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DETERMINATION OF THE OPTIMAL PHENOLIC EXTRACTION YIELD IN RED WINES USING THE GLORIES METHOD

Master Thesis presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* as part of the European Master of Science Degree in Food Science, Technology and Nutrition

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Abstract

This work addresses the phenolic composition and colour parameters in red wines throughout the vinification. The main purposes were to test the effectiveness of the Glories method in the evaluation of the maceration process and to observe the evolution of phenolic compounds during different stages of the winemaking process.

The experiments were carried out with two red dry table wines and one Port wine. The Glories method was modified to test the ripeness of the grapes and the pomace during maceration. Furthermore other spectrophotometric methods were used to assess the content of anthocyanins and total polyphenols. The same parameters and other phenolics were also quantified using the Skogerson-Boulton model. Classical colour parameters and CIELab values were determined by the methods recommended by OIV.

It was recognized that the application of the Glories method to the grape pomace during the winemaking process could potentially be used for the evaluation of the maceration. Regarding the polyphenol compounds, their extraction mostly occurs during the initial maceration stages. Moreover, alcoholic fermentation and malolactic fermentation do not seem to extensively affect the colour parameters of the wine, as well as the content of different phenolics including anthocyanins. Colour parameters remained relatively stable during the initial winemaking stages following maceration, although the increase of all parameters except hue after malolactic fermentation suggests changes in the content and structure of pigments. Furthermore it was found that the Skogerson-Boulton model can be used as tool to rapidly quantify different classes of phenolic compounds.

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1. Introduction

The phenolic composition of the grapes is extremely important for red wine quality. The increasing demand of consumers for highly coloured wines, with a good structure and body leads to the necessity for efficient polyphenol extraction in order to ensure intensity of colour and stability during ageing. Moreover, the techniques and the parameters in the vineyard and the processes in the winery will both affect the quality of the wine in term of its phenolic composition and quality. Given the many classes of phenolic compounds, the several methods for measuring their content and the effects exerted on the physical, chemical and sensorial properties of wines, it is very difficult to find one method that would help the winemaker to know when to stop the extraction before the grape seed tannins start to be extracted which can result in some green, unpleasant taste. Therefore, it would be interesting to know the evolution of extractable anthocyanins and tannins (particularly seed tannins) during the winemaking process. The Glories method was developed in 1990 and is used in the wine industry for the assessment of the phenolic maturity of grapes during maturation process, it has not been used yet as a method of evaluation of the maceration process thus the present experiment presents a novelty in the evaluation of the winemaking.

The main purpose of this thesis is to find an analytical parameter that would help the winemaker to determine the moment when to stop the maceration process using the Glories method and to correlate the obtained results with the data obtained from the UV-Vis spectra. Other, more specific objectives include:

- To observe the evolution of total polyphenols and anthocyanins during winemaking
- To observe the evolution of colour parameters during winemaking
- To test the Skogerson-Boulton model and compare its results with the ones of the classic tests

The present thesis consists of five chapters. First a literature review is presented focusing on the description of different classes of phenolic compounds, the techniques used in the vineyard and in the winery for the improvement of their presence in the wine and the methods used for the assessment of their content and their effect on sensorial properties. The chapter “Materials and Methods” describes the three main methods used during this project. In the following chapter the results are presented with the respective discussions. In the end the conclusions summarize the most important findings.

2. Literature review

2.1 Description of phenolic compounds in grapes and wines

Phenolic compounds (phenols or phenolics) are a class of organic molecules which contain at least one hydroxyl group (-OH) attached to an aromatic ring. The simplest molecule of this class is phenol (C_6H_5OH), also known as hydroxybenzene or carbolic acid (Campos, 2009). Phenolic compounds can be divided broadly into two groups, according to their chemical structure:

1. non-flavonoids which do not have a distinctive flavan structure (hydroxybenzoic and hydroxycinnamic acids, aldehydes, alcohols, coumarines, soluble tannins and stilbenes)
2. flavonoid compounds which possess a distinctive flavan (C6-C3-C6) structure (anthocyanins, flavan-3-ols and flavonols) (Campos, 2009), (Rodriguez Montealegre et al., 2006).

In this chapter only the phenols important for the colour and the astringency of the wine shall be described in more detail. The building blocks of phenolic compounds in wine can be separated into anthocyanins and tannins (proanthocyanidins) - oligomers containing between two and five monomeric units of flavanols (Campos, 2009). The anthocyanins are responsible for the colour of red wine. Furthermore, the intensity of colour in wine is related to wine quality (Roediger, 2006).

Anthocyanins are only present in grape skins (Kontoudakis et al., 2011). The exception is a class of red flesh grapes known as teinturier. Anthocyanins are the compounds that contribute to red-bluish colour of young red wines, and by reacting with other phenolic compounds they produce stable pigments responsible of the reddish-brown colour of aged wines (Torchio et al., 2011).

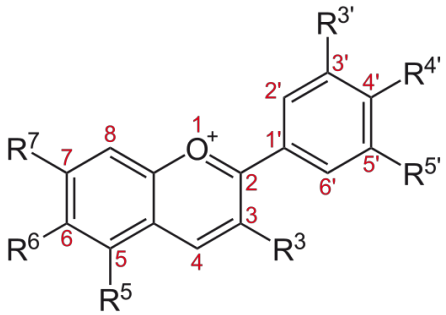


Figure 2.1 The basic chemical structure of anthocyanins (StaVin, 2014)

Table 2.1 Main anthocyanidins found in wine and their substitutions (StaVin, 2014)

Anthocyanidin	$R_{3'}$	$R_{4'}$	$R_{5'}$	R_3	R_5	R_6	R_7
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH
Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH
Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH
Malvidin	-OCH3	-OH	-OCH3	-OH	-OH	-H	-OH
Peonidin	-OCH3	-OH	-H	-OH	-OH	-H	-OH
Petunidin	-OH	-OH	-OCH3	-OH	-OH	-H	-OH

Their synthesis in the same vineyard is influenced by many factors such as temperature, sunlight, vigour and leaf surface area (Torchio et al., 2010). Only 3-O-monoglucoside anthocyanins and 3-O-acylated monoglucoside anthocyanins have been identified in Vitis vinifera grapes and wines. Anthocyanins are one of the most reactive wine constituents. Their colouring properties are unstable in the absence of other phenolics in the solution (Somers, 1998). Their stability depends on pH of the medium, temperature, light, solvent, oxygen, self-association interactions and their reactions of coordination with metallic ions or with other colourless compounds (co- pigmentation) (Bettini, et al., 2012).

During grape ripening, a biphasic accumulation of anthocyanins can be observed from the onset of ripening to maturity. All individual anthocyanin concentrations increase rapidly from veraison to intermediate stage and considerably more slowly thereafter (stabilization phase) (Lorrain et al., 2011). This stabilization phase could be explained either by a phenolic biosynthesis reduction either by chemical reactions occurring between anthocyanins and proanthocyanidins (Lorrain et al., 2011).

Flavan-3-ols are the structural monomers of proanthocyanidins and condensed tannins. This class comprises a large number of polyphenolic compounds responsible for the astringency, bitterness and structure of wines. The main monomeric flavanols found in grapes and wines are catechins [(+)-catechin, (-)- epicatechin] and gallocatechins [(+)-gallocatechin and (-)-

epigallocatechin] (Campos, 2009). **Proanthocyanidins** (PAs), polymeric compounds of flavan-3-ol subunits (between two and five), are also the second most abundant natural phenolic compounds after lignin. They can be divided in two classes: procyanidins (with terminal units composed of dihydroxylated flavanols [(+)-catechin or (-)-epicatechin]) and prodelphinidins with terminal trihydroxylated units [(+)-gallocatechin or (-)-epigallocatechin]) (Campos, 2009). A significant correlation between the concentration of proanthocyanidins and the antiradical activity was found (Bordiga et al., 2011). Grape seeds contain procyanidins only and higher concentrations of galloylated flavanols than skins (Bordiga et al., 2011). On the other hand, grape skins contain both procyanidins and prodelphinidins and lower amounts of galloylated derivatives (Campos, 2009), (Lorrain et al., 2011). Proanthocyanidins structures vary in the nature of their constitutive sub-units, mean degree of polymerisation (mDP) and linkage position. Three groups of proanthocyanidins are present in the skin tissues – the free proathocyanidins in solution in the vacuolar sap, the procyanidins bound to the proteins of the internal face of the tonoplast, and the procyanidins bound to cell wall polysaccharides. In the seeds, proanthocyanidins can be found both in outer coat and in the endosperm (Bordiga et al., 2011). The variety of proanthocyanidins and their extractability have to be considered especially when grapes in different maturity stages are used for vinification (Bordiga et al., 2011).

Tannins are a broad group of polymeric phenolic compounds which can precipitate proteins from aqueous media, generally classified in condensed (insoluble) and hydrolysable (soluble). Grape tannins can be classified into two categories, namely skin tannins and seed tannins. Hydrolysable tannins, extracted from wood cooperage, can also have an impact on wine bitterness and astringency. They tend to be more astringent than condensed tannins and their role in generally greater in white wines (Guadalupe et al., 2008). Tannins need to polymerise with anthocyanins to form stable flavouring compounds. Thus, the research of phenolic ripeness could focus on anthocyanin analysis, tannin analysis, or the polymerization process (Roediger, 2006). The perception of astringency is conditioned not only by proanthocyanidin content, but also by different levels and combinations of acids, alcohol, polysaccharides, monosaccharides and pH of the wine (Lee et al., 2008). Still, the association of tannins with anthocyanins will cause the decrease of astringency during different ageing stages (Torchio et al., 2011), (Lorrain et al., 2011).

A general decline in seed tannins has been noticed during ripening. This may be due to their attachment to the insoluble matrix of the seed, making them un-extractable during winemaking. During berry ripening the extractability of tannins normally decreases because of the covalent bond among the polymer flavan-3-ols sub-unit (Torchio et al., 2010). In the seeds, the tannins are either in a "free" state or they are esterified with gallic acid. The greater the degree of polymerization and galloylation, the more intense will be the sensation of astringency (Kontoudakis et al., 2011). On the other hand, tannins have the tendency to associate with parietal elements (e.g. polysaccharides) (Vivas, 1998). Different studies have established that the degree of proanthocyanidin polymerisation increases with maturity (Kontoudakis et al., 2011), (Vivas, 1998). The study of Kimberly et al. (2011) showed that the phenolic concentration will also increase in Pinot Noir variety if warm temperatures are maintained from budburst to bloom. However warm temperatures during ripening appear to offset this outcome (Nicholas et al., 2011). According to the research of Bordiga et al. (2011) the seed proanthocyanidins had a higher content of galloylated derivatives.

The seeds are protected by a lipidic layer which is destroyed by alcohol therefore seed tannins will only be extracted at the end of alcoholic fermentation (Bautista-Ortin et al., 2007), (Lee et al., 2008). The sensorial properties of tannins depend on their combination state (Nicholas et al., 2011), (Vivas, 1998). Generally tannins acquire their optimal sensorial quality when the levels of sugar and acids in the grapes have gone beyond their optimal ripeness levels. During berry ripening tannins get a rounder and more velvety sensorial quality, becoming less astringent (Roediger, 2006). However if only the phenolic ripeness is considered in order to determine the moment of harvest, there would be the risk of harvesting overripe grapes resulting in wines with high alcohol concentrations and with aromas of fruit jams (Roediger, 2006).

The smaller catechins and procyanidins are generally considered to be more bitter than astringent. Small condensed tannins are normally both bitter and astringent. The larger condensed tannins have little influence on taste. They seem to be too large to react well with taste receptors or to precipitate proteins (Rodriguez Montealegre et al., 2006). In general, the main compound found in seeds was catechin, except for Riesling and Viogner which contained more procyanidin B1 and Shiraz in which the main compound was epicatchin (Rodriguez Montealegre et al., 2006).

Tannins and anthocyanins share several stages in the flavonoid biosynthesis pathway, but tannin biosynthesis starts earlier (before veraison) while anthocyanins will be produced with the onset of veraison (Bucchetti et al., 2011). The ratio anthocyanin-tannin concentration is very important for the quality of the wine (Roediger, 2006). Mature grapes are usually characterized by skins rich in tannins and anthocyanins and seeds relatively poor in tannins. Therefore the lack of ripeness will result in low accumulation of pigments in the skins and in difficulty to extract them, as well as in a high accumulation of astringent tannins in the seeds and a low accumulation of the non-astringent ones (Vivas, 1998).

Flavonols are slightly bitter but, since their concentration in wines is relatively low, they have a substantial sensory impact only in white wines (Roediger, 2006). Their colour ranges from white to yellow depending on their structure. In red wines they are normally masked by anthocyanins (Castillo-Munoz et al., 2007). Furthermore flavonols which are a class of flavonoids are the most effective co-pigments of anthocyanins (Marquez et al., 2012). The small flavonoid content of white wines usually comprises flavan-3-ols (catechins) and flavan-3,4-diols (leucoanthocyanins). They contribute to the mouthfeel, and can add to the perceived quality of the wine. Nevertheless, their role in bitterness (~40 mg/L) sets an upper limit on their desirability. The flavonols namely quercetin, myricetin, kaempferol, isorhamnetin and their glycosides also contribute to wine bitterness (Rodriguez Montealegre et al., 2006).

Protocatechuic acid was the only **hydroxybenzoic acid** found in grape skins and only in red grapes. Other acids were part of the group of tartaric esters of caffeic, coumaric and ferulic hydroxycinnamic acids (Rodriguez Montealegre et al., 2006). The study of Rodriguez Montealegre et al. (2006) found that grape skins contain tartaric esters of hydroxycinnamic acids (6–45 mg/kg of grape), monomeric and dimeric flavan-3-ols (9–96 mg/kg) and flavonols (25–197 mg/kg). Regarding phenolic acids such as **caftaric acid** - they occur at concentrations below detection thresholds. However their combinations with other compounds have inferior thresholds to the ones of the original acids which become even lower in the presence of alcohol (Roediger, 2006).

The study of Rockenbach et al. (2011) found a greater content of phenolic compounds in the seeds than in the skins. Also some grapes such as Cabernet Sauvignon have a greater content in phenolic compounds than others (Rockenbach et al., 2011).

