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Predicting and controlling the colour of rosé wines and their pigments during alcoholic fermentation and storage

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Abstract

The visual and spectrophotometric measurement tools used routinely are nowadays insufficient to understand, predict and control changes in the color of rosé wines. This project aimed at developing finer tools and improve knowledge of the mechanisms impacting color during vinification process in order to control the impact of fermentation, which is the stage of the process that generates the most variations, predict post-fermentation developments and guarantee control over time.

Keywords: phenolic compounds, pigments, yeast, fining, grape variety

1. Introduction

1.1 Professional and social context

The colour of rosé wines and its evolution are fundamental for market positioning. Exports and consumer demands are imposing new criteria in terms of shelf life (colour changes as a result of longer distribution channels and transport times), and limiting inputs (organic and natural trends, etc.). In order to take these new parameters into account, we need to improve our knowledge of the pigments in rosé wines, so as to enhance our expertise in predicting colour and obtain more refined control tools.

In rosé wine making, fermentation is a "big bang", marking a major discontinuity in colour during the vinification process. In the vineyards, producers regularly comment on the colour drop during fermentation, without being able to explain this variability or control it.

Predicting the development of post-fermentation colour is also a demand from the profession. The aim is to control these changes over time after bottling and when using oenological treatments such as sulphites and fining agents. The goal is to better manage and reduce the use of these inputs, which have a major impact on the colour of wines, in response to societal demand. Ideally, a wine producer should be able to predict the colour of the wine at the end of fermentation and during the post-fermentation stages, based on the initial colour and composition of the juice (grape variety, terroir), so as to be able to control the transformation process in line with the target colour.

Numerous studies have been carried out to identify the factors in the process which influence the colour of rosés. Nowadays, winemakers have some keys to control the colour. However, detailed knowledge of the underlying mechanisms is required, in particular the structure and development of the pigments that contribute to the colour. The visual and spectrophotometric measurement tools routinely used are not yet



sufficient to understand, predict and control its evolution. The aim of this programme was to develop more sophisticated tools to:

- control the impact of fermentation, which is the stage in the process that generates the most variation;
- predict post-fermentation developments and guarantee control over time.

The main goal of this programme was to provide a pragmatic response to the winemaker's need to control the process. The first part of the project, on fermentation, condenses the results of a doctoral thesis focusing on the knowledge and behaviour of pigments. The second part, on post-fermentation, focuses on the impact of processes. Finally, the third part deals with the transfer tools deployed to professionals.

1.2 Partnership

The PigRosé project, funded by the Compte d'Affectation Spécial Développement Agricole et Rural (CASDAR) and led by the Institut Français de la Vigne et du Vin (IFV), is based on a partnership between the Centre du Rosé, the IFV and the Institut National (Français) de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE).

A doctoral student recruited by the IFV was hosted and supervised by the Sciences for Oenology Joint Research Unit (UMR SPO, INRAE, Institut Agro, Université de Montpellier) in Montpellier (34), in conjunction with technicians and engineers from the Centre du Rosé and the IFV based in Viduban (83). The latter supplied the raw materials and took charge of the technical experiments under pilot conditions, as well as the development of distribution and transfer tools.

The analytical resources mobilised for this project include the traditional oenological and colour analyses carried out at the Centre du rosé, supported by the resources of the ACTIA Minicave/Oenotypage UMT run by INRAE and IFV, as well as the cutting-edge analytical methods made available by the UMR SPO for the study of pigment composition during alcoholic fermentation and the fining stage.

On completion of her thesis, the doctoral student was recruited by INRAE as a research engineer at the Pech rouge experimental unit. This site is unique in that it operates with a joint INRAE/IFV team, which means that the skills acquired can be preserved and mobilised on a long-term basis through the IFV/INRAE/Centre du rosé collaboration.

The project will therefore continue to be developed, with articles to be written in 2023 and forecasts for 2024. Scientific and technical collaboration will also enable programmes on other subjects (innovative varieties, etc.) to be developed in rosé. It should be noted that the thesis was awarded a prize by the Académie d'Agriculture de France.

2. Summary of knowledge about colour and pigments in rosé wines

A detailed literature review was carried out in the first chapter of the thesis (Leborgne, 2022a). The first part lists the different phenolic compounds in grapes that can have an impact on the colour of rosé wines. The second part looks at the specific vinification of rosé wines and, more specifically, the alcoholic fermentation stage in relation to colour and the formation of derived pigments. The final section looks at the analysis of perceived colour and the phenolic compounds responsible for it.

The colour of Rosé wines is mainly due to the presence of phenolic compounds from the grape berry and their derivatives formed during vinification. The three main molecular families are anthocyanins (red pigments), hydroxycinnamic acids (colourless) and flavanols (monomers and tannins). These compounds are located in various parts of the grape berry, as shown in Figure 1.

