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Chapter

The Role of UV-Visible Spectroscopy for Phenolic Compounds Quantification in Winemaking

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Abstract

Phenolic compounds are bioactive substances present in a large number of food products including wine. The importance of these compounds in wine is due to their large effect on the organoleptic attributes of wine. Phenolic compounds play a crucial role in the colour as well as mouthfeel properties of wines. UV-visible spectroscopy appears as a suitable technique for the evaluation of phenolic compounds' properties and content. The ability of the phenolic ring to absorb UV light and the fact that some of the phenolic substances are coloured compounds, i.e. show absorption features in the visible region, make UV-visible spectroscopy a suitable technique to investigate and quantify grape and wine phenolic compounds. A number of analytical techniques are currently used for phenolic quantification. These include both simpler approaches (spectrophotometric determinations) as well as more complex methodologies such liquid chromatography analysis. Moreover, a number of spectroscopy applications have also been recently reported and are becoming popular within the wine industry. This chapter reviews information on the UV-visible spectral properties of phenolic compounds, changes occurring during wine ageing and also discusses the current UV-visible based analytical techniques used for the quantification of phenolic compounds in grapes and wine.

Keywords: UV-visible, spectrophotometry, phenolic compounds, anthocyanins, tannins, liquid chromatography, spectroscopy, chemometrics, fluorescence

1. Introduction

Phenolic compounds are bioactive molecules that are involved in some of the most relevant wine organoleptic attributes. Phenolic substances have been reported as being responsible for wine colour, mouthfeel perception and flavour. The appropriate management of the phenolic accumulation in the berry, extraction during the skin contact phase as well as the evolution during ageing in barrels or bottles will ensure a desired phenolic content and composition that will lead to a good quality wine [1]. Furthermore, the ability of phenolic molecules to act as antioxidant has placed this group of compounds in the spotlight of a considerable amount of research. Phenolic compounds have been reported as effective antioxidants and their preventive role against inflammatory, neurodegenerative, cardiovascular

diseases or even against cancer has been widely acknowledged [2]. The quantification of phenolic compounds is thus of high importance and UV-visible spectroscopy has proven to be one of the most suitable and reliable techniques to quantify these substances during the winemaking process.

The accumulation of the amino acid phenylalanine is the first step towards the biosynthesis of phenolic compounds. Phenolic substances or polyphenols are thus secondary metabolites that contain at least one aromatic ring and one or several hydroxyl groups. Two main families of phenolic compounds are generally classified as the non-flavonoids and the flavonoids. Phenolic acids, including hydroxycinnamic and hydroxybenzoic acids and stilbens are part of the structurally less complex non-flavonoid group (**Figure 1**). Flavonoids share a common C3-C6-C3 structure and contain flavonols, anthocyanins and flavanols, with the latter also known as proanthocyanidins or more widely as tannins [3]. The biosynthesis and accumulation of these key substances is due to a number of plant biological functions which include growth, plant reproduction and plant protection roles against environmental signals as well as biotic and abiotic stresses [4].

Phenolic compounds are released from the solid parts of the berries into the must during the winemaking process. The contact period refers to the period of time that the must is in contact with the skins and seeds and generally coincides with the alcoholic fermentation. The presence or absence of the solid parts during the winemaking process will determine the phenolic content and composition. In white winemaking the skin contact period is limited to a minimum and the levels of phenolic compounds found in wines are thus lower than in red wines (where the fermentation takes place in the presence of skins and seeds). Due to its location in the flesh, hydroxycinnamic acids are therefore the main phenolic compounds found in white wines. On the contrary, red wines contain high levels of tannins, anthocyanins and flavonol compounds that are extracted from the solid parts of the berries during the aforementioned skin contact phase [5].

Among the subclasses of phenolic compounds found in grapes, two of the subfamilies are mostly of importance to wine production. Anthocyanins are coloured compounds responsible for the red wine colour attributes. The state of the anthocyanins and the wine medium conditions have a major impact on the final wine colour. Anthocyanins are found in red grapes and wines in five mono-glucoside forms. The 3-glucoside forms of delphinidin, cyanidin, petunidin, peonidin and malvidin are present in *Vitis vinifera* cultivars (**Figure 2**). Monomeric anthocyanins are highly reactive substances involved in a large number of reactions and interactions. Simple anthocyanins are acylated with a number of grape components such as acetic acid, *p*-coumaric or caffeic acid, they are also able to combine with themselves through intra- and intermolecular copigmentation interactions [6]. During the winemaking and ageing processes further reactions to form pyranoanthocyanins have also been documented, in combination with several associations with tannins, some of them through acetaldehyde mediated reactions. Anthocyanin interactions and reactions lead to a number of complex pigments with increased
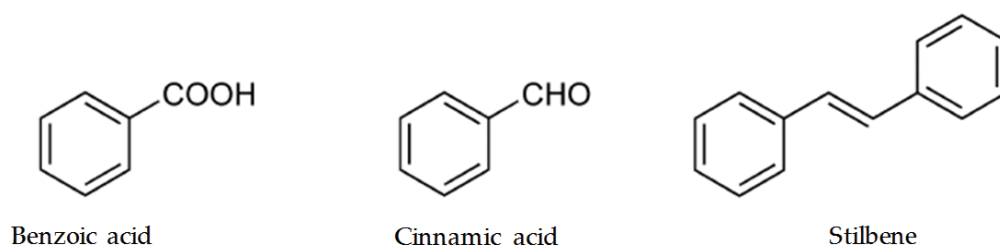

| Benzoic acid | Cinnamic acid | Stilbene |

Figure 1.
Chemical structure of the non-flavonoid group of phenolic compounds found in grapes and wines.

Cyanidin R1 = OH; R2 = H
Peonidin R1 = OCH$_3$; R2 = H
Delphinidin R1 = OH; R2 = OH
Petunidin R1 = OCH$_3$; R2 = OH
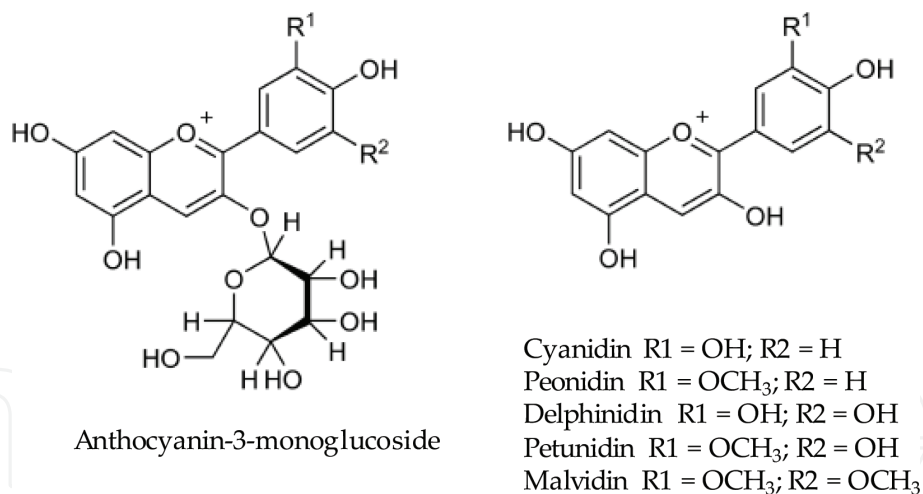Malvidin R1 = OCH$_3$; R2 = OCH$_3$

Figure 2.
Chemical structure of the main anthocyanins found in grapes and wines.

stability during wine ageing. These combinations also entail a modification of the anthocyanin coloration, phenomena that gives rise to the large variety of red and brown based colours found in red wines [7]. Additionally, tannin-anthocyanin interactions give rise to a decrease in the ability of tannins to elicit astringency [8].

Proanthocyanidins or tannins are the most abundant class of phenolic compounds. Tannins are polymeric compounds of varying size and structure, containing a combination of five flavanol monomers. The polymerisation of catechin, epicatechin, gallocatechin, epigallocatechin and catechin gallate subunits gives rise to larger and more polymerised tannin compounds (**Figure 3**) with varying ability to elicit astringency and bitterness [3, 9]. The reactivity of the hydroxyl groups towards salivary proteins creates a macromolecular complex that precipitates from solution and leads to a puckering and drying sensation, also known as astringency. Small molecular weight tannins are initially bitter and they became more astringent as the molecular size increases [9]. Young wines, initially more astringent, contain tannins that have been polymerising and have therefore and increased ability to react with salivary proteins. During the ageing process several phenomena explain why the wines become softer (less astringent). When a certain molecular size is reached the tannin molecule may become insoluble, thus precipitating from solution and lowering the tannin content of the wines. Moreover, as molecules grow in size, its conformation might hinder the tannin protein interactions which will also lead to decreased astringency intensity. It is also possible that large tannin molecules cleavages give rise to smaller and less astringent tannins. Finally, the tannin-anthocyanin combinations that take place during the ageing process may also be involved in the decrease of the astringency intensity experienced in older wines [1, 10].