2.2 Factors affecting the polyphenol content of grapes and wines

Significant variations in the levels of phenolic compounds are found both in skins and seeds of different grapes. These variations can be attributed to factors such as climate, degree of ripeness, berry size and grapevine variety. For example, the concentration of anthocyanins can be increased by limiting the water supply (Rockenbach et al., 2011), (Roediger, 2006). The research of Roediger (2006) found that there are enormous fluctuations of anthocyanin content during ripening. This is attributed to either heat stress or water stress. The comparison of two extremes vintages 2007 and 2009 in the study of Lorrain et al. (2011) seems to indicate that vine water status at the flowering stage and both temperatures and sunlight exposure during grape maturity would impact the phenolic levels of grape seeds and skins (Lorrain et al., 2011). Furthermore increased sun exposure can lead to enhanced levels of both total phenolics and anthocyanins (Valladao et al., 1995). Other agroclimatic factors, but also the oenological practices also caused variations in the phenolic compounds in Cabernet Sauvignon wine from different Balkan regions according to the study of Radavanovic et al. (2010). Additionally, during ripening there is a phenomenon of parietal degradation which influences the cell wall cohesion, allowing the leakage of vacuolar content and therefore the liberation of anthocyanins. This phenomenon is partially provoked by the enzyme attack of the cell walls. Furthermore, the grape cell walls are a genetic characteristic of grapes, thus different varieties of grapes will have a different response to enzymatic attacks. Thus, the extraction of anthocyanins will be determined by the quantity of these molecules which are synthesized in the skin cells (dependent on the climatic conditions, thus terroir), but mostly on the degradation state of the cells at ripeness (variety characteristic) (Vivas, 1998). Other study also found that high temperatures (35ºC) stopped the production of anthocyanins and led to their degradation (Mori et al., 2007). Also, humid climatic conditions will lead to the development of microorganisms and will, therefore, favour the degradation of cell walls. The extractability of anthocyanins is one of the most important factors affecting their future concentration in the wine. Moreover, grape skins and seeds also contain other components that are incorporated in the wine during maceration (Kontoudakis et al., 2010).

The study of Perez-Lamela et al. (2007) found that pruning and training system used with the vines was found to affect the colour characteristics of the wine; precisely, cordon systems increased the proportion of anthocyanins by 10%, 20% and 70% in the Souson, Mencıa and Brancellao variety, respectively. Furthermore, the study of Bucchetti, et al. (2011) showed that

water deficit reduced berry weight and increased anthocyanin concentration in Merlot grapes, although tannin content remained unaffected. Therefore vine water management deficit during ripening seems to be more effective tool to increase anthocyanin than tannin content of the grapes. However, anthocyanin degradation also occurred during ripening, especially at high temperatures (Bucchetti et al., 2011).

Normally, technological maturity is not reached at the same time as the phenolic one and they tend to separate depending on factors such as grape variety (Lorrain et al., 2011), cultivar, adverse weather conditions, soil, water availability and cultural practices (Obreque-Slier et al., 2012). Besides, this divergence is enhancing as a result of the climate change (Melendez et al., 2013). Physicochemical differences do occur between grape seeds and skins of different varieties at some particular stages of maturation. Among these differences total phenols, clarity, chroma, hue, weight, total tannins, polymeric and the monomeric fraction can be mentioned. Moreover, several low molecular weight phenolic compounds undergo a gradual decrease during ripening (Obreque-Slier et al., 2012). Other works that deal with Shiraz and Cabernet-Sauvignon skin tannin accumulation and composition, suggest that polymer length varies throughout berry development but without any obvious trend (Hanlin et al., 2009).

*2.*3 Colour of wine in relation with phenolic compounds

The colour of a wine is one of the most important visual features potentially providing a substantial amount of information. Colour is a sensation perceived visually from the refraction or reflection of light on the surface of objects. Colour is light—as it is strictly related to it—and depends on the type of incident light (illuminating or luminous stimulus) (Somers, 1998).

Wine absorbs a part of the radiations of light that falls and reflects another, which reaches the eyes of the *observer*, making him/her experience the sensation of colour. For instance, the sensation of very dark red wines is almost entirely due to the fact that most incident radiation is absorbed by the wine (OIV, 2013).

The colour of the wine is one of the sensory attributes that can be described objectively and while the perceived colour is due to the sensory detection of selective light transmission in the visible spectrum, much more information can be acquired by instrumental analysis of the UV-Vis spectrum. Usually the range of 220-1000 nm is used for spectral analysis of the wines. Many molecular compounds found in wines absorb light wavelengths between 220 and 250 nm,

while the visible light absorbance concerning the range 400-700 nm is used not only for the compositional analysis of wine, but also for the description of its colour parameters. It is very important to note that more of the 95.5 % of wine composition is completely transparent to radiation in the range 250-700 nm. These light-transparent compounds are water, glycerol, residual sugars, organic acids, mineral salts, most of the residual amino acids and peptides, and most of the many volatile constituents (Somers, 1998). Most of the absorbance in the chosen UV-Vis region is due to phenolic pigments and other phenolics. The actual colour of a red wine is a result of complex physical and chemical interactions that mainly involve anthocyanins. These processes include proton transfer and hydration of anthocyanins, co-pigmentation complexes between anthocyanins and other (usually non-coloured) phenolic compounds, and formation of anthocyanin-derived pigments (Gomez Gallego, 2012).

Table 2.2 Maximum absorption wavelengths for different compounds of wine (Marquez et al., 2012, Somers, 1998)

Wavelength, nm	Compounds that absorb light
280	Complexes between flavan-3-ols and anthocyanins
315	Esters of hydroxycinnamic acids (ci-caftaric, trans-caftaric, cis-coutaric, trans-coutaric, cis-fertaric and trans-fertaric acids)
360	Flavonols such as 3-glucuronide and 3-glucoside derivatives of quercetin and kaempferol, 3-glucoside derivatives of laricitrin, isorhamnetin, syringetin, and myricetin, kaempferol and isorhamnetin
369	C15-phenolics related to the flavan-3-ols
500-600	Anthocyanins and anthocyanin-derived pigments

Co-pigmentation is the phenomenon responsible for the characteristic vinous hues of red wines which are different from the colour of anthocyanins alone in a model wine solution. It represents the enhancement of visible colour due to formation of complexes between

anthocyanins and colourless co-factors (Harbertson et al., 2006). Authors describe two mechanisms of co-pigmentation:

- Intramolecular interactions in which the pigment (anthocyanin) is bound covalently to the copigment, usually via acylation reactions
- Intermolecular interactions in which the pigment and the copigment interact via weak p–p interactions (Malaj et al., 2013), (Gonzalez-Manzano et al., 2009).

These interactions are the origin of the stabilization of the flavylium ion, thus modifications in the colour tendency and intensity which are accompanied with increases of absorbance in the visible range (hyperchromic shift) and a shift of the wavelength of the maximum absorbance toward higher values (bathochromic shift) (Malaj et al., 2013). It has been suggested that these reactions are the first step towards more stable covalent linking (Gutierrez, Lorenzo et al., 2005). Co-pigmentation complexes adopt a sandwich configuration that protects the flavylium chromophore from the nucleophilic attack of water reducing the formation of colourless hemiketal and chalcone forms (García-Marino et al., 2013), (Gonzalez-Manzano et al., 2009).

The co-pigmentation process is dependent on the chemical structure of the co-pigment, its concentration, on the pH and temperature of the medium. It results from the fact that there is fairly high concentration of anthocyanins (around 1 g/L) in new red wines along with higher quantities of other flavonoid phenolics. Generally it is considered that the flavylium ion is the main coloured species that participates in co-pigmentation. However some authors suggest that the quinonoidal base is the major species involved (Gonzalez-Manzano et al., 2009). Non-phenolic compounds such as certain amino acids will have a smaller similar effect. Still the major co-pigments are the phenolic compounds coming mainly from the seeds (Somers, 1998). Syringic acid showed the highest contribution to the hyperchromic and bathochromic shifts followed by the vanillic acid with a slightly lower contribution and by the p-coumaric acid exhibiting the lowest one (Malaj et al., 2013).

The new formed pigments will have different colours: purple polymeric pigments (association of anthocyanins with flavanols), orange pyranoanthocyanins (anthocyanins and other wine components such as pyruvic acid, vinylphenols, or vinylflavanols) and blue portisins (formed by reactions between the anthocyanin-pyruvic acid adducts and flavanols or hydroxycinnamic acids) (Campos, 2009).

Anthocyanins can also react with small molecules originating in yeast metabolism, such as acetaldehyde, pyruvic acid, acetoacetic acid, vinyl phenols, and phenolic acids (coumaric, caffeic acids) resulting in the formation of compounds that belong to the family of pyranoanthocyanins. These compounds are considered partly responsible for the orange hues observed during maturation and ageing (Oliveira et al., 2013). Over the last years, several families of pyranoanthocyanin-derived compounds with unusual chromatic features were identified in red Port wines, such as Portisins A and B formed from the reaction of carboxypyranoanthocyanins with flavanols mediated by acetaldehyde and phenolic acids (caffeic, coumaric, ferulic and sinapic acids). Portisins A (vinylpyranoanthocyanin–catechin) and B (vinylpyranoanthocyanin– phenol) present curious spectroscopic features, resulting in a bluish colour in acidic conditions (Oliveira et al., 2013). Moreover, recently the research of Lambert et al. (2011) has shown that self-association of malvidin-3-glucoside is more important than co-pigmentation in young red wine. In the respective study the most effective co-pigment was quercetin, while caffeic acid and catechin were poor co-pigments.

Usually for both consumers and professionals the intensity of colour is related to the quality of the wine. A higher colour obtained by more efficient extraction usually involves higher concentrations of tannins, thus astringency which will contribute to the body of the wine (Roediger, 2006). Moreover in contrast with free anthocyanins the polymeric wine pigments are resistant to pH change and bleaching by SO_2 (Somers, 1998).

2.4 Control and optimization of phenol content and composition during oenological treatments

Several studies show that winemaking practices exert little influence on the basic composition of the wines, but they significantly modify its colour and polyphenolic and volatile composition (Rodriguez Montealegre et al., 2006). Considering the fact that modern market requires deeply coloured and full-bodied wines the risk to obtain very bitter and astringent wines increases if the maceration technique causes an over-extraction of tannins. On the other hand, anthocyanins are not always easily extracted from skins, and low levels of extraction can lead to a poor coloration of wines, although the anthocyanin concentration in the grapes is sufficient (Kontoudakis et al., 2010). Apart from the content of anthocyanins and tannins present in the fruit, there are other several factors which affect the fraction extracted into the wine such as anthocyanin and tannin composition, tannin interactions with cell wall components, as well as

winemaking technique and technologies (Bucchetti et al., 2011). The pumping-over periods, their intensity, the temperature and the fermentation time are factors that can be adapted each year to the type of grapes. This way, the best part of the grapes can be extracted respecting their real phenolic potential (Vivas, 1998). It has been shown that the extraction of phenolic compounds is conditioned by winemaking parameters such as maceration time, temperature, solvent composition, proportion of skin and seed present, fruit ripeness (Lee et al., 2008), density of the grapes (Kontoudakis et al., 2011), intensity of pressing, yeast and SO_2 doses (Gomez Gallego, 2012). However the study of Kontoudakis, et al. (2011) showed that proanthocyanidin molar concentration did not vary significantly in wines made from grapes that had different densities, leading to the conclusion that all grapes regardless of their density release a similar number of tannin molecules during winemaking.

The length of skin contact, the concentration of ethanol and the temperature of the fermenting musts are the most important factors which influence the diffusion of anthocyanins and tannins from pomace and their solubilisation into the must (Rodriguez Montealegre et al., 2006). Since not all grapes present an optimal content of polyphenols, the co-fermentation of red grapes of different cultivars has been introduced when any of the grapes do not present a good balance between the concentrations of anthocyanins and other polyphenols (Boulton, 2001).

Regarding seed tannins, it must be mentioned that their extractability depends on the used winemaking procedure. Indeed, the extraction from seeds is favoured by more intensive remontages at the end of alcoholic fermentation, allowing tannin solubilisation of less polymerized tannins called extractible tannins. Also relatively high temperature (around 30 ºC) can modify their structure (favouring polymerization reactions) and therefore soften their sensorial characteristics, making them less astringent (Vivas, 1998), (Guadalupe et al., 2008). One of the techniques very much used nowadays to soften the tannins is called microoxygenation and consists in the introduction of oxygen into wine to assist the polymerisation (Roediger, 2006).

The kinetic of anthocyanin extraction is very important for varieties of grapes rich in 3-hydroxylated (free) anthocyanins since they are preferentially extracted in the initial maceration phase (Torchio et al., 2011). Incomplete extraction of anthocyanins occurs and normally the transfer of anthocyanins to wine varies between 40 and 70 % of their concentration in the grapes. Higher transfer rates have been observed during the production of wines from premium grape

varieties and wines that are intended for ageing. Prolonged time on skins, up to several weeks may be applied in order to obtain higher extraction yields, yet no clear advantage has been established by such practice (Somers, 1998). Rotary and other fermenter designs are more efficient for the extraction than the traditional practice of "plunging the cap and pumping over" (Somers, 1998). The study of Bautista-Ortin (2007) showed that "the wine produced by running-off part of the juice had the highest colour intensity during the first steps of winemaking, but it showed very low stability and, at the moment of bottling, a dramatic decrease in colour was observed". Moreover maceration time is also critical for obtaining highly coloured and stable wines. Previous studies have shown that best young red wines need at least 10 days of maceration, shorter times leading to poor anthocyanin extraction and unstable colour (Bautista-Ortin et al., 2007). Still, high and stable polyphenol content is necessary for the production of wines that will withstand maturation. For these types of wines, a maceration period of 15 days was proven to be efficient, whilst periods longer than 15 days lead to the production of poor and unstable wine colour characteristics (Bautista-Ortin et al., 2007).

New vinification techniques such as employing a continuous pre-fermentative maceration system and using dry ice for low temperature maceration have been offered in order to improve anthocyanin extraction (Torchio et al., 2011). Cold pre-fermentative maceration is normally used to obtain the extraction of compounds from skins in aqueous medium. Therefore the preferential solubility of water-soluble compounds will be promoted and the release of anthocyanins and tannins of low molecular weight will be increased. High-weight tannins are more soluble in alcoholic solutions, so their extraction depends on alcoholic fermentation. This technique was proven to be inefficient with regards to extraction of phenolic compounds and it even caused colour reduction when compared with the control sample produced by classical vinification (Perez-Lamela et al., 2007).

