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UNIVERSITÀ DEGLI STUDI DI UDINE
Department of Food Science

Master of Viticulture and Enology

Effect of different fermentation management practices on the composition of the phenolic fraction of Cabernet Franc wines produced in North-East Italy

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Abstract

In the present thesis were studied four different vinification protocols, connected with different fermentation management practices in order to evaluate possible alternative process to improve the organoleptic qualities of the variety Cabernet Franc from the area DOC Colli Orientali.

It was found that the type of yeast and lactic acid bacteria which are involved or are inoculated, were determine the profile of the aromatic wine and the stability of the final product.

Refer to the results of wine measurements; No differences were found among the samples concerning total phenolics, intensity, and the color hue of the samples. It was clear that the higher amount of anthocyanins was concentrated in two the samples.

The results of this study show that the use of commercial strains gave the best results, concerning both the concentration of color compounds and tannins and the intensity of fruity aroma. Differences in color, were perceived from the analytical point of view, rather than from the sensory one, confirming that also spontaneous fermentation gave good results in the experimental conditions tested.

Finally, it was obviously that the choice of yeast and bacteria inoculums, affects wine aroma composition and sensory properties but further experiments will be useful to enhance this information between the differences in spontaneous and non spontaneous fermentation.

Key words: spontaneous alcoholic fermentation, spontaneous malolactic fermentation , organoleptic qualities, aromas

Περίληψη

Στην παρούσα διατριβή μελετήθηκαν τέσσερα διαφορετικά πρωτόκολλα οينوποίησης, που συνδέονται με διαφορετικές πρακτικές διαχείρισης της ζύμωσης, προκειμένου να αξιολογηθούν οι πιθανές εναλλακτικές διαδικασίες για τη βελτίωση των οργανοληπτικών ιδιοτήτων της ποικιλίας Cabernet Franc από την περιοχή DOC Colli orientali.

Διαπιστώθηκε ότι ο τύπος της ζύμης και βακτηρίων γαλακτικού οξέως τα οποία εμπλέκονται ή εμβολιάστηκαν είχαν καθοριστικό ρόλο στο αρωματικό προφίλ του οίνου και τη σταθερότητα του τελικού προϊόντος.

Όσον αφορά τα αποτελέσματα των μετρήσεων δεν βρέθηκαν διαφορές μεταξύ των δειγμάτων που αφορούν τα ολικά φαινολικά, την ένταση, το χρώμα και την απόχρωση των δειγμάτων. Ήταν σαφές ότι η μεγαλύτερη ποσότητα ανθοκυανινών συγκεντρώθηκε σε δύο δείγματα.

Τα αποτελέσματα αυτής της μελέτης δείχνουν ότι η χρήση των εμπορικών στελεχών έδωσε τα καλύτερα αποτελέσματα, όσον αφορά τόσο τη συγκέντρωση του χρώματος, τις τανίνες και την ένταση του φρουτώδους άρωματος. Οι διαφορές στο χρώμα, εντοπίστηκαν από την αναλυτική άποψη, και όχι από τον οργανοληπτικό έλεγχο, επιβεβαιώνοντας επίσης ότι η αυθόρμητη ζύμωση έδωσε καλά αποτελέσματα στις πειραματικές συνθήκες που εξετάστηκαν.

Τέλος, ήταν προφανές ότι η επιλογή της ζύμης και των βακτηρίων, επηρεάζει τη σύνθεση άρωματος του κρασιού, αλλά περαιτέρω πειράματα θα ήταν χρήσιμα για την ενίσχυση αυτών των πληροφοριών μεταξύ των διαφορών στην αυθόρμητη και μη αυθόρμητη ζύμωση.

Λέξεις κλειδιά : αυθόρμητη αλκοολική ζύμωση, ελεγχόμενη αλκοολική ζύμωση, αυθόρμητη μηλογαλακτική ζύμωση, αρωματικό προφίλ.

To my mother,

Acknowledgements

The preparation of this thesis would not have been possible without the support, hard work and endless efforts of a large number of individuals and institutions.

My heartfelt thanks to the University of Udine and to its faculty for equipping me all the tools and skills I needed for my thesis.

I am grateful to my supervisor, Prof. Piergiorgio Comuzzo, whose expertise, understanding, generous guidance and support made it possible for me to work on a topic that was of great interest to me. It was a pleasure working with him.

I am hugely indebted to my Erasmus supervisors Prof. Roberto Zironi and Prof. Lucilla Iacumin for accepting me in university of Udine and for being ever so kind to show interest in my research.

