When quoting a color using this system, it is usual to include the un-normalised Y value. The x and y data can be plotted against each other to produce a graph – the CIE color space diagram.

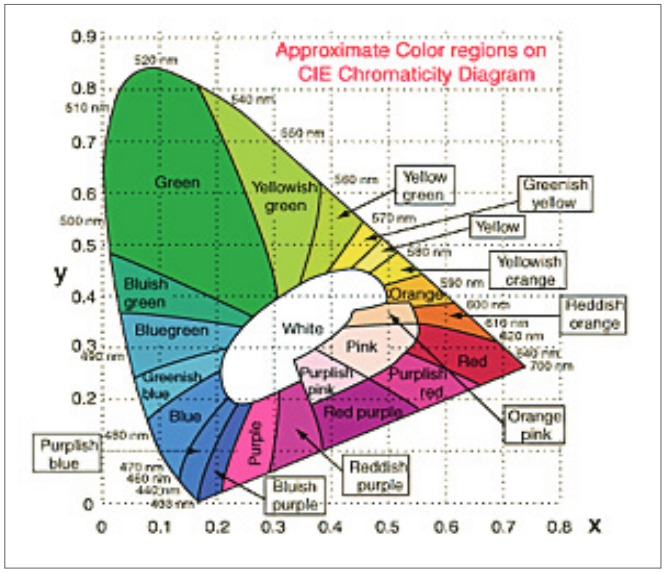

Figure 1. CIE Color Space Diagram

This diagram can represent any possible color (the "impossible" ones being outside of the colored area). There is also an area in the center which is called the white point.

One further refinement of the color measuring system was made by CIE in 1976. This is the L* a* b* system. This system is basically a way of representing color in three dimensional space using Cartesian coordinates. The transform to L* a* b* is as follows. (Xn, Yn, and Zn are the white point values of X, Y, and Z and for a 10 ° observer are 94.811, 100, and 107.304, respectively).

$$L^* = 116 \cdot \left(\frac{Y}{Y_n}\right)^{1/3} - 16 \qquad (8)$$

$$a^* = 500 \cdot \left[\left(\frac{X}{X_n}\right)^{1/3} - \left(\frac{Y}{Y_n}\right)^{1/3}\right] \qquad (9)$$

$$b^* = 200 \cdot \left[\left(\frac{Y}{Y_n}\right)^{1/3} - \left(\frac{Z}{Z_n}\right)^{1/3}\right] \qquad (10)$$

There is a special case to the equation if either X/Xn, Y/Yn, or Z/Zn is less than 0.008856. In this case, the calculation becomes:

$$7.787 \cdot \left(\frac{X}{Xn}\right) + \frac{16}{116} replaces \left(\frac{X}{Xn}\right)^{1/3} \qquad (11)$$

$$7.787 \cdot \left(\frac{Y}{Yn}\right) + \frac{16}{116} replaces \left(\frac{Y}{Yn}\right)^{1/3} \qquad (12)$$

$$7.787 \cdot \left(\frac{Z}{Zn}\right) + \frac{16}{116} replaces \left(\frac{Z}{Zn}\right)^{1/3} \qquad (13)$$

The L* a* b* values can then be represented in three-dimensional space as

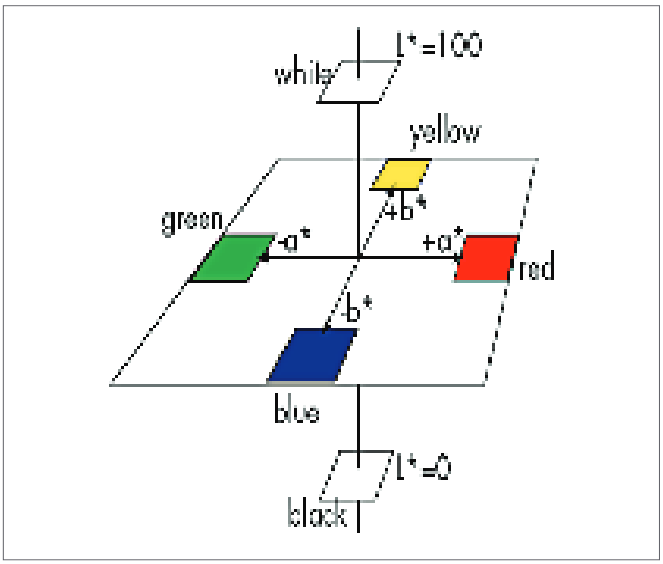

Figure 2. L* a* b* Color Space

The final part of the calculation is to calculate the Chroma (C*ab) and Hue Angle (h*ab). These are a conversion of L* a* b* values from Cartesian to polar coordinates. This is also termed L*C*h* color space.