The use of UV and visible light for the quantification of chemical compounds is a widely used technique [1]. Due to their biochemical and molecular properties, phenolic compounds are highly suitable to be quantified with UV-visible light. The ability of the phenolic ring to absorb UV light is exploited to quantify these compounds [11]. In addition to this, visible light can also provide valuable information due to the coloured nature of some of the phenolic compounds (e.g. red anthocyanins or yellow flavonols). The UV-visible spectra of a wine is thus attributed to the electronic transitions occurring within the hydroxyl groups of the phenolic molecules, with different transitions corresponding to the different phenolic subclasses [12]. A number of UV-visible applications have been exploited to quantify phenolic compounds. Among these the use of UV-visible spectrophotometry to estimate the content of phenolic compounds stands out as the most widely used approach. A number of methods have been optimised for the different phenolic subclasses, making nowadays the efficient estimation of
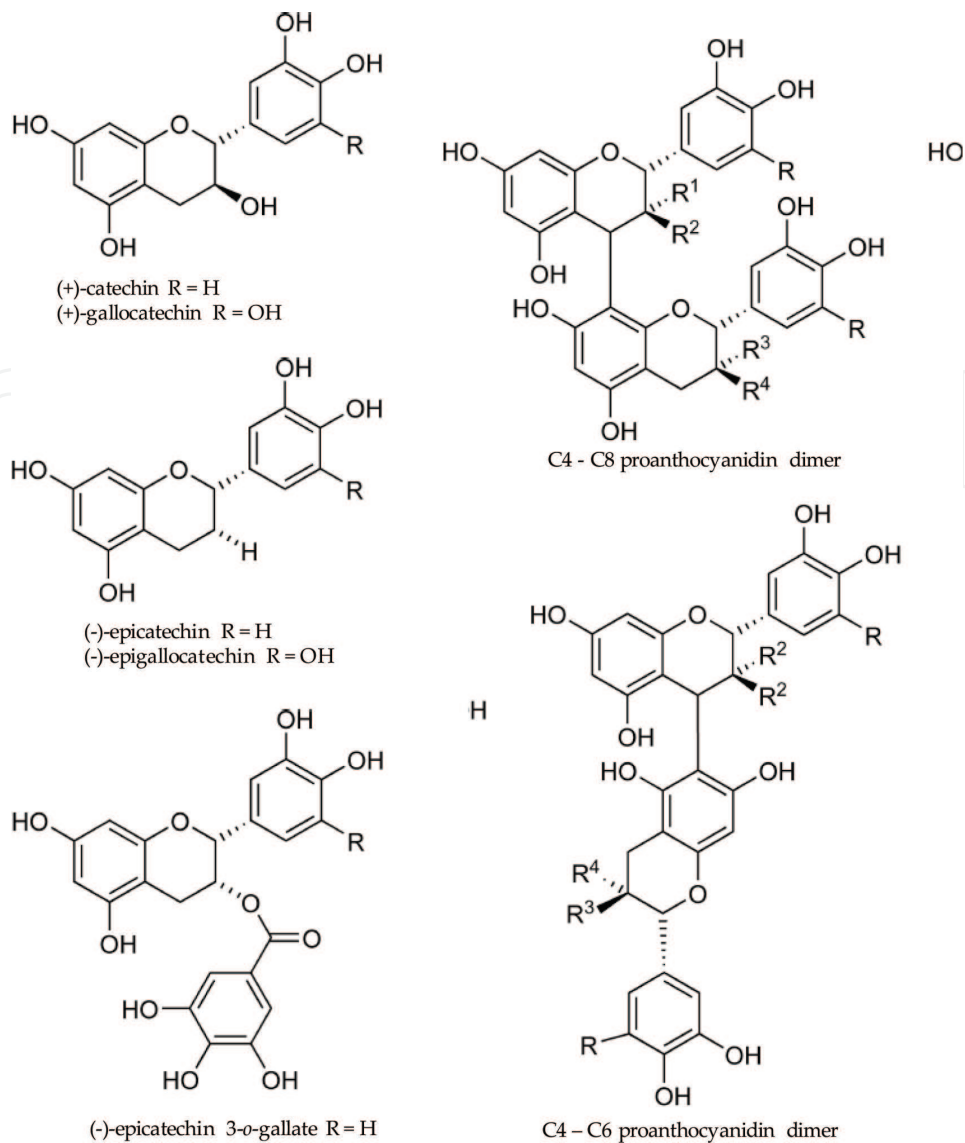
Figure 3.
Chemical structure of flavan-3-ol compounds found in grapes and wines.

phenolic content using a simple UV-visible spectrophotometer possible [1]. However, UV-visible spectroscopy is also used in more advanced separation techniques, such as liquid chromatography, that allows for the quantification of individual phenolic compounds [13]. The quantification of phenolic compounds is thus achieved through the UV-visible signal given by the individually separated phenolic compounds. On the other hand, fluorescence spectroscopy also makes use of the UV-visible spectral features of the excited substances. After the excitation process a coloured fluorophore is quantified based on its absorption intensity projected in the visible region [14]. Finally, UV-visible spectroscopy combined with chemometrics is also included in the techniques used for phenolic compounds' quantification [15]. In this case the spectral properties are used to predict the phenolic content of a given grape phenolic extract or wine [16]. This approach makes use of partial least squares regression analysis to correlate spectral information with reference data (phenolic levels). If successfully performed, a validated calibration can provide accurate predictions of phenolic content by only measuring the UV-visible spectral properties of wines.

This manuscript, in its different sections, reports therefore the current status of the different analytical techniques available for the quantification of phenolic content in grapes and wines. Moreover, the UV-visible spectral features observed in wines during the winemaking process, from the early stages of fermentation and through the ageing process are also reported and discussed.

2. UV-visible features of wines during winemaking and ageing

Among other analytical techniques, UV-visible spectroscopy appears to be suitable for the quantification of phenolic compounds. This is due to two main reasons. First of all, phenolic substances have the ability to strongly absorb UV light [11] and secondly, certain compounds due to the coloured nature can lead to absorption features in the visible range [17]. Polyphenols are biological compounds containing π conjugated systems with hydroxyl-phenolic groups. The π type molecular orbitals electronic transitions provide the UV-visible spectrum of this group of compounds. UV-visible spectroscopy is used in winemaking to quantify different sub-groups within the phenolic family [18]. The most common procedures for phenolic analysis are reported to quantify anthocyanins, phenolic acids (including hydroxycinnamates and hydroxybenzoates), stilbenes, flavonols and flavanols or tannins.

The main absorption feature of the flavanol monomers is a strong absorption band around 280 nm (**Figure 4c**). These colourless compounds do not show absorption features in the visible region of the electromagnetic spectrum. The flavanol monomers may contain a galloyl molecule attached to the flavan-3-ol structure. A galloylated flavanol has been reported to have higher absorption intensity, when compared to its non-galloylated form, it also shows a shoulder at 310 nm, characteristic of the galloyl group (see gallic acid as example in **Figure 4d**) [11]. For flavanol polymers or tannins the absorption features remain the same despite the degree of polymerisation (number of monomers) of the proanthocyanidin structure with a predominant absorption band at 280 nm.