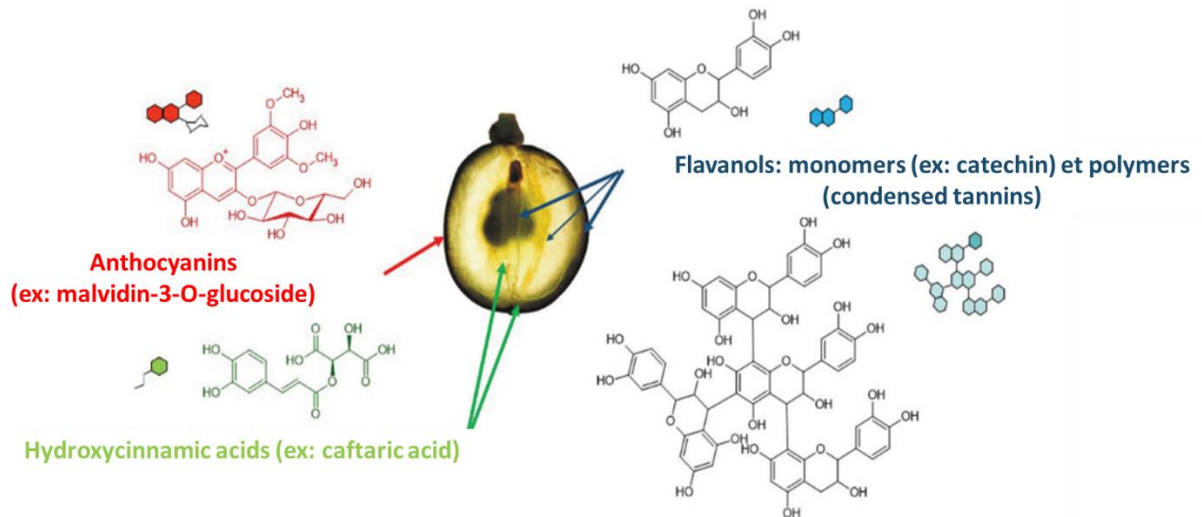


Figure 1: The three main classes of phenolic compounds in grapes and their location in the berry

These differences in their location in the berry have an impact on their extraction during the winemaking process. Hydroxycinnamic acids (present in the pulp) are extracted by simply pressing the berries, whereas the extraction of anthocyanins, located in the skins, requires a phase of maceration of the skins in the juice. The specific vinification of rosé wines, with controlled pre-fermentation maceration as described in Figure 2, limits the extraction of compounds from the skins (anthocyanins and flavanols), unlike vinification of red wines.

This typicality is reflected in the composition of the resulting wines. Studies carried out on rosé wines report anthocyanin levels ranging from less than 1 mg/L to 50 mg/L (Gil *et al.*, 2017, 2019; Lambert *et al.*, 2015; Salinas *et al.*, 2003; Wirth *et al.*, 2012) and very high levels of hydroxycinnamic acids compared to other types of phenolic compounds (Figure 3, Lambert *et al.*, 2015; Wirth *et al.*, 2012).

A multitude of derived pigments have also been detected in this work. Some of these pigments, such as caftaric acid-anthocyanin adducts, result from an enzymatic oxidation phenomenon generally observed during the pre-fermentation stages of white winemaking (must browning), while other compounds, such as tannin-anthocyanin adducts, are specific to ageing during red winemaking (Figure 4). Pyranoanthocyanins result from the reaction between anthocyanins and metabolites produced by yeast during alcoholic fermentation (Figure 4).

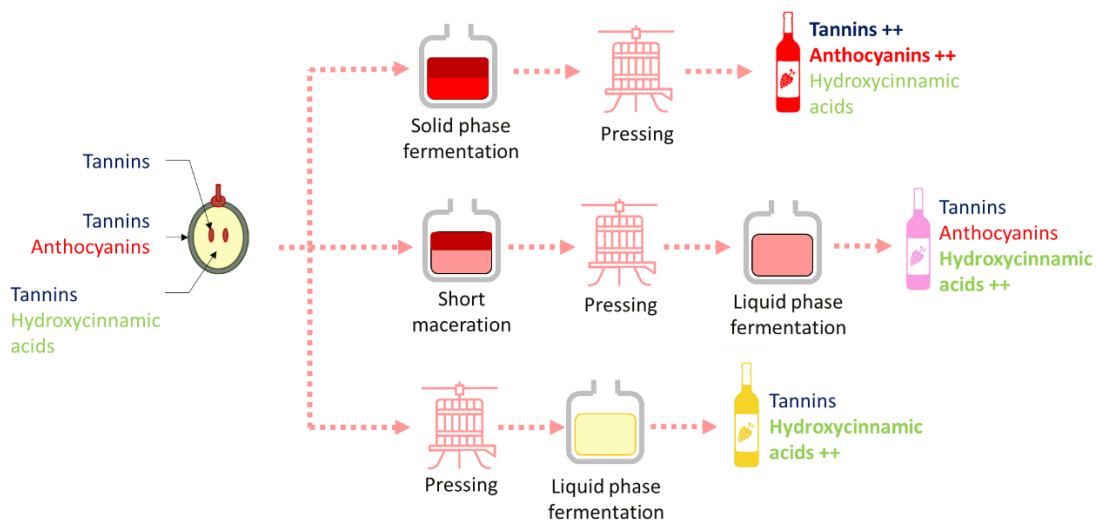
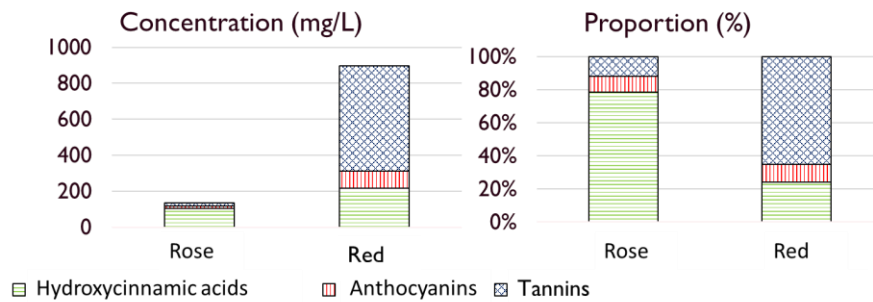


Figure 2: The different types of vinification and their impact on wine composition.