The study of Gonzalez-Neves et al. (2013) concluded that different alternatives of winemaking can change the colour, polyphenolic and volatile composition of wines. In the respective study the proanthocyanidin content was enhanced by cold pre-fermentative maceration in Merlot (27.6 %) and in Tannat (13.7 %) in comparison to the control wines. Still, this treatment had no effect on the proanthocyanidin levels of Syrah wines (Gonzalez-Neves et al., 2013). Other authors have also suggested that the effectiveness of cold pre-fermentative maceration is conditioned by the grape variety since the composition of the skin cells could

change the release of different compounds during winemaking (Ortega-Heras et al., 2012). Normally the fermentation and the pressing will lead to a loss of colour in the wine. The addition of alcohol will also lead to a decline in colour intensity (Somers, 1998).

The use of maceration enzymes and oenological tannins and their influence on wine colour were also investigated. Macerating enzymes that help phenolic extraction can also change the stability, taste and structure of red wines due to higher extraction of tannins bound to the cell walls which may be extracted using hemicellulases and cellulases (Gonzalez-Neves et al., 2013), (Bautista-Ortin et al., 2007), (Perez-Lamela et al., 2007), (Revilla et al., 2003). Furthermore the use of oenological tannins has shown some positive effects like colour stabilisation, improved wine structure, control of lacase activity and elimination of reduction odours. However, oenological tannins should be used with great care since depending on the wine characteristics they may provoke the loss of equilibrium, this effect being more observed when hydrolysable tannins are also used (Perez-Lamela et al., 2007)**.**

The study of Marquez, et al. (2012) showed that the drying process which is used for the production of certain liquourous wines causes skin rupture and therefore facilitates the access of phenolic compounds to the pulp. Some non-conventional winemaking techniques have also been researched. For example, the study of Puértolas et al. (2010) showed that wine from grapes treated by pulsed electric fields presented at the end of alcoholic fermentation higher colour intensity, total polyphenol index and total anthocyanin content than control wine. An obvious decrease in the values of the chromatic parameters was observed after cold stabilization and bottling possibly provoked by polymerization and precipitation of phenolics and adsorption onto proteins, tartrates and dead yeast (Bautista-Ortin et al., 2007).

The research of Lee, et al. (2008) showed that seed removal will cause minor differences in the proanthocyanidin content of the wines. Only the percentage of seed proanthocyanidin was found to be higher in the wine made by traditional winemaking due to higher seed tannin extraction. Furthermore little difference was observed when the coulour parameters were measured (Lee et al., 2008). The addition of sulphur dioxide during certain stages of winemaking will have an important impact on tonality since free anthocyanins can be bleached by this additive (Torchio et al., 2011). The addition of alcohol during the production of fortified wines will stop the diffusion of red pigments from skins before its completion (Figuereido-Gonzalez et al., 2013).

Given the limited amount of studies on the impact of seed and skin tannins in winemaking and the contradiction among the results obtained when using different winemaking techniques there is much confusion about best vinification strategies in order to obtain smooth, full-bodied wines.

2.3 Methods used for the determination of phenolic compounds and colour in grapes and wines

There are several methods for measuring phenolic maturity, therefore estimating the content of polyphenols in the grapes. These methods use different techniques such as skin texture measurement or direct measurement of colour absorption. However these methods remain experimental and are not usually used by winemakers. Presently the methods most used are based on obtaining extracts from grapes by maceration in different solvents. Glories and ITV methods are the most employed, although these methods are slow and laborious. The cellular maturity index, also called anthocyanin extractability (EA%) is currently one of the most used indices to assess the extractability (Torchio et al., 2010). Recently Cromoenos method has been proposed, it uses two commercial reagents and specific equipment in order to extract phenolics and give results in just 10 minutes (Kontoudakis et al., 2010). Despite the fact that these methods are employed by some wineries there is not a clear criterion for their selection due to the lack of information regarding their accuracy, precision and their real predictive capacity (Kontoudakis et al., 2010). The study of Kontoudakis et al. (2010) has determined that ITV, Glories and Cromoenos methods can be used to predict the future characteristics of the wine. Spectrophotometric analysis usually overestimates the total anthocyanin concentration since it also detects other pigments (Kontoudakis, et al., 2011).

The interest in phenolic maturity has led to the introduction of new techniques for its evaluation such as the skin texture measurement, in vineyard spectrophotometric visible and near-infrared (VIS-NIR) measurements, mid infrared spectroscopy or fluorescence. Moreover, the relation between phenolic maturity and appearance (colour and morphology) of grape seeds has been studied by image analysis (Melendez et al., 2013). The correlation between physicochemical and organoleptic variables can be studied by different statistical methods which can define better the two aspects (phenolic and technological) of grape maturity (Melendez et al., 2013).

Methods based on tasting grape berries and seeds have been introduced in recent years. Sensory analysis allows following the evolution of both grapes maturity (phenolic and technological) at the same time. Presently, the tasting of the grapes is used often by wine professionals directly in the vineyard, making it a good decision tool. At the moment several procedures are available in order to make the sensory analysis of the grape ripening (Melendez et al., 2013). Rousseau (2001) has proposed a method for grape tasting based on the segmentation of the analysis according to the three main tissues: pulp, skin and seeds. The mechanical methods are rapid and inexpensive, and can be considered as routine tool for monitoring the grape quality. Moreover, since there is a good correlation between the mechanical parameters and the sensory descriptors, the instrumental indices can be used for the assessment of grape ripeness. The break skin force is an important parameter that should be considered for the evaluation of anthocyanin extractability (Torchio et al., 2010).

CIELab parameters are less used by oenologists, but they define more clearly the colour of the wine. The parameters the CIELab space are red/green colour component, yellow/blue colour component and clarity from which parameters correlated with colour perception are obtained such as chroma and hue angle (Torchio et al., 2011), (OIV, 2006).

The Adams-Harbertson assay is based on the capacity of tannins to bind or precipitate proteins and is a direct adaptation of Hagerman-Butler assay. The method has been criticized in the article of Brooks et al. (2008) being characterized as invalid. However the UC Davis statement (2008) has justified the employment of the method and has explained the negative results of the validation. Kenedy et al. (2006) noticed that protein precipitation, namely Harbertson-Adams assay had the highest correspondence with perceived astringency therefore it can act as a relation tool between laboratory and consumer experiences. Skogerson et al. (2007) used this method for comparison when they created the statistical model for rapid determination of phenolic compounds.

Concerning proanthocyanidins, various methods for their quantification have been described. The most popular are the colorimetric assays relying either on the reaction of the A-ring with an aromatic aldehyde (vanillin) or on their oxidative de-polymerisation into anthocyanidins. Moreover, the concentration of proanthocyanidins can be measured using several chromatographic separation methods, while reverse-phase HPLC has been used for separation of proanthocyanidins with lower molecular weight (Bordiga et al., 2011). Larger

compounds cannot be separated by this method since the presence of many isomers with similar polarity will result in overlapping. The direct phase HPLC separation of monomeric up to dodecameric procyanidins was obtained in cocoa and chocolate. De-polymerisation in the presence of acid and nucleophile compounds (e.g. phloroglucinol) followed by HPLC analysis can be a good instrument for quantification and characterisation of proanthocyanidins. The respective method will also allow the determination of the nature and concentration of terminal units (Bordiga et al., 2011).

The Folin-Ciocalteu method involves the measurement of the total phenols, including tannins and many other phenols that are not tannin. This is a colorimetric oxidation/reduction assay which assesses all phenolic molecules with no differentiation between gallic acid, monomers, dimmers and larger phenolic compounds (OIV, 2012). Roedinger (2006) found no correlation between the total polyphenols determined by the Glories method and the gallic acid equivalent content determined by Folin-Ciocalteu method. However, good results for phenolic ripeness were found in South Africa using the Glories method (Roediger, 2006). The study of Bordiga et al. (2011) also showed that there is a good correlation between the spectrophotometric assay (vanillin) and acid-catalysed cleavage methods. Habertson et al. (2006) summarized the most popular methods for measuring phenolics and colour in the winery:

Table 2.3 Methods for measuring phenolics in the winery (Haberston et al., 2006)

Measured parameter	Methods	Author (or most recent reference)
Color	Tri-stimulus values Cielab coordinates Somers assay Copigmentation assay Intensity and Hue	Pérez-Caballero 2003 Somers, Evans 1977 Levengood, Boulton 2004
Total phenols	Absorbance at 280 nm Folin-Ciocalteau assay Iron-chloride assay Enzymatic method	Singleton 1999 Harbertson 2004 Stevanto 2004
Tannins	Glories Gelatin index Llaudy method UC Davis Tannin assay	Glories 1984 Llaudy 2004 Harbertson, Picciotto, Adams 2003

3. Materials and methods

3.1 Sampling

The experiments were carried out in association with a Portuguese producer of wines. Two red table wines and one Port wine were chosen and sampled at different stages of the winemaking process. Six sampling points were set for the table wines:

1. Sampling at grape maturity
2. Sampling during the transfer from the lagares to the fermentation tanks (analysis of liquid and pomace)
3. Sampling at the end of fermentation
4. Sampling during malolactic fermentation
5. Sampling at the end of malolactic fermentation
6. Sampling after 2 months of storage in stainless steel vats

Six sampling points were established for Port wine

1. Sampling at grape maturity
2. Sampling during maceration (analysis of liquid and pomace)
3. Sampling after the addition of alcohol and before pressing (analysis of liquid and pomace)
4. Sampling after pressing
5. Sampling during storage in stainless steel vats
6. Sampling after 2 months of storage in stainless steel vats

The grapes and the pomace were analysed using the Glories method (Vivas, 1998) in order to determine the extractability and the percentage of seed tannins that can be extracted during further steps of winemaking. The absorbance at 280 nm and the method employing bleaching with SO_2 were used to determine the total polyphenol index and the anthocyanin content in the liquid fraction and the fermenting wine. Furthermore, the L*a*b* parameters (OIV, 2013) and the Skongerson-Bolton parameters were evaluated in the last two.

The experiments were done in triplicate using three different wines at different winemaking stages. Firstly, the grapes used for each wine were analyzed. The percentages of each grape variety were the same as in the vinification process. The measurement of TPI (Total Polyphenol

Index) and classic colour parameters were also performed in the laboratory of the company during the maceration in lagares and during the first days of fermentation.

Table 3.1 Varietal composition of the wines used in this work

Lagar number	Varieties used	Percentage, %
2	Sousão	18
	Touriga Nacional	33
	Horta-Touriga Francesa	32
	Margaridas-Touriga Nacional	17
3	Sousão	40
	Touriga Nacional	29
	Margaridas-Pombal	31
5	Sousão	68
	Touriga Nacional sobre enxertia	66
	Margaridas Pombal-Touriga Francesa	66

3.2 Modified Glories method

The method involved the collection of 200 berries at random. The sample was crushed with a blender for 2 min in order to yield a mash. Two equal 50 g portions of the mash were weighed using the analytical balance Mettler PM 2000 (USA), the excess being discarded. The procedure was changed for the analysis of the pomace. Two portions of 50 g of pomace were weighed. Then, 50 mL of pH 3.2 solution were added to one portion and 50 mL of pH 1 (0.1 N HCl) solution to the second portion. The pH 3.2 solution was prepared by dissolving 5 g of tartaric acid and 22.2 mL of 1 N NaOH in one litre of water. The pH was adjusted to 3.2 by using either 1 N HCl solution or 1 N NaOH solution and measuring the pH with the pHmeter Crison micro pH 2002 (Spain). The pomace samples were mixed and homogenized with the blender after the addition of pH 1 and pH 3.2 solutions. The mixtures were allowed to homogenize and for extraction to occur, for 4 h prior to centrifugation for 10 min at 3000 x g in a centrifuge Centromix Selecta (Spain). After centrifugation, samples were filtered through Whatman filter paper nr 1 and 22 mm syringe nylon filter 0.45 μm (VWR International, USA). The filtrates were marked as filtrate pH 3.2 and filtrate pH 1.0. A small volume of filtrate pH 3.2 was diluted 100

times (1 mL in 100 mL) and the absorbance of this diluted solution was measured at 280 nm. This absorbance value was multiplied by the relative dilution values of the mash, i.e. 200 times. The anthocyanin concentration was measured on the filtrate pH 3.2 and filtrate pH 1.0. In the case of wine analysis the 100 times dilution was considered.

One mL of each filtrate (pH 3.2 and 1) was added to a beaker and 1 mL of ethanolic HCl solution (prepared by the dilution of 0.1 mL of hydrochloric acid in 100 mL of 99.6 % ethanol) and 20 mL of 2% HCl solution were added to each beaker. Then 10 mL of each of the pH solutions were pipetted into two test tubes. 4 mL of distilled water were added to the first test tube and 4 mL of 15% bisulphite solution were added to the second tube. The solutions were allowed to stand for 20 min. The absorbance of each solution was then measured at 520 nm with the UV/VIS spectrophotometer Nicolet evolution 100, Thermo Electron Corporation (United Kingdom) using distilled water as reference. In the case of wine analysis only this last step was performed.

Calculations and interpretation:

Calculation of the concentration of anthocyanin [A]:

$$A\ mg/L = Abs\ sodium\ bisulphite - Abs\ distilled\ water * 875 * dilution \quad (3.2.1)$$

The calculation of the total polyphenol index [TPI] was made following the method described by Ribereau-Gayon et al. (2006). This test presents a number of advantages such as speed and reproducibility. Still some molecules such as chalcones and cinnamic acids do not have an absortion maximum at this wavelength (Ribereau-Gayon et al., 2006).

$$TPI = Abs280 * dilution \quad (3.2.2)$$

Abs280 – absorbance of the solution at 280 nm

The value “875” is a mathematical constant which was established from data obtained from various red grape cultivars (Ribereau-Gayon et al., 2006).

Interpretation:

In the pH 1 solution an important extraction of anthocyanins will take place. The respective extraction does not depend on the extractability, presenting the total potential of anthocyanins while the pH 3.2 solution will contain only the anthocyanins that can be extracted into the future wine (Vivas, 1998).

Therefore the extractability of anthocyanins is expressed by the following equation:

$$EA\% = \frac{ApH1 - ApH3.2}{ApH1} * 100 \text{ (Ribereau-Gayon et al., 2006) (3.2.3)}$$

EA% - "cell extractability", capacity of the grapes to release anthocyanins

ApH1 – "total potential of anthocyanins", it is considered that all anthocyanins present in grapes will be extracted at this pH

ApH3.2 – the content of anthocyanins that can be extracted at wine pH

The higher the value of anthocyanin extractability, the higher is the proportion of easily-extractable anthocyanins in the grape. It increases normally as the grape ripens (Ribereau-Gayon et al., 2006), (Roediger, 2006).