Anthocyanin compounds co-exist under different forms and its colour intensity and tonality depends on the proportion of the different molecular structures
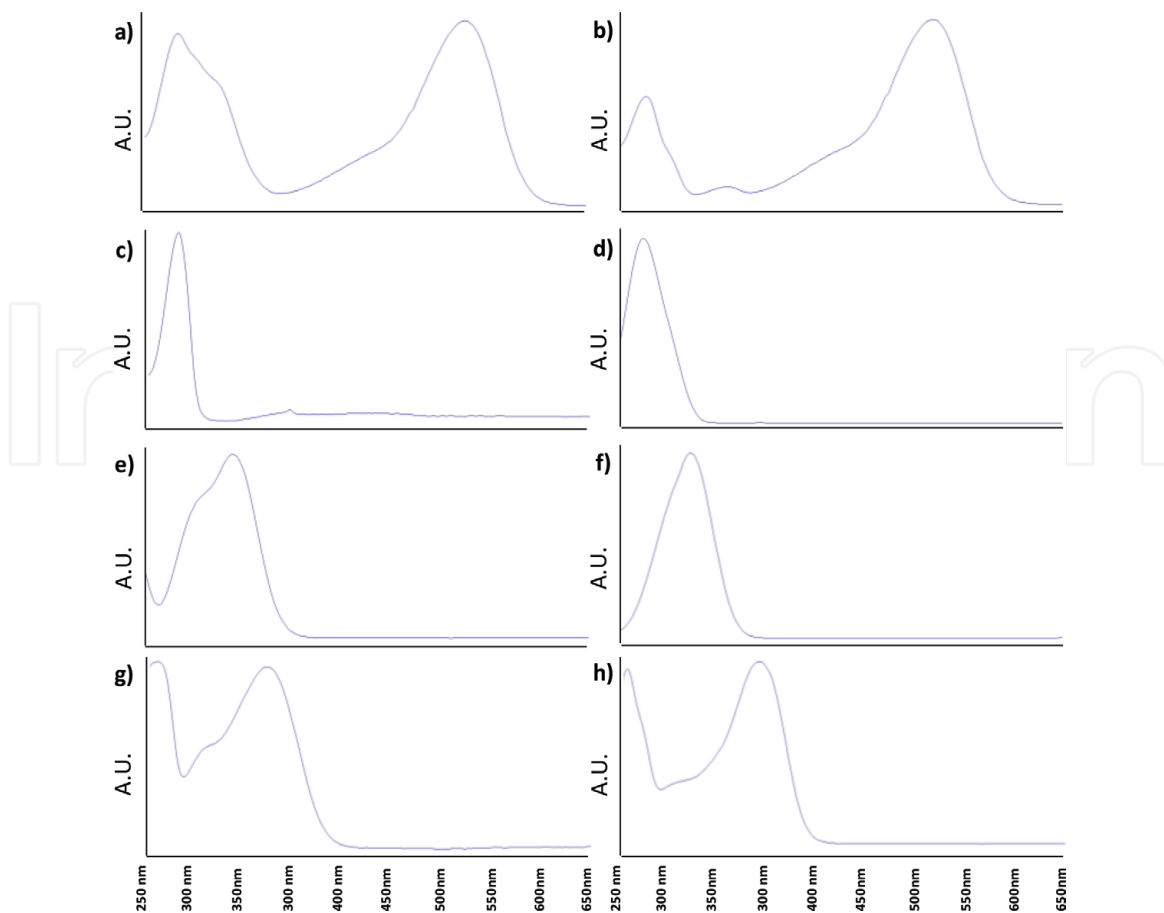

Figure 4.
UV-vis spectral properties of individual phenolic compounds. (a) Malvidin-3-glucoside, (b) malvidin-3-p-coumarylglucoside, (c) catechin, (d) gallic acid, (e) caftaric acid, (f) coutaric acid, (g) rutin, (h) quercetin.

present at the time of evaluation. The main absorption features of this phenolic sub-family are given by an intense absorption band at 280 nm, common to all phenolic substances, and by a characteristic absorption intensity around 520 nm characteristic of red colouring substances (**Figure 4a**). In addition, the anthocyanins are found in grapes and wines acylated with a number of other wine components, including some phenolic acids such as caffeic or *p*-coumaric acids [3]. In this case the anthocyanin molecule will also show a characteristic broad band around 320 nm (see malvidin-3-*p*-coumarylglucoside in **Figure 4b**). Anthocyanins are highly reactive phenolic compounds strongly influenced by the pH conditions and by the presence of SO_2 [19]. Lower pH values increase the proportion of anthocyanins present in the red flavylium form, leading to increased colour intensity, through an hyperchromic effect in the visible region. The opposite behaviour is thus observed if the wine's pH increases to higher values, leading to a decrease of the absorption intensity at 520 nm (hypochromic effect). On the other hand, the ability of anthocyanins to exist in its red forms is highly dependent on the SO_2 content. Sulphur dioxide has the ability to interact and combine with the anthocyanin molecule in position 4 of the central phenolic ring, causing the decolouration of the chromophore, leading to a colourless flavilium sulphonate [6]. The protective role of sulphur dioxide is due to its ability as antioxidant. In the case of the anthocyanins, SO_2 protects the non-coloured anthocyanin in solution until, due to the reversible nature of this reactions, the red anthocyanin chromophore is liberated.

Phenolic acids in grapes and wines include both hydroxycinnamic and hydoxy-benzoic acids. Hydroxybenzoic acids, such as gallic acid, show a single intense absorption band at 280 nm, common to all phenolic substances (**Figure 4d**). On the other hand, hydroxycinnamic acids show an absorption band around 320 nm, characteristic of this group of compounds (**Figure 4e** (caftaric acid) and f (coutaric acid)). Finally, the flavonol group show also particular UV-visible absorption features with an additional absorption band around 360 nm (**Figure 4g** (rutin) and h (quercetin)). This absorption band together with the 280 nm absorption features define the UV-visible spectra of the flavonol group.

Grape phenolic compounds are released into the must after the crushing operation. Phenolic compounds are initially located in the solid parts of the berries. Seeds, skins and to a lesser extent, stems, are the main sources of phenolic compounds found in wines. During crushing the juice contained in the berries comes in contact with skins and seeds. Subsequently, during the maceration step this contact will lead to the diffusion of the phenolic substances into the must. While tannins are found in both skins and seed tissues, the anthocyanins are only located in the skins (also found in the flesh of a few tenturier cultivars). Hydroxycinnamic acids are, on the contrary, the only group of phenolic compounds that is found in high levels in the flesh, whereas hydroxybenzoic acids (seeds), stilbenes (skins) and flavonols (skins) are found mainly in the solid parts of the grape berries [4]. The winemaking strategy i.e. presence or absence of skin contact, length and conditions of the skin contact and grape characteristics will define the pool of phenolic compounds that will be present in the wine after the fermentation. Due to this phenolic extraction, important changes in the UV-visible spectral feature take place.

Figure 5 shows the average UV-visible spectral features of 13 different red wines during the first 15 days of the fermentation that included cultivars such as Cabernet Sauvignon, Shiraz, Grenache or Pinotage. Three main absorption bands are observed in the UV-visible spectral features. The first and more prominent band is observed around 280 nm. Following this, broad high intensity absorption properties are also observed around 320 nm. Finally, a third intense absorption band is identified in the visible region around 520 nm. As can be observed in **Figure 5**, right after crushing (Day 0) low absorption intensity bands are observed in the 280 and 320 regions,
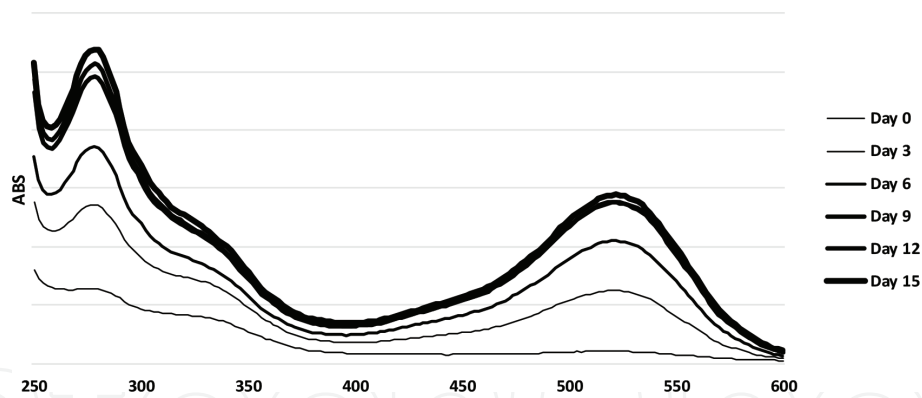
Figure 5.
UV-visible spectral features (250–600 nm) of red wines during fermentation.

whereas no absorption is observed at the visible anthocyanin absorbing 520 nm region. This can be explained by the instant release of some of the phenolic compounds located in the flesh such as the hydroxycinnamic acids. As fermentation progresses a hyperchromic effect is rapidly observed during the first days after crushing. The absorption band around 280 nm rapidly increases until Day 9 of fermentation. From then on, an increase is still identified but to a lesser extent than that initially observed. A different behaviour is observed for the absorption features around 320 and 520 nm. For these two regions, the intense hyperchromism is observed until Day 9 with no subsequent significant increase until the completion of fermentation.

