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ANALYSIS OF THE COLOUR PROFILE OF PORT WINES USING DIFFERENT INSTRUMENTAL METHODS *AND VISUAL APPROACHES*

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Thesis presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* to fulfill the requirements of Master of Science degree in Food Engineering

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Abstract

Colour is an important quality parameter in wines and is the result of a complex mixture of pigments (including anthocyanins and their derivatives, quinones, xanthyllium compounds, etc.). Red wine colour changes over time, as pigments react between themselves and with other wine macromolecules (particularly polyphenols). During wine tasting, colour is normally assessed on the outer rim of the wine in a tilted glass, since most wines are too opaque to be analysed in the middle of the glass. Therefore, depending on the depth of observation considered, the perception of wine colour can be different.

The current official (OIV) method for measuring wine colour is based on UV/Vis spectrophotometric determination of the spectrum. In the current official (OIV) method, the transmittance spectrum of a particular sample is measured and used to calculate CIE-L*a*b* parameters (L*, a* and b*), which define a single (predominant) colour for a particular wine.

Reflectance colorimetry is an alternative well-established method for measuring colour in foodstuffs, which can also be used in translucent samples (such as wines) as long as a reflective background is used.

In this work, a reflectance colorimeter was used to measure CIE-L*a*b* colour parameters of Port wine samples of different categories at different depths, in Petri dishes. The obtained results were compared with the parameters obtained using the OIV method. In addition, the colour profile of Port wine samples was analysed using the colorimetric approach described above. An (untrained) panel was asked to assess the colour hue of the wine samples in Petri dishes, using an unstructured line scale, with the goal of establishing a correlation with the colorimetric readings at the same depths.

The results suggest the colorimetric method can be used as an alternative to the OIV method for estimating the L* and H* parameters (the most important for wine colour definition), being quicker and more informative. In addition, there is a good correlation between H* parameter of colorimetric readings and the visual assessment of colour hue at all tested depths, being slightly better at lower depths. The colorimetric determination of wine colour at different depths can be conveniently used to characterize the visually perceived hue of Port wines.

Sumário

A cor é um parâmetro de qualidade importante em vinhos e é o resultado de uma mistura complexa de pigmentos (incluindo antocianinas e os seus derivados, quinonas, compostos xanthyllium, etc.). A cor do vinho muda ao longo do tempo, assim como os pigmentos reagem entre si e com outras macromoléculas presentes no vinho (especialmente polifenóis). Durante prova de vinhos, a cor é normalmente avaliada na orla do copo de vinho inclinado (45º), uma vez que a maioria dos vinhos são muito opacos para serem analisados no meio do copo. Por conseguinte, dependendo da profundidade de observação considerada, a perceção de cor de vinho pode ser diferente.

O método oficial (OIV) para medir a cor de vinho é baseado na determinação espectrofotométrica UV / Vis. Neste método, o espectro de transmitância de uma determinada amostra é medida e usada para calcular os parâmetros CIE L * a * b *, que definem uma única cor (predominante) para um determinado vinho.

Colorimetria pode ser considerado alternativo para a medição de cor em géneros alimentícios, também podendo ser utilizado em amostras translúcidas (tais como vinhos), desde que um fundo refletor seja usado.

Neste trabalho, um colorímetro foi utilizado para medir parâmetros de cor CIE-L*a*b* de amostras de vinho do Porto de categorias diferentes em diferentes profundidades, em placas de Petri. Os resultados obtidos foram comparados com os parâmetros obtidos usando o método OIV. Além disso, o perfil de cor de vinho do Porto amostras foi analisada utilizando a abordagem colorimétrica descrita acima. Além disto, um painel (não treinado) foi convidado a avaliar a tonalidade de cor das amostras de vinho em placas de Petri, usando uma escala linear não estruturada, com o objetivo de estabelecer uma correlação com as leituras colorimétricas com as mesmas profundidades.

Os resultados sugerem que o método colorimétrico pode ser utilizado como uma alternativa ao método OIV para estimar os parâmetros L * e H * (importantes na definição da cor de vinho), sendo um método mais rápido e informativo. Além disso, boas correlações foram obtidas entre as medições com o colorímetro do parâmetro H* (CIE-L*a*b*) e a avaliação visual pelo painel da tonalidade da cor a todas as profundidades testadas, sendo ligeiramente melhor em profundidades inferiores. A determinação colorimétrica da cor do vinho a diferentes profundidades pode ser convenientemente utilizada para caracterizar a cor percebida visualmente em vinhos do Porto.

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1. Introduction

1.1. Port wine

Port wine is a fortified wine produced in a Douro demarcated region; only 26.000 ha are authorized to produce Port wine (about 2/3 of vineyard area). During Port production, the alcoholic fermentation is stopped by the addition of spirits, thus obtaining a sweet and alcoholic wine. The fermentation occurs with skin maceration, in order to extract aroma and phenolic from the grapes. Port wine can be obtained from red or white grapes, the former being much more important in terms of volume of production. Red Port wines can be broadly divided into two groups.

Ruby Port wines (aged in bottle) seek to restrain the evolution of their deep red colour, more or less intense, and maintain the fruit and strength of a young wine. There are several categories for these wines, Ruby standard, Reserve Ruby, Late Bottled Vintage (LBV), Crusted and Vintage (IVDP 2016).

Tawny Port wines are obtained by the blending of different matured wines, led by ageing in wood casks. Colour shows evolution and should integrate in the sub-classes of red-tawny colour, tawny or tawny light. Aromas are reminiscent of dried fruits and wood; when older the wine, the characteristics of dried fruits and wood gain form and tonality become yellower. The existing categories are: Tawny standard, Reserve Tawny, Tawny with an Indication of Age (10 years, 20 years, 30 years and 40 years) and Colheita. These wines are obtained from blends of wines with different ages, except for Colheita, which must be produced from a single vintage, which is indicated on the label. The following scheme shows the difference between styles ports and the existing categories.

Table 1 Categories of Port wine (IVDP 2016)

Categories of Port wine	*Chromatic characteristics*
Ruby standard	Ruby Port resembles the ruby gemstone, because it has an ageing process with little or no oxidation (usually up to three years in wooden barrels).
Reserve Ruby	Being the product of a selection of the best Port wine made each year, display a more complex structure than Ruby with a “full-bodied, rich and deep ruby red”.
Late Bottled Vintage (LBV)	LBV Port is a “deep ruby red” and “extremely full-bodied” with good ageing potential. Presents a harvest date and generally is obtained from a lot of wines from that harvest. The ageing process takes place in large vats, oak barrels or stainless steel tanks for the oxidative evolution to be extremely slow. The LBV is bottled between the 4th year and 6th year after harvest.
Vintage	Port wine that matures in the

	bottle; produced from the grapes of a single year and bottled two to three years after harvesting, gradually evolving for 10 to 50 years in bottle. Vintage Port, in the first five years holds the ruby intensity of the original colours and after ten years began to create an average deposit and develops “red garnet” tones. With maturation, vintage colour evolve to a “golden brown” tones
Tawny standard	Port wine obtained from stocking rate wines, usually with an average age of three years and aged in wine seasoned wood casks. During the ageing process, oxidation process is forced to give a golden hue.
Reserve Tawny	Aged in oak, apparent their “médium golden brown colour”. Their tones vary according to the winemaking processes: they can be red next rubies, or brownish colour similar to the older tawny ports. Reserve Tawny is obtained from the blending of wines with an average age of five to seven years.
Ageing indication	Good Port wine quality and allowed to use age designation. Indications are age 10 years, 20 years, 30 years of age and 40 years of age. This Port is a tawny achieved by blending wines from several harvests in order to join different organoleptic characteristics (colour, aroma and flavour). **Tawny 10 years**

	old Port present a more developed colour than Reserve Tawny, very similar wine but with the added assurance that it bears the characteristics of a ten years old Port. **Tawny 20 years old Port** has colours ranging from a “reddish to golden Tawny”, these exceptional wines are full of fruit and their flavours are more developed and concentrated due to the fact that the wine was aged in small oak casks. **Tawny 30 years old Port** are set aside to age longer in wood. The gradual exposure to air concentrates and intensifies the original fruit of these wines and develop the colour to golden shades. **Tawny 40 years old Port** is the oldest Tawny Port, which usually present the goldest tone.
Colheita	Tawny Ports from a single harvest are aged in casks for a minimum of seven years, resulting in wines with colour amplitudes ranging from red to Golden-brown, depending on the age.

1.2. Wine colour and phenolic components

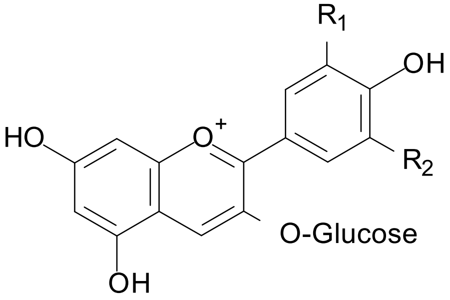
About 99.5% of the wine composition is completely transparent to light radiation. In this composition will encounter water, ethanol, glycerol and residual sugars, organic acids, minerals, and most of amino acids. The remaining 0.5% of wine composition absorbs light in the UV-Vis region and largely consists of phenolic pigments which are responsible for the existence of the different colours found in wines, from white wine to red wine (Somers 1998).

Phenolic compounds (phenols or phenolic) are a class of organic molecules which contain at least one hydroxyl group (-OH) attached to an aromatic ring. Phenolic can be divided in two groups according to their chemical structure, flavonoids and non-flavonoids. Flavonoids has a basic structure composed with two aromatic rings linked to each other and to a chain of three carbons, which form a benzopiranic central ring. This ring is known by C ring. Adjacent ring is called A ring and substituent phenolic ring is called B ring. The other group, non-flavonoids do not have the flavan structure described before (C6-C3-C6). Examples of non-flavonoids are phenolic acids, stilbenes or hydrolysable tannins (Aron and Kennedy 2008, Campos 2009). Since flavonoids are responsible for the colour of red wines, only these compounds will be discussed in this thesis in more detail.

The most important families of flavonoids found in wine are: flavonols, flavon-3-ols and anthocyanins (Gutiérrez, Lorenzo et al. 2005). In each of this families, there are several compounds which differ in their B-ring substituents (hydroxyl and methoxy group) (María, Pedro et al. 2006, Campos 2009, Fei, Lin et al. 2010).

The most important groups of pigments present in red Port wines are anthocyanins. Colour exhibited by anthocyanins was first explained by Linus Pauling in 1939, who proposed that the resonant structure of the flavylium ion caused the intensity of their colour (Wrolstad, Durst et al. 2005). These phenolic molecules are relatively water-soluble, and usually linked to a glucose molecule (Wrolstad, Durst et al. 2005, Castañeda-Ovando, Pacheco-Hernández et al. 2009). Anthocyanins are responsible for many colours in various plants and fruits, from red to blue (Ribéreau-Gayon, Glories et al. 2006, Castañeda-Ovando, Pacheco-Hernández et al. 2009, Oliveira, da Silva et al. 2013). Anthocyanins release anthocyanidins by hydrolysis, which are less chemically stable (Hutchings 1994, Ribéreau-Gayon, Glories et al. 2006).

There are five types of anthocyanidin's in grapes type *Vitis vinifera*, cyanidin, delphinidin, peonidin, petunidin and malvidin. It can be seen the different anthocyanins in the following figure.



Name	R1	R2
Cyanidin-3-*O*-glucoside	OH	H
Delphinidin-3-*O*-glucoside	OH	OH
Peonidin-3-*O*-glucoside	OCH_3	H
Petunidin-3-*O*-glucoside	OCH_3	OH
Malvidin-3-*O*-glucoside	OCH_3	OCH_3

Figure 1 Structural formulae of anthocyanins commonly found in grapes and wines (Campos 2009)

Free anthocyanindins are very unstable, and usually react with other wine components to become more stable. Auto-association between anthocyanindins, complexion with metals and co-pigmentation are example of such reactions (Boulton 2001, Berké and de Freitas 2005, Campos 2009, Oliveira, da Silva et al. 2013).

During fermentation and ageing, anthocyanins will react with (uncoloured) products of yeast metabolism and flavonoids, forming polymeric pigments, in a process known as co-pigmentation which is responsible of hue changes in colour of wine. (Hutchings 1994, Boulton 2001). Copigmentation results of hydrophobic interactions between coloured forms of the anthocyanins and other molecules (i.e., copigments) originating complexes which adopt a sandwich configuration, thus protecting the flavylium chromophore from nucleophilic attacks and stabilizing its colour (Boulton 2001, González-Manzano, Dueñas et al. 2009).

The decrease of the concentration of free anthocyanins and formation of polymeric pigments causes a change in Port wine colour from bright red colour of a young Port wine to an old brownish red Port wine (Somers 1998).

The colour evolution will be different in Port wines depending on the ageing process (bottle or wood cask). In the case of bottle-aged wines, anthocyanin concentration will decrease slower than wood-aged wines since the latter process will contribute to several reactions with anthocyanin, due to controlled oxidation (air present inside the cask) and reactions with wood components (Marquez, Serratosa et al. 2014, Galante 2015). These reactions, which occur over time will be ultimately responsible for the differences in tonalities and colour intensities found in Tawny and Ruby Port wines (Heredia, Francia-Aricha et al. 1998, Monagas, Bartolome et al. 2006).

There are more or less distinct colour differences between the previously described categories of Port wines. As Tawny Port ages, its hue becomes progressively more yellow whereas in Ruby Port there is a change in both colour intensity and hue, which becomes less red

1.3. Colour

Colour perception is the result of the interaction between a light source, an object and a human observer (Wyszecki and Stiles 1982, HunterLab 2016). The light source emits radiation, which is a form of energy, to the object. The most common light source is the sunlight but there also other light sources such as tungsten and fluorescent lamps (Wyszecki and Stiles 1982, HunterLab 2016). Object will absorb/refract or reflect the radiation emitted from the source to the observer. The colour's sample will depend on the absorption of some wavelengths of the incident light by colourants such as pigments in the object, and the reflection or transmission of other wavelengths to the observer (HunterLab 2016). The human eye has rod and cone cells in the retina which are responsible for colour perception. Rods are responsible for perceiving low luminosity while cones are responsible for colour vision at high levels. There are three types of cone sensitivities: red, green and blue (Wyszecki and Stiles 1982, HunterLab 2016).

Therefore, the perceived colour will depend on the light source, the sample and the visual perception of the reflected and refracted radiation by the observer. Sunlight is the most common light source used in wine tasting in a social environment (Hutchings 1994).

The first attribute normally evaluated during wine tasting is its visual appearance which includes its colour. Different perceptions of colour may occur when a glass of wine is tilted 45º since the wine profile adopts an oval shape. Depending upon the observed depth of the wine in the glass, colour perception changes since wine is a translucent product (Huertas, Yebra et al. 2003, Martin, Ji et al. 2007, Hernández, Sáenz et al. 2009).

Chromatic characterization is very important for assessing the quality of Port wine. In

Port wines, colour is associated with the age and the particular style of the wine. Ruby-styled Ports are aged (mostly or exclusively) in the bottle, which results in deep red tones, more or less intense depending on age and style.

Due to the subjective nature of visual evaluation, spectrophotometrically methods are normally used nowadays to characterize the colour of a particular wine. The current official method recommended by the (OIV 2006) is based in a molecular absorption (UV-VIS) spectrophotometry reading of absorbance/transmittances of a particular wine sample followed by the calculation of the CIE-L*a*b* parameters from the resulting spectra (OIV 2006). A limitation of this method is the measurement of colour at a single depth by using a fixed-path length spectrophotometric cuvette.

1.3.1. CIE-L*a*b* system

There are several colour reference systems that can be used to study the chromatic characteristics of objects, such as the CIE-L*a*b* from the ‘‘Commission Internationale de L’Eclairage” (CIE), RGB which is the abbreviation of the additive colour system formed by Red (Red), Green (Green), blue (Blue) and the CMYK which is a subtractive colour system consisting in Cyan, Magenta, Yellow and Black. The current system used for colour quantification in wine is the system established by the CIE which is based on the determination of tristimulus (x, y and z) coordinates in a three-dimensional space.