Mp is the concentration of tannins extracted during Glories method that are derived from the seeds. As the Abs 280/anthocyanin ratio of extracts at pH 3.2 is between 35 and 45 for ripe grapes from all varieties investigated, an average of 40 is used (Ribereau-Gayon et al., 2006). Once the concentration of total phenolic compounds (Abs 280) and anthocyanin (A) in the extract at pH 3.2 is known, it is possible to calculate the proportion of the phenolic compounds derived from the skins. The remaining of the polyphenols thus originates from the seeds (Vivas, 1998).

Abs280 = Abs (anthocyanins)+Abs(tannins from skins)+Abs(tannins from seeds)

Phenols from skin = Abs (anthocyanins)+Abs(Tannins from skins)=ApH 3.2*40

Mp% ={A280-[(A3.2 water-A3.2 bis)* 4/100]/A280}* 100 (Roediger, 2006)(3.2.4)

A280 – absorbance of the pH 3.2 extract with 100 fold dilution

The higher the contribution of the tannins from the seeds the greater is the risk that there will be negative consequences on the flavour of the wine, therefore the value of Mp should be at minimum. Usually in grapes this value varies between 60 and 0, depending on the grape variety, the number of seeds in the grapes and their ripeness (Ribereau-Gayon et al., 2006).

Roedinger (2006) also suggested that when the Glories method is used the extraction step should be extended to five hours for scientific purpose and accuracy.

3.3 Colour parameters in wines

Absorbance measurements were made in a Nicolet evolution 100 UV/VIS double-beam spectrophotometer (illuminant D65 and observer placed at 10º, with scan and resolution higher than 5 nm as recommended by the OIV methods) using a glass cuvette with 1 mm path length.

The samples were previously filtered through the 22 mm syringe nylon filter 0.45 μm (VWR International, USA). Visible and UV spectra were collected for the grape extracts, fermenting wines and pomace extracts. Spectra of grape and pomace extracts were collected within 12 hours after extraction. Wine spectra were collected following 4 to 7 days of refrigeration at 4 ºC. UV spectra were also collected using a 10 mm glass cuvette with prior 100-time dilution with model wine solution containing 6g/L of tartaric acid and 12% of ethanol. If the linear range of the spectrophotometer has been exceeded the sample was diluted using the model wine solution. Both spectra of diluted and undiluted samples were taken. The absorbance and the transmittance were measured from 200 nm to 950 nm every 1 nm using distilled water as reference in a cuvette with the same optical thickness, in order to establish the base line or the water line. When the cuvette of 1 mm optical path was used, the transmittance was transformed to 10 mm before calculating the CIELab parameters. All spectra were introduced in special tables created with Microsoft Excel programme in order to perform all the calculations.

3.3.1 Determination of classic colour parameters

Colour intensity was calculated as the sum of absorbance at 620, 520, and 420 nm (OIV, 2013).

$$I = A420 + A520 + A620 \quad (3.3.1.1)$$

The hue or tone is conventionally given by:

$$N = A420/A520 \text{ (OIV, 2013)} \quad (3.3.1.2)$$

3.3.2 Determination of colour characteristics according to CIELab

The scope of this spectrophotometric method is to measure and calculate the chromatic characteristics of wines and grape extracts derived from *trichromatic components*: X, Y and Z, according to the *Commission Internationale de l'Eclairage* (CIE, 1976), with the attempt to imitate the colour perception of a human observer.

The colour of a wine can be described using 3 attributes or specific qualities of visual sensation (OIV, 2013):

- tonality
- luminosity
- chromatism.

Tonality—colour itself—is the most characteristic: red, yellow, green or blue. *Luminosity* is the attribute of visual sensation according to which a wine appears to be more or less luminous. However, *chromatism*, or the *level of colouring*, is related to a higher or lower intensity of colour. The combination of these three concepts enables us to define the multiple shades of colour that wines present.

The *chromatic characteristics* of a wine are defined by the *colorimetric* or *chromaticity coordinates* (Figure 2): *clarity* (L*), *red/green colour component* (a*), and *blue/yellow colour component* (b*); and by its *derived magnitudes*: *chroma* (C*), *tone* (H*) and *chromacity* [(a*, b*) or (C*, H*)] (OIV, 2013).

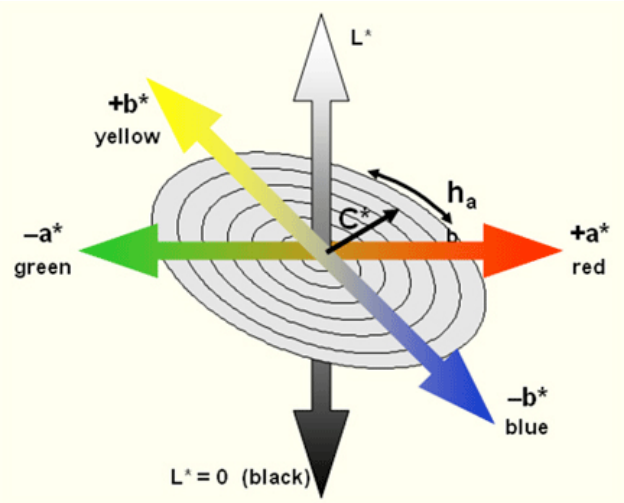


Figure 3.1 CIELab parameters (IHS Global Spec, 2013)

Clarity

It is directly related to the visual sensation of luminosity. Its symbol is L* and it is defined using the following mathematical function:

$$L^* = 116(Y/Yn)1/3-16 \quad (3.3.2.1)$$

Red/green colour component

Its symbol is a* and it is defined using the following mathematical function:

$$a^* = 500[(X/Xn) - (Y/Yn)] \quad (3.3.2.2)$$

Yellow/blue colour component

Its symbol is b* and it is defined using the following mathematical function:

$$b^* = 200 - [(Y/Yn)1/3 - (Z/Zn)1/3 \quad (3.3.2.3)$$

$$X = K \sum_{(\lambda)} T_{(\lambda)} S_{(\lambda)} X_{m(\lambda)} \Delta_{(\lambda)} \quad (3.3.2.4)$$

$$Y = K \sum_{(\lambda)} T_{(\lambda)} S_{(\lambda)} Y_{m(\lambda)} \Delta_{(\lambda)} \quad (3.3.2.5)$$

$$Z = K \sum_{(\lambda)} T_{(\lambda)} S_{(\lambda)} Z_{m(\lambda)} \Delta_{(\lambda)} \quad (3.3.2.5)$$

$$K = 100 / \sum_{(\lambda)} S_{(\lambda)} Y_{m(\lambda)} \Delta_{(\lambda)} \quad (3.3.2.6)$$

$T_{(\lambda)}$ - the measurement of the transmittance of the wine measured at the wavelength λ expressed at 1 cm of optical thickness

$\Delta_{(\lambda)}$ - the interval between the value of λ at which $T_{(\lambda)}$ is measured

$S_{(\lambda)}$-coefficients that are function of λ and of the illuminant

$X_{m(\lambda)}$, $Y_{m(\lambda)}$, $Z_{m(\lambda)}$-coefficients that are a function of λ and of the observer

Xn=94.825; Yn=100; Zn=107.381

The values of Xn, Yn and Zn represent the values of the perfect diffuser under an illuminant and a given reference observer. In this case, the illuminant is D65 and the observer is higher than 4º.

Chroma

The chroma symbol is C* and it is defined according to the following mathematical function:

$$\mathbf{C^* = \sqrt{a^{*2} + b^{*2}}} \quad (3.3.2.7)$$

Tone

The tone symbol is H*, its unit is the sexagesimal degree (º), and it is defined according to the following mathematical function:

$$H^* = tg^{-1}\left(\frac{b^*}{a^*}\right) \quad (3.3.2.8)$$

Difference of tone between two wines

The symbol is ΔH* and it is defined according to the following mathematical function:

$$\Delta H^* = \sqrt{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2}$$ (3.3.2.9)

Overall colorimetric difference between two wines

The symbol is ΔE* and it is defined according to the following mathematical functions:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} = \sqrt{(\Delta L^*)^2 + (\Delta C^*)^2 + (\Delta H^*)^2}$$ **(OIV, 2013) (3.3.2.10)**

Table 3.2 Expression of results of CIELab parameters (OIV, 2006)

Colorimetric coordinates	Symbol	Unit	Interval	Decimals
Clarity	L*		0-100 0 black 100 colourless	1
Red/green colour component	a*		>0 red <0 green	2
Yellow/blue colour component	b*		>0 yellow <0 blue	2
Chroma	C*			2
Tone	H*	Degrees	0-360	2

3.4 Rapid Determination of Phenolic Components in Red Wines from UV-Visible Spectra and the Method of Partial Least Squares

The spectral data were corrected for dilution and the analysis using the Skogerson-Boulton model was performed. Skogerson et al. (2007) used the spectra of 200 samples of wine to construct a mathematical model for rapid determination of colour and phenol components in fermenting and fermented wines. Furthermore other 200 samples were used to test the respective model. The authors have used multivariate statistics to correlate information from sample spectra to the results of a reference analytical method. Partial least squares regression was applied to the UV-visible spectra of 200 red wine samples at various stages of fermentation and the concentrations of several groups of phenols as determined by the Harbertson-Adams assay. The multivariate methods allowed obtaining prediction functions for each of the phenolic classes. (Skogerson, 2007). The method requires minimal sample preparation and produces rapidly results of numerous parameters.

4. Results and Discussion

The data from the laboratory of the company shall be presented first in order to see which methods are currently used for the evaluation of the extraction process. Thus tables 4.1; 4.2 and 4.3 summarize the respective data.

Table 4.1 Evolution of maceration and fermentation in Lagar 2

Date	Hour	Density g/L	T ºC	Abs 420	Abs 520	Abs 620	Abs 280	Colour intensity	TPI
27/09	19:00	1.102	17	0.5918	1.2814	0.3018	0.6647	2.1749	66
28/09	10:00	1.100	18.5						
28/09	20:00	1.085	22						
29/09	10:00	1.066	22						
29/09	20:00	1.052	19						
30/09	10:00	1.045	21	1.2145	2.7727	0.6148	1.264	4.6148	126
30/09	20:00	1.025	24						
01/10	10:00	1.022	23						
02/10	10:00	1.005	23.5	1.3550	2.9179	0.6774	1.43	4.9503	143
02/10	20:00	1.004	24						
03/10	10:00	1.002	25	1.2459	2.6736	0.6064	1.455	4.5259	146

Table 4.2 Evolution of maceration and fermentation in Lagar 3

Date	Hour	Density g/L	T ºC	Abs 420	Abs 520	Abs 620	Abs 280	Colour intensity	TPI
27/09	19:00	1.100	17	0.4504	0.8324	0.2202	0.5009	1.5031	50
28/09	10:00	1.112							
28/09	17:00	1.097	20						
28/09	20:00	1.093	21						
29/09	10:00	1.068	23	1.4607	3.3016	0.8562	0.925	5.618	93
29/09	12:20		25						
29/09	16:00	1.035	22.5	1.361	2.3856	0.8859	1.050	4.6331	105
29/09	20:00	1.036	18						
30/09	10:00	1.037	19	1.2534	2.5132	0.6631	1.287	4.4297	129
30/09	16:00			1.4738	2.8117	0.8861		5.1716	138
30/09	20:00	1.032	19.5	1.2337	3.1264	0.6117	1.401	4.9718	140
01/10	10:00	1.039	17	1.222	2.9733	0.6026	1.070	4.7982	147
01/10	14:00			1.1540	2.5203	0.5687	1.440	4.2430	144

Table 4.3 Evolution of maceration and fermentation in Lagar 5

Date	Hour	Density g/L	T ºC	Abs 420	Abs 520	Abs 620	Abs 280	Colour intensity	TPI
27/09	19:00	1.110	16	0.3047	0.5198	0.1536	0.512	1.9781	51
28/09	10:00	1.103	17						
28/09	20:00	1.102	16						
29/09	10:00	1.097	18						
29/09	20:00	1.098	17						
30/09	10:20	1.080	19	1.4680	2.5620	0.8050	0.862	4.8350	86
30/09	20:00	1.072	24						
31/09	10.00	1.055	26						
02/10	10:00	1.022	23	1.3699	3.1097	0.6390	1.410	5.1187	141
02/10	20:00	1.024	25						
03/10	10:00	1.022	26	1.2759	2.8657	0.6464	1.131	4.7880	113
03/10	20:00	1.007	24						
04/10	09:00	1.007	23	1.3391	2.8137	0.6878		4.8406	137
04/10	22:00	1.007	27	1.3073	2.9580	0.6119	1.398	4.8770	140
05/10	09:00	1.005	25	1.3025	2.9207	0.6283	1.494	4.8516	149
06/10	09:00	1.003	24	1.2965	2.8779	0.6222	1.445	4.7967	144

As it can be seen many parameters are used presently in the wine company for monitoring the maceration and fermentation. However it is hard to predict content of polyphenols, their type and the colour and taste characteristics that they would confer to the future wines just by simple observation of these parameters.

4.1 Analysis of the results of the modified Glories method

Firstly the results of the modified Glories method shall be discussed, since the assessment of this method is the main objective of this thesis. The numerical values of the obtained data are in the same range as the ones obtained by other authors (Roediger, 2006). The figures below provide information for comparative analysis for different samples at different stages of the winemaking process.