Anthocyanins are water soluble compounds that are extracted during the early stages of fermentation. Alongside with the anthocyanins, the extraction of other skin-localised phenolics, such as flavonols and flavanols or tannins also takes place. However, as alcohol content increases, seed phenolics, mainly flavanols and tannins, are released into the must. The later extraction of seed flavanols and tannins requires the hydrolysis of the lipidic layer around the seed as well as the hydration of the seed tissue itself. Seed tannins have been defined as more astringent and bitter tannins while skin tannins have been described as softer or less reactive towards proteins [10]. The flavanol content in terms of individual composition (procyanidins or prodelphinidins), galloylated subunits and mean degree of polymerisation will provide the intensity and sub qualities of the bitterness and astringency perception of wines [9]. The intense absorption band at 280 nm is due to the extraction of flavonols, hydroxycinnamic acids, flavanols and the UV absorption part of the anthocyanins. The band observed around 320 nm is purely ascribed to the hydroxycinnamic acids. Finally, the band observed at 520 nm is due to the anthocyanin extraction during fermentation. The further increase in the absorption intensity at 280 nm after Day 9 may be due to further extraction of seed tannin content material.

Phenolic compounds are highly reactive and a large number of interactions and reactions can take place during wine ageing and storage. Some of these phenolic reactions benefit from the presence of oxygen during the barrel ageing period. This is the case for some of the direct tannin-anthocyanin complexes as well as the indirectly acetaldehyde mediated tannin-anthocyanin reactions. On the other hand, the absence or shortage of oxygen during the bottle ageing period will stimulate tannin polymerisation reactions and also some direct tannin-anthocyanin combinations. **Figure 6** shows the average UV-visible spectra of a number of commercial red wines after the fermentation process was completed as well as after a year of barrel ageing (12 months after fermentation is completed), followed by a year of bottle ageing (24 months of fermentation completion). In this case an average spectra of a large number of wines including Cabernet Sauvignon, Pinotage, Shiraz, Merlot,
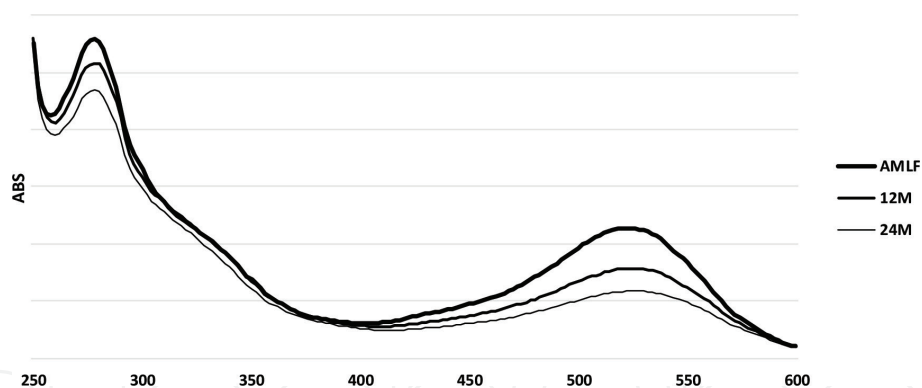
Figure 6.
UV-visible spectral features of wines after malolactic fermentation completion (AMLF) and at 12 months of barrel ageing (12M), followed by 12 months of bottle ageing (24M).

Ruby Cabernet, Petit Verdot, Cinsault, Malbec, Grenache, Pinot Noir and Cabernet Franc was evaluated. The most important features are observed at 280 and 520 nm, whereas the broad band at 320 nm remained constant over the ageing period. It is also important to mention that the bigger decrease in absorption intensity was observed at the 520 nm region which corresponds to the visible absorption part of the anthocyanins.

After reaching maximum levels during the fermentation process, anthocyanin content starts decreasing. Anthocyanins are involved in a large number of phenomena, such as degradation, oxidation, reabsorption into grape and yeast cell walls, precipitation with tartaric salts, interaction with SO_2 or reaction with tannins, among others [7]. Despite this, red wines still maintain an intense colour during ageing which is due to the transformation of anthocyanins into longer term stable polymeric pigments. Anthocyanins give rise to a number of pigments from acylation with diverse grape components, intra and intermolecular copigmentation reactions and interactions, occurring early during the process, to more complex reaction leading to pyranoanthocyanin or tannin-anthocyanin complexes formation [7]. The limited decrease observed around 280 nm is attributed to a larger extent to the decrease of the UV absorption ability of the anthocyanins and to a lesser extent to a decrease of tannin compounds through precipitation. Tannins are also highly reactive substances with high affinity for proteins and polysaccharides, which can lead to tannins precipitation. In addition, the polymerisation ability of these compounds may result in insoluble larger molecules that also precipitate from solution, thus reducing its content in wine. Finally, the absorption band around 320 nm remains stable during ageing, indicating stability of this region absorbing compounds during barrel and bottle ageing.

3. Spectrophotometric methods for phenolic analysis

3.1 Total phenolic content

Total phenolic index (TPI). The measurement of UV-visible absorption light to quantify phenolic compounds was first proposed in the late 1950's. The absorbance at 280 nm was selected as the best indicator of the phenolic content in wine due to the ability of phenolic substances, and more specifically the phenolic ring, to absorb UV light [20]. A simple wine or grape extract dilution is used to quantify the total phenolic content or total phenolic index (TPI). The TPI corresponds to the A280 nm times the dilution factor. The dilution factor might change depending on the sample under evaluation, as well as the path length of the cuvette. Dilution factors of 100

and 50 have been reported for red wines. Depending on the extraction methods dilution factors between 50 and 20 for grape extracts have been proposed. In the case of white and rose wines, with lower phenolic levels, the dilution factor needs to be adjusted. In this case dilution factors from 5 to 20 have been used. The TPI can also be expressed as gallic acid equivalents when used as a standard. This method has been reported to be simple, fast and reliable, although overestimation of the total phenolic content occurs due to the ability of other grape component that also absorb UV-light. A value of 4 units, that can be subtracted from the index, has been proposed to account for the interferences caused by these other UV absorbing material. Additionally, some other phenolic compounds such as cinnamic acids or chalcones do not show absorption features at 280 nm, however due to its low content in wines the expected differences are considered negligible [21].

Folin-Ciocalteu. The Folin-Ciocalteu assay for total phenolic content relies on the ability of the Folin-Ciocalteu reagent to strongly react with phenolic compounds. A mixture of two acids, namely phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMO_{12}O_{40}$) acids, react with mono and dihydroxylated phenolic substances due to their high ability to donate electrons. This reaction creates a blue coloured complex that is quantified at 750 nm [22]. After a simple wine dilution, the Folin-Ciocalteu reagent is added. A 20% $NaCO_3$ solution is then added to the mixture with some additional distilled water. The sample is then incubated for 30 min before absorbance measurement can be performed. Moreover, it is of crucial importance to maintain the order of the additions to ensure that the reaction takes place under alkaline conditions. In order to preserve accuracy, the A750 nm needs to be around 0.3 A.U. If this is not achieved, a different wine dilution needs to be performed. A blank with distilled water, to account for background interferences, is also included [23]. The results are commonly reported as gallic acid equivalents. Although the method is very often used, the ability of some other wine component to also donate electrons leads to potential overestimation of the phenolic content. This compromises the comparison of different samples containing varying phenolic and wine composition. In addition, the comparison of the Folin-Ciocalteu with the TPI is also possible by multiplying the A750 nm times the dilution factor times 20. A strong correlation between the two methods has been reported, thus making the total phenolic content between these two methods comparable [1].