The first CIE reference system was developed in 1931 when x, y and z functions were determined at a 2º-observation angle. After experiments, x, y and z functions were recalculated at 10º observation angle in 1964, which simulate the perception of the observer looking to a sample of wine (HunterLab 2016). The differences between an observation of 2º and 10º angle can be seen in the following figure.

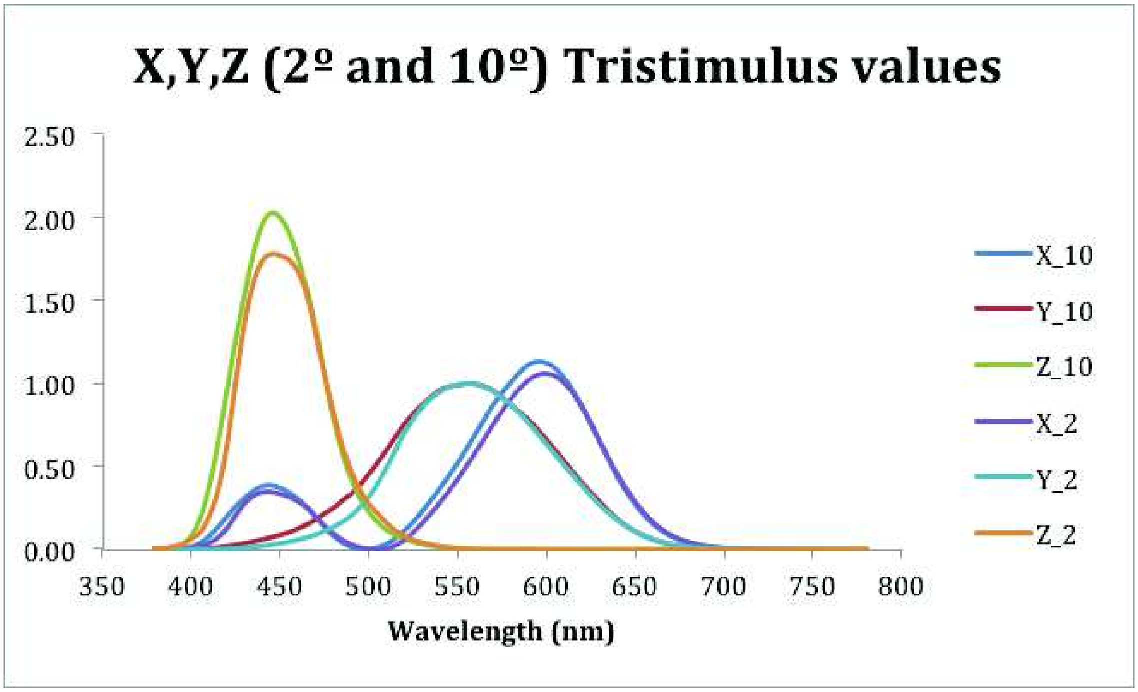


Figure 2 Tristimulus values with 10º-observation angle are higher than 2º-observation angle, especially in Z values (Lab 2015).

The tristimulus values, in the CIE system, can be used to calculate the parameters L * (which represents the brightness of the sample), a * (representing the red/green colour component) and b * (representing the blue/yellow colour component).

In addition to the parameters mentioned above, colour can also be characterized by its "purity" or chroma (C *) and hue (H *), which are calculated using the a * and b * values. Chroma represents the saturation or brightness of the colour and is usually observed by how intense the colour is. C* value is the perpendicular distance from the lightness axis (figure 3).

Tone or Hue is one of the main parameters described as "the degree to which a stimulus can be described as similar to or different from stimuli, which are described as red, green, blue, and yellow" (Birse 2007). Tone is the first attribute described of colour that is observed. Hue (H*) values can be represented in degrees (from 0º to 360º), with 0º corresponding to a pure red colour and 90º to a pure yellow colour. Low hue values represent young wines and high hue values represent aged wines, so with tonality it is possible to represent the ageing of a wine (Birse 2007).

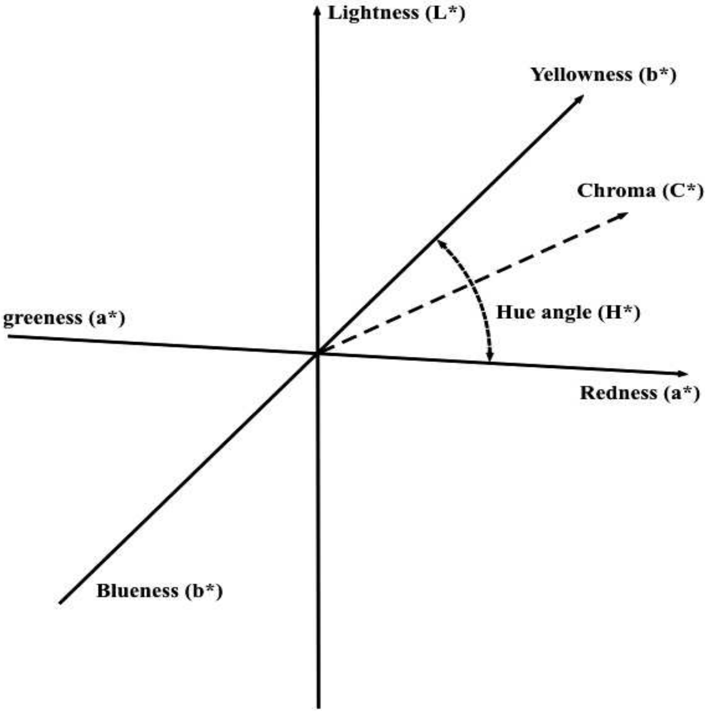


Figure 3 Colorimetric coordinate diagram in accordance with the Commission Internationale de l'Eclairage (OIV 2006)

1.4. Relation between polymeric pigments and chromatic characteristics

As mentioned previously, anthocyanins are the main molecules responsible for the colour of wine. During ageing, anthocyanin concentration decreases due to the formation of polymeric pigments, which will change the wine colour. Several researchers had studied the relation of polymeric pigments and the chromatics characteristics.

In a study of Francisco Heredia, several CIE colour spaces and chromatic systems were determined and submitted to multivariate statistical techniques such as discriminant analysis in order to obtain a mathematic model to predict and classify ageing status in red wines (Heredia, Troncoso et al. 1997).

The fastest changes in colour composition generally occur during the first year of storage (Somers and Evans, 1986). Purple-red colour of young red wines turns to orange- red hues with storage time. These changes are mainly caused by the displacement of the monomeric pigments of anthocyanins by more stable oligomeric forms (Gómez-Plaza, Gil-Muñoz et al. 2000).

The relationship between the colour parameters (colorimetric indexes and CIE-L*a*b* variables) and phenolic components such anthocyanins, pyranoanthocyanins, hydrobenzonic, hydroxycinnamic acids and flavanols were studied by (María, Pedro et al.

2006). Both anthocyanins (simple glycosides and acetyl-glycosides) and pyranoanthocyanins were selected by polynomial regression as the variables that best described the different colour parameters during ageing in bottle (María, Pedro et al. 2006).

(Pérez-Magariño and González-San José 2006) worked with several red wines in order to investigate the influence of the grape harvesting date on their chromatic characteristics and polyphenolics contents. Their results showed that harvesting date of grapes was correlated with degree of maturity and had influence in chromatic characteristics of red wines.

(Fu-Liang, Wen-Na et al. 2008) found negative correlations between monomeric anthocyanins and CIE parameters L*(lightness), b*(yellowness) and H*(hue angle). Conversely, positive correlations were obtained with a*(redness) and C*(Chroma) CIE-L*a*b* parameters.

(González-Manzano, Dueñas et al. 2009) investigated the importance of the co-pigmentation process between anthocyanins and flavanols on colour expression of red wines. Divergences were found between evaluation of copigmentation process based on chromatic characteristics in CIE-L*a*b* system. It was concluded that qualitative phenolic composition is essential to control and analyse the copigmentation process.

(Sen and Tokatli 2016) showed practical and rapid methods for classification purposes using Spectra of UV–Vis and colour parameters of wines with one variety with orthogonal partial least square discriminant analysis (OPLS-DA). Different wavelength (UV-vis) regions were found to be effective in classification with respect to variety and vintage. This study demonstrated the potential of combination of UV–visible spectra and colour characteristics to be used in the authentication of wines.

According to these researchers cited in this chapter, a good correlation can be obtained with CIE-L*a*b* system and anthocyanin concentrations, which in turn CIE-L*a*b*measurements appear to be related with several reactions during ageing.

1.5. Physical-chemical methods for measuring colour parameters:

Radiation can be refracted or reflected from the surface of the sample or be transmitted through the sample. Therefore, the perceived colour depends on the used light source, the incidence angle, the sample used and the radiation will be received (transmitted or reflected/refracted) (Hutchings 1994). The radiation emitted by the sunlight, which is the most common light source (D65) used in wine tasting in a social environment, has energy in the visible (380-700 nm), ultraviolet (<380 nm) and infrared (700-1000 nm) ranges.

Several works have been done by researchers in order to obtain a correlation between measured physical-chemical colour parameters and visual assessments. These studies are mostly intended to try to understand the contribution of the different types of radiation (reflection/refraction and transmitted) to colour perception.

(Almela, Javaloy et al. 1995) made comparisons between colour measurements with tristimulus values (XYZ) and CIE-L*a*b* and found that a precise definition of the wine colour could be obtained with lightness (L*) and redness/greenness (a*) parameters.

(Huertas, Yebra et al. 2003) performed colour measurements with a spectroradiometer at 26 regular spaced points of a wine sample, poured in a standard glass of wine. This method allowed the description of colour changes in different areas of the wine using CIE-L*a*b* parameters. Greater differences were noticed in colour (ΔE) in red wine samples, followed by rosé wines and white wines. It was concluded that hue displayed insignificant changes in colour in all spaced points tested in red, rosé and white wines, although hue colour is usually assessed in the rim when the glass is tilted 45º. However, for lightness and chroma, it was demonstrated that the colour changes depending on which area is being measured. The main reason for the colour change was the depth that the wine has in the measurement area.

(Martin, Ji et al. 2007) used two different methods for measuring colour appearance, a digital camera and a tele-spectroradiometer. For tele-spectroradiometer a cocktail glass was used for a better observation of colour differences in different depth, meanwhile a digital camera was used with proper software to measure colour in Petri dishes at different depths. Good correlations were obtained between these two methods.

(Niskanen, Mutanen et al. 2008) also studied the optical properties of red wine. Incorporating different types of optical measurements, the authors were able to characterize different stages of the winemaking process and aid in authenticating the wine.

(Hernández, Sáenz et al. 2009) used a spectroradiometer to measure the colour of wine in a glass tilted 45º with D65 light source. The colour of the samples set was measured in the centre of the glass and the rim. In the rim, the spectroradiometer was able to differentiate red wines classified in different colour categories, while in the centre it wasn't possible to differentiate the wine samples.

(Sáenz Gamasa, Hernández et al. 2009) conducted a similar work, also using a spectroradiometer, to measure the colour of white and rosé wines. The authors concluded that the measurements of white wines were more precise in the centre region of the glass than in the rim

Recently, (García-Marino, Escudero-Gilete et al. 2012) tried to establish a relationship between colour measurements by transmission and reflection radiation in order to evaluate which methodology would offer a better interpretation of the results in visual terms. The authors found that spectroradiometric measurements were more suitable for comparing with visual results.

As mentioned above, several authors have tried different approaches for measuring wine colour such the chromatic measurements at different depths and the use of different types of radiation such as the reflected and refracted radiation instead only the transmitted radiation (which is used in spectrophotometric methods). These new approaches allow for a more realistic characterization of the colour perception of a wine and will be explored further in this dissertation.

1.6. Spectrophotometric (UV-Vis) methods

Wine colour has been classically analysed using spectrophotometric measurements in the Ultraviolet and Visible ranges of the spectrum (normally from 300nm to 800nm, which corresponds to the absorbance range of wine pigments). As mentioned before, the colour of a red wine is of complex physical and chemical interactions, which mainly involve anthocyanins and their derivatives. The mathematical model normally used is Beer-Lambert-Bougier law which assumes (within limits) a linear relationship between the absorbance of light of a specific (monochromatic) wavelength with the concentration of light-absorbing species, according to the equation: $\boldsymbol{Abs = abc}$; Where $\boldsymbol{Abs}$ is absorbance of the sample, $\boldsymbol{a}$ is the molar absorptivity coefficient of the light-absorbing species, $\boldsymbol{b}$ is the optical pathlength and $\boldsymbol{c}$ is concentration of the sample.

Spectrophotometer readings may be expressed as absorbance (Abs) or transmittance ($\boldsymbol{T}$) values, which correspond to the percentage of radiation transmitted through the sample. The following formula represents a relation between absorbance and transmittance: $\boldsymbol{Abs = -\log(T)}$;

For wine colour measurements, spectrophotometric cells of 1 to 10 mm pathlengths are normally used in order to ensure absorbance readings in the linear zone of the Beer-Lambert-Bouguer law. Dilutions are not normally done since they could change the chemical balance of the wine samples and consequently have an impact on its colour.

The most traditional method of analysing wine colour consists of measuring the absorbance at three reference wavelengths: 420, 520 and 620 nm (corresponding to the absorption zones of yellow-brown, red and purple pigments, respectively) and calculating the Glories' parameters from the measurements. (Heredia, Troncoso et al. 1997, Sáenz Gamasa, Hernández et al. 2009). These parameters are: Colour Intensity (represented by the sum of the three absorbance's), Colour hue or tonality (obtained by dividing the absorbance at 420 and at 520 nm) and the percentage of yellow, red and purple pigments (obtained by dividing each of absorbance's by the colour intensity). These parameters are useful for comparing wines but not for an objective characterization of the wine colour.

Although still not very much implemented in the wine industry, the method recommended nowadays by the OIV (Office International de la Vigne et du Vin) for measuring wine colour uses the CIE-L*a*b* colour system. L*a*b* values are calculated from transmittance measurements made at specific wavelengths in the visible spectrum range using a spectrophotometer (OIV 2006). Although this method is more complicated and involves some mathematical calculations, it allows for establishing a predominant colour for a particular wine and thus to compare different wines.

1.7. Colorimetric method

Colorimetry is nowadays the most common instrumental technique used for measuring colour in foodstuff (Pathare et al. 2013). As alternative method to determine chromatic characteristics of wines can be the use of reflectance colorimetry, which is based on the measurement of the reflected radiation from the sample, using a colorimeter. This equipment has been used to measure colour of many different foodstuff and can be used in both with opaque and translucent samples as long as a reflective background is used. Basically, a colorimeter has an incorporated light source, which flashes over the sample, and the reflected radiation (at a specific fixed angle) is measured and expressed as colour parameters.

The use of a colorimeter allows the study of chromatic characteristics of a particular wine sample at different depths, by adjusting the amount (volume) of sample in a cylindrical container such as a Petri dish., as can be seen in figure 5. This method is simple and approaches the visual assessment when tasters are analysing the colour of the wine.