Wirth et al., 2010, 2012

Figure 3 : Differences in the phenolic composition of Grenache vinified as rosé or red wine (Wirth *et al.* 2010, 2012)

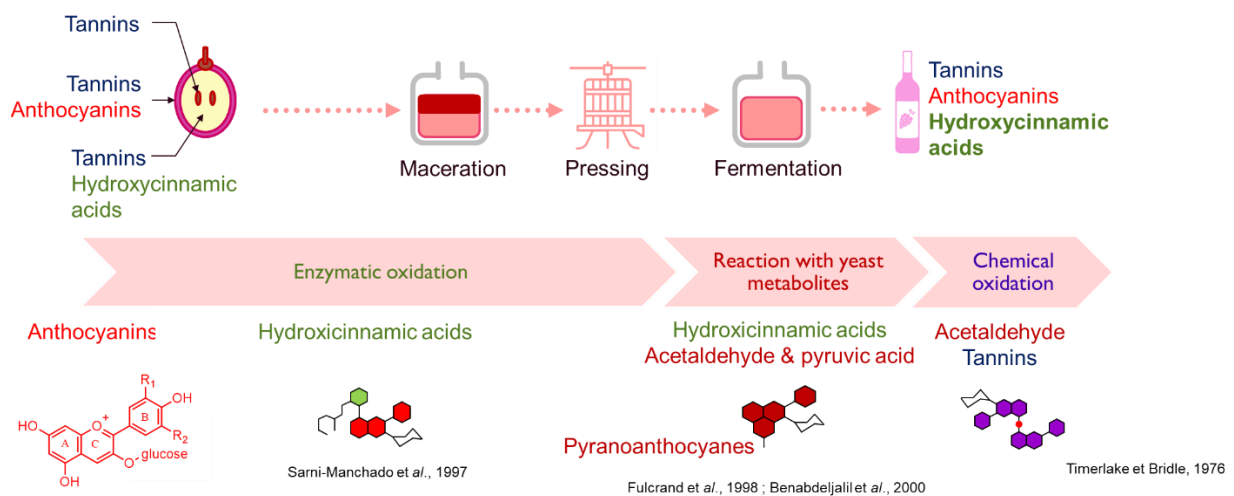


Figure 4: Pigments derived from native grape anthocyanins during the specific vinification of rosé wines.

3. Behaviour of pigments during fermentation

The literature review highlighted the lack of available data on rosé wines, which are at the interface of white and red vinification. This particular vinification process has an impact on the proportion of phenolic compounds extracted from the grapes and therefore on the resulting chemical reactions. The initial hypothesis was therefore as follows: the atypical composition of rosé wines, due to their particular vinification, has an impact on the chemical reactions and in particular the nature of the pigments formed. The research work on this thesis was therefore directed towards an initial large-scale analysis of the colour and phenolic composition of commercial rosé wines. Then, the work focused on the alcoholic fermentation stage to investigate its impact on colour and composition.

3.1 Study of commercial wines

An initial study was carried out on 268 commercial wines selected to cover a wide range of rosé wine colours and styles (Leborgne, 2022a - Chapter 3 of the thesis). These wines were collected by the Centre du Rosé from the Union des Œnologues de France, which organises the "Mondial du Rosé®" competition.

Communicating and transferring the results to the winemakers involves tackling the problem by positioning the samples on the Centre du Rosé's official paper colour chart (Figure 4).

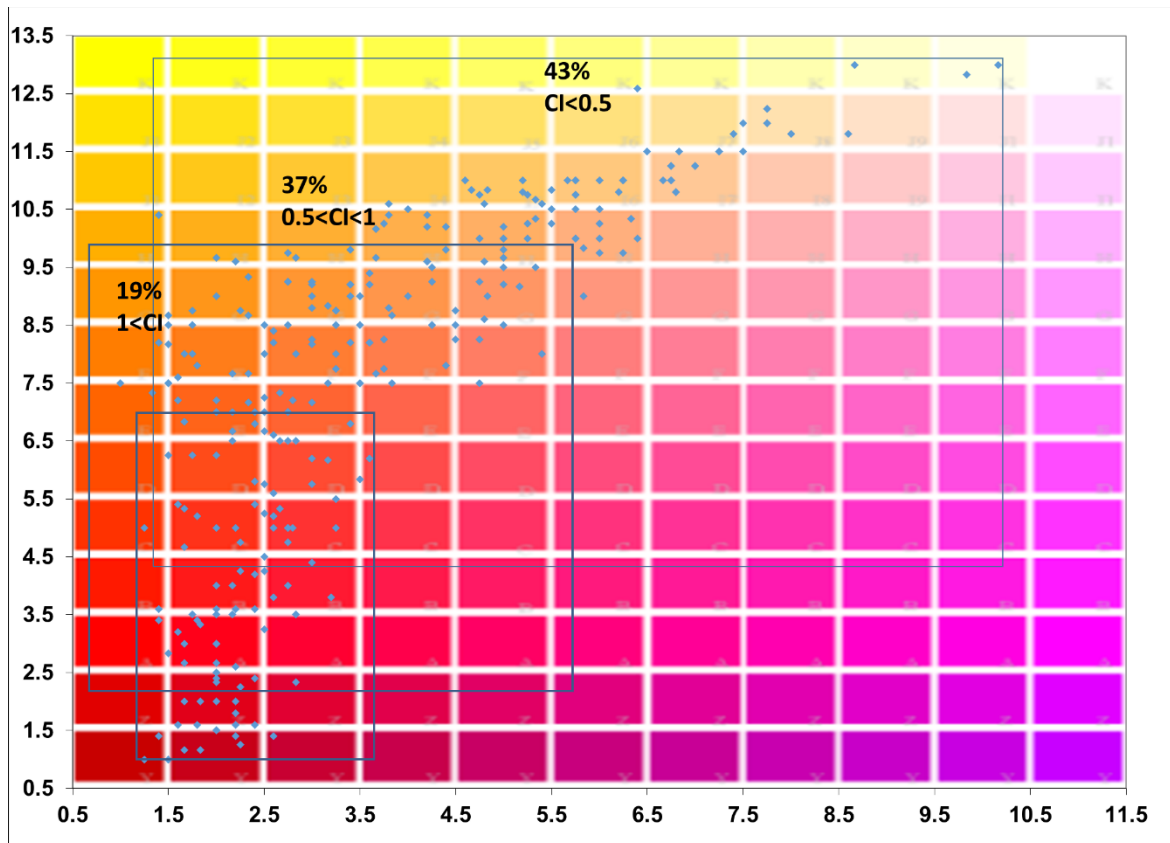


Figure 4: Positioning of the world's 268 rosés on the Centre du Rosé's paper colour chart, CI = Colour Intensity

On this panel of wines, the concentration of 125 phenolic compounds was determined by ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS) in MRM (Multiple Reaction Monitoring) mode, using equipment from INRAE's Polyphenols Platform. Colour was characterised spectrophotometrically.