3.2 Total anthocyanin content

Hydrochloric acid method. The estimation of the total concentration of anthocyanins in wine or grape extracts is possible due to the characteristic absorption band of this group of compounds around 520 nm. The coloration of anthocyanins are highly influenced by pH, with lower pH values leading to a higher proportion of anthocyanins in the red flavilium ion form. This property is thus exploited in this method to quantify the total anthocyanin content. Due to its ability to decrease pH, the method makes use of hydrochloric acid (HCl) i.e. the sample is diluted with a 1 M HCl solution. After a waiting period, to allow the free monomeric forms of the anthocyanin to be transformed into their red coloured forms, the A520 nm is measured [24]. The waiting period was initially reported to be longer than 3 hours but shorter than 24 hours, however later research confirmed that a waiting period of 1 hour is sufficient [25]. The values can be reported as A.U. or as malvidin-3-glucoside equivalents by making use of the molar extinction coefficient (commonly used 28,000 L/cm*mol) and the molecular weight (MW = 529 g/mol) of the major anthocyanin found in grapes and wines i.e. malvidin-3-glucoside.

Bisulphite bleaching method. Another property of the anthocyanins is in this case used to quantify this group of compounds. Sulphur dioxide is able to combine with the anthocyanin in the position 4 of the central phenolic ring, giving rise to a non-coloured flavene sulphonate. The decolouration ability of SO_2 is thus used to estimate the total content of free anthocyanins in the wine. The method also makes use of HCl with the aim of transforming the anthocyanins to their red coloured flavilium form. Two test samples are in this case compared. The control sample, with no SO_2 addition is compared against a treatment sample where the anthocyanins have been bleached by the SO_2 addition. After a waiting period, the A520 nm of both samples are compared and the total anthocyanin content calculated [26]. However, the ability of SO_2 to react and bleach some pigmented forms might lead to an overestimation of the total content [27].

pH differential method. Another method that exploits the effect of pH on the anthocyanin coloration was reported by Giusti and Worldstad [17]. This methodology compares a red flavilium form sample at pH 1 against a sample where the anthocyanins are transformed to its non-coloured hemiketal from at pH 4.5. Instead of measuring the anthocyanin content of both pH 1 and pH 4.5 samples at a fixed wavenumber (520 nm), the method measures the A_{max} observed around the 520 nm absorption band, which may not coincide with 520 nm. In addition, the method also includes the measurement of the A700 nm that is subtracted from the A_{max}, with the aim of accounting for possible light scattering caused by other sample components. By doing this the method ensures that the recorded absorption values only correspond to the anthocyanin content in the samples. The results are reported as malvidin-3-glucoside equivalents, by also using the molar extinction coefficient and the molecular weight of this anthocyanin. In addition, the method also allows for the calculation of additional indices by using the ability of sulphur dioxide to combine and bleach anthocyanins. A more complete picture of the anthocyanin content and composition is thus obtained after the inclusion of the pigment degradation, polymeric colour and browning indexes. In this case the method makes use of the absorbance at 420 nm to account for the polymeric anthocyanin material with colour properties closer to this region of the visible spectrum (orange colouration). The polymeric pigment colour is calculated as the proportion between the colour observed in the bleached samples at 420 nm and the A_{max} around 520 nm and that measured at the same wavelengths in the non-bleached samples. In order to ensure accuracy, measurements need to be taken between 15 min and 1 hour in line with what was reported earlier to avoid increased absorption properties at longer times [17].

Modified Somers assay. This methodology is based in the original method reported by Somers and Evans [21]. More recently a modified protocol, adapted to a high throughput format, using a microplate reader spectrophotometer was reported for both grape extract and wine samples [28]. The method presents a number of parameters and provides a broad overview of the status of the anthocyanin's equilibria in the sample. The method relies on the effect of hydrochloric acid, acetaldehyde and sulphur dioxide on the anthocyanins. Sulphur dioxide is added with the aim of calculating the levels of non-blanchable pigments, which includes more stable pigments such as tannin-anthocyanin complexes as well as pyranoanthocyanins. Moreover, acetaldehyde is used to negate the bleaching effect of SO_2 on anthocyanins and thus measure the total content of coloured anthocyanins. Finally, hydrochloric acid is added to account for those free anthocyanins that were not bleached or were derived from copigmentation complexes. The main advantage of the method relies on the fact that the pH adjustment, crucial to accurately estimate the state of the anthocyanins, is done by adjusting the pH of a buffer solution [28]. In the original protocol the pH of the samples was individually adjusted, with a

considerable extension of the time of analysis. This method provides information on the wine "chemical age", which provides an estimation of the extent that the polymeric pigments has displaced the monomeric anthocyanins. Additional parameters report on the percentage of anthocyanins in its flavilium red form (% of ionisation), SO_2 resistant pigments (polymeric pigments), colour intensity, hue as well as total phenolic content.

Copigmentation assay. Anthocyanins interact with other wine components including other phenolic substances to form pigmented molecules through weak hydrophobic forces. The sandwich-like structure is composed of copigment molecules in between the anthocyanins [29]. The newly formed structure places the sugar moieties of the anthocyanin towards the external part of the complex, thus protecting the copigmented pigment from decolouration by water. These interactions account for a large part of the colour of young red wines with its contribution to wine colour decreasing over time, due to the weak nature of the copigmented structure [29]. Two main effects are characteristic of these complexes, which includes an increased absorption intensity in the visible absorption region of the anthocyanins (hyperchromic effect) accompanied by a shift into the absorption maxima towards higher wavelengths (blue colouration) through a bathochromic effect. The copigmentation assay was developed by Boulton and it is the only available method for the quantification of the colour due to copigmentation in red wines. The method relies on the ability of the anthocyanin complexes to avoid decolouration by water at constant pH i.e. measures the decolouration of the anthocyanins due to the dissociation of the copigmented forms [29].

3.3 Colour measurements

Colour density. Coloured anthocyanins and anthocyanin derived pigments are responsible for the colour properties of red wines. During the early stages of winemaking the colour properties of wines are mainly due to less complex monomeric forms of anthocyanins, however as the wine ages and anthocyanins start interacting with other wine components, more stable pigmented polymeric forms are responsible for the colour properties of red wines. The wine colour density was initially measured through the addition of the absorption values at 420 and 520 nm, which corresponds to the yellow and red colorations of wine [30]. Using this information, the hue of a wine samples was defined as the ratio between these two absorption values (A420 nm/A520 nm). More recently the absorption at 620 nm, which accounts for the blue wine colouration, was also added to the colour density parameter [31]. The method relies on a simple measurement (without dilution) and provides an estimation of the colour intensity of the wine. The results are often reported as %yellow, %red and %blue providing thus a more complete interpretation of wine colour properties. On the other hand, the A420 nm or A440 nm are commonly used to measure the colour properties of white wines including the brownish wine colour (browning index) [32].

CIElab colour space. Wine colour can also be measured through the information contained in the visible spectra of wines. Three colour components result from the integration of the visible absorption features. The Commission International de l'eclairage [33] proposed a method that uses three chromatic coordinates X, Y and Z to determine the chromatic characteristics of wines (also applicable to other beverages). The method aims to simulate the perception that real observers have for the colour properties of a sample. The calculation of the CIElab coordinates is based on measurement conditions given by a spectrophotometer with illuminant D65 and observed placed at 10°. The colour of a wine is thus described by the intensity of the wine colour (chromaticism), the luminosity of the wine and the colour itself based on the

red, yellow, green and blue components (tonality). The colorimetric measurements are defined by the chromatic coordinates red/green component (a*) (a* > 0 red, a* < 0 green), blue/yellow component (b*) (b* > 0 yellow, b* < 0 blue), clarity (L*) (L* = 0 black and L* = 100 colourless) and its complementary magnitudes tone (H*) and chroma (C*). The ability to compare the colorimetric differences between two colours (ΔE*) makes it possible to directly compare the colour properties of wines. Moreover, it has been established that a colour difference higher than 2.7 indicates that the colour of two samples can be perceived different by the human eye [34].

3.4 Total tannin content

Acid hydrolysis. Due to the complex nature of proanthocyanidins or tannins the determination of these compounds is a difficult undertaking and has been challenging researchers for a long time. However, a number of methods, albeit with certain limitations, have been reported and will be discussed. The acid hydrolysis method is based on the transformation of proanthocyanidins in carbocations that are partially converted into anthocyanidins when exposed to heating under acidic conditions (Bate-Smith reaction). The total tannin content is thus estimated by using the red coloration of the resulting anthocyanin compounds at 550 nm and expressing it in cyaniding-3-glucoside equivalents. Although the method is widely used, a number of limitations have also been reported. First of all, the tannin concentration seems to be overestimated with higher values for tannins reported than those for total phenolic content. Moreover, it is also common to observe an increase in the total tannin content of wine during ageing and finally the method does not provide any information on the structure of the tannins [35].