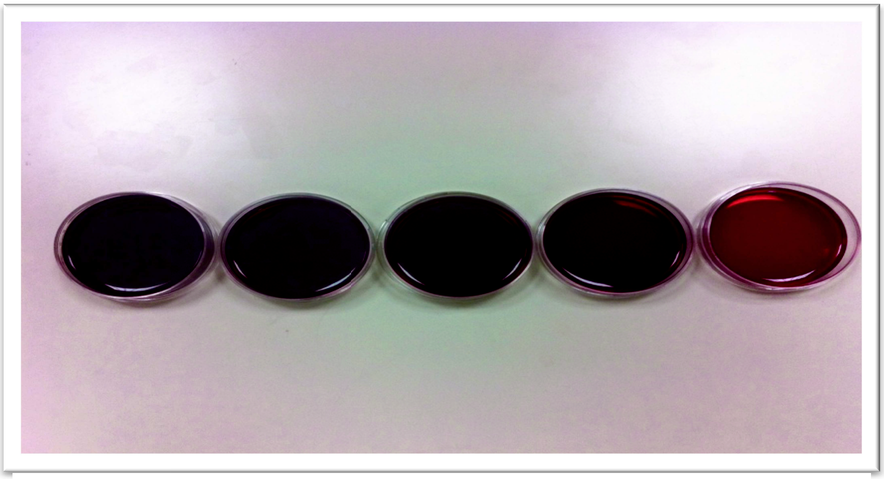


Figure 4 Port wine sample in five petri dishes with a decrease in depth (from left (6mm) to right (2 mm))

The proposed method has the advantage of measuring colour at different depths, contrarily with the spectrophotometric methods which depends on the (fixed) pathlength of the spectrophotometric cells.

1.8. Sensory analysis

The Sensory Evaluation Division of the Institute of Food Technologists define sensory evaluation as a scientific discipline used to evoke, measure, analyse and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste and hearing (Bruwer 2014). Sensory tests are used for several occasions such as: new products development, quality assessment of products, cost reduction in process or in raw material, research and storage time/quality control (Hutchings 1994).

Colour in wine is perceived by visual assessment and makes part of a quality control during tasting. Since sight is the first sense to be used during wine tasting it can affect the assessment of the other senses. Several experiments were made to enhance the importance of colour assessments in translucent samples. As example, a test was performed with hundreds of students to assess the sweetness of a lemon-and-lime drink in various degrees of colour intensity. Students believed the stronger the colour the sweeter the drink and it was quite the opposite: the stronger the colour the more sour the drink became (Lindstrom 2005). In 2001, (Morrot, Brochet et al. 2001, Parr, White et al. 2003) invited 57 wine experts and asked them to give their impressions about two glasses of wine, one red and one white wine. The wines were actually the same white wine, one of which had been tinted red with food colouring. The invited experts described the "red" wine in language typically used to describe red wines.

These studies showed that sight is one of the most important senses of all five and then colour is an important attribute to be analysed, mostly in Port wines (Parr, White et al. 2003).

The first task in sensory assessment of wines is the judgment of its visual characteristics and its colour. Wine is poured inside a standard tasting sampler filling one third of its capacity (75 cc). The wine taster then assesses the colour of wine holding the tasting sampler in front of a white background and tilting the glass 45º. In this way, the wine surface adopts a rather oval shape inside the tasting glass; the thickness of wine varies from 30 mm in the centre to the rim. This reveals a complete range of colour nuances, which can be distinguished by the taster looking through the glass. Colour perception will be different when looking to inner rim and outer rim of a glass of wine as can be seen in the following figure (Hernández, Sáenz et al. 2009).

Figure 5 Colour perception in a sample of Port wine tilted 45º

Discriminant and descriptive tests are normally used in sensory analysis in wine. A descriptive test allows a characterization of each product to be evaluated and see differences between products (Lawless and Heymann 2010). These tests provide selective information on characteristics of food by scoring critical attributes. For comparison of sensorial results and colorimetric readings, an unstructured line scale can be used. This latter method consists in the quantification of colour across a scale (from 0 to 100), being able to compare and deploy the wines depending on the colour display of the wine samples. It is necessary to use anchors to guide the assessors during the assessment. Unstructured line scale allows to test directly the similarities through correlations between visual assessment and instrumental methods. This technique of colour assessment has been used in previous studies for comparison of instrumental and sensorial colour assessments of foodstuff (Kane, Lyon et al. 2003). For our goal, the most adjustable method to make a relation between colorimetric readings from CR-400 and human vision is the unstructured line scale method. Allows a quantification of the samples, similar to scientific measurements (CIE-L*a*b*).

1.9. Comparison of instrumental measurements and visual colour perception

All wines possess a number of visually perceived attributes and all of them contribute to their colour and appearance as well their overall quality. Specifically, colour appearance of wines consist in the colour as it appears in the static glass, colour change as the depth of the wine is increased (Hutchings 1994, Martin, Ji et al. 2007, Hernández, Sáenz et al. 2009). Hue and depth are the generally considered the most important characteristics in colour quality in wine (Somers 1998).

Conventional measurements such as the recommended by OIV can only quantify (transmittance or absorbance) colour in small areas on food sample, which is not appropriate for inhomogeneous food products such as Port wine (Huertas, Yebra et al. 2003, Sáenz Gamasa, Hernández et al. 2009). Since traditional instrumental colour assessment based in absorbance measurements are not appropriate for inhomogeneous samples, correlation between visual and instrumental colour measurement of wine is normally not very high (Huertas, Yebra et al. 2003, Sáenz Gamasa, Hernández et al. 2009).

As mentioned above (physico- chemical methods for colour measurements chapter), new studies were made to correlate and compare the colour of wine as it appears in visual assessments. This previous studies enhance the importance of colour perception in the analysis of quality in wines. Therefore, it is important to study and suggest a simple and effective method for colour measurements that can explore more information than the conventional methods. An important parameter, which is not explored by conventional measurements of wine, for colour perception is “colour depth” (Hutchings 1994, Somers 1998).

1.10. Framework and objectives of the study

The importance of wine colour as a quality parameter demands accurate colour measurement procedures correlated with visual colour assessment by wine tasters. In some wines, like Port, colour is even more important since it is one of the parameters used to classify the wine in categories (which may eventually reflect on its market value).

As, mentioned before, the current spectrophotometric methodology shows some limitation in characterizing the colour of wines, since spectrophotometric measurements only correspond to a single depth and do not reflect the complexity of colour perception on the glass.

The main goal of this dissertation was to test an alternative colorimetric method for measuring the colour profile of Port wines (at different depths) with the objective of obtaining a colour profile capable of distinguish Port wines from different categories. To accomplish this, colour measurements were done in two sets of Port wine samples (kindly supplied by IVDP). Colour measurements were done using the spectrophotometric method suggested by the OIV (OIV 2006) and using a colorimeter (Konica Minolta CR-400) to compare the results obtained using the two methods. For this dissertation, sensory tests should be made for better correlation with psychical measurements mentioned before (colorimetric method and UV-Vis method). Visual assessments of the colour of the samples were made by an untrained panel, using an unstructured line scale, with the goal of establishing a correlation with the colorimetric readings at the same depths.

In addition to the correlations between visual assessment, colorimeter (CR-400) and UV-Vis spectrophotometer, multivariate analysis was made in order to test the proposed method to classify and predict the Port wine samples in the corresponding categories predetermined by IVDP.

2. Material and methods

2.1. Samples

During the experimental work, two set of Port wine samples were used for the colour profile analysis. Representative samples from the different Port wine categories were kindly supplied by IVDP. Colorimetric and spectrophotometric readings were done in both sets of samples, while visual assessment was done using the second set. The following figures describe the layout of the two sets of samples used.

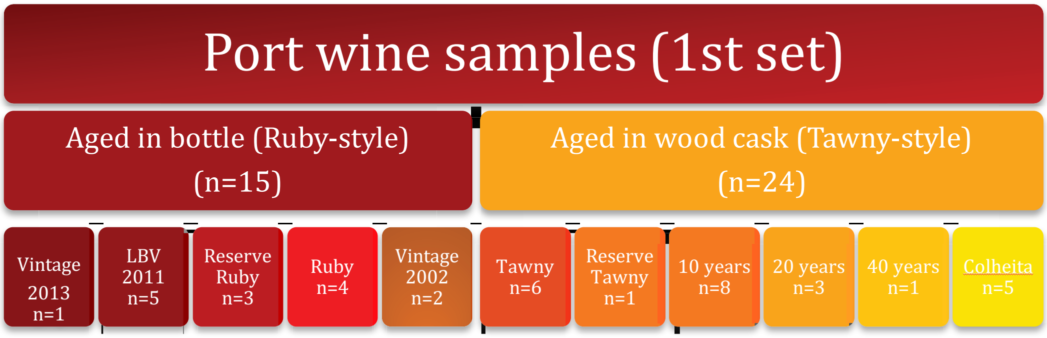


Figure 6 First set of Port wine samples (used for the colorimetric and spectrophotometric readings)

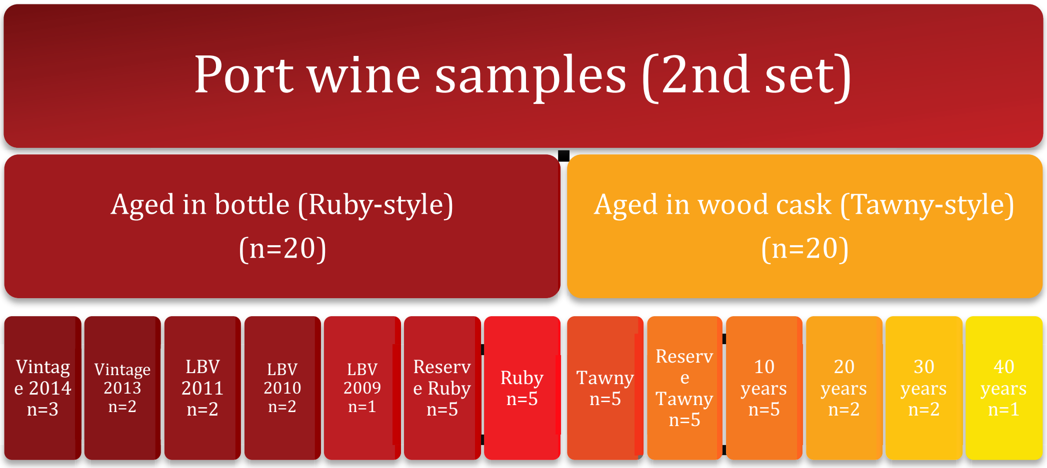


Figure 7 Second set of Port wine samples (used for the colorimetric and spectrophotometric readings and colour assessment)

2.2. Measurements

2.2.1. Spectrophotometrical measurements

For the spectrophotometric (transmittance) measurements, a Helios Alpha UV-Vis double-beam spectrophotometer from Thermo-Scientific (Waltham, MA, USA) was used. UV-Vis spectra were collected at wavelengths between 300 and 800 nm (in 1 nm intervals) using spectrophotometric cells with 1 mm width (pathlength).

The obtained spectra were used to calculate the CIE tristimulus values (assuming a D65 illuminant and an observation angle of 10º) as described in the OIV method (OIV 2006). The theoretical transmittances at other pathlengths (2 mm; 3.2 mm; 4 mm; 4.8 mm; 6 mm) were calculated from the Beer-Lambert-Bouguer law (assuming a linear response in this pathlength range)

The CIE-L*a*b* parameters were calculated from the tristimulus values (X, Y, Z) obtained from spectra readings using the following formulas:

$$L = 116\left(\frac{Y}{Yn}\right)^{\frac{1}{3}} - 16$$

$$a = 500(\left(\frac{X}{Xn}\right) - \left(\frac{Y}{Yn}\right))$$

$$b = 200 - (\left(\frac{Y}{Yn}\right)^{\frac{1}{3}} - \left(\frac{Z}{Zn}\right)^{\frac{1}{3}}$$

Where Xn, Yn and Zn correspond to the values of a perfect diffusing object under a D65 illuminant at an observation angle higher than 4 degrees:

Xn = 94.825;   Yn = 100; Zn = 107.381

The tristimulus values X, Y and Z result from the integration, throughout the visible range of the spectrum, of the functions obtained by multiplying the relative spectral curve of the colour stimulus by the colorimetric functions of the reference observer. Since the true X, Y and Z values can only be determined experimentally; a mathematical approach is used for the calculus of the approximate values using the following formulas:

$$X = K \times \sum T_{(\lambda)} \times S_{(\lambda)} \times \bar{X}_{n\,(\lambda)} \times \Delta_{(\lambda)}$$

$$Y = K \times \sum T_{(\lambda)} \times S_{(\lambda)} \times \bar{Y}_{n\,(\lambda)} \times \Delta_{(\lambda)}$$

$$Z = K \times \sum T_{(\lambda)} \times S_{(\lambda)} \times \bar{Z}_{n\,(\lambda)} \times \Delta_{(\lambda)}$$

$$K = 100/\sum S_{(\lambda)} \times \bar{Y}_{n\,(\lambda)} \times \Delta_{(\lambda)}$$

Where T (λ) represents the transmittance value with 2 mm; 3.2 mm; 4 mm; 4.8 mm and 6 mm of thickness optical route at a particular wavelength λ (recorded from 380 nm to 780 nm at 5 nm intervals); S (λ) represents the spectral power distribution of the illuminant (D65) at wavelength λ; $\bar{X}_{n\,(\lambda)}$, $\bar{Y}_{n\,(\lambda)}$ and $\bar{Z}_{n\,(\lambda)}$ represent the CIE 1964 standard observer functions at wavelength λ and $\Delta(\lambda)$ represents the interval at which the transmittance values were recorded (1 nm).

2.2.2. Colorimetric method

The colorimetric (reflectance) measurements were made using a Konica Minolta Chroma meter 400 (CR-400). This equipment focuses incident radiation to the object, which can be absorbed, refracted or reflected. The reflected radiation component is detected by the colorimeter and converted to the CIE-L*a*b* parameters. The Konica Minolta CR-400 colorimeter has a pulsed-xenon light source (corresponding to a D65 illuminant) with diffuse illumination at a 0º angle geometry. This illumination method illuminates the specimen from all directions with almost completely equal brightness, and receives the reflected light vertically (2º observation angle) from the specimen surface (Minolta). The following figure clarifies the illumination optics of the CR-400.

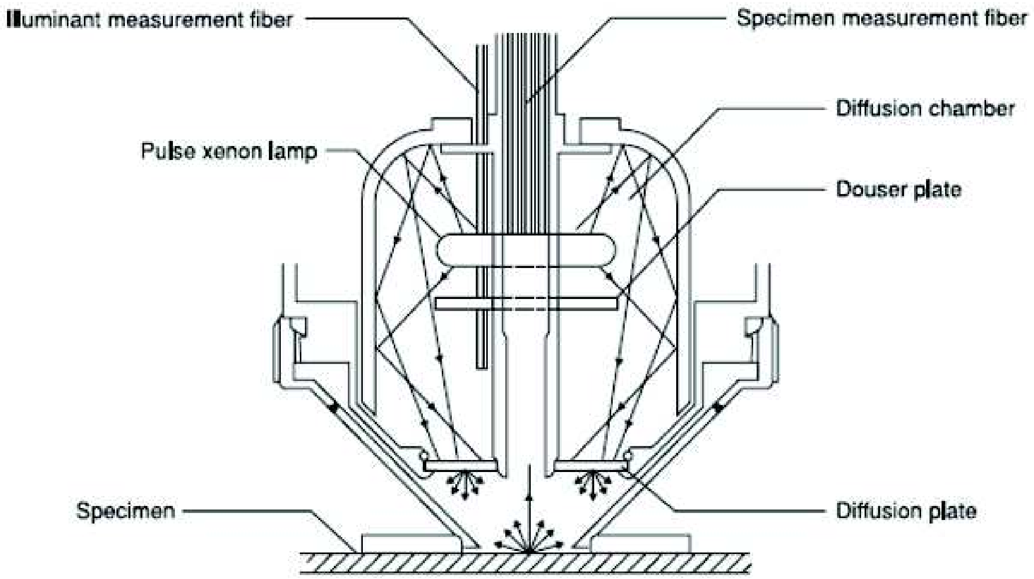


Figure 8 Illumination optics of the CR-400 (Minolta).