I would like to express my gratitude to my professors of University of Athens, Prof. Stamatina Kallithraka and Prof. Kotseridis Yorgos for encouraging me to travel abroad for my research and also their kind help to any problem.

Thanks also to my colleagues Alexandra Vitou , Federico Rosini and Panagiota Vassileiou who helped me and became part of my study.

Lastly, I would like to thank my family and friends. My father, for understanding and helping me overcome all the problems I had in the previous year and not letting me give up. I am most grateful to my mother, who had always helped and encouraged me to pursue my own goals.

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UNIT 1: INTRODUCTION

1.1 Red Wine Production

Red wine is a type of wine made from dark-colored (black) grape varieties. The actual color of the wine can range from intense violet, typical of young wines, through to brick red for mature wines and brown for older red wines.

The stylistic differences are based on differences in wine characteristics such as grape variety, color, flavor, body, mouth feel, and aging potential. The styles range from simple, fruity, fresh, light colored blushes and rosés to complex, full-bodied, rich and dark red, with long aging potential. Many factors such as a variety, soil, climate, growing conditions, and viticultural practices influence the fruit composition, and therefore, the style of wine that can be produced. In addition to fruit composition, winemaking techniques also play an important role in determining the wine style.

General speaking, it is easier to produce a low-input red wine than a white one. Red wines often have higher alcohol than whites and their tannins play a double role of antimicrobial and antioxidant agents. The modern consumer is looking for red wines with a smooth palate, low astringency and ripe fruit aroma and the presence of off-flavors can drastically reduce competitiveness of the wines in the market. These consumer demands are pushing wine-makers to look for a full maturity of grape in order to obtain intense varietal fruitiness, absence of vegetal notes and softer tannins. A side effect of this trend is the general increase of pH in red wines, which requires more attention to the management of spoilage micro-organisms.

There are many available varieties of red wine production. The wines are usually produced as varietals, or as blends containing several varieties. A list of commonly used red wine varieties is given in Table 1.

Table 1: Red wine varieties

Vinifera Group	Labrusca	French hybrids	Others
Cabernet Sauvignon	Concord	Baco Noir	Northon/Cynthiana
Merlot	Steuben	Chambourcin	St. Vincent
Pinot noir		Chancellor	Vincent
Zinfandel		Foch	
Syrah (Shiraz)		Rougeon	
Grenache		Villard noir	
Cabernet Franc		Colobel	
Barbera			
Gamay			

Varieties from the Vinifera group are most widely used for winemaking. In regions where Vinifera grapes are not grown, French hybrids, Labrusca, and other varieties are often used. Among the Vinifera group, Cabernet Sauvignon alone, or in combination with Merlot and/or Cabernet Franc is used in premium red wine production. Pinot noir, the famous grape of Burgundy, makes excellent red wine. When grown in other parts of the world, the wine does not always attain the same level of quality as found in Burgundy.

The juice from most purple grapes is greenish-white; the red color comes from anthocyan pigments (also called anthocyanins) present in the skin of the grape; exceptions are the relatively uncommon varieties, which produce a red colored juice.

Much of the red-wine production process therefore involves extraction of color and flavor components from the grape skin.

So anthocyanins are the red pigments found in the skin cells that produce the red color in red wines. Tannins are polymerised catechin molecules that produce the astringency in wines which gives the wines 'mouth-feel' or 'structure'. Roughly speaking, the smaller the tannin, the more bitter it is, but if they become too large, they stop being dissolved in wine and form a precipitate at the bottom of the bottle.

Also, anthocyanins can react with tannins and form more stable color compounds in young wines. Over time (months and years) these compounds polymerise (join together) further and precipitate out.

In fact, red wines contain phenolic compounds, a large number of which may act as antioxidants, with mechanisms involving both free radical scavenging and metal chelation (Chimi et al., 1991; Iwahashi et al., 1990; Frankel, 1993; Nardini et al., 1995).

Phenolic compounds are also important group of wine constituents, and they greatly contribute to the sensory properties by affecting the color and taste (Cyzowska & Pogorzelski, 2002). These compounds are natural antioxidants, and possessing neuroprotective and potent cancer-preventive properties, which are considered as beneficial and proved (Lee, Hur, Lee, & Lee, 2005; Yoo, Al-Farsi, Lee, Yoon, & Lee, 2010). Wine, in comparison with other sources, contains relatively high amounts of highly diversified polyphenols. Most of the phenolic compounds pass from berry to the wine during extraction and fermentation, and a few are newly formed, such as some free phenolic acids and flavonic isomers (Wulf & Nagel, 1980).