Methylcellulose precipitable (MCP) tannins assay. This method falls under the precipitation based methods category as it uses the tannin precipitation ability of a methylcellulose polymer to estimate the total tannin content of grape extracts and wines. As mentioned the method relies on tannin-MCP interactions in the presence of ammonium sulphate, giving rise to an insoluble polymer-tannin complex that precipitates and is further separated by centrifugation [36]. This method has also been lately adapted and validated into a high throughput format leading to a considerable reduction of the analytical time [28]. A control sample without MCP addition (absence of tannin precipitation) is compared against a treated sample where the tannins have been removed after precipitation with MCP. The absorption difference measured at 280 nm is then used to quantify the total tannin content of a sample. The total tannin content is in this case estimated as epicatechin equivalents. In addition, one of the main benefits of precipitation based methods is that a theoretic positive correlation with astringency intensity is foreseen [37–39]. The hypothesis is based on the assumption that the method simulates the phenomena that naturally occurs in wine when it becomes in contact with the salivary proteins. An insoluble macromolecular complex is then formed that precipitates from solution causing the drying and puckering sensation known as astringency.

Bovine serum albumin (BSA) tannin assay. This precipitation based method exploits the ability of proteins to combine and precipitate tannins. The precipitation is achieved through the incorporation of bovine serum albumin protein. The precipitated protein-tannin complexes are then redissolved and quantified at 510 nm after the addition of ferric chloride [40, 41]. The accuracy of the method is based on obtaining the appropriate wine dilution as concentrated or very diluted samples tend to underestimate the tannin content. The BSA tannin assay, as part of the precipitation based methods for tannin analysis, has also been found to positively correlate with astringency intensities given by sensorial evaluation [37–39]. The total tannin content is in this assay calculated as catechin equivalents. In addition,

the method also allows for the determination of additional parameters related to the anthocyanin and polymeric pigment fraction. Specifically, the method makes use of SO_2 to obtain information on the nature of the polymeric pigments by dividing them into small (SPP) (pigments that do not precipitate with BSA) and large polymeric pigments (LPP) that do precipitate with the protein. On the other hand, the comparison of both precipitations based methods has shown that MCP tannin values are on average three time higher than those found for BSA. However, a strong correlation (0.8) between the values obtained with the two methods has also been reported [42], whereas no correlation was observed between these two methods and the tannin content obtained with the acid hydrolysis method [37]. Finally, despite the differences in absolute values, attributable to the differences in both procedures, it has also been stated that both precipitants (BSA and MCP) precipitate the same amount of tannins when tested under the same conditions [39, 43].

4. UV-visible role in liquid chromatography

High liquid pressure chromatography (HPLC) is a suitable method to quantify individual phenolic compounds in grape extracts and wines. HPLC instruments make use of a diode array detector that allows for the quantification of phenolic substances at different wavelength within the UV-visible regions. The benefit of using diode array detectors in liquid chromatography is beyond using retention times for peak identification as it adds qualitative information by the incorporation of the UV-visible spectral features of a specific peak or compound [44]. It is thus nowadays possible to obtain a number of individual phenolic compounds by direct injection of wine samples without any sample pre-treatment. Based on its spectral features, phenolic compounds will be quantified at their absorption maxima, i.e. sub-families of phenolics are quantified at 280 nm for flavanol monomers and polymers and some phenolic acids, 320 nm for hydroxicinnamic acids, 360 nm for flavonols and finally 520 nm for anthocyanins. Although a considerable number of individual phenolics can be quantified using HPLC, the majority of the methods are not able to separate larger molecular structures such as polymeric phenols and pigments [13]. These two groups of compounds are commonly identified as broad absorption bands at later elution times at 280 nm for the polymeric phenols and at 520 nm for the polymeric pigments. Furthermore, in a previous study, the composition of the broad absorption band observed at 520 nm theoretically attributed to polymeric pigment material was investigated and confirmed [13]. Additionally, the polymeric pigments peak was also found to correlate with the spectrophotometric measurements of phenolic compounds and with wine age. In terms of polymeric phenols, it is believed that the phenolic compounds forming part of this broad absorption band correspond to a large extent to proanthocyanidins or tannins of high degree of polymerisation. The strong correlation (0.83) observed for a significant number of wines between the polymeric phenol peak area and the total tannin content, obtained with the MCP tannins assay, confirmed this [16]. HPLC methods for quantification of phenolic substances can also incorporate mass spectrometers. Mass spectrometry provides information about the molecular weight of the compounds and it is used to discern the identity of unknown compounds. The identification of phenolic compounds in chromatographic techniques using DAD is limited by co-elution (impure UV-visible spectra) or by similarities in the UV-visible properties of phenolic compounds belonging to the same phenolic family. These factors combined with similar elution times of some of the phenolic substances complicates the accurate quantification of chromatographic peaks. The use of mass-spectrometry provides thus a valid tool to confirm the identity of phenolic substances as well as the identification of novel compounds.

5. Fluorescence spectroscopy

An interesting and more recent technique to quantify phenolic compounds makes use of the ability of this group of substances to emit fluorescence light after the excitation/emission process. Fluorescence spectroscopy is able to measure the analyte concentration through its fluorescence properties, being thus suitable to measure compounds in solution, such as phenolics found in grape extracts or wines [14]. If phenolic compounds are excited at the appropriate light intensity and wavelength, generally through UV light exposure, the energy change occurring at electronic level will cause a light emission in the visible region of the electromagnetic spectrum [45]. Phenolic molecules are initially at ground levels at low energy state until light exposure elevate the vibrational levels to an elevated high energy state. After a period of time (in the order of milliseconds) the excited molecule while returning to its non-excited electronic state emits light (so-called fluorescence) at higher wavelengths than those absorbed during the excitation process. During the excitation/emission sequence both the absorbed and emitted light can be measured, with higher emission intensity corresponding to higher concentration of the analyte. Fluorescence spectroscopy has been commonly applied to the quantification of phenolic compounds in combination with liquid chromatography techniques. The main benefit of these applications rely on the increased sensitivity and selectivity of the method [45]. Additionally, fluorescence spectroscopy has been defined as a fast, non-destructive, easy to perform technique that can also be used for process monitoring purposes due to the versatility of the fluorescence spectrometers. Excitation emission spectral (EEM) properties might potentially be correlated with reference analytical data to establish regression calibrations for the quantification of phenolic compounds in a similar manner than what is reported for UV-visible or infrared spectroscopy calibrations.

6. UV-visible spectroscopy with chemometrics

The UV-visible spectra can alternatively be used in combination with powerful chemometric analysis to obtain spectroscopic calibrations for the prediction of phenolic content in grapes as well as in wines during the winemaking process [15]. In this case the totality or parts of the UV-visible spectra are correlated through multivariate regression approaches with reference phenolic data. After the spectral and phenolic content acquisition of a significant number of samples and in the case that strong correlations are found between the spectral data and the phenolic levels, a reliable prediction calibration can be obtained after the corresponding calibration and validation procedure. The advantage of these spectroscopy calibrations relies on the possibility of estimating the total content of phenolic substances through a simple spectral measurement, therefore avoiding the tedious reference method procedures. The main advantage of the spectroscopy calibrations is due to the rapidness, simplicity, reliability and cost-effectiveness ascribed to these techniques. Moreover, due to the multi-parametric nature of this approach a single spectral measurement is able to provide the levels of a number of phenolic compounds. Spectroscopic applications are also highly suitable to perform online measurement during the process of winemaking, allowing for improved process control strategies, through process monitoring, in line with a process analytical technologies (PAT) approach [46].

The first indication of the use of UV-visible spectroscopy calibrations to quantify some of the most important phenolic parameters was reported in 1995 [47]. In this first approach the total tannin and anthocyanin content was predicted using

a limited number of samples. The UV-visible spectra was collected from 200 to 650 nm at 6 nm intervals. Errors in prediction (root mean standard error of prediction (RMSEP)) of 0.35 g/L (14% RMSEP%) and 29 mg/L (8%) where reported for tannins and anthocyanins respectively. Despite the relative small sample set and the limitations of the analytical reference method investigated (mainly due to non-specificity for phenolic compounds of the employed procedures) this publication reported for the first time the suitability of UV-visible spectroscopy to quantify phenolic content in wines through partial least squares (PLS) regression analysis.