The measurement time was one second with three seconds of interval between each measurement. All colour parameters were measured in triplicate for each sample. A prior calibration of the colorimeter (blank reading) was performed using the white calibration plate provided with the equipment. Different volumes of Port wine sample (2.5 ml, 4.0 ml, 5.0 ml, 6.0 ml and 7.5 ml) were transferred to a glass Petri dish (with a diameter of 4.0 cm) in order to measure colour at different depths (2.0 mm, 3.2mm, 4.0 mm, 4.8 mm and 6.0 mm). The Petri dish (containing the sample) was placed over the white calibration plate and the colorimeter was placed directly above the Petri dish in a fixed horizontal position.

The CIE-L*a*b* parameters (using D65 illuminant and 2º observation angle) were provided directly by the colorimeter. The coordinate L*, represents the clarity/luminosity of the subject, where L*= 0 corresponds to black and L*= 100 corresponds to white. The parameter a* represents the red/green colour component (wherein a*> 0 red and a* <0 green) while the b* parameter represents the blue/yellow colour component (b*> 0 yellow, b*< 0 blue).

From the a* and b* values, two other parameters were calculated: colour “purity” or saturation (Chroma C *) and colour tone or hue (H *), which were calculated using the following formulas:

$$C = \sqrt{a^2 + b^2}$$

$$H = tg^{-1}(b/a)$$

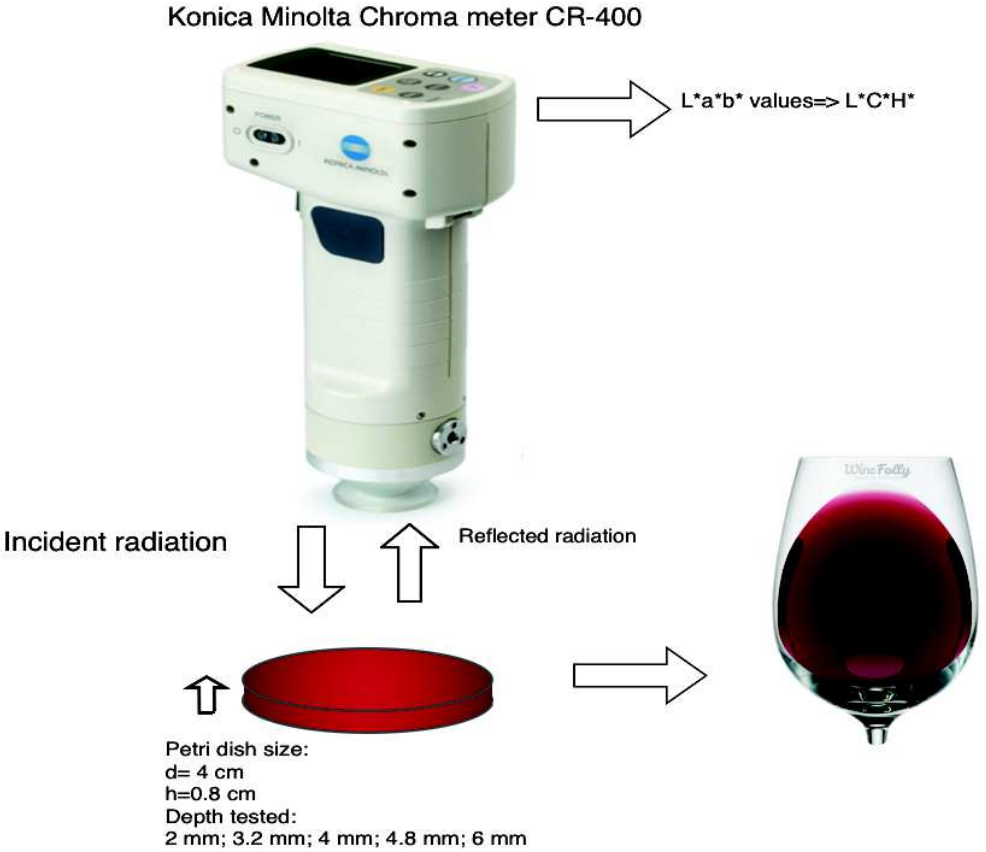


Figure 9 Layout of colorimetric readings of wine samples at different depths

2.3. Visual Assessment

A previously untrained panel of 12 volunteers from Universidade Católica Porto-Escola Superior de Biotecnologia were asked to assess visually the colour of the Port wine samples. The basic categories used by IVDP can be found in this second set, as Tawny (5), Reserve Tawny (5), 10 years (5), 20 years (2), 30 years (2) and 40 years (1) for Tawny styled wines and Ruby (5), Reserve Ruby (5); Late bottled Vintage from 2011 (2), 2010 (2) and 2009 (1); Vintage from 2013 (2) and 2014 (3) for Ruby styled wines. The sensory test consisted of placing the Petri dishes on a one-meter horizontal scale according to their hue using two “anchors” in the extremities of the scale (which represented the lowest and highest values of H* obtained in the colorimetric readings). The visual assessment test was done in a sensory room with controlled luminosity and using a white background. Plastic Petri dishes (with a diameter of 6 cm) were used for this essay. Two separate tests were made, one for Ruby-styled wines and another for Tawny-styled wines. Each test was performed with an established depth of 2 mm, 3.2 mm, 4 mm, 4.8 mm and 6 mm in order to compare the results obtained in the colorimetric readings at the same depths.

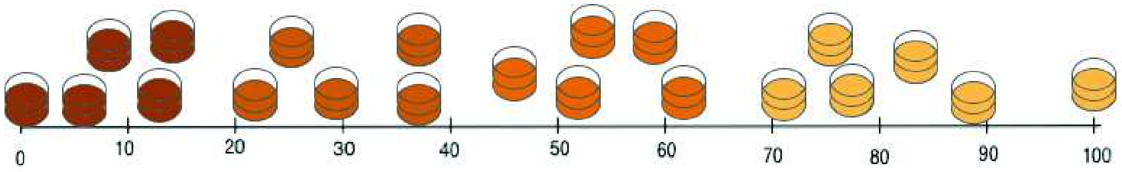


Figure 10 Layout of the (unstructured) visual scale test used for sensory assessment of wine colour. Reference samples ("anchors") were placed at "0" and "100" points and the participants were asked to distribute the samples between the two according to the wine hue.

2.4. Multivariate analysis

A multivariate analysis was performed in order to predict and classify the Port wine samples into the categories used in IVDP. To accomplish this, a principal component analysis (PCA) and a discriminant analysis (DA) was executed, where the software used was SPSS statistics 23.

For PCA, varimax rotation was used. For DA, it was used stepwise method (wilk's lambda). In statistics descriptive was performed the box's M test. In matrices, within-groups correlation and in function coefficients it was used "fisher's and unstandardized. In classify, the prior probabilities are "all groups equal". Finally, the predicted group membership, discriminant scores and probabilities of group membership were also performed in discriminant analysis.

3.Results and Discussion

3.1. Colorimetric (CR-400) results

The samples set were grouped to their colour and ageing according to the names used by IVDP (Instituto Vinho Douro e Porto) to classify each Port wine. Ruby categories are known as Ruby standard, Reserve ruby, LBV and Vintage. Tawny categories are branded as Tawny standard, reserve Tawny, 10 years, 20 years, 30 years 40 years and Colheita.

Chromatic characteristics were measured using the colorimeter at different depth (2.0 mm; 3.2 mm; 4.0 mm; 4.8 mm and 6.0mm) in Port wines for observation of differences in colour of each sample with an increase in depth.

The obtained results are presented in the following figures (fig. 11 to 16) which correlate the lightness (L*), hue (H*) and chroma (C*) parameters with wine depth.

Lightness, hue and colour purity are interesting parameters for Port wine assessments, since Port wine categories have different descriptions in transparency, tonality and colour intensity. An example is standard Ruby, which normally presents a purer and light red colour than the special categories of Ruby-styled wines such as Vintage, Late bottled vintage (LBV), Crusted and Reserve. Also, in tawny Port wines, the ageing will allow a gradual evolution in colour tonality (increase over time) and purity (decrease over time).

3.1.1. Ruby Port wines

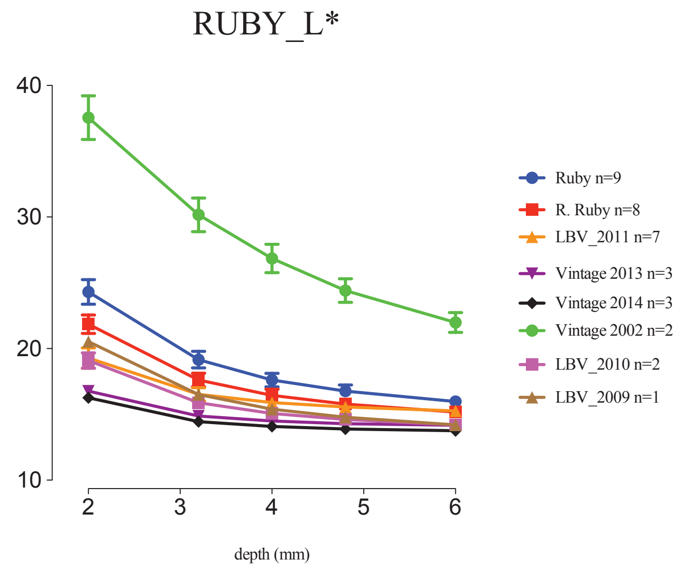


Figure 11 Lightness (L) values for ruby Port wines grouped by category in an increasing depth (mm); L* values represent the mean of each Port wine category and standard error of mean used for dispersion in case of number of samples higher than one.*

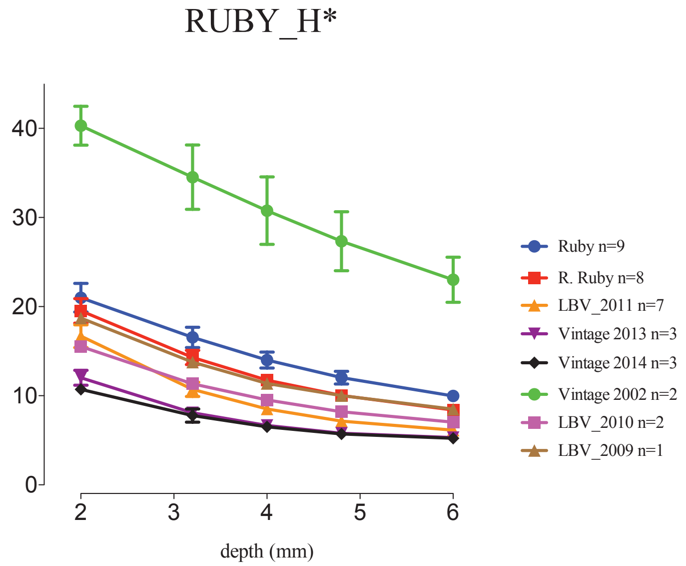


Figure 12 Hue (H) values for ruby Port wines grouped by category in an increasing depth (mm); H* values represent the mean of each Port wine category and standard error of mean used for dispersion in case of number of samples higher than one.*

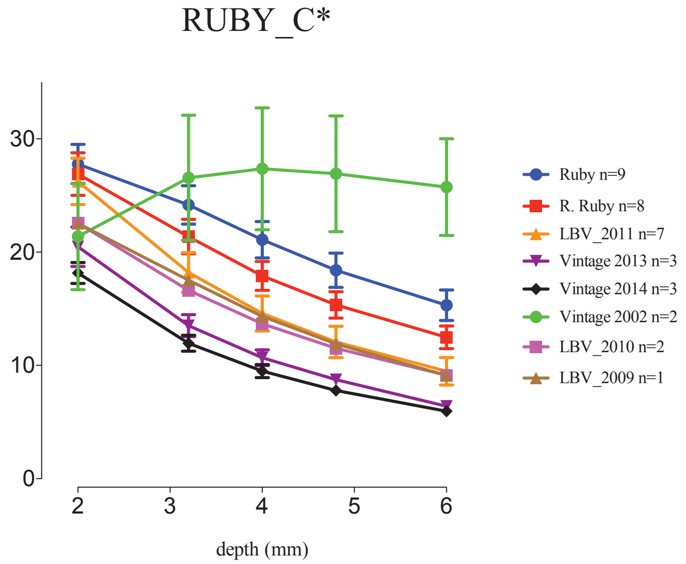


Figure 13 Chroma (C) values for ruby Port wines grouped by category in an increasing depth (mm); C* values represent the mean of each Port wine category and standard error of mean used for dispersion in case of number of samples higher than one.*

For Ruby Port wines, a decrease in lightness, hue and chroma was noticed with an increased depth, except for the Vintage 2002 sample that presented a different behaviour in all parameters (figures 11; 12 and 13). Vintage 2002 displayed higher values of lightness and hue compared to the other categories, although maintaining the decrease of L* and H* values with an increased depth (figures 11; 12). However, C* values increased until 4 mm in depth followed by a decreased on the following depths (figure 13).

On the other hand, Ruby standard and Reserve samples presented a higher decrease in lightness comparatively to the other Ruby-styled categories.

In general, the results obtained using the colorimeter, correspond with the characteristics that each category usually display in colour such purity and hue (Porto 2004). Usually, in the Ruby-styled Port wines, the colour hue evolves from darker red to ruby red and evolves to a less “pure” colour (Porto 2004). For instance, Vintage Port wines from recent harvests such 2013 and 2014 presented “red darker” colours (low H*values) followed by LBV 2011 and 2010, which have similar colour, LBV 2009, Reserve Ruby and Ruby standard (Figure 11 and 12). With CIE L*C*H* parameters obtained from CR-400, this evolution can be noticed clearly in Hue parameter and also in chroma.

Lightness (L*) is a parameter that measures the amount of light that can pass through the sample and Ruby Port wines are known for their dark colour due to wine pigments. Hence, pigment concentration will interfere with the amount of light passed through the wine sample. It can be observed that the L* parameter does not allow to differentiate wines from different Ruby-styled categories, since all samples presented similar L* values at all depths with an exception of vintage 2002 (Figure 11). This evolution could be noticed at lower depths (2mm, 3.2mm and 4mm) in hue (H*) and chroma (C*) parameters. A different behaviour was observed in vintage 2002, which had a linear decrease in hue and an increase in chroma at the lowest three depths tested which can be explained by the fact that this wine has undergone an ageing in the bottle of at least 10 years. During this time, the wine has suffered a decrease of the concentration of anthocyanidins, with the formation of polymeric pigments that exhibit more orange colouration comparing to the vintage from a recent year as vintage 2013.