The L* term is the same as for L*a*b* color space.

The Chroma value is the perpendicular distance from the Lightness axis.

The Hue Angle is the angular component.

The wine industry also uses these values to produce some additional parameters based on the L*a*b*and C*ab values. These are:

$$S^* = \frac{C^*ab}{L^*} \qquad (14)$$

$$Q^* = (0.15 \cdot L^*) \cdot \log(Yn) + (0.6 \cdot L^*) + 40 \qquad (15)$$

Experimental

Wine samples were measured a Lambda 25, 35, or 45 using a standard quartz cuvette. The pathlength depends on the type of wine being measured. For full bodied red wines a 1 mm cuvette should be used. For white wines, a 10 mm pathlength is fine. The instrument was autozeroed using a distilled water blank using the same pathlength cuvette as the test sample. For short pathlength cells, the use of a suitable spacer is recommended.

The measurement was carried out using the Wine Analysis method which is supplied as an example method with UV WinLab version 6 software.

Table 1. Part numbers of cuvettes and spacers.

Part Number	Description
B0631025	1 mm pathlength quartz cuvette with PTFE stopper (pk/2)
B0631008	5 mm pathlength quartz cuvette with PTFE lid (pk/2)
B0631009	10 mm pathlength quartz cuvette with PTFE lid (pk/2)
N9302471	Spacer for 1 mm pathlength cuvette (pk/2)
N9302743	Spacer for 5 mm pathlength cuvette (pk/2)

UV WinLab v6 software is compatible with the LAMBDA 20, 40, 25, 35, 45, 650, 750, 800, 850, 900, and 950 instruments. The program is designed to work with the medium performance instruments (LAMBDA models with double-digit numbers – eg. LAMBDA 25), but data can be imported into the method from all the listed models.

Results

A variety of different wines was analyzed using a LAMBDA 25 with UV WinLab version 6.0 and the results are shown in the table.

In terms of wine tasting and appreciation, wine can be classified under the following headings. These were taken from a Spanish publication, the classification is subjective and will vary slightly from country to country.

Soft straw yellow: Very young wines with a light body and alcohol content. This color can also indicate a defective wine that has been over-filtered or that uses too many clarifying agents.

Straw yellow with green tones: A very fresh white wine with greenish reflections because of the presence of chlorophyll. The wine tends to retain the green pigment of the grapes.

Straw yellow: A term for white wines that are at peak maturity.

Golden yellow: Indicates that a white wine is aging and oxidizing.

Yellow amber: Typical of wines made from partially dried grapes or fortified grapes. When straw yellow wines develop this color they are losing their quality due to oxidation.

Pink: Wines with a reddish color and soft, lighter reflections.

Pale pink: Wines with a soft red color, almost transparent.

Cherry red: Wines with no more than 100 milligrams per liter of anthocyanins (plant pigments that produce a blue to red color) and that are made with longer skin contact.

Purple red: A red wine with purple reflections towards the edge of the glass.

Ruby red: Describes a healthy red wine in the early stages of maturity.

Garnet: Burgundy color typical of mature red wines with a remarkable structure.

Mahogany: Wines with a warm orange color at the rim of the glass during their prime maturation period.

Table 2. Wine analysis using LAMBDA 25.

Wine Type	Type	Color Intensity	Color Hue	X	Y	Z	x	y	L*	a*	b*	C*ab	h^*_{ab}	S*	Q*
Spanish Tempranillo	Full Red														
Australian Shiraz	Full Red														
Chilean Merlot	Full Red														
French Cabernet Sauvignon															
Californian White Zinfandel															
French Beaujolais	Light Red														
French Rose D'Anjou															
Portugese Vinho Verde															
Australian Oaked Chardonnay															
New Zealand Sauvignon Blanc															
South African Unoaked Chenin Blanc	Straw Yellow with green tones	0.1045	4.0241	91.12	96.42	95.94	0.3214	0.3401	98.60	-0.53	4.91	4.94	96.14	0.05	118.74

L* a* b* values can be used to produce a color difference from a known standard (or ideal value) by calculating the ΔE value. This is calculated as follows:

$$\Delta E = \left((L_1 * - L_2 *)^2 + (a_1 * - L_2 *)^2 + (b_1 * - b_2 *)^2 \right)^{1/2}$$

This value assumes color space is uniform (which it is not) but is still widely used in the wine industry and is useful for the small color differences as would be expected in this type of analysis.