In a further study the UV-visible spectral properties of a large dataset (400) were used to quantify phenolic content of samples collected at different stages of the winemaking process. The sample set included samples from a variety of different regions and cultivars. Spectral data was collected over the 230–900 nm at 0.17 nm intervals. The parameters derived from the BSA tannin assay including the anthocyanin and total phenolic related parameters were in this case evaluated. RMSEP of 87 mg/L (20% RMSEP%) for total anthocyanin content; 0.37 (26.4%), 0.46 (76.7%) and 0.48 (24%) A.U. for small, large and total polymeric pigments, respectively; 66 mg/L (30.1%) for tannin content; 99 (17.2%) mg/L for non-tannin phenols and 130 mg/L (16.4%) for total phenols were reported [48]. Later on, the same phenolic parameters were again investigated. A 100 samples of Cabernet Sauvignon and 100 samples of Shiraz were collected during the fermentation process over a single vintage to provide calibration that can be used for the prediction of phenolic content in must. In this case an adaptation of the BSA tannin assay was used for phenolic analysis. UV-visible spectral properties were collected in the 200–900 nm range. The results showed calibrations able to predict the phenolic content of Cabernet Sauvignon samples, but not for Shiraz, suggesting cultivar specificity of the predicted calibrations. Standard errors in cross validation (RMSECV) of 102.22 mg/L (23.8% RMSECV%) and 211.38 mg/L (25.6%) were reported for total tannin and iron reactive phenolic content, respectively for Cabernet Sauvignon samples. In terms of anthocyanin measurements, error in cross validation of 101 mg/L (43.3% RMSECV%), 0.46 A.U. (26.1%) and 0.48 A.U. (41.4%) for total anthocyanins, small and large polymeric pigments were observed [49].

Due to its characteristic absorption band at 280 nm the UV spectral properties of wines have also been used for the determination of phenolic content and more specifically for total tannin content. The MCP tannin levels of a significant number of samples from a variety of different locations, cultivars and during different steps of the winemaking process were successfully predicted with the use of multiple linear regression (MLR) and partial least square regression (PLS). In this case spectral properties were collected between the 230 and 350 nm range of the UV part of the electromagnetic spectrum. Errors in cross validation (RMSECV) of 0.2 g/L (9.3% RMSECV%) were reported for MLR models using the above-mentioned UV region. Moreover, the authors also reported calibrations but in this case using only a limited number of key wavelengths. Further calibrations were investigated using the UV absorption values at 250, 270, 280, 290 and 315 nm. The external validation calibrations showed errors in prediction (RMSEP) of 0.18 g/L (9.2%) which confirmed the suitability of the UV region spectral properties to quantify tannin content in wine samples [50].

In a more recent study the ability of UV-visible spectroscopy to predict tannin content in finished wines was reported. In this case two precipitation based methods, namely MCP and BSA tannin assays, were used to generate the spectral data. A large number of samples containing a varying number of cultivars from different regions as well as vintages were included in the model optimization procedure. UV-vis spectra was measured in the 260–610 nm region at 2 nm intervals. The best

calibrations were found for both reference methods where the spectral properties in the UV region were used as spectral data (260–310 nm). A RMSEP of 0.16 g/L (9.9% RMSEP%) and 0.08 g/L (13.3%) were reported for MCP and BSA tannin content, respectively. In agreement with previous studies, accurate calibrations were also observed when a reduced number of wavelength were used as spectral data. Models optimised using the absorption values at 270, 280, 290, 300 and 314 nm lead to errors in prediction of 0.18 g/L (11.2% RMSEP%) and 0.11 g/L (18.3%) for MCP and BSA tannin content, respectively. Also in agreement with previous findings, cultivar and vintage specificity issues influenced to a certain extent the accuracy of the calibrations [42].

In a more recent study PLS calibrations based on UV-visible spectral data for the quantification of phenolic content in grapes, fermenting samples and wines have been reported. A large number of fermenting samples from 13 different vinifications over two consecutive vintages were included into the calibrations. Moreover, a number of finished wines from varying vintages and from a number of cultivars were also included. PLS validation calibrations showed prediction errors of 209 mg/L (14.3% RMSEP%), 14 mg/L (3.2%), 1.6 (3.2%) and 2.6 (14.7%) for total tannin content (MCP tannin assay), total anthocyanins, total phenolic content and colour density, respectively. In addition, individual phenolic compounds quantified using a HPLC method to generate the reference data were also reported, including flavanol monomers and the dimer B1, phenolic acids, flavonols as well as monomeric and acylated anthocyanins. Calibration for the estimation of polymeric phenol and pigment content were also reported. On the other hand, the same study reported PLS calibrations for determination of phenolic content in grapes extracts obtained through two extraction protocols. A phenolic extraction in high solvent content and after the entire berries being finely blended lead to successful calibrations for total tannin content, anthocyanins levels, total phenol index and colour density. The RMSEP reported was 0.22 mg/g (7% RMSEP%), 0.034 mg/g (3.1%), 0.17 (1.32%) and 0.72 (6.61%), for the above mentioned parameters, respectively. In addition, an alternative method with phenolic extraction performed under wine-like ethanol levels from hand crushed grapes was also reported. Validation errors of 0.12 mg/g (10.7% RMSEP%), 0.03 mg/g (8.33%), 0.42 (1%) and 6.2 (20%) for total tannins, total anthocyanins, total phenolic index and colour density, respectively were reported [16].

7. Conclusions

The role of UV-visible spectroscopy in wine science appears to be of high importance. A number of applications can be used to quantify the levels of phenolic compounds in grape extracts and wines. Apart from the conventional routine spectrophotometric methods for phenolic analysis, more advanced analytical techniques such as liquid chromatography can be also used to quantify individual phenolic substances using UV-visible spectroscopy. Moreover, fluorescence spectroscopy, making use of the ability of phenolic molecules to emit fluorescence light, appears to be a promising technique that can also be used to quantify phenolic content at different stages of the winemaking process and under different conditions. Finally, UV-visible spectroscopy calibrations are also a valid alternative as they allow for the efficient measurement of phenolics in grape extracts as well as wines during fermentation and ageing. These new developments in phenolic monitoring during the winemaking process opens exciting new possibilities for wine producers in their bid to obtain wines of a certain composition and style in a more controlled manner.

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References

[1] Aleixandre-Tudo JL, Buica A, Nieuwoudt H, Aleixandre JL, du Toit W. Spectrophotometric analysis of phenolic compounds in grapes and wines. Journal of Agricultural and Food Chemistry. 2017;**65**:4009-4026

[2] Aleixandre R, Aleixandre-Tudó JL, Bolaños-Pizarro JL, Aleixandre-Benavent M. Mapping the scientific research on wine and health (2001–2011). Journal of Agricultural and Food Chemistry. 2013;**61**:11871-11880

[3] Cheynier V, Schneider R, Salmon J, Fulcrand H. Chemistry of wine. In: Comprehensive Natural Products II. The Netherlands: Elsevier Ltd.; 2010. pp. 1119-1172

[4] Teixeira A, Eiras-Dias J, Castellarin SD, Gerós H. Berry phenolics of grapevine under challenging environments. International Journal of Molecular Sciences. 2013;**14**:18711-18739

[5] Casassa LF, Harbertson JF. Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. Annual Review of Food Science and Technology. 2014

[6] He F, Liang NN, Mu L, Pan QH, Wang J, Reeves MJ, Duan CQ. Anthocyanins and their variation in red wines. I. Monomeric anthocyanins and their color expression. Molecules. 2012;**17**:1571-1601

[7] He F, Liang NN, Mu L, Pan QH, Wang J, Reeves MJ, Duan CQ. Anthocyanins and their variation in red wines. II. Anthocyanin derived pigments and their color evolution. Molecules. 2012;**17**:1483-1519

[8] Gomez-Plaza M, Cano-Lopez E. A review on micro-oxygenation of red wines: Claims, benefits and the underlying chemistry. Food Chemistry. 2011;**125**:1131-1140

[9] McRae JM, Kennedy JA. Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. Molecules. 2011;**16**:2348-2364

[10] Smith PA, Mcrae JM, Bindon KA. Impact of winemaking practices on the concentration and composition of tannins in red wine. Australian Journal of Grape and Wine Research. 2015;**21**:601-614

[11] Boulet JC, Ducasse MA, Cheynier V. Ultraviolet spectroscopy study of phenolic substances and other major compounds in red wines: Relationship between astringency and the concentration of phenolic substances. Australian Journal of Grape and Wine Research. 2017;**23**:193-199