The raw data showed that colour intensity (CI) is mainly determined by the extraction of phenolic compounds from the grapes. The major compounds identified are anthocyanins and flavanols (tannins) present in the skin of the berry (Figure 1 and 2) and therefore subject to an extraction factor.

Chemometric analysis of the data was then used to refine these processes. This method involves using mathematical tools, particularly statistics, to obtain the maximum amount of information from chemical data. The 268 rosé wines were classified into three colour groups according to their colour intensity (CI): light rosés (group 1: $CI < 0.5$), intermediate rosés (group 2: $0.5 < CI < 1$) and dark rosés (group 3: $CI > 1$). Comparison of the concentration of phenolic compounds in these three groups revealed a difference in concentration for tannins and anthocyanins (Figure 5), which come from the grape skin and are therefore extracted to a greater or lesser extent depending on the grape variety and type of vinification. The link with colour is obvious: the higher the extraction, the more colourful the wine. Conversely, the concentration of hydroxycinnamic acids does not vary according to the three colour groups. However, these compounds represent different proportions for each of the three colour groups, thus impacting the chemical reactions that occur, as illustrated in Figure 6. The pigments derived from the reaction of anthocyanins with hydroxycinnamic acids (phenyl-pyranoanthocyanins) are found in equivalent quantities in the three colour groups. In contrast, the pigments derived from the reaction of anthocyanins with yeast metabolites (pyranoanthocyanins and carboxypyrananthocyanins) are correlated with the extraction phenomenon, meaning that the higher the colour intensity (CI), the greater the quantity of these anthocyanin derivatives. These derivative pigments are essential for wine colour because, unlike native

anthocyanins which are coloured between 10% and 30% at the pH of the wine (pH = 3-4) and decoloured by SO₂, these pigments are resistant to decolouration (pH and SO₂) and are fully coloured.

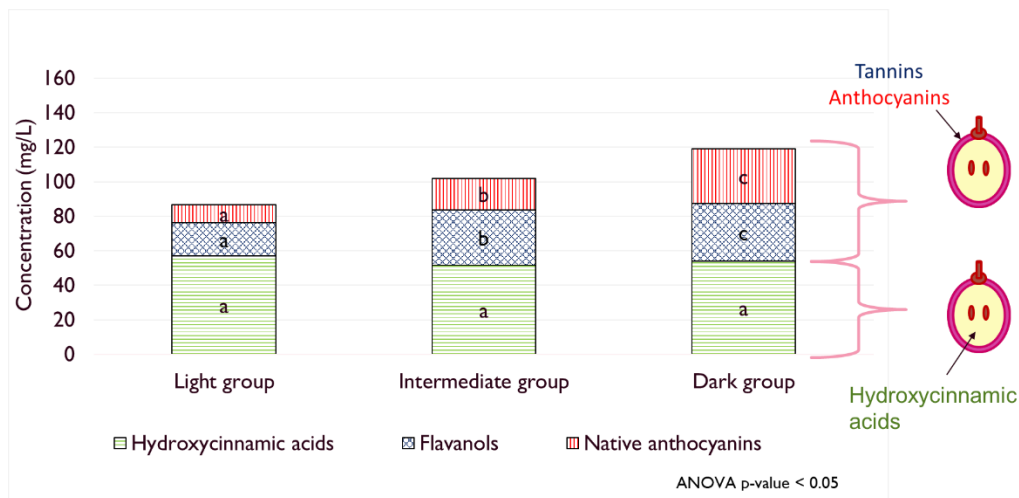


Figure 5: Distribution of the main families of grape phenolic compounds (i.e. anthocyanins, flavanols and hydroxycinnamic acids) in the three colour groups (group 1: light rosés; group 2: intermediate rosés, group 3: dark rosés). Different superscript letters indicate significant differences between colour groups for a given parameter (ANOVA with SNK test for $p < 0.05$). (Leborgne *et al.*, 2022b)