It is possible to use L* a* b* color data to analyze individual pigments for identification purposes. Some pigments, such as malvidin 3-glucoside, exhibit a large color change up to acidification whereas others, such as vinylcatechol pyranoanthocyanin are virtually unchanged.

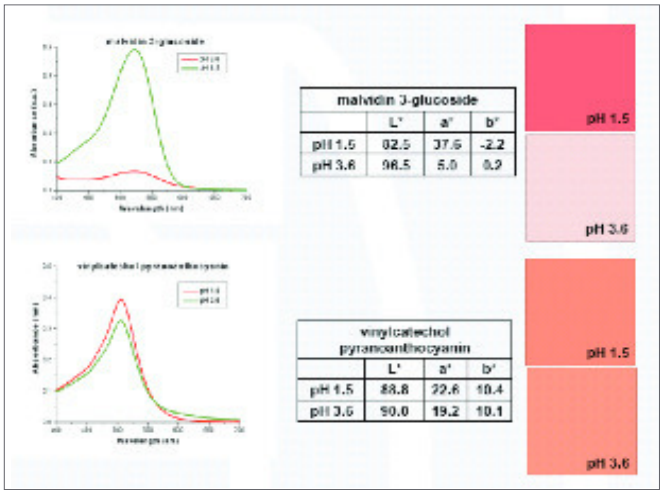

Figure 3. L* a* b* color data to analyze individual pigments.

Other Wine Assays Using UV/Visible Spectrophotometers

Absorbance at 280 nm

This is a very non-specific assay for phenolics which all absorb around 280 nm due to their aromatic nature. Its simplicity is countered by the fact that it will measure other aromatic compounds (eg nucleic acids and aromatic peptides/proteins) and will not differentiate between the different phenolic types. This assay requires a spectrophotometer that is capable of measuring in the UV region (ie below 320 nm)

Folin-Ciocalteu Assay for Phenolics

This assay is used to measure phenols and is based on the fact that phenols ionize completely under alkaline conditions and can, therefore, be readily oxidized by the Folin-Ciocalteu reagent. This oxidation causes a color change from yellow to blue, which is easy to measure in a UV/Visible spectrophotometer at 765 nm. The Folin-Ciocalteu reagent is very reactive and can oxidize unintended components in the wine such as fructose, ascorbic acid, bisulfite, and amino acids. This can be prevented by adding acetaldehyde (to bind the bisulfite) or, for a sweet wine, applying a correction factor.

Enzymatic Methods for Phenolics

A recent method involving horseradish peroxidase (HRP) has been introduced as an alternative to the Folin-Ciocalteu Assay. It works by converting phenolics into quinine-imine products. The assay is fast (less than five minutes). The method is still in development and has not been shown to work well with all types of phenolics.

Iron Chloride Assay for Polyhydroxylated Phenolics

Iron Chloride will bind to certain phenolics that contain more than one hydroxyl group, so all phenolics in wine can be measured except anthocyanins and other monohydroxylated phenolics.

Tannin Assays

There are three main assays for the measurement of tannins in wine. These are the Glories Gelatin Index, the Llaudy Method, and the UC Davis Assay. These three methods are all based on the precipitation of tannin by proteins and vary in their complexity. The Glories Assay (which uses gelatin as a protein) suffers from the fact that the precipitation step is very long. The Llaudy method uses ovalbumin and is quicker and more reproducible that the Glories method but still requires a total of twelve measurements to be made for each wine sample. The UC Davis Assay uses Bovine Serum Albumin (BSA) for the precipitation. These are then reacted with Iron (III) Chloride and measured at 510 nm.