As a result, the quality and characteristics of the phenolic compounds in the vintage vary according to the grape variety and its maturity. Some phenolics may be; highly coloured, stable, soft, full. Others may be; herbaceous, bitter, astringent. Much of the art of red winemaking is in controlling the extraction and development of grape phenolics.

One of the possibilities of the occurrence of new polyphenols in wine may be transformation of substances contained in fruit. Enzymes of fruit origin and microorganisms responsible for fermentation may lead to oxidation or hydrolysis of native components (Nagel & Wulf, 1979), therefore, when fermenting the same must, the employment of whatever yeast strains are straightly associated with the composition and content of polyphenols in the final product.

Another important key is that, in red wines oxidation is less common phenomenon than in whites. Tannins consume significant amounts of oxygen which are required in the polymerization which results in more stable pigments and soft polyphenols. Dissolution of oxygen also reduces the appearance of reduced odors. This oxygen presence must be controlled as an excess can cause a loss of color and aroma. In some varieties, poor in red pigments, oxygen can cause significant loss of color and consequent depreciation of the wine.

Finally, aroma is one of the most important quality factors of wine and is one of the key determinants of consumer acceptance (Lockshin & Corsi, 2012; Rapp, 1998; Saénz-Navajas, Ballester, Pêcher, Peyron, & Valentin, 2013). Wine aroma is a complex sensory characteristic that is determined by more than 1300 volatile compounds, including alcohols, esters, acids, aldehydes, isoprenoids, lactones and ketones, with a wide concentration range (Villamor & Ross, 2013).

In the aroma of wines terpenes play an important role, being a group of flavor compounds characteristic for specific grapes used for wine production. Terpene compounds in wine are prone to changes during winemaking process. Especially monoterpene alcohols can undergo several reactions during wine production and storage, induced by the time of storage, relatively low pH and a presence of compounds that can interact with them. Monoterpene alcohols can easily isomerize and oxidize, forming oxides and aldehydes. Monoterpenes occur in grapes and wines in a free form, however it is known that majority of them are also bound with sugars as glycosides

The type and concentration of these volatile compounds are responsible for the characteristic aroma of wine. In particular, concentration usually explains variation in aroma between certain types of wine which contain the same volatile compounds (Boido et al., 2003). Differences in the aromatic profile of wines are determined by changes in the type, proportion and concentration of these volatile compounds (Atanasova et al., 2005).

And so, red winemaking (picture 1) can be broken down into 4 main steps:

- Harvest, Pre-fermentation processing
- The alcoholic fermentation with skin contact and phenolic extraction
- Draining and pressing
- The malo-lactic fermentation (MLF)