3.1.2. Tawny Port wines

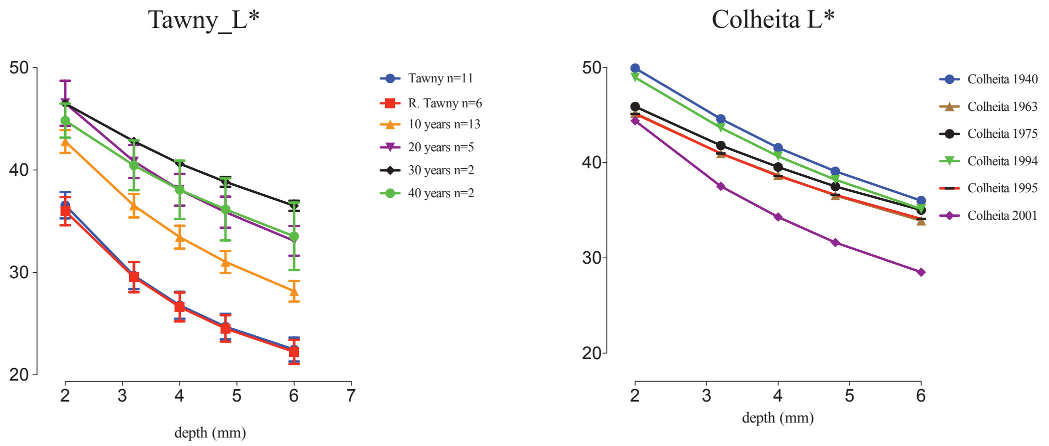
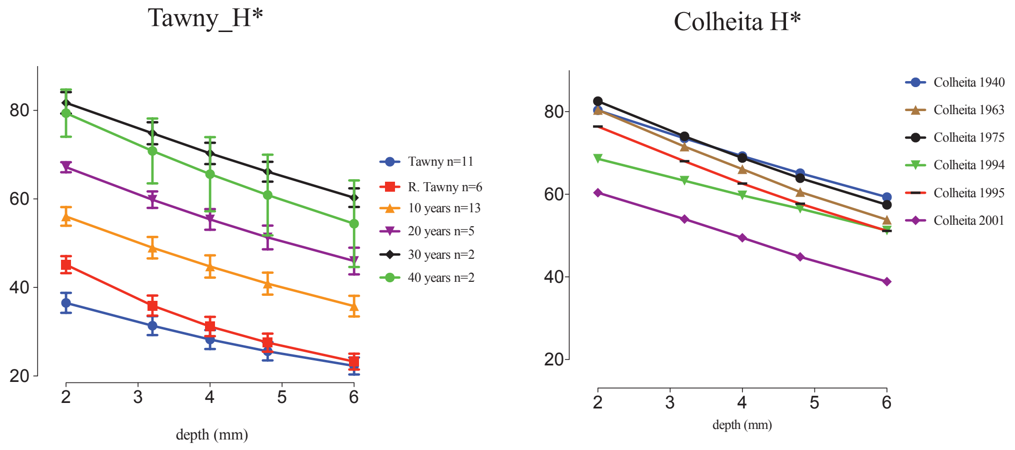
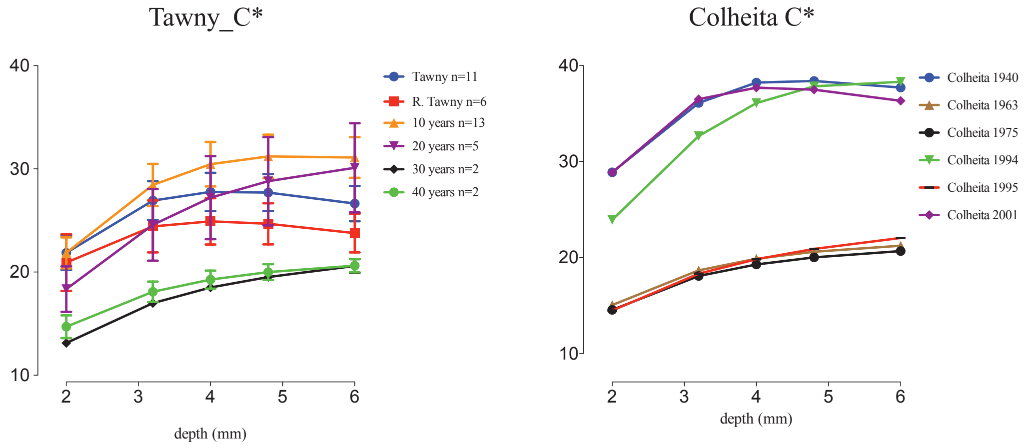


Figure 14 Lightness (L) values for Tawny Port wine samples grouped by category in an increasing depth (mm); L* values represent the mean of each Port wine category and standard error of mean used for dispersion in case of number of samples higher than one*


Tawny_H*

Colheita H*

Figure 15 Hue (H) values for Tawny Port wine samples grouped by category in an increasing depth (mm); H* values represent the mean of each Port wine category and standard error of mean used for dispersion in case of number of samples higher than one.*


Tawny_C*

Colheita C*

Figure 16 Chroma (C) values for Tawny Port wine samples grouped by category in an increasing depth (mm); C* values represent the mean of each Port wine category and standard error of mean used for dispersion in case of number of samples higher than one.*

Wood-aged wines normally evolve differently from bottle-aged wines due to reactions of anthocyanins with oxygen (due to the controlled oxidation which occurs in casks) and with wood components (Boulton 2001, Castañeda-Ovando, Pacheco-Hernández et al. 2009, González-Manzano, Dueñas et al. 2009, Tao, García et al. 2014). For this reason, Tawny-styled wines, normally have a greater transparency in its colour and higher levels of brightness than Ruby-Port wines.

According to the colorimetry results, it can be seen that the colour in each category displays more reddish shades (lower H*), purer (higher C*) and darker colour (lower L*) with increasing depth (Fig. 14; 15 and 16). These results reflect in a quantitative way what is normally observed in a glass of wine tilted 45 degrees.

Tawny Port samples showed a decrease in lightness with increased wine depth, linear decrease in hue and an increase at lower depths (2.0, 3.2 and 4.0 mm) for chroma. Since Tawny Port wines suffer are aged in wood casks, a greater transparency (higher L*) and a more orange hue (higher H*) was noticed in these categories comparatively to Ruby-styled wines (Fig 11 to 16). However, it was observed that lightness (L*) alone cannot differentiate the various categories of tawny as can be seen in the parameters of hue and colour purity (Fig 14).

It is well known, that during ageing, the colour of Port wines changes from red to a more yellow-brown. This fact can be more easily observed in the Tawny categories, since the primary difference between categories resides in the age of each wine. During this process, polymerization and co-pigmentation of anthocyanins with other phenolic compounds will take place with the consequent formation of more stabled pigments (Boulton 2001, Sen and Tokatli 2016) which have a more orange-yellow colour (higher H*). This can be observed in figure 15, where higher H* values were obtained for more aged Port wines. A clear distinction in H* values can be seen between wines of different categories at all tested depths (Figure 15). In the Colheita samples, however, there were similarities in the hue of older wines from 1940, 1975 and 1963.

Different factors can influence the colour changes during the ageing process such as the pH, the rate of anthocyanin loss during ageing and the rate of oxidation reactions (Boulton 2001). In addition, the effect of copigmentation would provide lower free concentrations and slower rates of oxidation, especially for the anthocyanins (Boulton 2001). Subsequently, copigmentation protects the wine pigments from further oxidation, which can influence the different colours of Port wines with similar age. Wines from different producers or aged in different locations can have different colour evolutions which could explain the differences found in wine samples with the same age

An interesting behaviour was found in Tawny-styled wines since different categories showed higher colour purity or chroma (C*) at different depth. For instance, Standard and Reserve Tawnies had highest C* at 4.0 mm depth, while tawnies with an age indication (10 years, 20 years and 40 years) and Colheitas (harvest from the years 1940, 1963, 1975, 1994 and 2001) had the highest chroma values at 6.0 mm.

Light Transmission and scattering usually occurs in translucent materials. It occurs between the extremes of transparency and opaqueness and a major part of the visual quality of wine and foods in general is caused by translucency (Martin, Ji et al. 2007). This light scattering and transmission changes with colour depth. Therefore, the obtained results (both in Ruby- and Tawny-styled wines), enhance the argument that wine colour should be measured at different depths, since different colour profiles exist in different Port wine categories. Since wine is translucent, wine colour changes with depth due to several factors (physical and chemical).

The colorimetric method proved to be a fast and easy alternative way of measuring wine colour at different depths, which is also more similar to the visual perception than the spectrophotometric readings. Although there are others methods already studied for measuring at different depth such as digital camera or tele-spectroradiometry (Martin, Ji et al. 2007), Hernandez et al. 2009, Saenz-Gamasa et al. 2009). These methods require more expensive equipment’s or sophisticated software. Due to its simplicity, the use of the colorimetry might be useful for the wine industry, particularly where wine colour is an important quality parameter for establishing age (and price), like the Port wine industry.

3.2. Comparison of transmittance and reflectance results

To understand the magnitude of the difference between the spectrophotometric (transmittance) and the colorimetric (reflectance) methods, linear correlations (R^2) and functions were used in order to compare readings at the same depth. The following figures (Figures 18, 19 and 20) illustrates the correlation of lightness (L*), hue (H*) and Chroma (C*), between spectrophotometric and colorimetric measurements at all tested depths. For the comparison of colour measurements at same depth, an extrapolation of the measured transmittance values was made from 1 mm pathlength to 2.0, 3.2, 4.0, 4.8mm and 6.0 mm pathlengths, assuming a linear relationship of the Beer-Lambert-Bouguer law in this interval. Each figure displays 12 correlations plots (6 for Ruby styled wines and 6 for tawny styled wines) that correspond to each depth tested. In addition to the R^2, each plot display a linear equation obtained from the relation of the methods in comparison (spectrophotometric vs colorimetric). The equations mentioned earlier, were presented in the following form, $UVVis = m * CR400 + b$; where *"UVVIS"* represents the values obtained by spectrophotometric method, *"m"* represents the slope, *"CR400"* represents the values obtained with colorimetric method and *"b"* represents the intercept when "x" = 0.

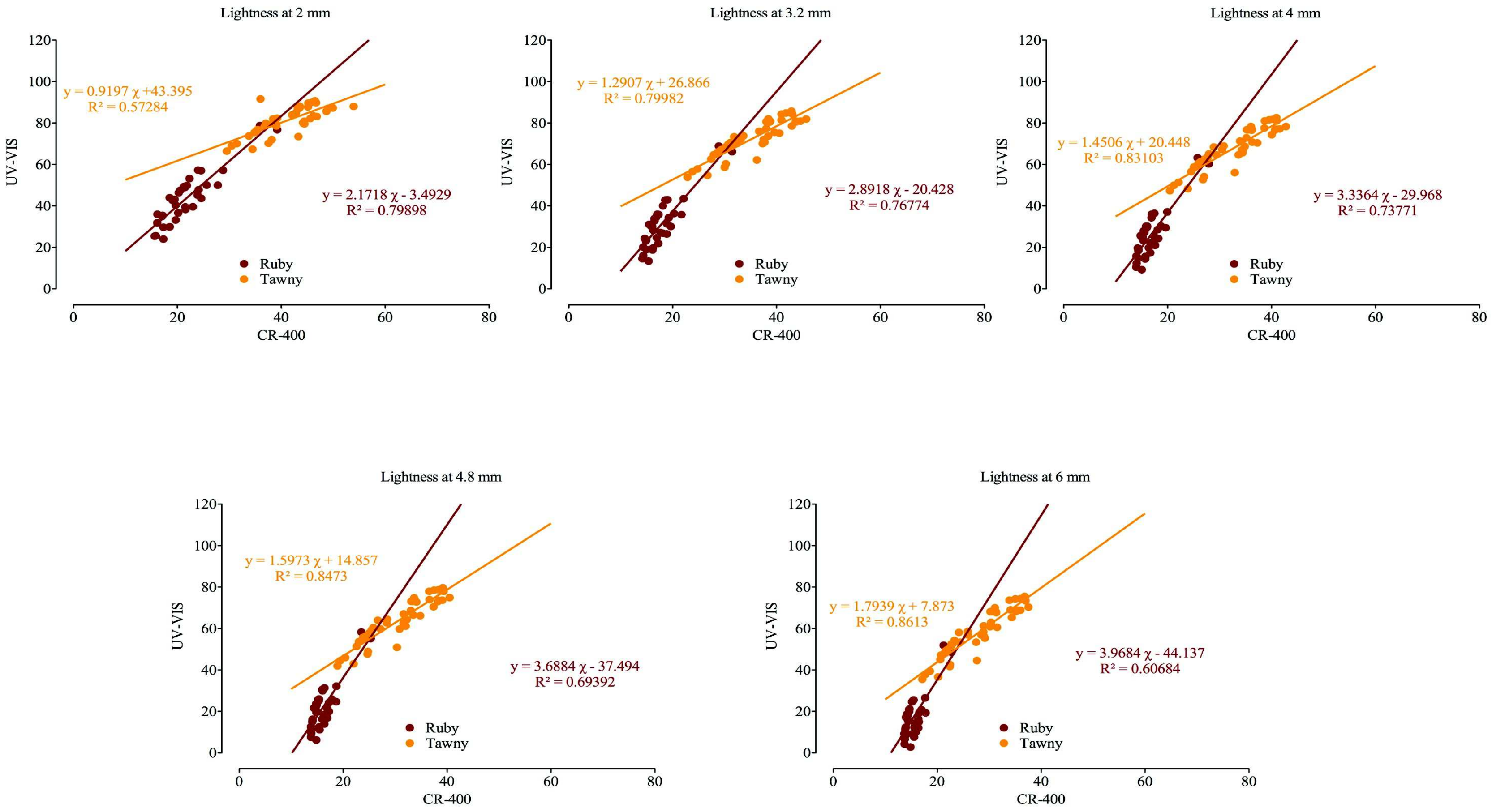


Figure 17 Correlations of Lightness (L) between spectrophotometric and colorimetric measurements at 2.0 mm; 3.2 mm; 4.0 mm; 4.8 mm and 6.0 mm*

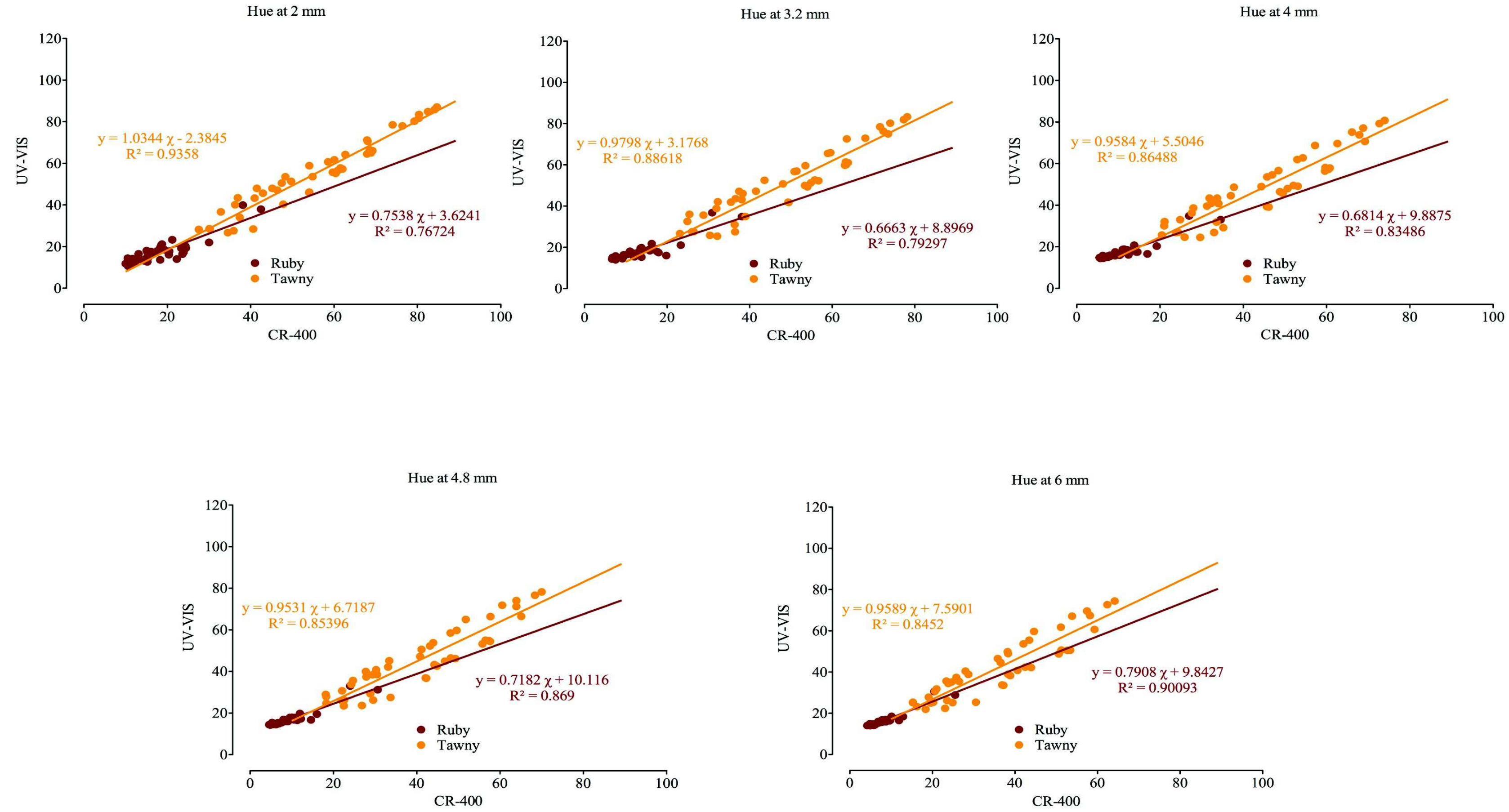


Figure 18: Correlations of Hue (H) between spectrophotometric and colorimetric measurements at 2.0 mm; 3.2 mm; 4.0 mm; 4.8 mm and 6.0 mm*