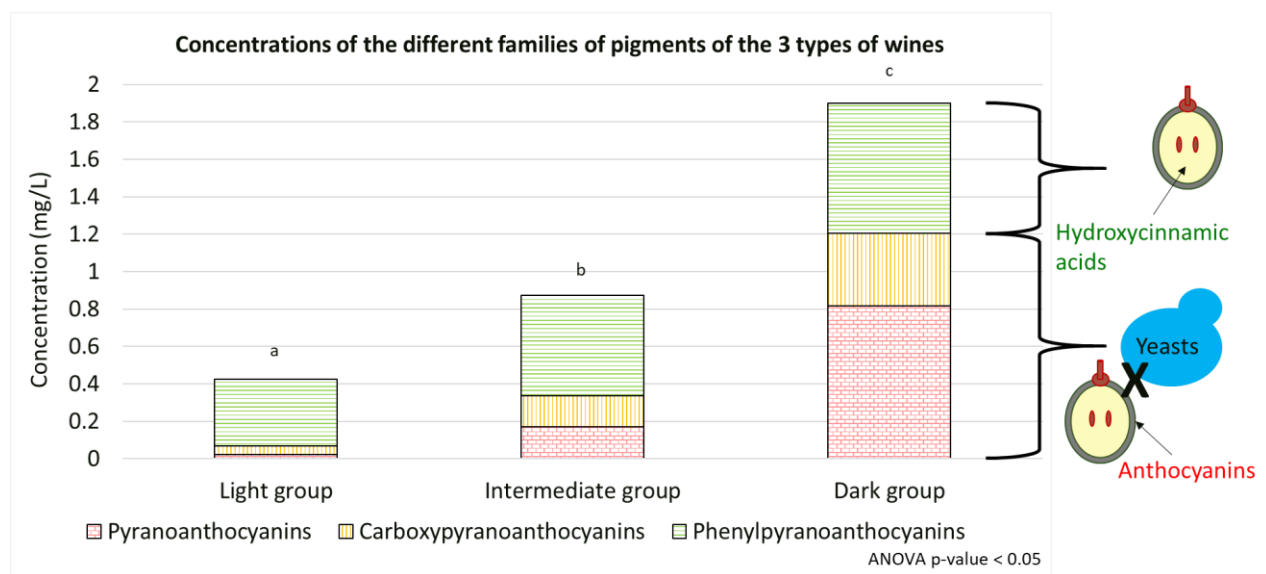


Figure 6: Distribution of the main families of derived pigments (i.e. pigments formed during vinification) in the three colour groups (group 1: light rosés; group 2: intermediate rosés, group 3: dark rosés). Different superscript letters indicate significant differences between colour groups for a given parameter (ANOVA with SNK test for $p < 0.05$). (Leborgne *et al.* 2022b)

The salmon hue of light rosé wines is mainly due to phenylpyrananthocyanins (Figure 6), which, unlike native grape anthocyanins, are fully coloured. These compounds result from the reaction of anthocyanins with hydroxycinnamic acids during enzymatic oxidation (pre-fermentation) and reactions with metabolites produced by yeast (alcoholic fermentation).

The colour of intermediate and dark rosé wines is divided into two components, red and yellow.



The red component of intermediate-coloured wines is linked to anthocyanins and carboxypyrananthocyanins. The red component of dark rosé wines is linked to the reaction products of anthocyanins with flavanols (tannins) and is therefore subject to an extraction factor.

The yellow component of dark and intermediate wines is associated with pigments resulting from chemical oxidation formed by reaction with acetaldehyde (ethyl bridges, pyrananthocyanes) (Figure 7).



Figure 7: Phenomena affecting the pigment composition, and therefore the perceived colour, of light and dark rosé wines (Leborgne *et al.*, 2022b).

These results have made it possible to obtain data on the pigment composition of rosé wines, which has been little studied in the literature, and to put forward hypotheses as to its variability. The formation of pyrananthocyanins is mainly observed during alcoholic fermentation, thus initiating further work aimed at understanding the mechanisms that occur during this crucial stage in the winemaking process.

This work was the subject of a publication entitled "Elucidating the Color of Rosé Wines Using Polyphenol-Targeted Metabolomics", which appeared in the journal *Molecules* in 2022 (Leborgne *et al.*, 2022b).

3.2 Impact of the physical and chemical parameters of fermentation

3.2.1 Temperature and sulphites

This work looked at the impact of temperature (12°C; 16°C; 20°C), pH (3.1; 3.5; 3.9) and sulphite dosage (20 mg/L; 50 mg/L; 80 mg/L) on the colour and phenolic composition of rosé wines during alcoholic fermentation. The methods used in this first study were optimised using experimental designs to maximise the number of experiments required. These designs were applied to three different grape varieties (Grenache, Syrah and Cinsault) widely used to make rosé wines, particularly in Provence. Samples were prepared at the Rosé Centre at two different maceration levels for each grape variety. In order to assess changes in colour and composition, this study combined spectrophotometry, ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry in MRM mode and high-performance steric exclusion chromatography.

The results of this first approach to the impact of pH, temperature and SO₂ concentration during alcoholic fermentation (K1 yeast, ICV, France) showed no impact on phenolic composition and a limited impact on colour in relation to the chemical balance of anthocyanins in solution and sulphites in the musts.

3.2.2 Adsorption on yeast

To go further, the pigments adsorbed on the yeast walls were analysed on the central modalities (T°C = 16°C; pH = 3.5; total SO₂ = 50 mg/L) of the six experimental designs carried out previously. The six conditions were compared in order to highlight the impact of the raw material on the chemical and physico-chemical phenomena involved in alcoholic fermentation.

Firstly, the results show that the colours and compositions of the wines produced by alcoholic fermentation of Grenache and Cinsault correspond to those of the light rosé group (group 1) in the study of the world's

268 rosés. In contrast, the wines produced by alcoholic fermentation of Syrah showed a composition and colour corresponding to the dark rosé group (group 3).

Analysis of the compounds adsorbed by the yeast walls revealed large quantities of oligomeric pigments derived from hydroxycinnamic acids and flavanols, resulting from enzymatic oxidation reactions in the Grenache and Cinsault musts. These oligomeric pigments, whose structure could not be determined, were largely lost during fermentation due to their adsorption to the lees.

In contrast, the colour of the Syrah musts was mainly due to native anthocyanins, which were converted into derived pigments by reaction with yeast metabolites, resulting in a limited loss of colour during fermentation.