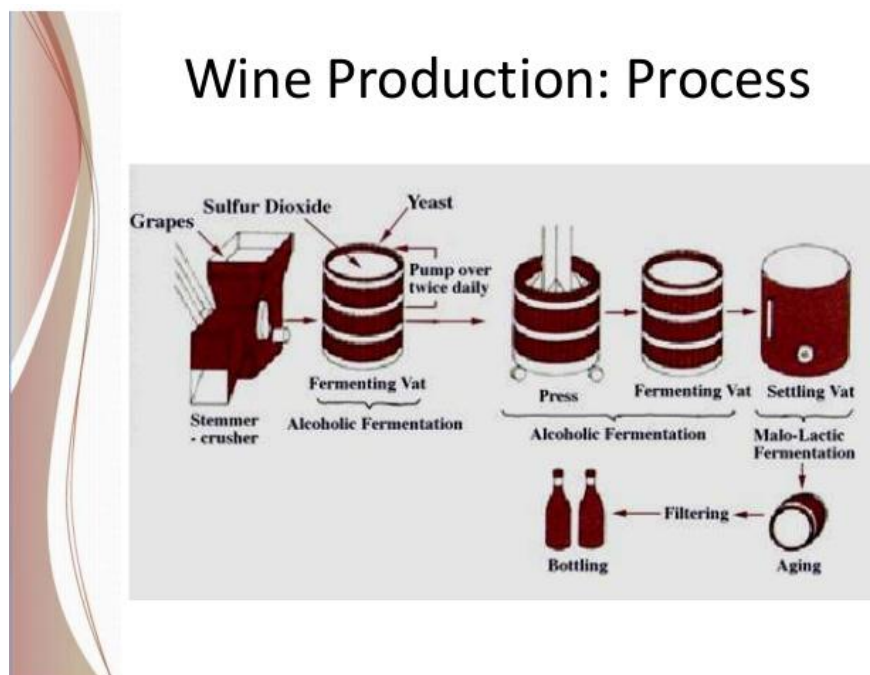


Figure 1: Process of red wine production

1.1.1 Harvest

The decision to harvest grapes with certain maturity parameters is guided by many factors. These include wine style, variety, and maturity criteria. Typically during the course of maturation sugars accumulate, titratable acidity declines, pH rises, color, and phenolic compounds increase and the formation of distinct varietal aroma components occurs. It would be highly desirable to have all these parameters in an ideal balance, in practice this can be difficult to achieve since these parameters are influenced by many factors.

An important prerequisite for optimal wine quality is the optimal physiological maturation of the grapes which is dictated by the grape variety, the environmental and climatic conditions as well as the type of wine that the wine-maker wants to produce. Thus a perfect knowledge of véraison conditions (the optimal relationship between sugar, acid content and pH of the juice as well as the color of the berries, the smell and taste of the grapes and juice) will allow the vine-grower to organize the harvest according to the various grape maturity periods.

The fruit is harvested based on sugar (^oBrix), acidity, and pH. It should be noted that for making red wine, following only these harvest criteria is not sufficient. Skin constituents such as color, tannins, and flavor strongly influence red wine character and, therefore, their level should also be evaluated when making harvest decisions. Because the skin is fermented with the juice, the skin condition and the proportion of skins to juice are also important considerations. The grape crop should be harvested by hand or mechanically.

Different methods are adapted of each winery to transport the harvest from grapevine to winery. The most common practice of handling harvested grapes is to separate the berries from the stems. This is achieved by using the machine called a stemmer/crusher. In most vineyards, the harvest is transported in shallow trailers or trucks. Whatever the container capacity, the grapes should be transported intact without being crushed.

Generally, red grapes can be harvested during the higher day temperatures. However red grapes are certainly less sensitive to maceration and oxidation phenomena than white grapes and microbial contamination is likely to occur in a partially crushed harvest, especially in the presence of sunlight. These risks must be avoided.

For this reason, the harvest transport is determined by the organization of harvest work (harvesting by hand or mechanical) and the winery's equipment. From the quality and wine-making viewpoint the grapes should arrive at the winery immediately. If necessary the grapes and the must should be protected from oxygen and microbial infection by using SO₂, carbon dioxide or dry ice.

1.1.2 Alcoholic fermentation

Alcoholic fermentation is an important step of the wine-making process. The process of fermentation in winemaking turns grape juice into an alcoholic beverage. During fermentation, yeasts transform sugars present in the juice into ethanol and carbon dioxide (as a by-product).



Sugar \rightarrow ethanol + carbon dioxide

The most important sugars in grape juice are the two six-carbon sugars glucose and fructose. These are the sugars that make the juice sweet and are fermented to alcohol by the yeast. In addition, small quantities of pentoses (five carbon sugars) and pectins (galacturonic acid polymers) are found.

The driving-force behind this reaction is the release of energy stored in the sugars to make it available to other biological processes. The biochemical process of fermentation itself creates a lot of residual heat which can take the must out of the ideal temperature range for the wine. In aerobic conditions, the reaction can proceed further and convert the ethanol to H₂O and CO₂, releasing all of the energy present in the original sugars (Figure 2).

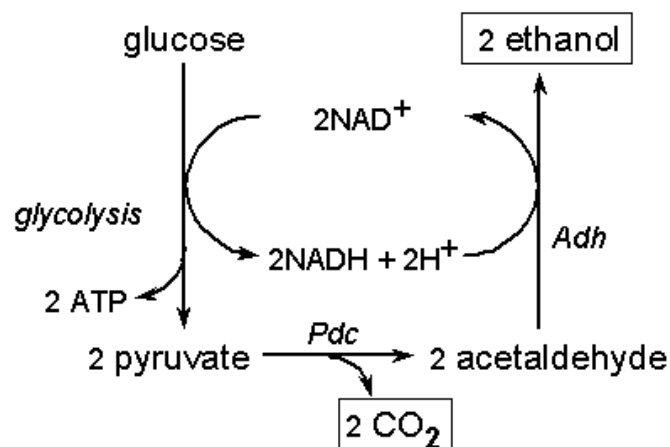


Figure 2: Alcoholic fermentation

In winemaking, the temperature and speed of fermentation are important considerations as well as the levels of oxygen present in the must at the start of the fermentation. The risk of stuck fermentation and the development of several wine faults can also occur during this stage.