[12] Sanna R, Piras C, Marincola FC, Lecca V, Maurichi S, Scano P. Multivariate statistical analysis of the UV-vis profiles of wine polyphenolic extracts during vinification. The Journal of Agricultural Science. 2014;**6**:152-162

[13] Peng Z, Iland PG, Oberholster A, Sefton MA, Waters EJ. Analysis of pigmented polymers in red wine by reverse phase HPLC. Australian Journal of Grape and Wine Research. 2002;**8**:70-75

[14] Airado-Rodríguez D, Durán-Merás I, Galeano-Díaz T, Wold JP. Front-face fluorescence spectroscopy: A new tool for control in the wine industry. Journal of Food Composition and Analysis. 2011;**24**:257-264

[15] Cozzolino D. The role of visible and infrared spectroscopy combined with chemometrics to measure phenolic compounds in grape and wine samples. Molecules. 2015;**20**:726-737

[16] Aleixandre-Tudo JL, Nieuwoudt H, Olivieri A, Aleixandre JL, du Toit W. Phenolic profiling of grapes, fermenting samples and wines using UV-visible spectroscopy with chemometrics. Food Control. 2018;**85**:11-22

[17] Giusti M, Wrolstad R. Characterization and measurement of anthocyanins by UV-visible spectroscopy. Current Prvtocols in Food Analytical. 2001:1-13

[18] Hassane E, Gierschner J, Duroux J, Trouillas P. UV/visible spectra of natural polyphenols: A time-dependent density functional theory study. Food Chemistry. 2012;**131**:79-89

[19] Bueno JM, Ramos-Escudero F, Sáez-Plaza P, Muñoz AM, Navas MJ, Asuero AG. Analysis and antioxidant capacity of anthocyanin pigments. Part II: Chemical structure, color, and intake of anthocyanins. Critical Reviews in Analytical Chemistry. 2012;**42**:102-125

[20] Flanzy C, Poux M. Les possibilités de la microvinification, application à l'étude de la macération. Annales de Technologie Agricole. 1958:390-394

[21] Somers TC, Evans ME. Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines. Journal of the Science of Food and Agriculture. 1974;**25**:1369-1379

[22] Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;**16**:144-158

[23] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. 1998;**299**:152-178

[24] Mazza G, Fukumoto L, Delaquis P, Girard B, Ewert B. Anthocyanins, phenolics, and color of cabernet franc, merlot, and pinot noir wines from British Columbia. Journal of Agricultural and Food Chemistry. 1999;**47**:4009-4017

[25] Bindon KA, Kassara S, Cynkar WU, Robinson EMC, Scrimgeour N, Smith PA. Comparison of extraction protocols to determine differences in wine-extractable tannin and anthocyanin in *Vitis vinifera* L. cv. Shiraz and cabernet sauvignon grapes. Journal of Agricultural and Food Chemistry. 2014;**62**:4558-4570

[26] Ribéreau-Gayon E, Stonestreet P. Le dosage des anthocyannes dans Je vin rouge. Bulletin de la Société Chimique de France. 1965;**9**:2649-2652

[27] De Beer D, Harbertson JF, Kilmartin PA, Roginsky V, Barsukova T, Adams DO, Waterhouse AL. Phenolics: A comparison of diverse analytical methods. American Journal of Enology and Viticulture. 2004;**55**:389-400

[28] Mercurio MD, Dambergs RG, Herderich MJ, Smith PA. High throughput analysis of red wine and grape phenolics—Adaptation and validation of methyl cellulose precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format. Journal of Agricultural and Food Chemistry. 2007;**55**:4651-4657

[29] Boulton RB. The copigmentation of anthocynins and its role in the color of red wine: Comments on a critical review. American Journal of Enology and Viticulture. 2001;**52**:67-87

[30] Sudraud P. Interpretation des courbes de'absorption des vin rouges. Annals of Agricultural Sciences. n.d.;**7**:203-208

[31] Glories Y. La couleur des vins rouges, 2eme partie, Connaiss. La Vigne Du Vin. 1984;**18**:253-271

[32] Singleton VL, Kramling TE. Browning of white wines and an accelerated test for browning capacity. American Journal of Enology and Viticulture. 1976;**27**:4-7

[33] Commission Internationale de l'Eclairage. CIE Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms; CIE Public. Commission Internationale de l'Eclairage; 1978

[34] Martinez JA, Melgosa M, Perez MM, Hita E, Negueruela AII, Martínez JA, Pérez MM. Note. Visual and instrumental color evaluation in red wines. Food Science and Technology International. 2001;**7**:439-444

[35] Ribéreau-Gayón P, Stonestreet E. Dógase des tannins du vin rouges et détermination du leur structure. Chemia Analityczna. 1966

[36] Sarneckis CJ, Dambergs RG, Jones P, Mercurio M, Herderich MJ, Smith PA. Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. Australian Journal of Grape and Wine Research. 2006;**12**:39-49

[37] Cáceres-Mella A, Peña-Neira Á, Narváez-Bastias J, Jara-Campos C, López-Solís R, Canals JM. Comparison of analytical methods for measuring proanthocyanidins in wines and their relationship with perceived astringency. International Journal of Food Science and Technology. 2013;**48**:2588-2594

[38] Kennedy JA, Ferrier J, Harbertson JF, Peyrot Des C. Gachons, analysis of tannins in red wine using multiple methods: Correlation with perceived astringency. American Journal of Enology and Viticulture. 2006;**57**:481-485

[39] Mercurio MD, Smith PA. Tannin quantification in red grapes and wine: Comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. Journal of Agricultural and Food Chemistry. 2008;**56**:5528-5537

[40] Harbertson JF, Yuan C, Mireles MS, Hanlin RL, Downey MO. Glucose, fructose and sucrose increase the solubility of protein-tannin complexes and at high concentration, glucose and sucrose interfere with bisulphite bleaching of wine pigments. Food Chemistry. 2013;**138**:556-563

[41] Harbertson JF, Mireles M, Yu Y. Improvement of BSA tannin precipitation assay by reformulation of resuspension buffer. American Journal of Enology and Viticulture. 2015;**66**:95-99

[42] Aleixandre-Tudo JL, Nieuwoudt H, Aleixandre JL, Du Toit WJ. Robust ultraviolet-visible (UV-vis) partial least-squares (PLS) models for tannin quantification in red wine. Journal of Agricultural and Food Chemistry. 2015;**63**:1088-1098

[43] Harbertson JF, Downey MO. Technical brief investigating differences in tannin levels determined by methylcellulose and protein precipitation. American Journal of Enology and Viticulture. 2009;**60**:246-249

[44] Lamuala-Raventós RM, Waterhouse A. A direct HPLC separation of wine phenolics. American Journal of Enology and Viticulture. 1994;**45**:1-5

[45] Cabrera-Bañegil M, Hurtado-Sánchez M d C, Galeano-Díaz T, Durán-Merás I. Front-face fluorescence spectroscopy combined with second-order multivariate algorithms for the quantification of polyphenols in red wine samples. Food Chemistry. 2017;**220**:168-176

[46] Aleixandre-Tudo JL, Nieuwoudt H, Aleixandre JL, du Toit W. Chemometric compositional analysis of phenolic compounds in fermenting samples and wines using different infrared spectroscopy techniques. Talanta. 2018;**176**:526-536

[47] Garcia-Jares C, Medina B, García-Jares C, Medina B. Prediction of some physico-chemical parameters in red wines from ultraviolet-visible spectra using a partial least-squares model in latent variables. The Analyst. 1995;**120**:1891-1896

[48] Skogerson K, Downey M, Mazza M, Boulton R. Rapid determination of phenolic components in red wines from UV-visible spectra and the method of partial least squares. American Journal of Enology and Viticulture. 2007;**58**:318-325

[49] Beaver CW, Harbertson JF. Comparison of multivariate regression methods for the analysis of phenolics in wine made from two vitis vinifera cultivars. American Journal of Enology and Viticulture. 2016;**67**:56-64

[50] Dambergs RG, Mercurio MD, Kassara S, Cozzolino D, Smith PA. Rapid measurement of methyl cellulose precipitable tannins using ultraviolet spectroscopy with chemometrics: Application to red wine and inter-laboratory calibration transfer. Applied Spectroscopy. 2012;**66**:656-664