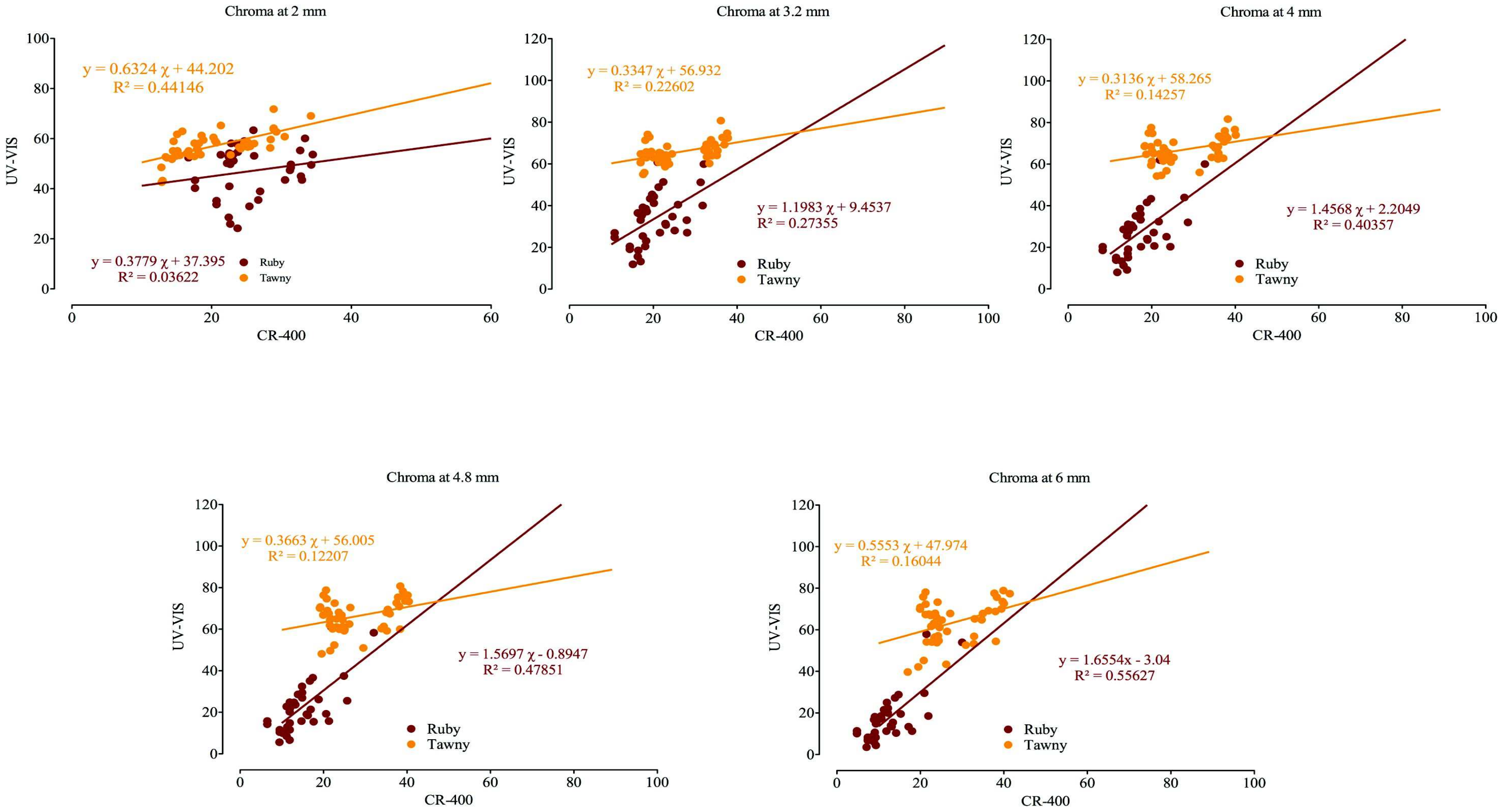


Figure 19: Correlations of Chroma (C) between spectrophotometric and colorimetric measurements at 2.0 mm; 3.2 mm; 4.0 mm; 4.8 mm and 6.0 mm*

It can be observed that good correlations (high R^2) were obtained in the Lightness (L*) parameter for Tawny- and Rub-styled wines (figure 17). In Tawny-styled wines, R^2 and the slope increased with depth. The slope has a value near to 1 at 2.0 mm and higher than 1 in the following depths, which means the values obtained by both methods were similar at 2.0 mm and that the spectrophotometric method gave higher L* (than the colorimetric method) at higher depths (figure 17). For Ruby-styled wines, R^2 decreased with increasing depth (figure 17). On the other hand, the slope increased with an increasing depth, with values higher than 1 (from 2 to 3), which means that the spectrophotometric measurements gave much higher L* values than the colorimetric method (figure 17).

As for the hue parameter (H*), very good correlations were obtained between the two methods in both Ruby- and Tawny- styled wines, with R^2 decreasing with depth in Tawny-styled wines (from 0.9358 to 0.8452) and increasing with depth in Ruby-styled wines (from 0.7672 to 0.9009) (figure 18). The slope values of the spectrophotometric vs. colorimetric graphs were found to be close to 1 in the case of Tawny-styled wines and somewhat lower in the case of Ruby-styled wines (figure 18)

In the case of the chroma parameter (C*) inferior results were obtained when comparing the spectrophotometric and colorimetric results. In fact, a poor relation (R^2) was observed between the results obtained by both methods in all samples (figure 19).

Since wine is translucent, its colour is expected to change with due to several factors. The quality and direction of illumination, viewing geometry and the inclusion/exclusion of specular component are some reasons related to the equipment in use for colour measurement (Hutchings 1994). Furthermore, sample preparation is also an important matter for the colour measurements. According to (Hutchings 1994), there is a complex continuous relationship between sample thickness, diameter, light intensity and direction, light absorption and scattering properties, which represents the colour, depth of colour, reflectance and transmission properties. These interactions between the sample preparation and instrument in use will be important for the result measurement (Hutchings 1994). In our study, differences (C* parameter) and similarities (L* and H* parameters) were obtained between the spectrophotometric and colorimetric measurements that could be related to the reasons mentioned above. In the case of colour measurement of a translucent wine sample using the reflected radiation with 0º geometry (specular radiation included) as occurred in the colorimetric method, some translucency errors could be detected by light trapping, which means not all the light can't reach the detectors instrument. Although, in the spectrophotometric method, a monochromatic beam of light is transmitted through the sample, which reduces light trapping (Hutchings 1994).

In addition to this problem, different depths affect colour measurements, which different proportions of light are reflected from different depths in the same sample (Hutchings 1994). These effects that occur in translucent samples as Tawny and Ruby Port wines may be responsible for the differences in L* and C* observed between the two methods. Also, because in the spectrophotometric method recommended by the OIV, the results are extrapolated (using the Beer-Lambert-Bouguer law) from a path-length of 1.0 mm to higher path-lengths (2.0 mm, 3.2 mm, 4.0 mm, 4.8 mm and 6.0 mm), possible refraction effects at these higher pathlengths are not taken into account in this method. On the other hand, the colorimeter used (Konica Minolta CR-400) is designed so that the sample is illuminated almost equally from all directions (measuring the vertically reflected radiation from the wine sample) which creates more realistic conditions, comparing to visual assessment of translucent liquids such as wine (Minolta).

These differences found between the results obtained by the spectrophotometric and the colorimetric method could be explained by the fact that different types of radiation (transmitted vs reflected) are measured by the two methods and also to the different geometric configuration of the equipment.

3.3. Multivariate analysis

Different physical-chemical and sensorial parameters are normally used for discrimination of categories of Port wines. Although it was not the objective of this work to discriminate categories of Port according to their colour alone, a multivariate analysis of the results obtained with the colorimetric method at different depths was done in order to evaluate a possible discriminatory power of each colour parameter (L*C*H*) used in the CIE-L*a*b* method. A Principal Component Analysis (PCA) was initially made in order to detect redundant variables and to understand the relationship between variables and samples from different categories. Also, a Discriminant Analysis was also done in order to obtain a discriminant function and observe the accuracy of these functions (validation). The main results will be summarized in this chapter.

3.3.1. PCA analysis

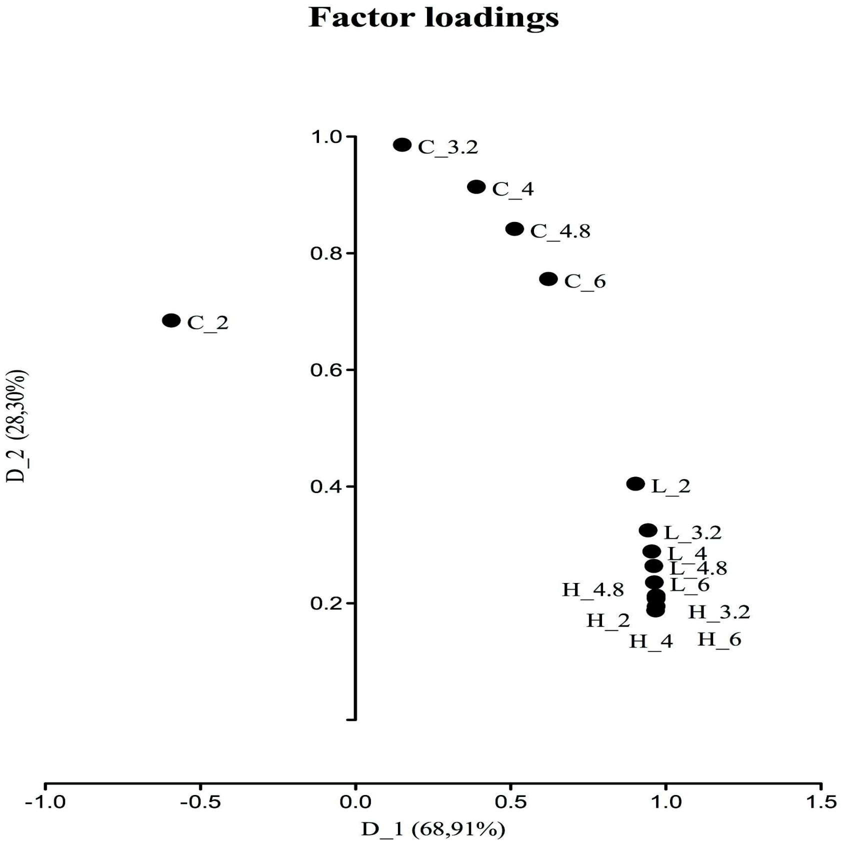


Figure 20: PCA plot of factor loadings (after dimension reduction of the variables and Varimax rotation)

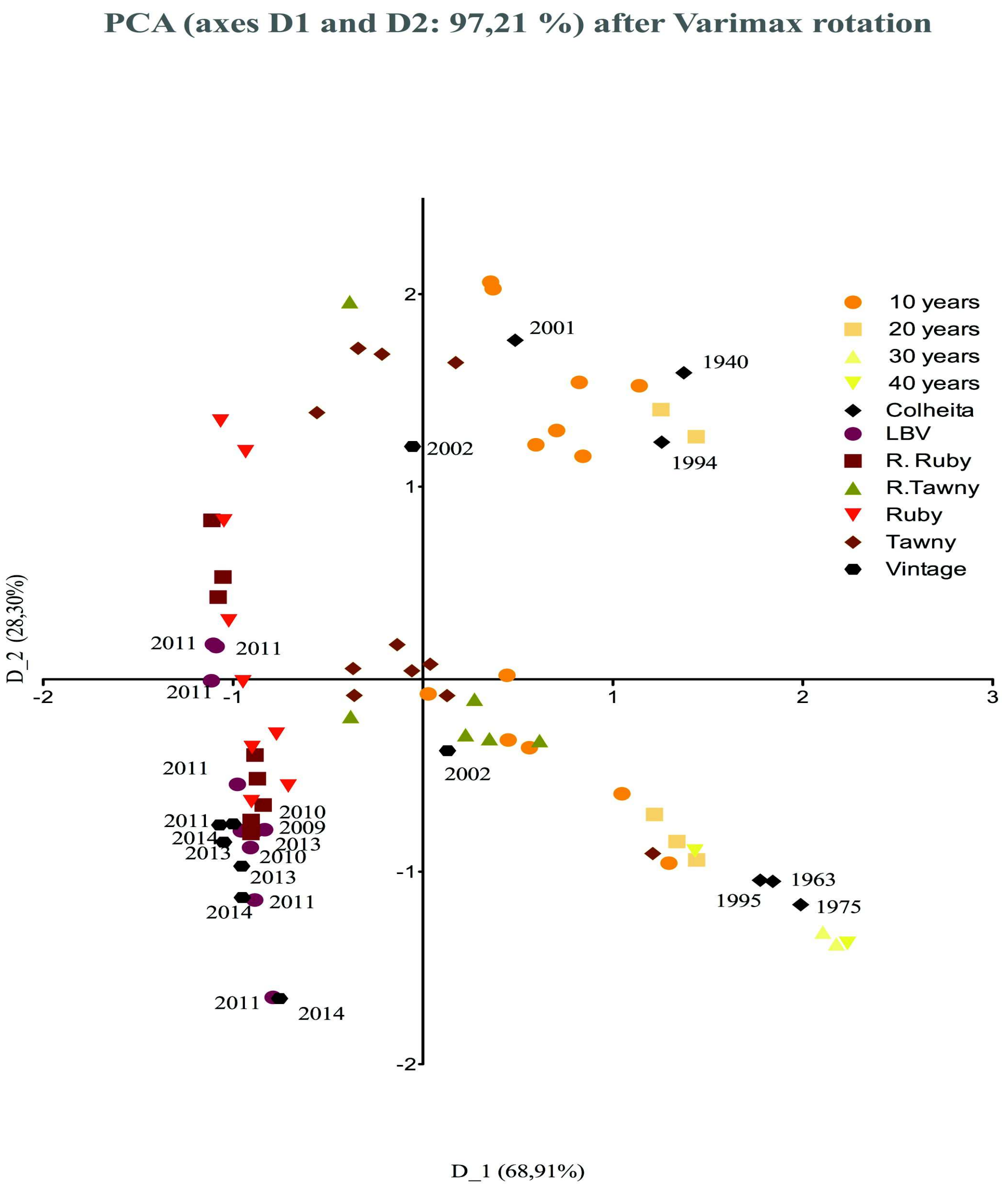


Figure 21 PCA plot of Port wine samples (after dimension reduction of the variables and Varimax rotation)

According to PCA results, two components (factors) were obtained with the variables in study. The first (horizontal) component explains 68,91% of all variance of the variables and the second (vertical) component explains 28,30 %, giving a total explained variance of 97,21% (figures 20 and 21). The first component is based on the variables lightness (L*) and hue (H*) at all depths, while the second component is based on chroma variables at all depths (see figure 20). Consequently, all hue and lightness values appear highly correlated in the PCA analysis. The first component also allowed to discriminate the Tawny-styled Port samples according to their age and style which seems to indicate that L* (lightness) and H* (hue) are the most important parameters for distinguishing wines of these categories. On the other hand, Ruby styled wines were more discriminated by the second (vertical) component (chroma or C* values) (see figure 21).

3.3.2. Discriminant Analysis

To confirm which variables are more important for discrimination the Port wine samples into different categories, a Discriminant Analysis was made using L*, H* and C* colour parameters at different depths. For this analysis "Colheita", "LBV" and "Vintage" samples were not used since at least two samples from the same year were necessary. The analysis results are interpreted bellow.