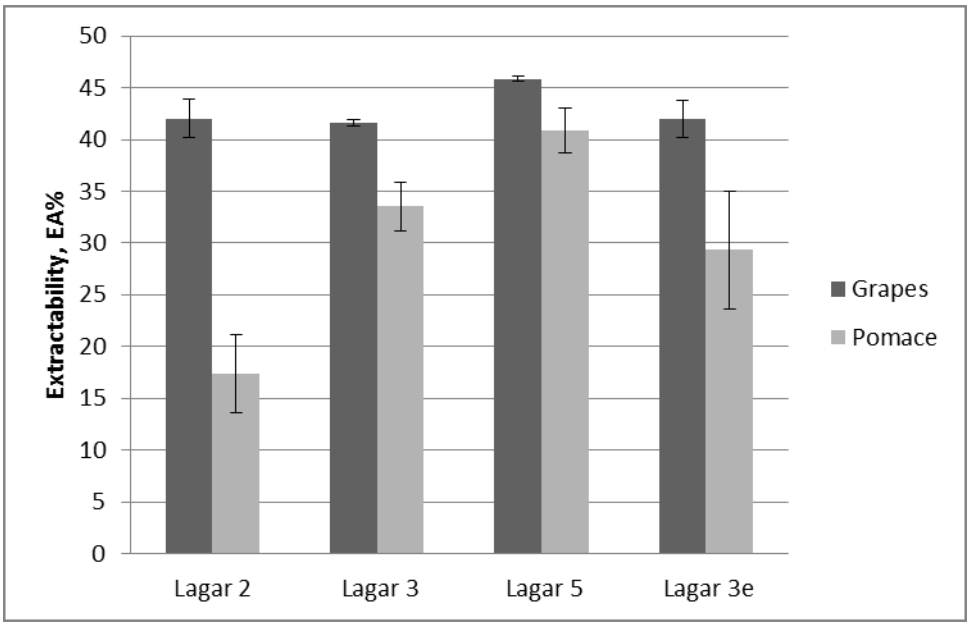


Figure 4.1 Comparative analysis and change of extractability in harvested grapes and pomace separated when maceration in lagares was ceased (error bars represent standard deviation of three determinations)

Figure 4.1 presents the extractability of freshly harvested grapes and the extractability of the pomace after maceration during three days. The highest extractability of both fresh grapes and pomace was observed in the mix of the grapes in Lagar 5. Furthermore this index has decreased after a certain period, the maximum decline being found in Lagar 2. Thus, the extractability decreases over time during maceration. Very low values of EA% in harvested grapes lead to difficulties in the extraction of anthocyanins from grape skins. Some authors suggest that this can lead to low effectiveness of cold pre-fermentative maceration (Rodriguez Montealegre et al., 2006).

The column Lagar 3e represents the extractability of the pomace used for the production of Port wine measured right before pressing, after the addition of alcohol to the fermenting mass and its transfer to the vat. The producer uses a procedure different to the classical one, the alcohol being added in the lagar with the fermenting mass. The mixture is then left for 24 hours to macerate, after which it is directed to pressing and transfer to the vat. One can observe the decline by 12 % in EA values given the fact that alcohol allows the extraction of compounds soluble in ethanol.

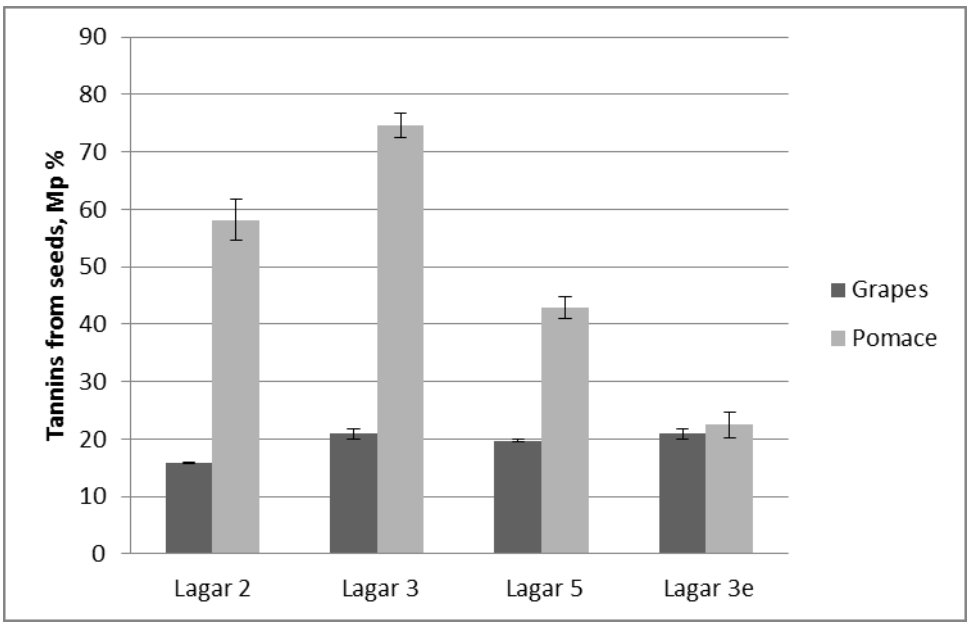


Figure 1.2 Percentage of seed tannins of grapes and pomace sampled after three days of maceration (error bars represent standard deviation of three determinations)

Figure 4.2 shows the percentage of seed tannins extracted from grapes and pomace. As it can be observed after maceration and a certain period of fermentation the percentage of extracted seed tannins increases, confirming that after a certain stage of the process it is mainly the tannins from the seeds that will be extracted if fermentation on skins will go on. The most obvious increase (256%) in percentage of seed tannins can be observed in Lagar 3 after 3 days of maceration. The columns Lagar 3e on Figure 4.2 refer to the values of seed tannins percentages obtained in Port wine right before pressing, after the addition of alcohol to the fermenting mass and its transfer to the vat. They show a decrease of this parameter after the addition of alcohol; this phenomenon could be explained by the fact that alcohol extracted the seed tannins into the wine. This variety of tannins is not normally favourable for the future wine especially if their over-extraction occurs (Vivas, 1998). This index can help the winemaker make decisions related to types of winemaking procedures and more important to determine the time when to stop the maceration. However more research is needed to evaluate the true potential of the Glories method when used for such purposes. Moreover the two parameters, extractability and percentage of tannin from seeds can be combined to create a new index. Furthermore increasing the extraction time of the Glories method would be useful as it was suggested by other authors (Roediger, 2006).

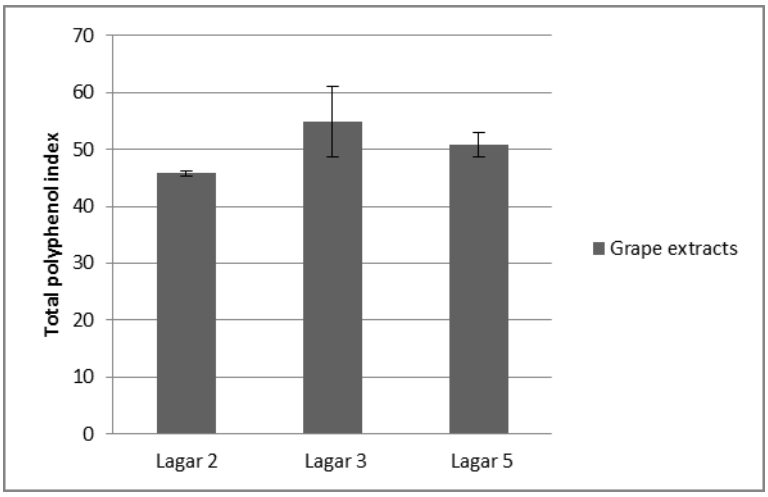


Figure 4.3 Total polyphenol index in grape extracts (error bars represent standard deviation of three determinations)

Additionally Figure 4.3 presents the total polyphenol index of the grape extracts. Although the levels are much lower than the ones found in future wines, this index could be used for the assessment of the potential of a certain mix of grapes.

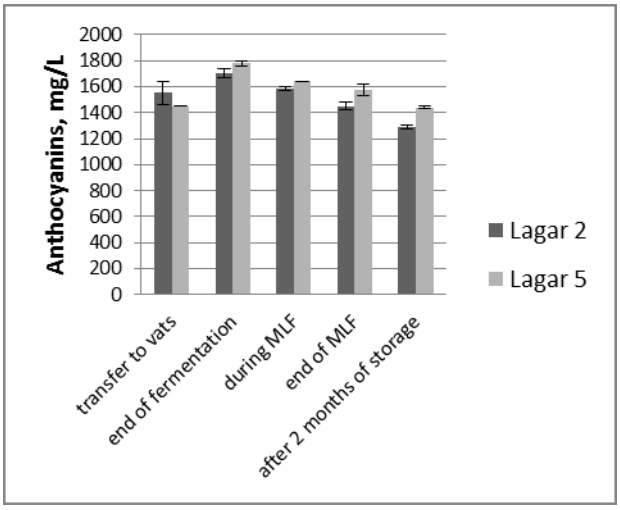


Figure 4.4 Anthocyanin content in dry red wines at different stages of the winemaking process (error bars represent standard deviation of three determinations)

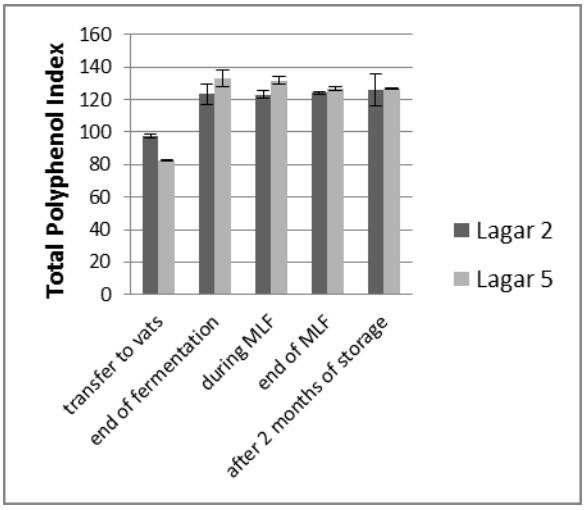


Figure 4.5 Total polyphenol index in dry red wines at different stages of winemaking process (error bars represent standard deviation of three determinations)

Looking at Figure 4.5 one can observe that the total polyphenol index remained relatively stable during fermentation and malolactic fermentation with higher values detected in Lagar 5, although the procedures for both wines were identical. However a slight increase during

fermentation can also be observed. This fact may be due to the production of alcohol which is a strong co-pigmentation factor, but also to the formation of larger, more stable pigments (Somers, 1998). Only during the transfer from lagares to the vats lower values of TPI can be noticed in Lagar 2 (see Figure 4.5). The grapes in Lagar 2 also showed higher extractability indexes. The same stable evolution trend applies to the anthocyanin content showed in Figure 4.4. One can conclude that malolactic fermentation did not affect the content of total polyphenols in the wines used for this study, as well as the one of anthocyanins. Furthermore the wines analysed at different sampling points had higher concentrations of anthocyanins than the extracts of the original grapes (see Tables 4.4 and 4.5) indicating that not all anthocyanins are liberated during the experimental stages of the Glories method. Once again the suggestion to increase the extraction time in the initial extraction stage of the Glories method can be made.

Table 4.4 Results (±standard deviation) of the modified Glories method

Nr.	Total polyphenol index		A1 mg/L (Anthocyanins extracted at pH 1)		A3,2 mg/L (Anthocyanins extracted at pH 3.2)		EA, Extractability %		Mp, tannins from seeds, %	
	Grapes	Pomace	Grapes	Pomace	Grapes	Pomace	Grapes	Pomace	Grapes	Pomace
L2	45.8±0.5	122.2±5.2	1223±47	864±8	732±18	756±32	42.0±1.8	17.4±3.7	15.8±0.1	58.2±3.6
L3	54.9±6.2	155.6±4.8	1306±75	1409±102	820±19	1085±66	41.6±0.3	33.6±2.3	21.0±0.9	74.7±2.1
L5	50.9±2,2	94.9±2.4	1253±43	1682±113	647±17	1053±54	45.9±0.2	40.9±2.2	19.8±0.2	42.9±1.8
L3e	54.9±0.2	55.9 ±3.0	1223±47	965±59	732±18	713±5	42.0±1.8	29.4±5.7	21.0±0.2	22.5±2.2

Table 4.5 Total polyphenol and anthocyanin contents (±standard deviation) during winemaking

Sample Sampling time	Total polyphenol index			Anthocyanins, mg/L		
	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5
Transfer to vats	97.4±1.3	126.0±1.0	82.6±0.2	1551±90	2268±60	1452±4
End of fermentation (Before pressing for Port wine)	123.5±6.2	122.1±2.0	133.2±5.3	1704±32	1919±25	1781±20
During malolactic fermentation (after pressing for Port wine)	123.2±2.4	128.1±2.2	131.8±2.2	1585±17	1713±37	1641±1
After malolactic fermentation (15 days storage Port wine)	124.2±1.0	115.3±2.1	127.1±1.2	1451±31	1699±39	1577±45
Storage 2 months	126.1±10.0	120.3±1.6	126.5±0.4	1292±13	1358±52	1441±11

Moreover several studies showed the anthocyanin content has decreased after reaching its maximum in the first days of fermentation. This decay has been attributed to different phenomena that occur at the same time such as oxidation reactions, condensation reactions of anthocyanins with other polyphenols and precipitation in the lees of anthocyanins absorbed by yeast (Bautista-Ortin et al., 2007). On the other hand, the study of Puértolas et al. (2010) showed as well that the content of anthocyanins remained approximately constant from the end of the maceration to the end of the alcoholic fermentation.

Normally, the maximum levels of anthocyanins are observed during the first few days of maceration, after which the extraction stops although 30-40 % of anthocyanins remain in the crushed skin (Marquez et al., 2012). The present study confirms this hypothesis since the content of anthocyanins has not changed a lot since the transfer to vats to the end of fermentation.

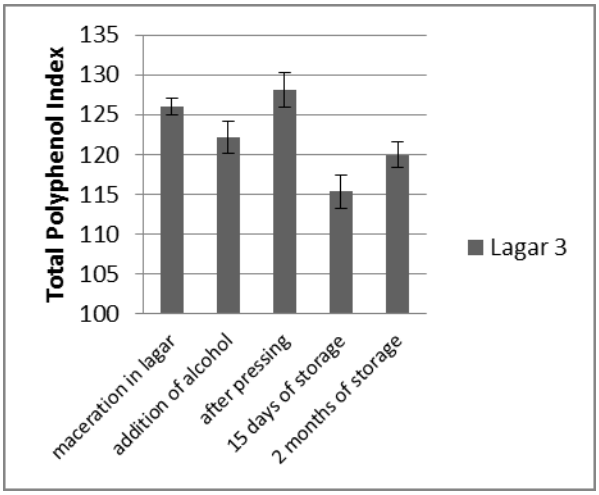


Figure 4.6 Total polyphenol index in Port wine at different stages of winemaking process (error bars represent standard deviation of three determinations)

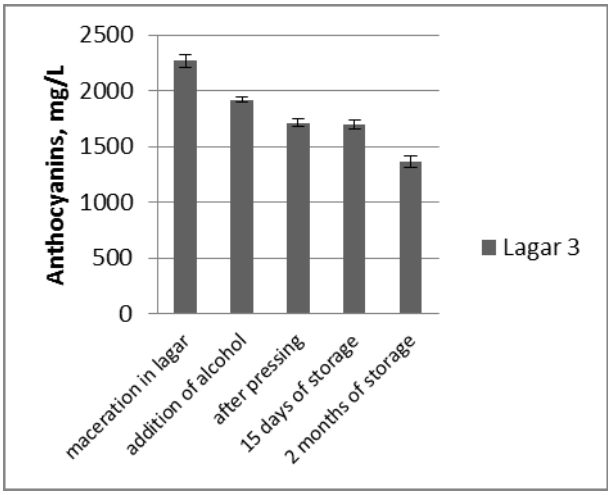


Figure 4.7 Anthocyanin content in Port wine at different stages of the winemaking process (error bars represent standard deviation of three determinations)

The same parameters were analysed in the Port wine and results are presented in Figure 4.6 and Figure 4.7. The content of anthocyanins has decreased by 15% after the addition of alcohol which can be explained by the dilution effect. Moreover, according to some authors, the addition of alcohol during the production of fortified wines stops the diffusion of red pigments from skins before its completion (Figuereido-Gonzalez et al., 2013). A slight decrease was observed in the following days of storage. The graph for the total polyphenol index shows variations with a slight decline after the addition of alcohol. There is an increase after pressing,

after which the value drops again by 10% in the following weeks of storage possibly because of the sedimentation and the stabilisation of the wine. After two months of storage the value of TPI starts to increase again, presumably due to the initial formation of co-pigmentation complexes.