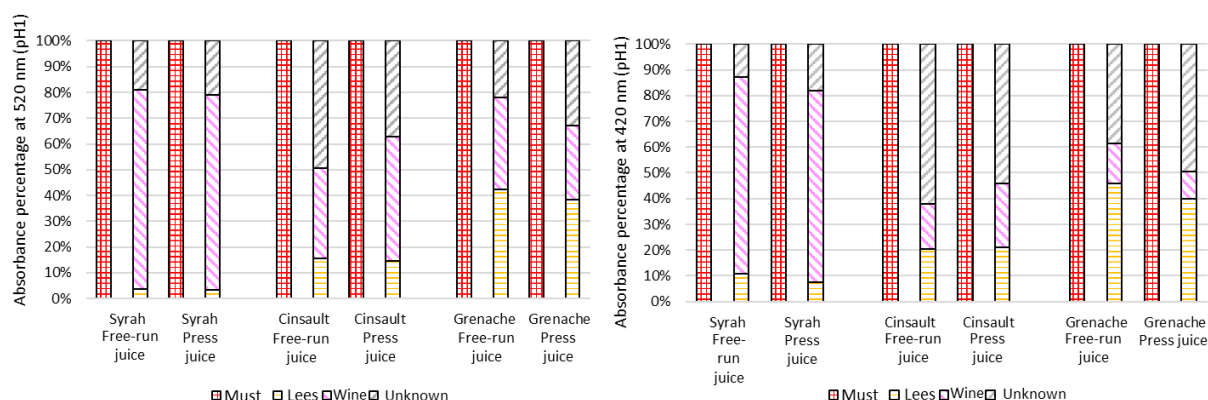


Figure 8: Absorbance at 520 nm (red) and 420 nm (yellow) of musts, wines and yeasts (pigments desorbed from lees) from three contrasting grape varieties at two extraction levels.

The pigments adsorbed by the yeast walls represent less than 5% of the colour of the final wine for Syrah, whereas they represent more than 50% for Grenache and Cinsault wines (Figure 8). The colour of rosé wine therefore depends on the level of extraction, the state of oxidation at the must stage and the grape variety, all of which determine the composition of the must and, consequently, the reactions and interactions of the phenolic compounds that occur during fermentation.

This work was the subject of a publication entitled "Multi-method study of the impact of fermentation on the polyphenol composition and color of Grenache, Cinsault, and Syrah rosé wines" in the journal *Food Chemistry* in 2023 (Leborgne et al., 2023).

4. Fate of pigments during post-fermentation stages

4.1 Impact of oenological inputs before packaging: sulphite adding, fining

The aim was to study the impact of different fining additives on the phenolic composition and colour of rosé wines. To achieve these objectives, two rosé wines made from grape varieties with different phenolic compositions (Grenache and Syrah) were produced and preserved after fermentation with two different doses of sulphite. After characterising the influence of grape variety and sulphite dose on the phenolic composition and colour of the wines in the first part of the study, the work was then devoted to studying the impact of fining. Two types of fining at two doses were tested on the four wines in order to compare their effectiveness and understand their influence on phenolic composition and colour.

The analyses initially revealed differences between the grape varieties (quantity and proportion of the different forms of anthocyanins, different HPSEC (*High Performance Size Exclusion Chromatography*) profiles) that had been produced using the same technical itinerary. These differences have an impact on



the colour observed by the naked eye. Syrah has a pronounced pink colour, whereas Grenache has an orange colour. These differences are due to the initial phenolic composition of the wines, which is linked to the initial composition of the must before vinification.

Grenache had virtually no native anthocyanins at the time of analysis. The analyses carried out on the musts showed that these concentrations were already very low for this grape variety. The wine also lost a significant proportion of its colour intensity during vinification, regardless of the dose of SO₂. This indicates the presence of chemical reactions leading to these losses: the formation of colourless compounds or compounds with different extinction coefficients.

Developments have been very different for Syrah, a variety where the colour is much more resistant, with or without sulphites.

Clarifying agents are used to improve wine stability and modulate wine colour by binding and precipitating phenolic compounds. In a second study, four different clarifying agents were investigated (Figure 9): two plant proteins, from potato (patatine) and pea, an animal protein, casein, and a synthetic polymer, polyvinylpolypyrrolidone (PVPP). The impact on the colour and phenolic composition of the wines was analysed. CIELab parameters were calculated to define the colour: L* transparent, a* red, b* yellow.

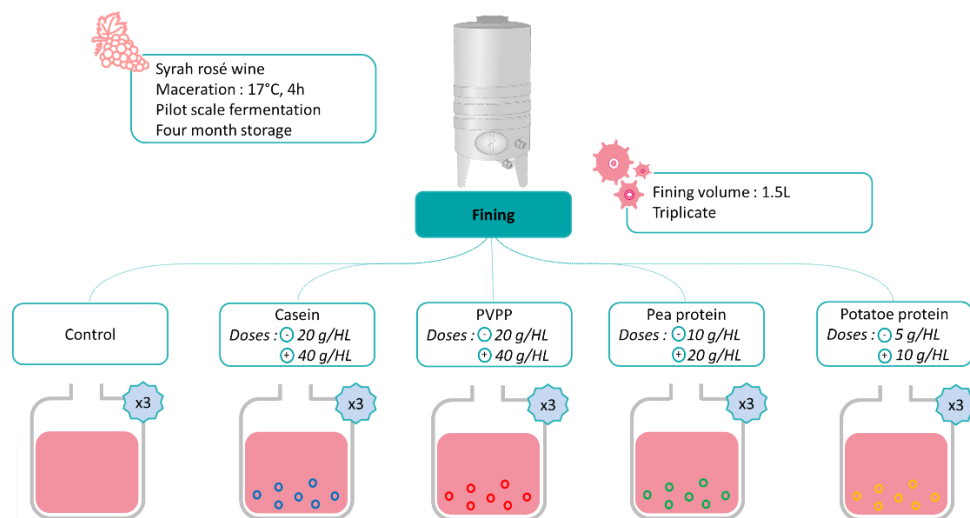


Figure 9: Bonding test protocol (Leborgne, 2021)

Fining tests highlight differences in behaviour between PVPP and other fining agents. It has been shown that PVPP is the sizing agent that generally has the greatest impact on pigments and colour, particularly on the flavylum form of anthocyanins, and therefore the red colour (parameter a* of the CIELab system) (Figure 10a). PVPP also has a strong impact on the yellow component of the colour (parameter b* of the CIELab system). This fining treatment therefore interacts strongly with a wide range of coloured compounds, as do casein and pea protein in lesser quantities (Figure 10). Conversely, patatin showed a particular affinity for yellow pigments (Figure 10b). These pigments turned out to be polymeric compounds that were difficult to analyse.