Enzymatic Measurement of Sulfite, Ethanol

As an alternative to chromatography, it is possible to measure several analytes in wine, such as sulfites and ethanol, by using kits like those sold by r-Biopharm (a division of Hoffmann La Roche). In the case of ethanol, the ethanol is oxidized to acetaldehyde in the presence of nicotinamide adenine dinucleotide (NAD+) in the presence of alcohol dehydrogenase. The assay is designed to work at 20 °C. In the case of sulfite, this is a two stage reaction. The first stage involves oxidation of the sulfite with sulfite oxidase (SO2-OD). The product of this reaction is hydrogen peroxide which is then reacted with NAD+ in the presence of NADH peroxidase (NADH-POD).

Other Useful Analytical Techniques for Wine Analysis

For the busy laboratory where sample throughput is an important factor, there are other techniques that can be considered including gas and liquid chromatography. Head Space gas chromatography is particularly useful as it allows for rapid analysis for volatile components such as alcohol in wine. For metal analysis (such as copper), atomic absorption (AA) or Inductively Coupled Plasma (ICP) instruments should be considered.

Conclusion

The LAMBDA 25, 35, or 45 provides an accurate simple means to acquire high-quality spectra of wine and to calculate the required parameters automatically without the need to export to a spreadsheet or external program. The dual-beam optics and low-stray light ensure excellent performance with highly colored wine samples and the flexible reporting allows for customized reports to suit both internal and external clients.

Appendix

We have documented the UV WinLab method calculation processing chain should you wish to modify it or check the calculations. The method, as supplied, is ready to run so no modification or adjustment should be necessary.

Table 3. UV WinLab method calculation processing chain.

No	Process	Description	Equation
1	Equation	Adjust Absorbance Spectrum to 1cm pathlength	All* 10/Pathlength
2	Equation	Absorbance at 420 nm	Yval[All,420]
3	Equation	Absorbance at 520 nm	Yval[All,520]
4	Equation	Absorbance at 620 nm	Yval[All,620]
5	Equation	Color Intensity (CI)	A420+A520+A620
6	Equation	Color Hue	A420/A520
7	Convert X	Convert absorbance to transmittance	No equation
8	Equation	Interpolate 380 to 780, Data Interval=5	No equation
9	Equation	K part 1 (not shown in results table)	" Illuminant_D65.asc" * " Y_1964.asc"
10	Equation	Final K calculation (not shown)	100/[[Mean[K part 1]]* 160]
11	Equation	X calculation part 1 (not shown)	Sample* " X_1964.asc" * " Illuminant_D65.asc"
12	Equation	Final X calculation	[[[Mean[X part1]]* 160]* Kj/100
13	Equation	Y Calculation part 1 (not shown)	Sample* " Y_1964.asc" * " Illuminant_D65.asc"
14	Equation	Final Y Calculation	[[[Mean[Y part 1]]* 160]* Kj/100
15	Equation	Z Calculation part 1 (not shown)	Sample* " Z_1964.asc" * " Illuminant_D65.asc"
16	Equation	Final Z Calculation	[[[Mean[Z part 1]]* 160]* Kj/100
17	Equation	x Calculation	X part 2/[X part 2+Y part 2+Z part 2]
18	Equation	y Calculation	Y part 2/[X part 2+Y part 2+Z part 2]
19	Equation	Y/Yn Calculation for 10 degree Cbs	Y part 2/100
20	Equation	X/Xn Calculation for 10 degree Cbs	X part 2/94.811
21	Equation	Z/Zn Calculation for 10 degree Cbs	Z part 2/107.304
22	Equation	L* Calculation	116* [[YYn]^ [1/3]]-16
23	Equation	a* Calculation	500* [[[XXn]^ [1/3]]-[[YYn]^ [1/3]]]
24	Equation	b* Calculation	200* [[[YYn]^ [1/3]]-[[ZZn]^ [1/3]]]
25	Equation	Chroma (C* ab) Calculation	Sqrt[[[Sqr[astar]]+[Sqr[bstar]]]]
26	Equation	Standard Hue Angle Calculation using PI variable (PI())	ATan[bstar/astar]* 180/PI[]
27	Equation	Conditional Hue Angle Calculation using IF statements	if [[astar] > 0 and [astar] > 0, hstar,if [[astar] < 0 , [180+hstar], if [[bstar] < 0 and [astar] > 0, [hstar + 270], hstar]]]
28	Equation	S* Calculation	Cstar/Istar

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