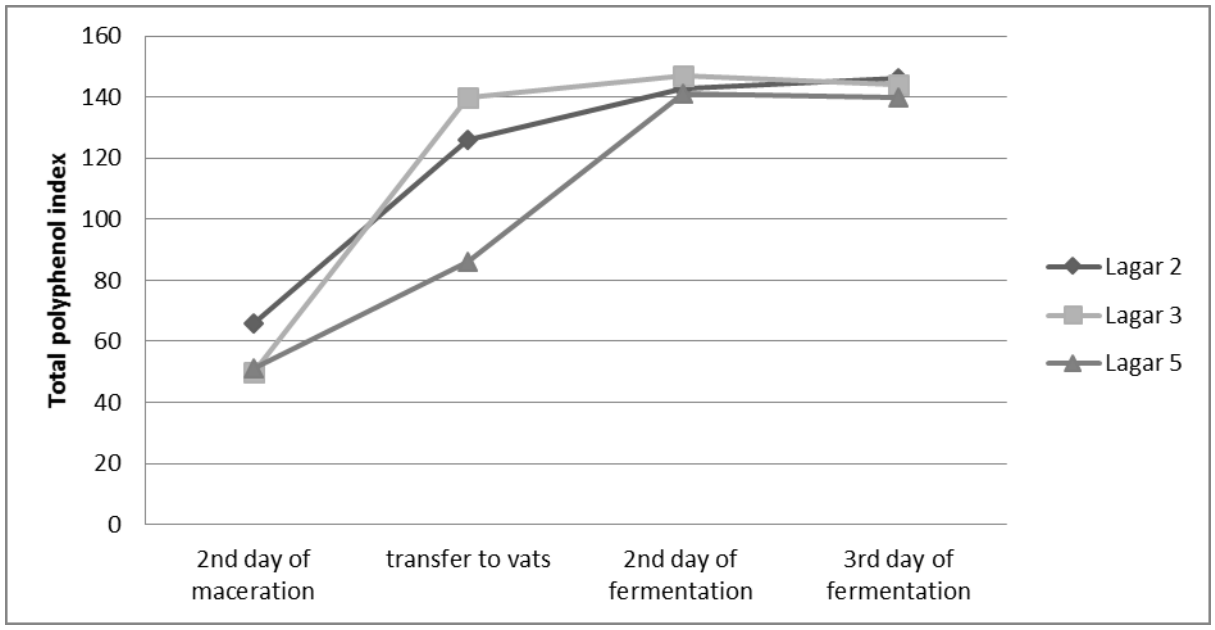
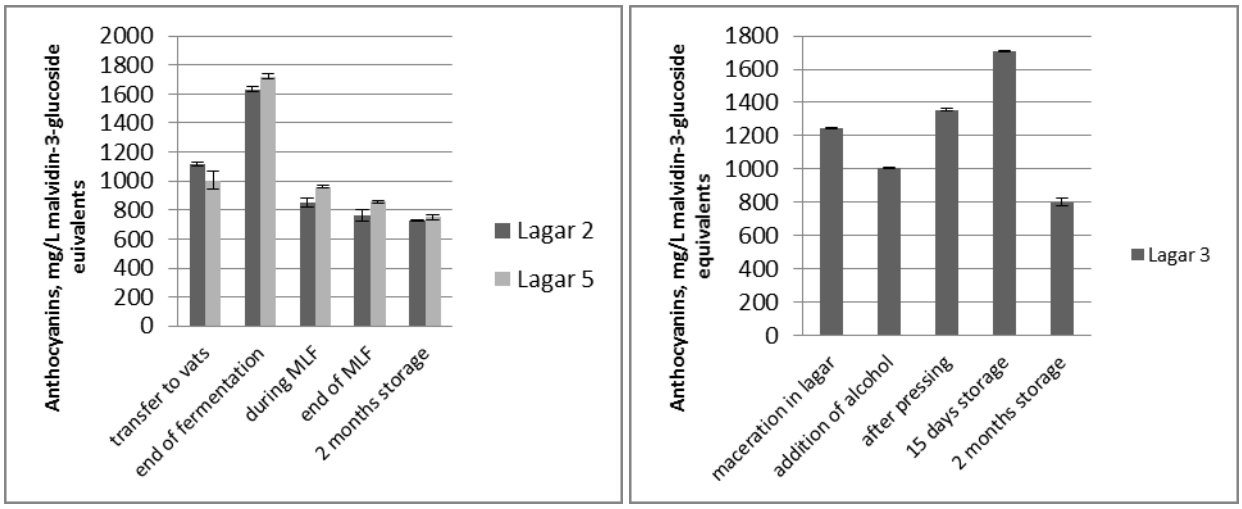


Figure 4.8 Evolution of total polyphenol index during maceration and onset of fermentation process (data provided by the producer's laboratory)

The TPI was also measured in the wine factory during the whole maceration time and in the first days of fermentation. It can be observed that TPI reached its maximum during maceration remaining stable after the onset of fermentation. Only in Lagar 5 the maximum was obtained after the second day of fermentation. However the values found during transfer to the vats in the factory are not similar to the ones obtained in the research laboratory (laboratory 83-97, factory – around 140, laboratory – around 140 at the end of fermentation). Two drawbacks were identified for this method: interferences from non-phenolic compounds that also contain aromatic rings (nucleotides, aromatic aminoacids, peptides, proteins) and it doesn't offer information about the type of measured phenolics (Harbertson et al., 2006).

4.2 Analysis of results obtained with Skogerson-Boulton model

The values for the anthocyanin content were also calculated using the Skogerson-Boulton model (Skogerson, et al., 2007).



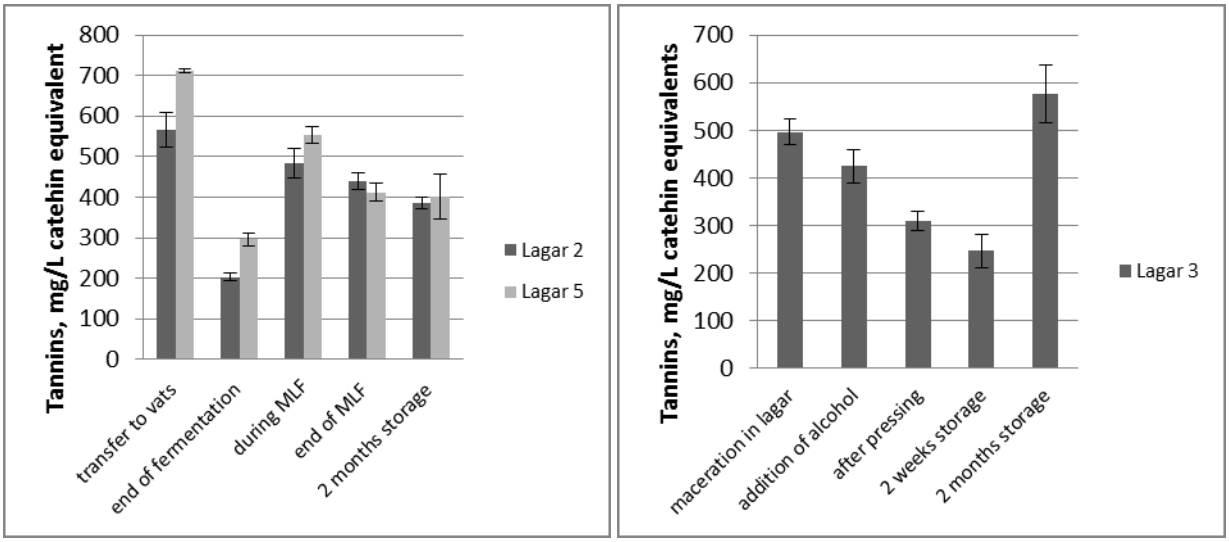
a. b.

Figure 4.9 Anthocyanin content obtained with Skogerson-Boulton model at different stages of the winemaking process (a. dry red wine, b. Port wine) (error bars represent standard deviation of three determinations)

Figure 4.9 shows the anthocyanin content values derived from the Skogerson-Boulton model. Contrary to the values obtained by bleaching with SO_2, the last ones are quite unstable showing a maximum at the end of fermentation and then a sudden decrease during malolactic fermentation. It must be pointed out that the values obtained at the end of fermentation by the two methods, SO_2 bleaching and Skogerson-Boulton model, are very similar (1704 mg/L and 1631 mg/L in Lagar 2 and 1721 mg/L and 1781 mg/L in Lagar 5 respectively). With this one exception the results of the two methods are contradictory and the values obtained with the Skogerson-Boulton model suggest that malolactic fermentation has a negative impact on the anthocyanins content as has been shown by (Guadalupe & Ayestaran, 2008). One explanation for the different results could be the fact that the anthocyanin concentration as defined in the Adams essay (used for the generation of the Skogerson-Boulton model) is just (monomeric) free anthocyanin (i.e. not considering polymers) and it is measured by shifting the wine pH while anthocyanin concentration as defined in the Glories (bleaching) method includes (monomeric) free anthocyanins plus anthocyanin-tannin complexes which can be bleached by SO2 (Ribereau-Gayon et al., 2006). Several authors suggest that anthocyanins can react with other components

such as acetaldehyde, keto-acids, tannins, and cinnamates forming the polymeric pigments as soon as the grapes are crushed and in the subsequent winemaking operations. They are a stable form of colour resistant to bisulfite bleaching and to changes in pH (Harbertson et al., 2006). Furthermore, catechins and proanthocyanidins are the main substrates for condensation with monomeric anthocyanins and their subsequent evolution to polymeric anthocyanins (Marquez et al., 2012).

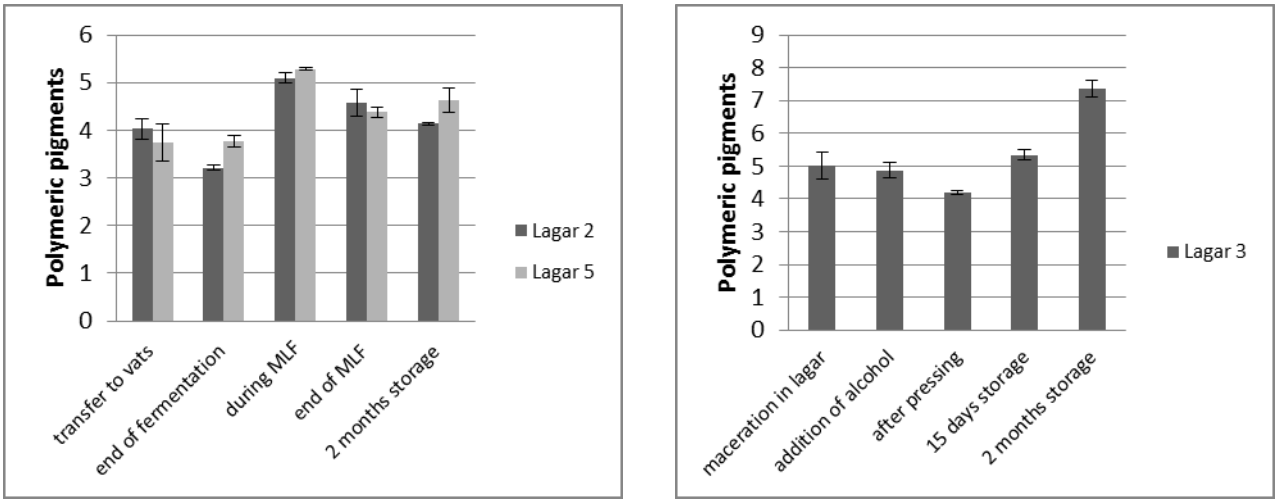
The slight decrease of anthocyanin content in wines is possibly provoked also by the polymerization of anthocyanins with other compounds such as tannins and the re-adsorbtion of coloured compounds by the solid parts of the berries (Marquez et al., 2012). On the other hand, Figures 4.10 and 4.11 show a clear decrease in the tannin and polymeric pigments content at the end of fermentation and after the addition of alcohol – opposite to what was observed for anthocyanins. An explanation to this could be the formation of combined larger pigments – a process that can be reversible. Other authors suggest a partial depolymerization of anthocyanin-tannin complexes after a certain period of time (Torchio et al., 2011).



a. **b.**

Figure 4.10 Tannin content obtained with Skogerson-Boulton model at different stages of the winemaking process (a. dry red wine, b. Port wine) (error bars represent standard deviation of three determinations)

Figure 4.10 presents the evolution of tannins during the winemaking of Port wine and dry red wine. It can be noted that the content of tannins decreased during the winemaking of Port wine, especially in the weeks following the addition of alcohol. Regarding the evolution of the same parameter in dry red wine, its evolution is unstable with a decrease by 64% in Lagar 2 and 58% in Lagar 5 by the end of alcoholic fermentation. In the case of short maceration generally there are lower amounts of extracted tannins and most of the extracted procyanidins come from the skins since they can be easily extracted, therefore it is expected that extracted proanthocyanidins are mostly of skin origin (Torchio et al., 2011).