Table 2: Box's M test result

Box's M		161.439
F	Approx.	4.278
	df1	30
	df2	2083.350
	Sig.	**.000**

One assumption of Discriminant Analysis is identical variability within groups. The variance and covariance matrices should be the same for all groups. The verification of this assumption is made with Box's M test which verifies that the different dispersions observed are not statistically significant. The results of the Box's M test showed that there was no equality of dispersion between Port wine categories (Table 2).

Another assumption for discriminant analysis is a normal distribution. The following table (table 3) presents the results of normality tests.

Table 3: Normality test result

	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Residual for D1	.130	56	.019	.929	56	**.003**
Residual for D2	.160	56	.001	.944	56	**.011**

The variables do not follow a normal distribution, however, as the sample set used is above 50, we consider that the variables approach asymptotically a normal distribution.

Table 4: Wilk's Lambda values for the discriminant functions

Test of Function(s)	Wilks' Lambda	df	Sig.
1 through 3	.012	21	**.000**
2 through 3	.153	12	**.000**
3	.785	5	.035

Using the Wilks' Lambda criteria, it was confirmed that the first 2 functions are highly discriminating, because they have low values (.012, .153) (Table 4). Thus, after exclusion of the first two functions, lambda increased greatly for 0.785 showing a decrease in discrimination of the third function, although still significant (0.035) (Table 4). All functions are significantly discriminant (sig <0.01). However, only the first two functions were used in discriminant analysis.

Table 5: Eigenvalues and percentage of explained variance for discriminant functions

Eigenvalues				
Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	11.521^a	72.3	72.3	.959
2	4.133^a	25.9	98.3	.897

The first discriminant function explained 72.3% of the total variability among model groups, being the one with greater discrimination power (Table 5). The second function explained 25.9% intergroup variance, allowing in conjunction with the first function to differentiate groups quite substantially. Thus, together the first two discriminant functions explain 98.3% of the total variability of the mathematical model (Table 5).

Equation 1: Discriminant function 1 (F1)

$$F_1 = 1{,}128 * H_2 - 0{,}210 * H_{4.8} + 0{,}062 * L_2$$

Equation 2: Discriminant function 2 (F2)

$$F_2 = 2{,}248 * L_2 - 2{,}167 * H_{4.8} + 0{,}101 * H_2$$

The most discriminant variable for function F1 was colour hue at 2.0 mm depth, followed by hue at 4.8 mm depth while for function F2 the most discriminant variable was lightness variable at 2.0 mm depth and hue at 4.8 mm depth. These variables allowed the separation of the samples between groups, as can be seen in the following figure (Figure 22) and table (Table 5).

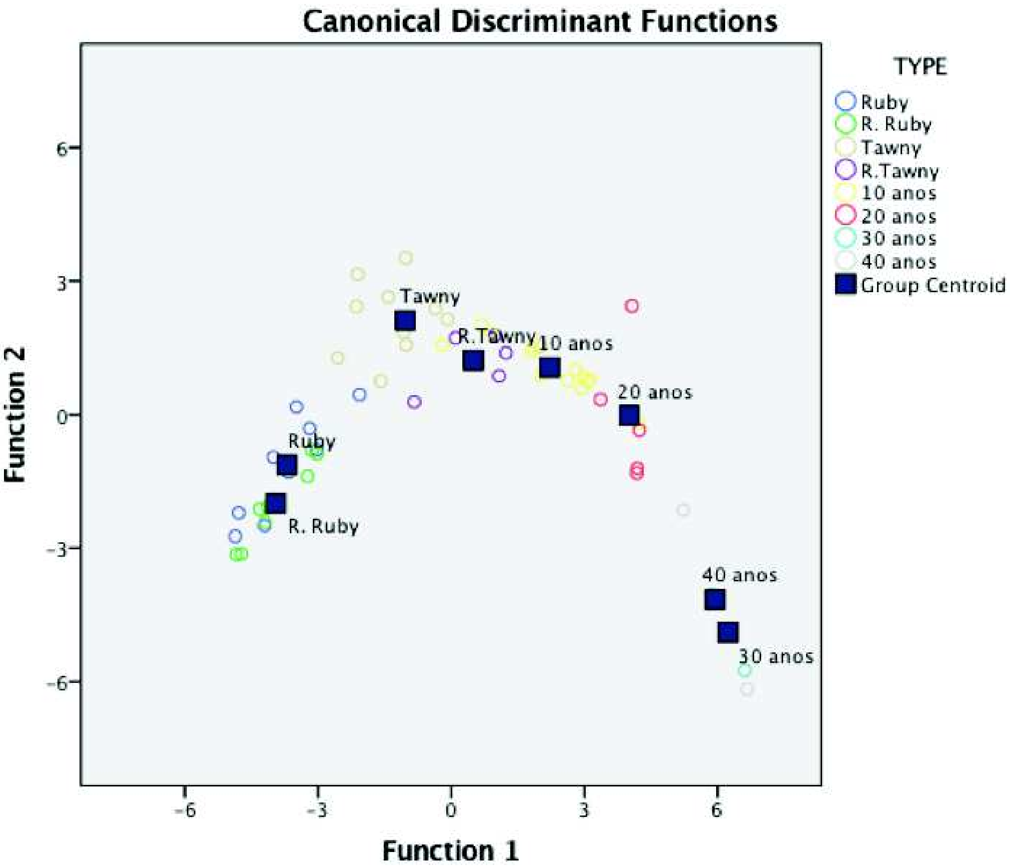


Figure 22: Port wines samples plotted by discriminant functions F1 and F2.

Table 6: Cross validation with the F1 and F2 discriminant functions using the predicted group membership

Original Categories	***Predicted Group Membership***								***Total (n)***
	Ruby	*R. Ruby*	*Tawny*	*R. Tawny*	*10 years*	*20 years*	*30 years*	*40 years*	
Ruby	**5**	**3**	**1**	0	0	0	0	0	9
R. Ruby	**3**	**5**	0	0	0	0	0	0	8
Tawny	0	0	**9**	**1**	**1**	0	0	0	11
R. Tawny	0	0	0	**5**	**1**	0	0	0	6
10 years	0	0	0	**3**	**9**	**1**	0	0	13
20 years	0	0	0	0	**2**	**3**	0	0	5
30 years	0	0	0	0	0	0	**2**	0	2
40 years	0	0	0	0	0	0	**1**	**1**	2
Ruby (%)	**55.6**	**33.3**	**11.1**	.0	.0	.0	.0	.0	100.0
R. Ruby (%)	**37.5**	**62.5**	.0	.0	.0	.0	.0	.0	100.0
Tawny (%)	.0	.0	**81.8**	**9.1**	**9.1**	.0	.0	.0	100.0
R. Tawny (%)	.0	.0	.0	**83.3**	**16.7**	.0	.0	.0	100.0
10 years (%)	.0	.0	.0	**23.1**	**69.2**	**7.7**	.0	.0	100.0
20 years (%)	.0	.0	.0	.0	**40.0**	**60.0**	.0	.0	100.0
30 years (%)	.0	.0	.0	.0	.0	.0	**100.0**	.0	100.0
40 years (%)	.0	.0	.0	.0	.0	.0	**50.0**	**50.0**	100.0
69,6 % of original grouped cases correctly classified									

Through the discriminant functions (F1 and F2), a separation was made of the different Port wines categories (Figure 22). It can be seen that F1 discriminated better the different categories than F2, with exception of Ruby and Reserve Ruby, which were better discriminated with F2, despite overlaps existed between these two categories (Figure 22). For a better analysis, a cross-validation needs to be made in order to understand the accuracy of discrimination of the discriminant functions. According to Table 5, 69.6 % of the original grouped cases (samples) were correctly classified. The categories that showed higher classification errors were Ruby (44,4 %) and Reserve Ruby (37,5 %), while the rest of the categories were less misclassified as can be seen in Table 5. This indicates a good discrimination of the colorimetric method which could be useful for help categorizing Port wine samples. However, since the number of samples used in this study was limited, an analysis with a larger sample size (n = 200-300) and more balanced groups (in terms of number of samples of each category) would be necessary to confirm the results. Nevertheless, the discriminant functions indicated the importance of using measurements of different colour parameters (particularly L* and H*) at different depths in order to discriminate Port wine samples.

3.4. Sensorial assessment

In order to correlate the colorimetric readings with the visual perception of colour the samples a sensorial assay was performed. For this assay, an unstructured line-scale method was used for the quantification of Port wine samples. The second set of samples was used for this colour assessment, which corresponds to 20 Ruby and 20 Tawny styled wines. These two type of Port wines were analysed separately, using the unstructured line scale method. As mentioned before, 10 sensory tests were performed, where five were to test Tawny Port wine samples and the other five were to test the Ruby Port wines. Each test corresponded to the depth used for the colorimetric readings (2mm; 3.2mm; 4mm; 4.8mm; 6mm). Untrained panellists (10-12) were asked to order the samples in terms of colour hue of the samples on an unstructured line scale in order to compare the sensorial scores with the H* values obtained from the colorimetric method at the same depth. In addition, the sensorial results showed the classification order of each sample at different depths and allowed a dispersion analysis of assessors on each sensory test, in order to understand at what depth it was no longer be possible to discriminate the different Ports wines categories.

The following figures, present the correlations between the scores obtained from the sensorial assessment and the H* values obtained from the colorimeter. In addition, these plots show the position of each sample in each sensorial test.

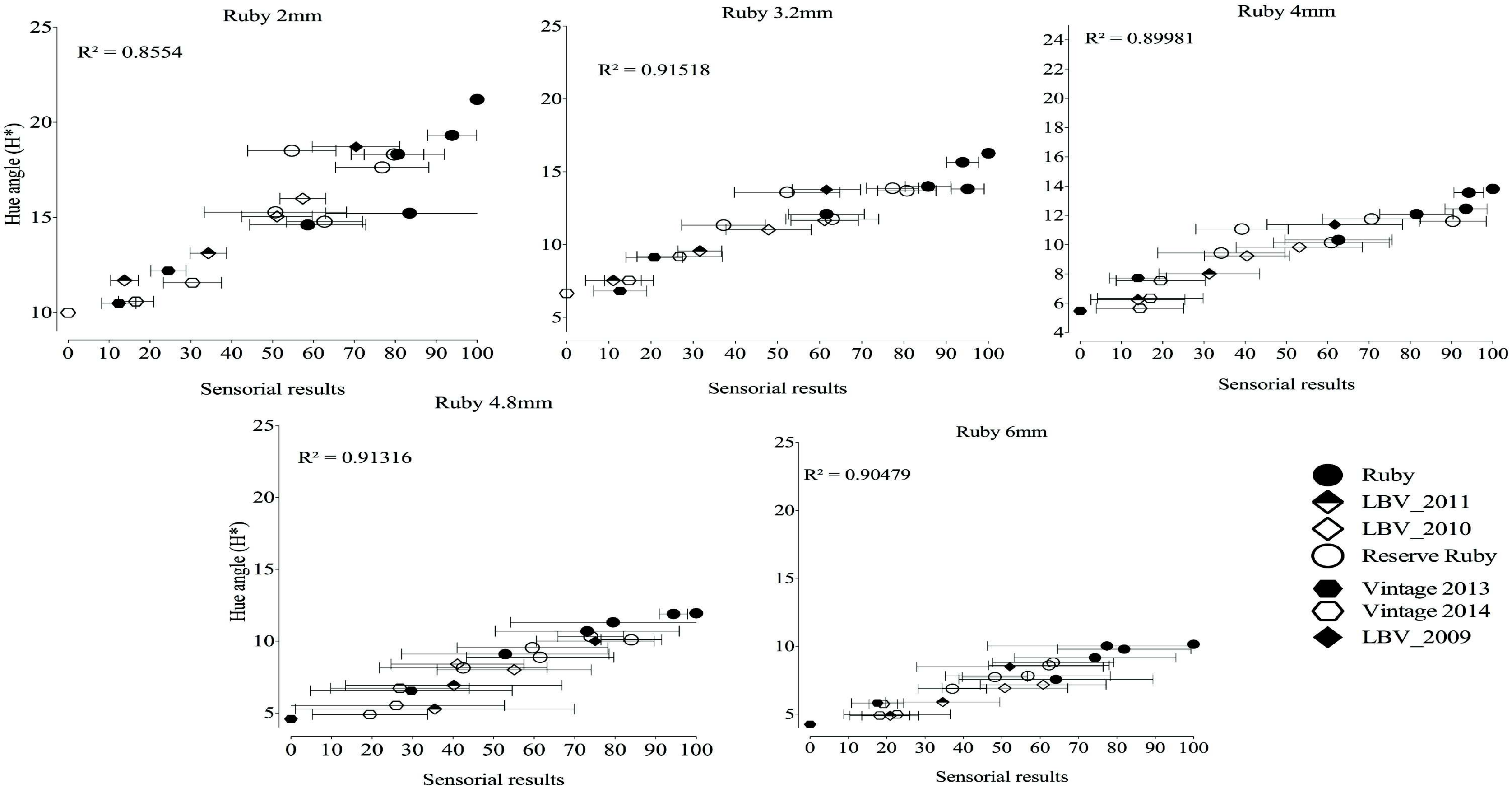


Figure 23 Plots of correlation between hue angle (H) and sensorial scores by the panellists for Ruby Port wines at different depths (error bars represent standard deviations; n=10)*

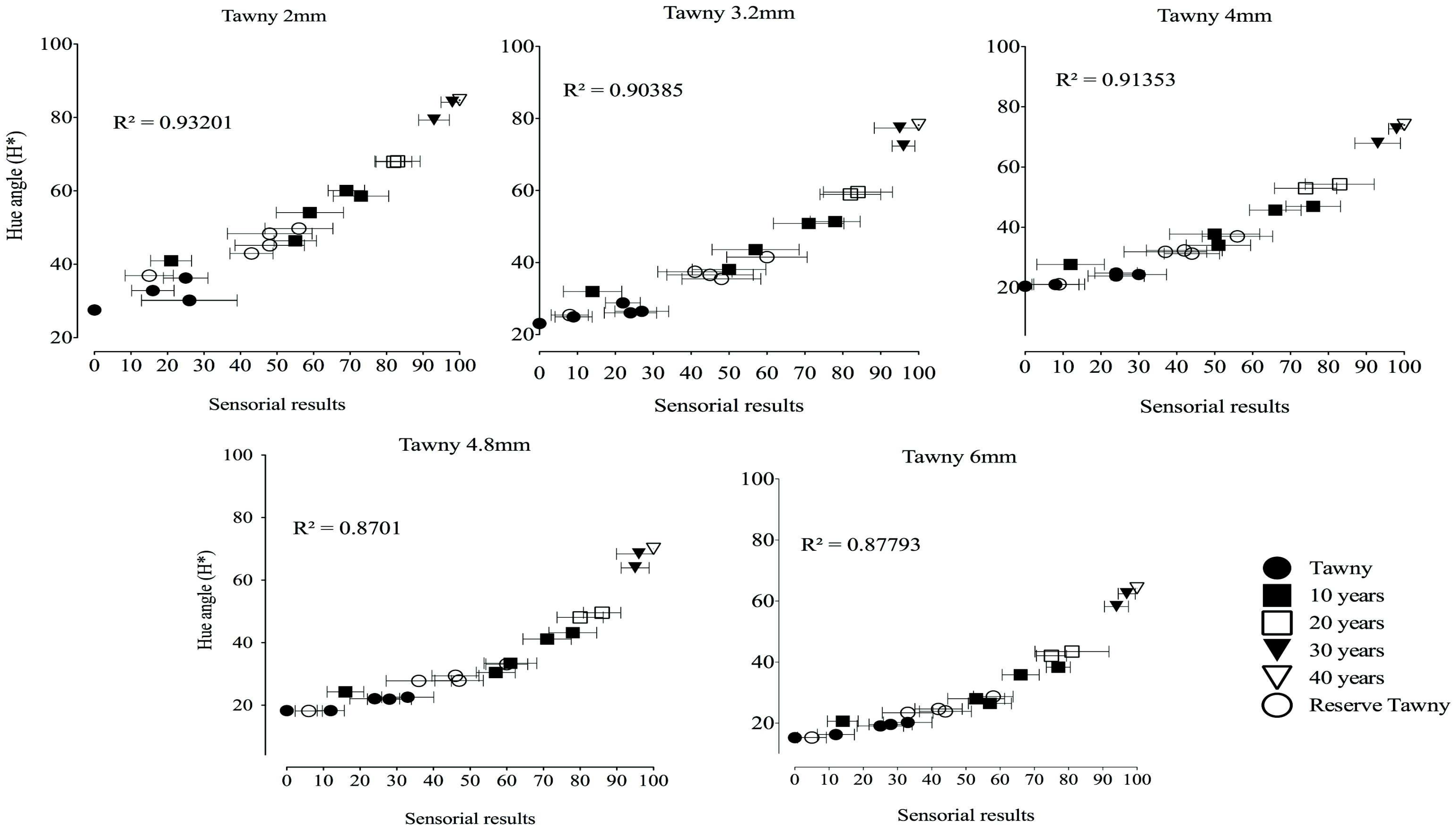


Figure 24: Plots of correlation between hue angle (H) and sensorial scores by the panellists for Tawny Port wines at different depths (error bars represent standard deviations; n=12)*