During fermentation, there are several factors that winemakers take into consideration, with the most influential to ethanol production being sugar content in the must, the yeast strain used, and the fermentation temperature.

The promotion of a healthy and fast development of good wine yeasts drastically reduces the risks of oxidation and microbial contamination without the addition of inputs. A healthy and suitable yeast population at the end of alcoholic fermentation offers different options of “on lees” practices, with direct favorable effects on wine quality and indirect advantages in terms of protection from oxygen.

Typically, white wine is fermented between 18-20°C (64-68°F) though a wine maker may choose to use a higher temperature to bring out some of the complexity of the wine. Red wine is typically fermented at higher temperatures up to 29°C (85°F). Fermentation at higher temperatures may have adverse effect on the wine in stunning the yeast to inactivity and even "boiling off" some of the flavors of the wines. Some winemakers may ferment their red wines at cooler temperatures, more typical of white wines, in order to bring out more fruit flavors.

Optimizing the control of alcoholic fermentations for winemaking is a difficult challenge. Unlike industrial fermentations, such fermentations do not aim to maximize the concentration or yield of a defined metabolite, or the productivity of the process. In winemaking, the main objective is to optimize product quality, which is very difficult to quantify. Wine tasting remains the best way to assess the characteristics of wine, but is difficult, imprecise and time-consuming (Tominaga & Dubourdieu, 2000).

The control of technological parameters, such as sugar exhaustion, the duration of the fermentation and the amount of energy required to regulate

fermentation temperature, is also of interest. Many works have shown that fast fermentations may be detrimental to wine quality, especially for white wines (Francis & Newton, 2005).. On the contrary, too long a fermentation both delays the subsequent processes and increases the risks of wine damage.

Today, most wine is produced using selected commercial strains of *Saccharomyces* sp and even small wineries select yeasts from their own environment for use as starter cultures. *Saccharomyces cerevisiae* is the main yeast used in winemaking, due to its high fermentation capacity. In spontaneous fermentations, other yeast species may affect the fermentation process and wine characteristics, but these effects are variable and difficult to predict. More than 200 different *S. cerevisiae* strains are currently available commercially, with highly diverse fermentation properties.

Certain criteria need to be met in order to guarantee the desirable features of the yeast strains selected. The most important of these are: tolerance to ethanol; exhaustion of sugar potential and high fermentation activity; growth at high sugar concentrations; resistance to, and low production of, sulfur dioxide; low production of hydrogen sulfide and low volatile acidity; resistance to killer toxin; good enzymatic profile (Nikolaou, Soufleros, Bouloumpasi, & Tzanetakis, 2006). All these characteristics should go together with adequate flavor wines (Lambrechts & Pretorius, 2000).

Generally, yeast strain impacts on fermentation .Colombie, Malherbe, and Sablayrolles (2005) observed only moderate differences in a comparison of 20 randomly chosen commercial strains (i.e. without taking their fermentative capabilities into account) cultured in an easily fermented synthetic medium. Much larger differences were reported by Blateyron and Sablayrolles (2001), in a comparison of 13 randomly chosen strains cultured in a difficult-to-ferment must (natural must leading to sluggish or stuck fermentations).

The choice of strain used by the winemaker is increasingly motivated by the potential impact of that strain on the wine characteristics. The very large number of strains commercially available, and the many complex mechanisms of interaction between strain, must and fermentation conditions make this choice difficult. The potential of strains to increase the geographical typicity of a wine remains a matter of debate, but specific strains are now widely recognized to be useful: (i) for increasing the fruity character, (ii) for improving some varietal characters in Sauvignon (Dubourdieu, Tominaga, Masneuf, Peyrot des Gachons, & Murat, 2006) and Chardonnay (Eglinton et al., 2000) wines and, more generally, for increasing the expression of varietal characters by the hydrolysis of glycoside-bound volatile compounds during fermentation (Ugliano, Bartowsky, McCarthy, Moio, & Henshke, 2006), (iii) for limiting the production of organic acids or increasing the production of glycerol (Scanes, Hohmann, & Prior, 1998) and (iv) for limiting off flavours, including those due to sulphur (Rauhut, 1993) and volatile phenols (Shinohara, Kubodera, & Yanagida, 2000). Some authors have also highlighted the value of specific strains for producing mannoproteins (Moine-Ledoux & Dubourdieu 2002), and for improving the color of red wines through their interactions with polyphenolic compounds (Medina, Boido, Dellacassa, & Carrau, 2005).