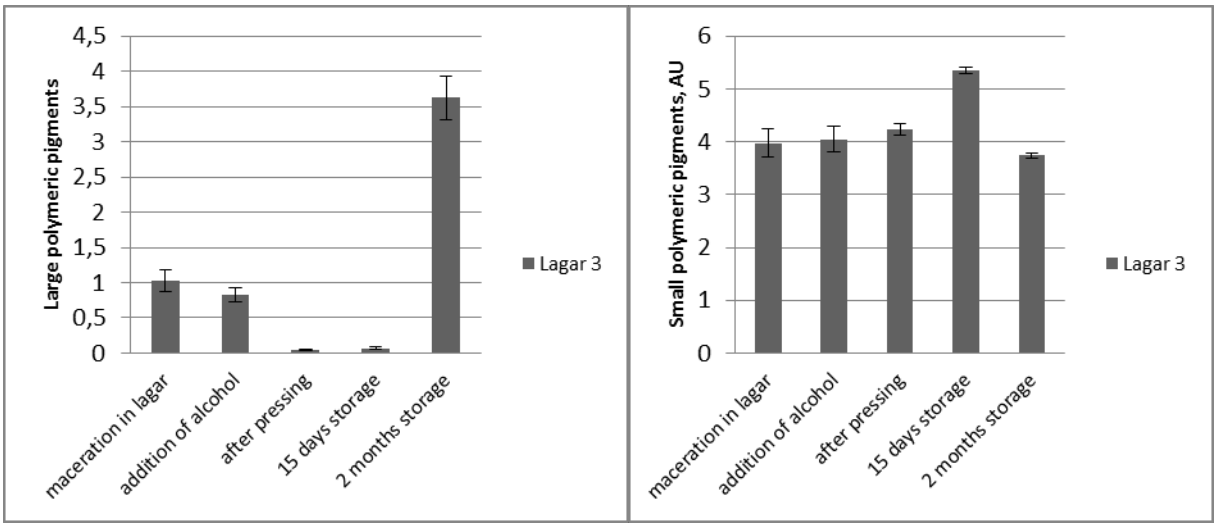


a. b.

Figure 4.11 Content of polymeric pigments obtained with Skogerson-Boulton model at different stages of the winemaking process (a. dry red wine, b. Port wine) (error bars represent standard deviation of three determinations)

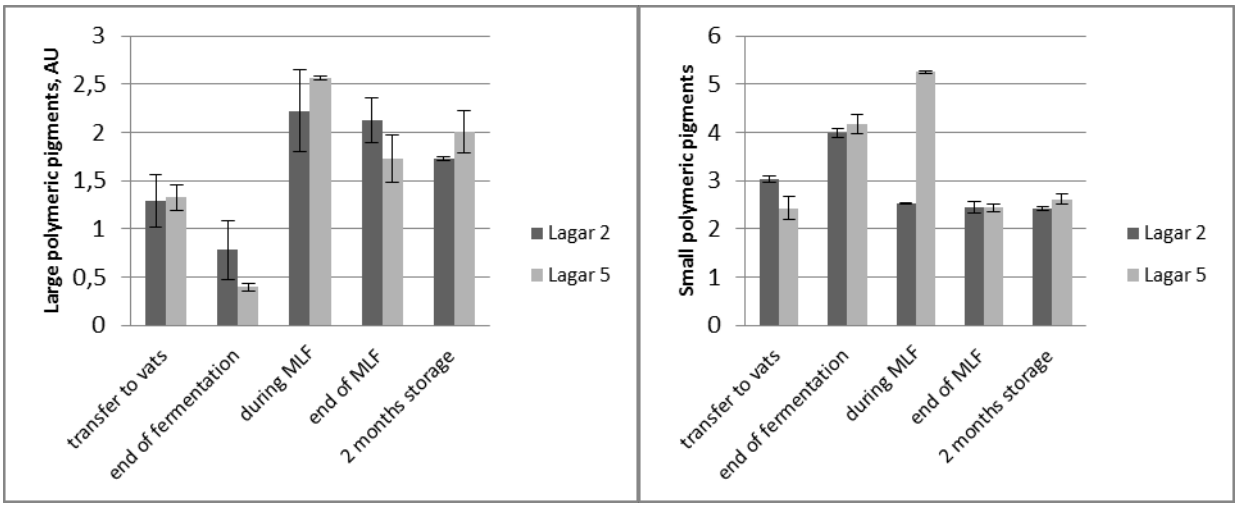
Figure 4.11 shows the evolution of the polymeric pigments which are quite stable throughout the vinification process especially for the dry red wines. In the case of Port wine, an increase is found during storage. A slight increase in the polymeric pigments content can be observed in dry wines in the stages following fermentation. Even if the name "polymeric pigments" is not the best one, the practice of measuring this parameter, normally identified with the colour fraction resistant to bleaching, is believed to be very useful for the winemakers (Harbertson et al., 2006). Two classes of polymeric pigments can be distinguished: small polymeric pigments (SPP) that do not precipitate with protein and large polymeric pigments (LPP) that can do so (Harbertson et al., 2003). The research of Harberston et al. (2003) also

showed that grape skins contain very small amounts of large polymeric pigments compared to small polymeric pigments. The same study also suggested that most large polymeric pigments are formed during fermentation. The present study found a maximum of LPP during malolactic fermentation in dry red wines (Figure 4.13 a). The quantity of LPP starts to increase after malolactic fermentation while SPP decline (Figure 4.13 a, b), therefore these results indicate changes in the structure of the wine pigments. In Port wine (Figure 4.12 a and b), however the content of SPPs is relatively stable during vinification and the concentration of LPPs increases drastically after two months of storage, therefore, most probably, the changes in the structure of pigments of Port wine appear mainly during storage. It must be pointed out that the Skogerson-Boulton model was developed recently and still needs further testing before it can be used by the winemakers.



a. **b,**

Figure 4.12 Large (a) and Small (b) Polymeric Pigments in Port wine during different vinification stages (error bars represent standard deviation of three determinations)



a. **b.**

Figure 4.13 Large (a) and Small (b) Polymeric Pigments in dry red wines during the winemaking process (error bars represent standard deviation of three determinations)

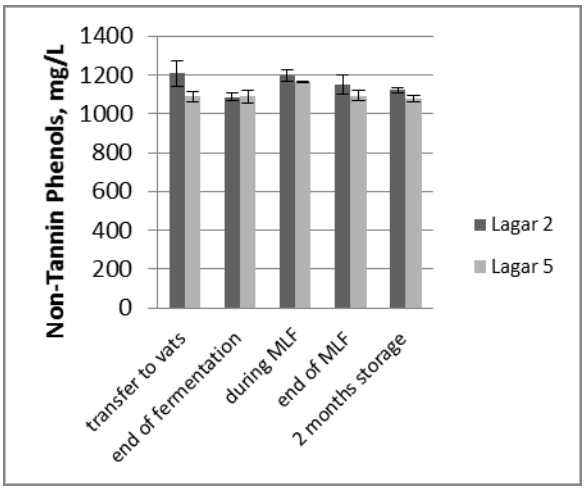


Figure 4.14 Evolution of Non-Tannin Phenols in dry red wine during winemaking process (error bars represent standard deviation of three determinations)

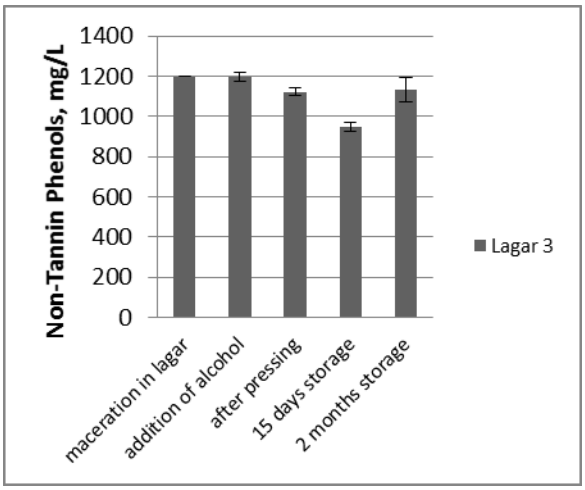
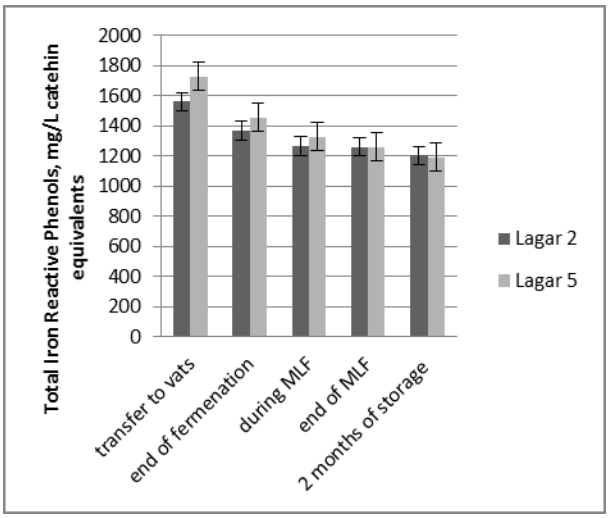
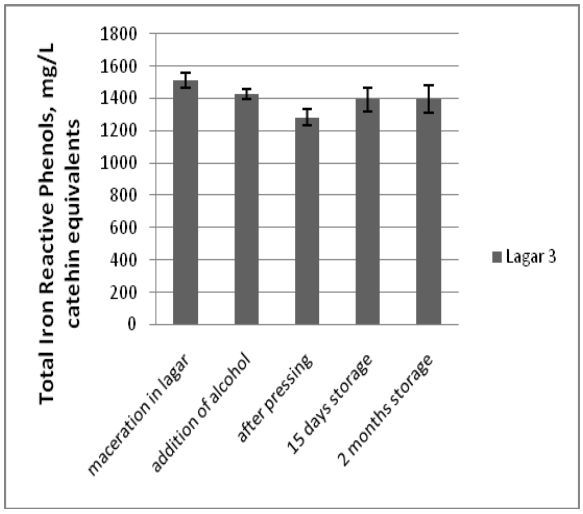


Figure 4.15 Evolution of Non-Tannin Phenols in Port wine during winemaking process (error bars represent standard deviation of three determinations)



a.



b.

Figure 4.16 Evolution of Total Iron Reactive Phenols during different stages of winemaking process (a. dry red wine, b. Port wine) (error bars represent standard deviation of three determinations)

Figure 4.16 which shows the evolution of total iron reactive phenols demonstrates a decrease in their content indicating a loss in reactivity of the phenols, meaning they are less susceptible for chemical interactions. The classic method for this measurement has been recently re-introduced for the measurement of the phenolics in the winery. It is based on the ability of iron to react with all the phenolics that have more than one hydroxyl group. Therefore, it is suitable to measure all phenolics in wine except anthocyanins and monohydroxylated phenols. It is, strictly speaking, a method for measuring "iron-reactive phenols", rather than "total phenols". However, it can be successfully combined with measurements of anthocyanins, polymeric pigment, and co-pigmentation, to provide a convenient assay of the main functional types of phenols (Harbertson et al., 2006).

4.3 Analysis of colour parameters

This chapter presents the classic colour parameters and the CIELab parameters.

Table 4.6 Classic colour parameters

Sample / Sampling time	Colour intensity			Hue		
	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5
Transfer to vats	26.3±0.9	33.4±1.6	25.6±0.4	0.47±0.02	0.50±0.01	0.44±0.01
End of fermentation (Before pressing for Port wine)	26.9±0.3	33.4±0.5	37.7±0.8	0.47±0.01	0.50±0.04	0.40±0.01
During malolactic fermentation (after pressing for Port wine)	28.1±0.2	39.8±0.5	32.4±0.3	0.53±0.01	0.46±0.04	0.52±0.02
After malolactic fermentation (except Port wine)	25.5±0.1	39.0±0.4	27.5±0.3	0.54±0.01	0.43±0.01	0.52±0.01
Storage 2 months	24.1±0.1	36.4±0.1	25.9±0.3	0.54±0.01	0.52±0.01	0.54±0.01

The colour intensity of dry red wines and Port wine is high as shown in Table 4.6. Its value is relatively stable during the winemaking processes. Only in Lagar 5 a marked increase in the value of colour intensity can be observed at the end of fermentation. However the values stabilize after malolactic fermentation suggesting the equilibrium of the wine colour. Wine hue values are comprised between 0.4 and 0.5 characteristic for a young wine (Ribereau-Gayon et al., 2006). The tonality of the wine shows a slight increase in the dry red wines after malolactic fermentation displaying already a shift towards red-orange tones. Further analyses are necessary in order to observe the evolution of colour intensity during storage. In the case of sparkling wine, the study of Torchio et al. (2011) showed that total anthocyanins, monomeric anthocyanins and total flavonoids underwent quantitative changes after secondary fermentation resulting in colour intensity decrease and tonality increase (Torchio et al., 2011).

Regarding tonality published data suggest normal values for a red wine comprised between 0.5 and 0.7, but may increase during ageing up to 1.3 (Torchio et al., 2011). In other studies the evolution of colour intensity followed a similar pattern to the evolution of anthocyanin content (Puértolas et al., 2010).

The following figures present the evolution of the CIELab parameters in each lagar.