As can be seen in Figure 23, a linear correlation between the visually perceived hue and the H* parameter measured by the colorimeter was observed for Ruby-styled wines at all tested depths. However, in Tawny-styled wines a curved pattern was observed, meaning that the panellists used the scale in a non-linear manner for more aged wines (with higher H* values) (Figure 24). Furthermore, higher linear correlations (H* vs sensorial scores) were found at greater depths (4.8mm and 6.0 mm) in Ruby-styled wines (Fig. 23) and at shallower depths (2.0 mm to 4.0 mm) in Tawny-styled wines (Fig. 24). Yet, in general, good correlations were found at all depths for all Port wine samples. These sensorial results show also some accordance between the categorization made by the IVDP and the sensorial scores. In fact, a correct sequence is seen in both styled wines (Ruby and Tawny) according to the official IVDP categories of Port. The panel was able to make a better distinction between the Tawny-styled wines than the Ruby-styled wines, as shown by the lower standard deviations in the sensorial scores (Figures 23 and 24). In Ruby-styled wines, only at lower depths (2.0 to 4.0 mm) there was some consensus between panellists (Figures 23). In general, sensorial results showed a better colour distinction on shallower depths (2.0 mm, 3.2 mm and 4.0 mm) for both styles of Port wine (Ruby and Tawny) (figures 23 and 24).

The pattern curve seen in Tawny styled wines with an increased depth could be related to contrast effects previously described in the literature (Pridmore et al. 2005) which are responsible for changes in colour into a translucent object with an increased /decreased luminance. These effects include the Bezold–Brücke shift which represent the changes in hue as luminance changes (an increased luminance, spectral colours shift more towards blue if below 500 nm or yellow if above 500 nm) and the Abney effect, which represent the changes in chroma as luminance changes (the translucent object become more saturated with an increased lightness)(Pridmore, Huertas et al. 2005). The *Bezold–Brücke shift* could have some interference in the scores obtained by the untrained panellists, once the curvilinear graphs show that the analysts showed a greater difficulty in the classification of wine 10 years and 20 years, classifying these as redder than colorimeters. Said effect may be responsible for the curve shown on the plots (4.0-6.0 mm) in figure 24.

Yet, it is difficult to obtain good correlations between physical measurements and visual assessment, since conventional measurements only measure small areas of inhomogeneous foods (Hernández, Sáenz et al. 2009). Hence, colorimetric method and visual assessment allowed to compare wine samples at different volumes (depth), which is important since depth is known to influence the colour of the sample.

4. Conclusion

Port wines from the same category exhibited similar colour (depth) profiles, with Tawny-styled wines showing a more linear profile with depth than Ruby-styled wines regarding the L* and H* parameters. For young Ruby-styled wines, the colour parameters seemed to stabilize at 4.0 mm depth, particularly for younger wines, which indicates that the visual observation of the colour should be done at depths equal to or below 4.0 mm. Chroma (C*) showed a different behaviour in Ruby and Tawny wines, with a maximum obtained at 2.0 mm depth for Ruby styled wines (except for aged vintage Port) and at 3.2-4.0 mm depth for Tawny wines. In general, chromatic characteristics of wines from different Port wine categories agreed well with the colour profiles obtained with the colorimetric method.

Good correlations between the colorimetric and spectrophotometric methods were obtained for the L* (Ruby: R>0.78; Tawny: R>0.89, except for 2.0 mm) and H* parameters (Ruby: R>0.86; Tawny: R>0.90) with the C* parameter giving inferior results, particularly in Tawny wines. The colorimetric method proved to be useful for estimating the colour profile of Port wines, being quicker and more informative than the spectrophotometric method.

Discriminant analysis showed that it was possible to discriminate the Port wine samples into their different categories using the H* and L* parameters at different depths. However, further work is necessary to obtain goo discriminating functions with more representative samples of the different Port wine categories.

A linear correlation was observed for Ruby-styled wines at all tested depths between the visual assessment and colorimetric readings. However, in Tawny-styled wines a curved pattern was observed, meaning that the panellists used the scale in a non-linear manner for more aged wines (with higher H* values). Higher linear correlations (H* values vs sensorial scores) were found at greater depths (4.8mm and 6mm) in Ruby-styled wines and at shallower depths (2 mm to 4 mm) in Tawny-styled wines. Yet, good correlations were found at all depths in all Port wine samples. The panel was able to make a better distinction between the Tawny-styled wines than the Ruby-styled wines (as shown by the lower standard deviations in the sensorial scores). In Ruby-styled wines, only at lower depths (2.0 to 4.0 mm) there was some consensus between panellists. In general, sensorial results showed a better colour distinction on shallower depths (2.0 mm, 3.2 mm and 4.0 mm) for both styles of Port wine (Ruby and Tawny).

5. Future work

The results obtained during the experimental work suggested new lines of research regarding the colour measurement and perception of Port wine which could be interesting to explore.

Measuring Port wine colour with other equipments such as optical fibres or spectroradiometer could be interesting to compare with the results obtained with the colorimetric method used in this work. These equipments allow the colour measurements at different depth in tilted glasses, which could be give a more accurate and realistic colour profile of the perceived wine colour. Thus, it can be perceived the similarities and differences between the colorimetric method and these methods.

Further studies on correlation of the (visual) perception of colour and colour parameters (L*, H* and C*) would be interesting since there is still a generalized lack of knowledge in the literature about this subject.

Finally, the development of mathematical models for categorization of Port wine based on their colour profiles could be an interesting tool both for official authorities (IVDP) and to Port wine producers.

6. Bibliography

Almela, L., S. Javaloy, J. A. Fernandez lopez and J. M. Lopez roca (1995). "COMPARISON BETWEEN THE TRISTIMULUS MEASUREMENTS YXY AND L-ASTERISK A-ASTERISK B-ASTERISK TO EVALUATE THE COLOUR OF YOUNG RED WINES." FOOD CHEMISTRY **53**(3): 321-327.

Aron, P. M. and J. A. Kennedy (2008). "Flavan-3-ols: Nature, occurrence and biological activity." Molecular nutrition & food research(1).

Berké, B. and V. A. P. de Freitas (2005). "Influence of procyanidin structures on their ability to complex with oenin." Food Chemistry **90**(3): 453-460.

Birse, M. J. (2007). The colour of red wine. Doctor of philosophy, The university of Adelaide.

Boulton, R. (2001). "The Copigmentation of Anthocyanins and Its Role in the Colour of Red Wine: A Critical Review." Enology and Viticulture **52**(2): 67-87.

Bruwer, N. (2014). Perceptions in wine.

Campos, F. (2009). Studies on the interactions between phenolic compounds and lactic acid bacteria from wine. PhD, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto (Portugal).

Castañeda-Ovando, A., M. d. L. Pacheco-Hernández, M. E. Páez-Hernández, J. A. Rodríguez and C. A. Galán-Vidal (2009). Review: Chemical studies of anthocyanins: A review. **113**.

Fei, H., M. Lin, Y. Guo-Liang, L. Na-Na, P. Qiu-Hong, J. Wang, M. J. Reeves and D. Chang-Qing (2010). "Biosynthesis of Anthocyanins and Their Regulation in Coloured Grapes." Molecules **15**(12): 9057.

Fu-Liang, H., Z. Wen-Na, P. Qiu-Hong, Z. Cheng-Rong, C. Hai-Yan and D. Chang-Qing (2008). Principal Component Regression Analysis of the Relation Between CIELAB Colour and Monomeric Anthocyanins in Young Cabernet Sauvignon Wines. **13**.

Galante, M. J. (2015). Evolução das antocianinas, atividade antioxidante e parâmetros de cor no Vinho do Porto ao longo do seu envelhecimento. Master, Faculdade de Farmácia da Universidade do Porto.

García-Marino, M., M. L. Escudero-Gilete, M. T. Escribano-Bailón, M. L. González-Miret, J. C. Rivas-Gonzalo and F. J. Heredia (2012). Colorimetric characteristics of the phenolic fractions obtained from Tempranillo and Graciano wines through the use of different instrumental techniques. **732**.

Gómez-Plaza, E., R. Gil-Muñoz, A. Martínez and J. M. López-Roca (2000). Colour and phenolic compounds of a young red wine. Influence of wine- making techniques, storage temperature, and length of storage time. **48**.

González-Manzano, S., M. Dueñas, J. C. Rivas-Gonzalo, M. T. Escribano-Bailón and C. Santos-Buelga (2009). "Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine." Food Chemistry **114**: 649-656.

Gutiérrez, I. H. n., E. S.-P. Lorenzo and A. V. Espinosa (2005). "Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars, Cabernet Sauvignon, Cencibel, and Syrah." Food Chemistry **92**(2): 269-283.

Heredia, F. J., E. M. Francia-Aricha, J. C. Rivas-Gonzalo, I. M. Vicario and C. Santos-Buelga (1998). Chromatic characterization of anthocyanins from red grapes—I. pH effect. **63:** 491.

Heredia, F. J., A. M. Troncoso and M. Guzman-Chozas (1997). "Multivariate characterization of aging status in red wines based on chromatic parameters." Food chemistry(1).

Hernández, B., C. Sáenz, J. F. de la Hoz, C. Alberdi, S. Alfonso and J. M. Diñeiro (2009). "Assessing the colour of red wine like a taster's eye." Colour Research and Application **34**(2): 153-162.

Huertas, R., A. Yebra, M. M. Perez, M. Melgosa and A. I. Negueruela (2003). "Colour variability for a wine sample poured into a standard glass wine sampler." COLOUR RESEARCH AND APPLICATION **28**(6): 473-479.

HunterLab. (2016). "Colour Theory." 2016, from https://support.hunterlab.com/hc/en-us/categories/201319586-Colour-Theory.

Hutchings, J. (1994). Chemistry of Food Colour. Food Colour and Appearance, Blackie Academic & Professional, an imprint of Chapman & Hall, Wester Cledens Road, Bishopbriggs, Glasgow G64 2NZ**:** 390-392.

Hutchings, J. B. (1994). Colour Measurements of Foods. Food Colour and Appearance. B. A. Professional**:** 239-252.

IVDP. (2016, 14/07/2016). "Categorias especiais Vinho do Porto." Instituto Vinho do Porto e Douro Retrieved 19/07/2016, 2016, from https://www.ivdp.pt/.

Kane, A. M., B. G. Lyon, R. B. Swanson and E. M. Savage (2003). Comparison of two sensory and two instrumental methods to evaluate cookie colour. **68**.

Lab, H. (2015). CIE 2° versus 10° Degree Standard Observer. C. S. O. and and Y. calculation of CIE X, Z colour values.

Lawless, H. T. and H. Heymann (2010). Sensory evaluation of food: principles and practices, Springer Science & Business Media.

Lindstrom, M. (2005). "Broad sensory branding." Journal of Product and Brand Management **14**(2): 84-87.

María, M., M.-Á. Pedro, B. Begoña and G.-C. Carmen (2006). Statistical interpretation of the colour parameters of red wines in function of their phenolic composition during aging in bottle. **222:** 702.

Marquez, A., M. P. Serratosa and J. Merida (2014). "Influence of bottle storage time on colour, phenolic composition and sensory properties of sweet red wines." Food Chemistry **146**: 507-514.

Martin, M. L. G.-M., W. Ji, R. Luo, J. Hutchings and F. J. Heredia (2007). "Measuring colour appearance of red wines." Food Quality and Preference **18**: 862-871.

Minolta, K. Chroma meter 400/410. http://www.konicaminolta.eu/en/measuring-instruments/technical-service/service-locations.html.

Monagas, M., B. Bartolome and C. Gomez-Cordoves (2006). "Updated knowledge about the presence of phenolic compounds in wine."

Morrot, G., F. Brochet and D. Dubourdieu (2001). "The Colour of Odors." Brain and Language **79**(2): 309-320.

Niskanen, I., J. Mutanen, P. Lehtonen, J. Raty, M. Yamaguchi, K.-E. Peiponen and T. Jaaskelainen (2008). Multifunction spectrometer for optical inspection of red wine. **2**.
OIV (2006). Determination of chromatic characteristics according to CIELab. L. O. I. d. l. V. e. d. V. (OIV).

Oliveira, J., M. A. da Silva, A. Jorge Parola, N. Mateus, N. F. Brás, M. J. Ramos and V. de Freitas (2013). "Structural characterization of a A-type linked trimeric anthocyanin derived pigment occurring in a young Port wine." Food Chemistry **141**: 1987-1996.

Parr, W. V., K. G. White and D. A. Heatherbell (2003). "The nose knows: influence of colour on perception of wine aroma." Journal of Wine Research **14**(2/3): 79-101.

Pérez-Magariño, S. and M. L. González-San José (2006). Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade. **96**.

Porto, O. I. d. V. d. D. e. (2004). 2016, from https://www.ivdp.pt/pagina.asp?codPag=70&codSeccao=2&idioma=0.

Pridmore, R. W., R. Huertas, M. Melgosa and A. I. Negueruela (2005). "Discussion on perceived and measured wine colour." Colour Research and Application **30**(2): 146-152.

Ribéreau-Gayon, P., Y. Glories, A. Maujean and D. Dubourdieu (2006). The Chemistry of Wine Stabilization and Treatments. Phenolic Compounds, in Handbook of Enology. L. John Wiley & Sons, Chichester, UK. **2**.

Sáenz Gamasa, C., B. Hernández, J. V. de Santiago, C. Alberdi, S. Alfonso and J. M. Diñeiro (2009). "Measurement of the colour of white and rosé wines in visual tasting conditions." Zeitschrift für Lebensmittel-Untersuchung und -Forschung. A, European food research and technology(2).

Sen, I. and F. Tokatli (2016). "Original Research Article: Differentiation of wines with the use of combined data of UV–visible spectra and colour characteristics." Journal of Food Composition and Analysis **45**: 101-107.

Somers, T. C. (1998). Phenolic Composition and Wine Spectrum. The wine Spectrum. winetitles**:** 49-66.

Somers, T. C. (1998). Variability of Wine Quality. The Wine Spectrum.

Tao, Y., J. F. García and D.-W. Sun (2014). Advances in Wine Aging Technologies for Enhancing Wine Quality and Accelerating Wine Aging Process. **54**.

Wrolstad, R. E., R. W. Durst and J. Lee (2005). "Review: Tracking colour and pigment changes in anthocyanin products." Trends in Food Science & Technology **16**: 423-428.

Wyszecki, G. and W. S. Stiles (1982). Colour science, Wiley New York.