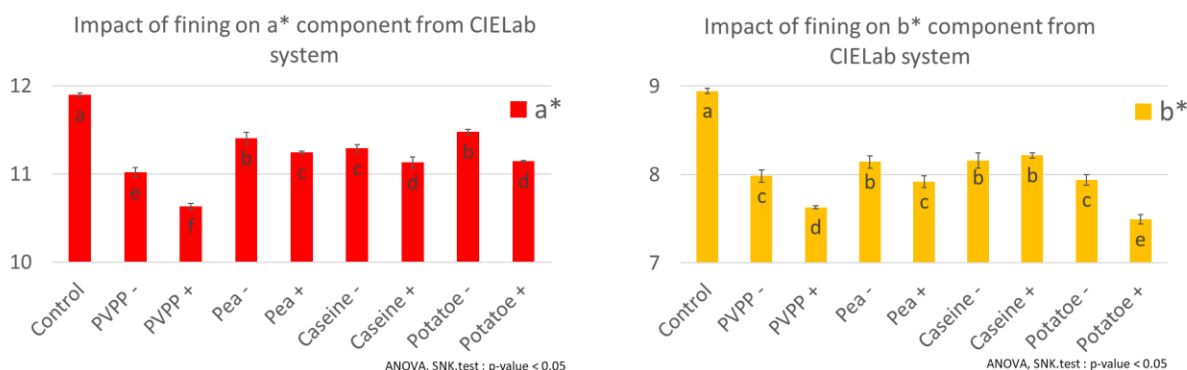


Figure 10: Impact of fining on the red (a*) and yellow (b*) components of the CIELab system (Leborgne, 2021)

4.2 Impact of physical and chemical parameters on the stability of wines over time after packaging: Temperature, sulphites, exposure to light

Two Grenache and Syrah wines were subjected to different storage conditions for 6 months, varying the level of sulphite, temperature and exposure to light. The wines were all bottled in standard 75 cL clear glass bottles.

Each method was carried out in triplicate. Analyses were carried out after 6 months' storage: colour, HPSEC on wine, HRMS (*High Resolution Mass Spectrometry*) composition.

The results obtained on pigment composition are in line with the literature. The effects of grape variety and temperature are strong, while the effects of exposure to light and sulphite dose are weak (Figure 11).

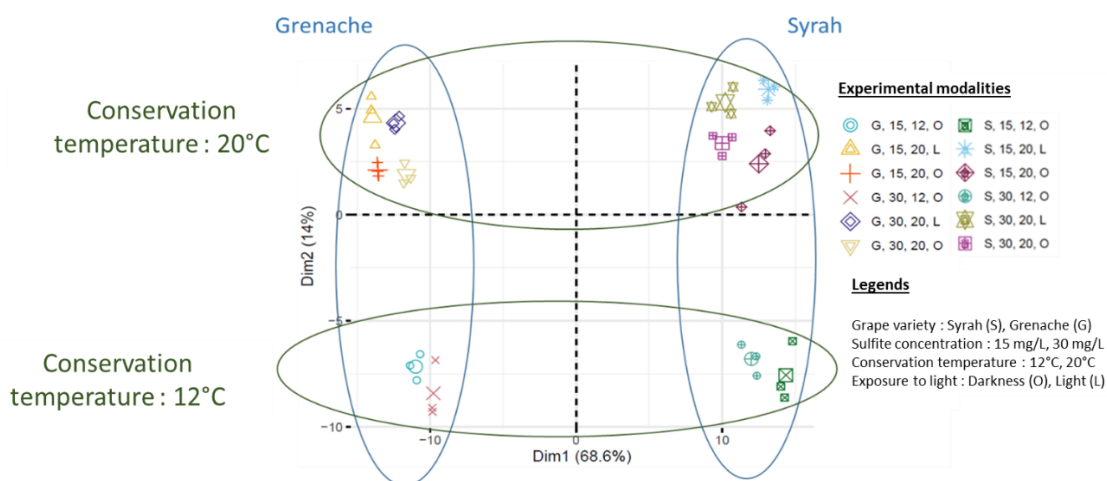


Figure 11: Representation of individuals on the first two axes of the Principal Component Analysis carried out on the chemical composition data acquired, illustrating the effects of temperature, sulphites and exposure to light on wines from Grenache and Syrah grape varieties.

From the point of view of producers' expectations, the programme confirms that conservation is optimal at the lowest temperature, at the highest level of sulphites concentration and in the dark. The compounds involved still need to be identified in more detail. Detailed publication of these results is scheduled for 2024 in the French journal of oenology.



5. Dissemination and transfer tools

These tools, developed by the Centre du Rosé, are intended for communication with professionals, to act as an interface to help them understand these complex subjects more simply. They have been used to communicate about the project and its results in Provence and at national level by the Centre du Rosé, IFV and INRAE. These tools will also be impacted in the short term by the results of the project, particularly as regards the reference colour chart.

5.1 The reference paper colour chart

The colour chart is available in various formats, including paper. This tool is usually used in the laboratory or sensory analysis room to characterise the colour perceived by tasters (Figure 12). A laminated educational version, which is easier to handle and more resistant, has been developed for use in the field, particularly in production cellars or for communicating product characteristics to consumers.



Figure 12: <https://centredurose.fr/nuanciers-vins-roses/> paper reference colour chart and fan colour chart

To ensure that as many winegrowers and winemakers as possible are able to make use of the results, it would be useful to translate them visually by positioning them on the reference colour chart. Ideally, a modelling tool could be used to position the initial colour of the must or wine and the proportion of different grape varieties, and then, depending on the planned itinerary, to display a "forecast path" for the colour between different boxes on the colour chart.