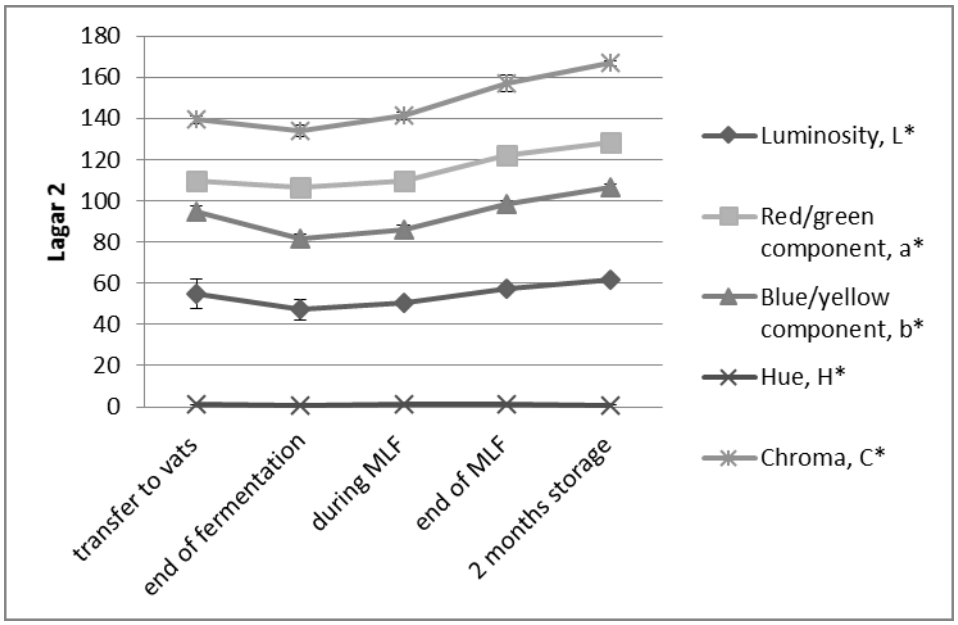


Figure 4.17 Evolution of CIELab parameters in Lagar 2 (error bars represent standard deviation of three determinations)

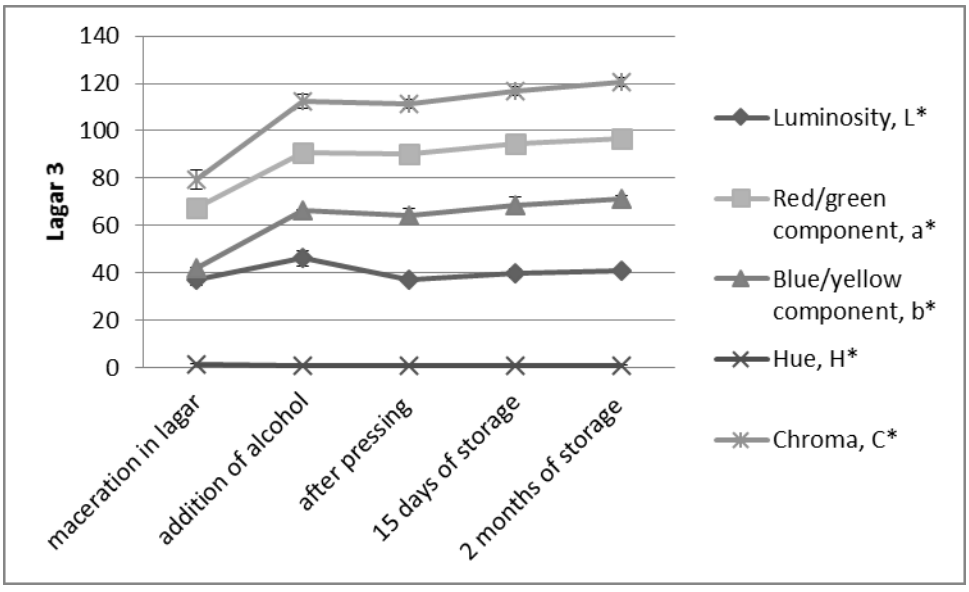


Figure 4.18 Evolution of CIELab parameters in Lagar 3 (error bars represent standard deviation of three determinations)

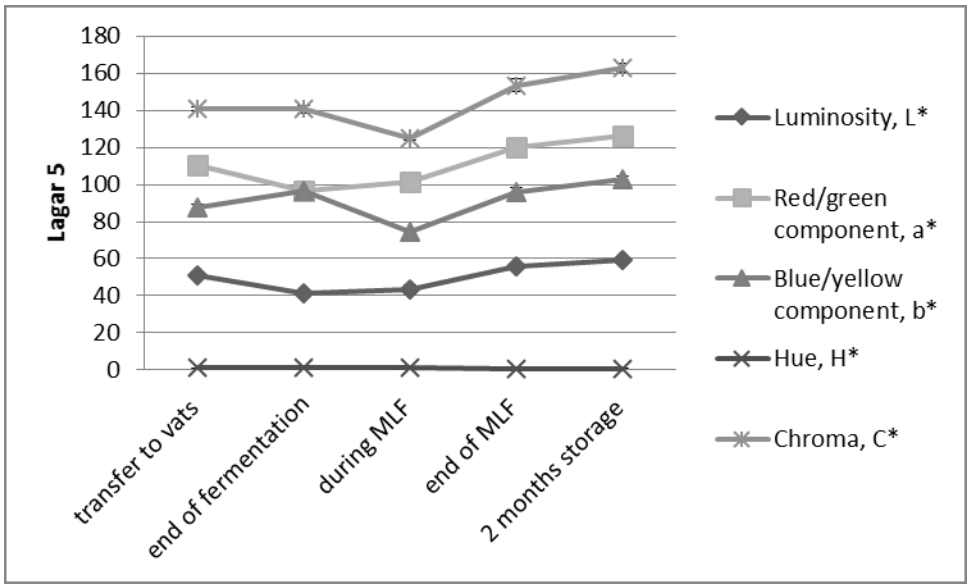


Figure 4.19 Evolution of CIELab parameters in Lagar 5 (error bars represent standard deviation of three determinations)

The luminosity of the wine is relatively low given the high levels of extraction and the high colour intensity (Figures 4.17; 4.18 and 4.19). The values remain quite stable during the whole winemaking process. The values of L* varied between 40 and 60 and increased in the final stages of the malolactic fermentation. It is expected for this index to increase during maturation.

Liang et al. (2011) found high values of L* in the grape extracts of cultivars with low total anthocyanins. On the other hand the values of a* were high in both red and pink cultivars even when the total anthocyanins were low (Liang et al., 2011). The authors also suggest that there is a very significant linear correlation between all CIELab parameters and anthocyanin content and composition.

The same figures show the evolution of red/green and blue/yellow components, which is relatively stable with values comprised between 67 and 129, and 41 and 99 respectively. All studied wines showed deep red-purplish colour, red being the predominant, characteristic for young red wines. Both parameters increased in time after malolactic fermentation suggesting an evolution towards deeper red tones and less blue ones. The higher anthocyanin content may be responsible for the higher contribution of the red colour component, while the higher hue value

will be a sign of contribution of other pigments which usually involves pyroanthocyanin formation resulting in red-orange hues (Torchio et al., 2011), Cretu et al., 2007).

It is generally accepted that tasters can distinguish the colour of two wines through the glass when ΔEab^* is higher than 5 units. Furthermore the differences that can be distinguished by the human eye also depend on the colour intensity (Kontoudakis, et al., 2011). Other authors report that the perceptibility threshold of CIELab colorimetric differences are ΔE^*=0.8-1 (Gonnet, 2001) and ΔE^* =3 (Martinez et al., 2011). The calculated ΔEab^* between Lagar 2 and Lagar 3, Lagar 3 and Lagar 5, and Lagar 2 and Lagar 5 are 52, 47 and 5 respectively. Therefore, the two dry red wines are practically indistinguishable, even though the level of extraction was different in the mix of grapes from Lagar 2 and Lagar 5.

The values of the chroma and the hue are also presented in the respective figures. The H* parameter indicates that the tone varies between violet and rose and it remains quite stable during winemaking. On the other hand chroma, which presents the colour quality, increases after the end of fermentation, phenomenon that could be explained by the appearance of other pigments during the process. The hue is used to describe one of the main attributes of the colour that is observed and the anthocyanin-derived pigments can contribute to orange hues observed in wine colour (Birse, 2007). The obtained values of hue in this study correspond to red-orange tones, although its low value indicates a young wine. Therefore when describing young red wines or mature red wines H* is one of the most accurate descriptors that can be used (Birse, 2007).

Other research suggests that the most important effect on chroma and on hue as well as on other CIELab parameters will be exerted by ageing time (Torchio et al., 2011). Moreover, authors suggest a partial depolymerization of anthocyanin-tannin complexes after a certain period of time (Somers, 1998). According to Gonzalez-Manzano et al. (2009) co-pigmentation can cause a decrease in lightness (L*) and an increase in chroma (C*). On the other hand the increase in anthocyanin polymerisation during ageing, and the decrease in the degree of co-pigmentation can lead to higher values of L* and lower values of C* (Gutierrez et al., 2005)

It must be pointed out that it has been confirmed by other authors that the OIV method leads sometimes to important errors especially with dark wines such as the ones used for the present study (Ayala et al., 1997).

The study of Garcia-Marino (2013) showed that a* (redness), b* (yellowness), C* (chroma) and H* (hue) tended to decrease from the end of fermentation to ageing in bottle for four months, while L* (lightness) was maintained stable. According to Birse (2007) the values of L*, a* and b* offer little information about colour to a beginner, thus it is quite difficult to describe a colour only providing the information about its “redness” or “yellowness”. Nonetheless L* and C* values are meaningful in defining colour in terms of its darkness and colourfulness as it is perceived by the human eye. In conclusion, the CIELab parameters, especially L* and C* need be monitored during ageing.

5. Conclusions

- The values of extractability and the percentage of seed tannins obtained by the Glories method on the macerating pomace can serve as an indicator to stop the maceration process. This would allow the production of standardized wines with regards to their anthocyanin and tannin content. However, it is necessary to establish a criterion based on the observation of the evolution of certain technical parameters. Also, as suggested by other authors, it would be useful to increase extraction time in the first stages of the procedure.

- The present study confirms the hypothesis that most of the anthocyanins are extracted during the first few days of maceration since the content of anthocyanins has not changed significantly from the transfer to vats to the end of fermentation.

- Alcoholic fermentation and malolactic fermentation do not affect extensively the colour parameters of the liquid obtained after maceration, as well as its content of different phenolics including anthocyanins.

- Colour parameters remain relatively stable during the initial winemaking stages following maceration, although the increase of all parameters, except hue after malolactic fermentation, suggest changes in the content and structure of pigments.

- The Skogerson-Boulton model is a fast and easy way to quantify different classes of polyphenols, but its potential for the prognosis of the quality of the future wine still has to be researched, while the measurement of CIELab parameters is so far the most complete method to describe the wine colour.

Future work comprising a higher number of samples taken during maceration is necessary in order to establish one index for its monitoring. Moreover, it is important to observe the evolution of polyphenols and colour parameters during the ageing of wine and to evaluate whether the decision to stop the maceration was taken at the right time.

6. Annex 1

All data was analysed using the functions of Microsoft Excel programme. The results are presented in the tables below. Graphs and tables were constructed using the respective results for conclusive reasoning. The results present the average of three experiments ± standard deviation.

Table 6.1 Content of tannins and anthocyanins obtained with the Skogerson-Boulton model

Sample	Anthocyanins, mg/L malvidin-3-glucoside equivalents			Tannins, mg/L catehin equivalents		
Sampling time	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5
Transfer to vats	1116±16	1247±5	1004±60	567±43	496±26	712±5
End of fermentation (Before pressing for Port wine)	1631±18	1004±3	1721±15	203±10	426±8	296±17
During malolactic fermentation (after pressing for Port wine)	851±28	1357±6	962±5	484±35	310±21	553±21
After malolactic fermentation (except Port wine)	762±38	1707±4	856±9	439±22	247±36	412±22
Storage 2 months	726±6	801±21	751±21	368±14	577±60	403±56

Table 6.2 Total Reactive Phenols and Polymeric Pigments contents obtained with the Skogerson-Boulton model

Sample	Total Iron Reactive Phenols, mg/L catehin equivalents			Polymeric Pigments		
Sampling time	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5
Transfer to vats	1562±76	1515±47	1729±18	403±0.21	5.01±0.42	3.76±0.39
End of fermentation (Before pressing for Port wine)	1367±43	1425±32	1456±33	3.21±0.05	4.87±0.24	3.78±0.12
During malolactic fermentation (after pressing for Port wine)	1269±13	1284±50	1329±1	5.10±0.10	4.21±0.06	5.29±0.02
After malolactic fermentation (except Port wine)	1262±49	1394±73	1262±17	4.58±0.28	5.35±0.15	4.38±0.11
Storage 2 months	1204±22	1396±83	1194±66	4.14±0.03	7.36±0.27	4.62±0.26

Table 6.3 The contents of small polymeric pigments, large polymeric pigments and non-tannin phenols obtained with Skogerson-Boulton model

Sample / Sampling time	Small polymeric pigments, AU			Large polymeric pigments, AU			Non-Tannin Phenols, mg/L		
	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5
Transfer to vats	3.03±0.08	3.98±0.26	2.43±0.24	1.29±0.27	1.03±0.15	1.33±0.13	1209±66	1197±1	1088±29
End of fermentation (Before pressing for Port wine)	3.99±0.10	4.05±0.24	4.18±0.20	0.78±0.30	0.82±0.10	0.40±0.04	1087±20	1198±24	1088±32
During malolactic fermentation (except Port wine)	2.53±0.01	4.23±0.11	5.25±0.03	2.22±0.42	0.05±0.01	2.57±0.02	1197±30	1154±21	1161±4
After malolactic fermentation (except Port wine)	2.47±0.11	5.35±0.07	2.44±0.09	2.13±0.24	0.07±0.01	1.73±0.23	1151±48	946±22	1093±24
Storage 2 months	2.41±0.04	3.74±0.05	2.61±0.10	1.73±0.02	3.62±0.31	1.73±0.23	1121±13	1133±61	1081±17

Table 6.4 L*a*b* parameters

Sample / Sampling time	Luminosity, L*			Red/green component, a*			Blue/yellow component, b*		
	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5
Transfer to vats	55.0±7.1	37.3±2.4	50.8±1.3	109.6±0.3	67.4±1.9	110.4±2.0	94.9±2.4	41.8±0.4	87.4±1.7
End of fermentation (Before pressing for Port wine)	47.3±5.0	46.2±3.2	41.2±0.2	106.4±2.1	91.0±2.2	96.4±1.2	81.6±2.2	66.4±0.3	96.4±2.5
During malolactic fermentation (after pressing for Port wine)	50.5±1.0	37.4±1.3	43.3±2.2	109.7±1.0	90.3±1.9	101.6±3.4	85.7±2.2	64.4±2.8	74.7±0.8
After malolactic fermentation (except Port wine)	57.2±0.6	39.9±2.1	55.6±1.5	122.3±2.5	94.5±3.4	119.7±2.3	98.6±1.1	68.8±3.5	95.9±2.3
Storage 2 months	61.5±0.3	41.0±0.1	59.6±1.0	128.7±0.5	97.0±1.1	126.0±1.6	106.6±1.5	71.4±1.1	102.8±1.7

Table 6.5 C* and H* parameters

Sample Sampling time	Chroma, C*			Hue, H*, degrees		
	Lagar 2	Lagar 3	Lagar 5	Lagar 2	Lagar 3	Lagar 5
Transfer to vats	139±2	80±4	141±1	1.00±0.01	1.43±0.01	0.98±0.04
End of fermentation (Before pressing for Port wine)	134±3	113±3	141±2	0.90±0.05	1.06±0.02	1.02±0.03
During malolactic fermentation (after pressing for Port wine)	141±2	111±2	125±1	1.00±0.01	1.07±0.13	1.06±0.03
After malolactic fermentation (except Port wine)	157±4	117±2	153±3	0.96±0.54	1.12±0.17	0.94±0.01
Storage 2 months	167±1	121±2	163±2	0.93±0.01	1.11±0.02	0.97±0.12

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