Figure 13 shows the changes in colour observed by the Centre du Rosé during vinifications carried out on 7 different estates and positioned on the original colour chart.

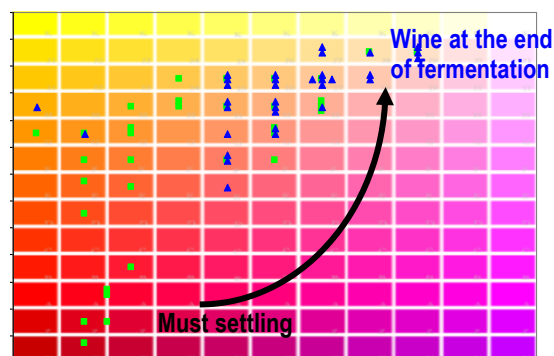


Figure 13: Changes in colour during vinification at 7 winemaking sites

Even if the intensity of the colour tends to decrease, it is not possible to accurately predict the trajectory of the colour during fermentation solely on the basis of the colour of the initial must. The PigRosé project

has enabled us to acquire a level of knowledge that allows us to better predict the evolution of this colour on the basis of different indicators in the molecular composition.

Another condition for rigorously achieving this objective would be to have elements that would enable the $L^*a^*b^*$ coordinates to be correlated with a colour perceived on the rosé wine colour chart, which is not currently the case. The colour chart was created empirically on the basis of colours perceived by the eye.

To compensate for the lack of correspondence between the colours perceived on the original colour chart and the $L^*a^*b^*$ coordinates measured, the Centre du Rosé undertook standardisation work with the scientific support of a colour specialist who monitored the thesis work. To this end, a Rosé Chair was created in partnership with Kedge Business School and the Centre du Rosé, and funded by the Comité Interprofessionnel des Vins de Provence.

The work carried out in the PigRosé programme has contributed to the development of a new colour chart, in particular by making contact with NCS (Natural Color System), which produces one of the two standardised universal colour charts. This partnership to develop new wine colour charts will make it possible to validate colour labels by grape variety and to use standardised references to predict wine colour from must (Figure 14).

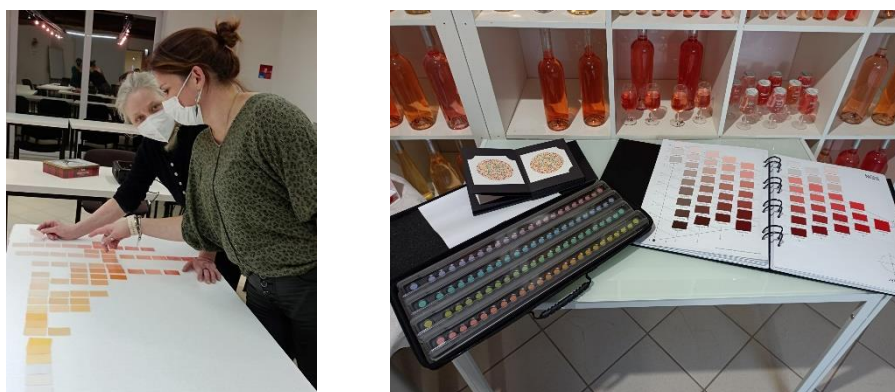


Figure 14: Creation of a new colour chart for rosé wines based on standard NCS (Natural Color System) colour labels.

5.2 Educational gel colour charts

New colour charts developed in 2019 and tested in the field by the Centre du Rosé have been available since early 2020. The colours have been adjusted on the gel formats to better reflect the reality of the wines marketed today (Figure 15). Other more traditional teaching aids, such as photographs of the various colour charts, have also been used to communicate about colour.



Figure 15: Gel colour chart for tubes and glasses

6. Conclusion

Rosé wine requires a high level of technical expertise to make. This exploratory work on the pigments in rosé wines was essential to lay the foundations for going beyond simple colour indicators and exploring the subject right down to the molecular level. The results of the PigRosé project will have an impact on



the control of winemaking, particularly in fermentation and conservation, but also on the analysis of commercial wines by giving initial indications of the specific characteristics of grape varieties and processes. All technologies and inputs will now be able to be questioned on the basis of this work, in particular other grape varieties and innovative varieties, but also the impact of the diversity of micro-organisms used in the process. Finally, the desire to gain an understanding of the fine mechanisms that determine the colour of rosés and the success of the collaboration undertaken in PigRosé to gain access to more advanced tools have stimulated the drive to increase expertise in the composition of the product. A similar strategy on the question of aromas has been initiated by the Centre du rosé, with a thesis due to start in 2021 in partnership with Nice Côte d'Azur University.

Ethics

The authors declare that the experiments were carried out in compliance with the applicable national regulations.

Declaration on the availability of data and models

The data supporting the results presented in this article are available on request from the author of the article.

Declaration on Generative Artificial Intelligence and Artificial Intelligence Assisted Technologies in the Drafting Process.

The authors used artificial intelligence in the translation process from French to English.

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Conceptualisation: G.M., A.C., A.V., V.C. and J.-R.M.; data acquisition: C.L. and M.-A.D.; writing - preparation of original version, C.L. and A.C.; writing - revision and editing: M.-A.D., M.M., A.V., J.-R.M. and V.C.; supervision: A.V., N.S., J.-R.M. and V.C.; project administration and acquisition of funds: G.M., A.C., A.V., J.-R.M. and V.C. All authors have read and approved the published version of the manuscript. Please refer to the CRediT taxonomy for an explanation of the term. Authorship should be limited to those who have made a substantial contribution to the work reported. All authors have read and approved the published version of the manuscript.

Declaration of interest

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