On the other hand, many commentators report that they feel that spontaneous fermentation promotes better mouth feel in wines. That is, the wines are thought to be softer and creamier than those made using single strain starter cultures. This suggestion however has not been conclusively demonstrated scientifically. The rationale for the suggestion is that the natural yeast flora on the grape is genetically heterogeneous, i.e. consists of multiple strains and that is the reason of the improved mouth feel. Moreover, spontaneous fermentation costs nothing to initiate. It is obviously that spontaneous fermentation has some disadvantages like long time and higher probability of spoilage.

Taking into consideration all these above, each winery choose spontaneous or non spontaneous fermentations. But in the last 30 years, the wine industry has tended to move away from spontaneous fermentations towards controlled fermentations initiated by inoculation, which are more reliable (70–80% of fermentations are now initiated by inoculation in France, for example).

1.1.3 Phenolic compounds

Phenolic compounds appear as the grape changes colour, substituting the chlorophyll. They are of great oenological importance and play a key role in determining the quality of the wine. Along with their nutritional and pharmacological properties they also account for characteristics like colour, aroma, taste and astringency (Bartolome, Nunez, Monagas, & Gomez-Cordoves, 2004; Harborne & Baxter, 1999). Their antioxidant properties also have positive effects on a wine's stability (Waterhouse, 2002). The total content of polyphenols is also a clue as to whether the wine can be aged.

Grapes of the *Vitis* type are relatively rich in phenolic compounds compared to other fruits. The grape essentially contains non flavonoid compounds in the pulp and flavonoid compounds in the skin , seeds and stems. It is estimated that seeds contain 65% of the polyphenols of the bunch, the stem 22%, the skin 12% and the pulp just 1% (Hidalgo Togores, 2003).

As it is already known, phenolic compounds are crucial components of red wines, which strongly affect a series of wine sensorial characteristics including color, flavor, palate fullness and body, as well as bitterness and astringency (Jaitz et al., 2010; Rastija, Srecnik, & Medic-Šaric, 2009). This group of chemicals is mainly composed of non-flavonoids, such as phenolic acids, stilbenes, and flavonoids including proanthocyanins, flavonols, flavan-3-ols and their condensed proanthocyanidins. As the

main pigments having hues from orange to purple, anthocyanins play an important role in the color formation of red wines.

On the one hand, the monomeric anthocyanins are the main contributors for the color of young red wines. They are responsible for diverse colors such as red, blue and purple, which are dependent on the wine pH values that could affect the equilibrium between different chemical forms of anthocyanins (Wrolstad, Durst & Lee, 2005). On the other hand, the polymeric anthocyanins, which are the major colourants in the aged wines, are considered to be more stable than the monomeric anthocyanins (Ivanova et al, 2011). It is well known that aldehydes in red wines play a significant role in the modification of anthocyanins, participating in the formation of pyranoanthocyanins and polymeric anthocyanins. Nevertheless, during the fermentation and ageing, many factors, such as wine pH, alcohol, SO₂, temperature, light and metal ions etc, have been found to be able to affect the concentration of anthocyanins (Alcalde-Eon, Escribano-Bailon, Santos-Buelga, & Rivas-Gonzalo, 2006; Marquez, Serratos, Lopez-Toledano, & Merida, 2012; Mateus, Pascual-Teresa, Rivas-Gonzalo, Santos-Buelga, & Freitas, 2002).

The phenolic content of red wines depends on many factors such as grape variety, growing conditions, canopy and crop management, yearly climatic variations, harvest time and winemaking methods (Cliff, King, & Schlosser, 2007; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999). It has been reported that a thermal flash release process before fermentation can not only result in an increased ratio of tannin to anthocyanin and an increased conversion of anthocyanins to tannin-anthocyanin polymers showing the same color properties as anthocyanins, but also an increased stability of wine during ageing (Morel- Salmi et al. , 2006).

It is worthwhile to mention that the phenolic compounds are an integral part of human diet and are considered to be non nutrient biologically active compounds (Subramani, Casimir & Krewer, 2002). Being polyphenolic compounds, flavonoids are able to act as antioxidants through a mechanism that removes free radicals and by chelation of metal ions

(Sellappan, Akoh & Krewer , 2002). The growing evidence for the role of radicals and antioxidants in health and ageing ant the importance of wine in the Mediterranean diet has aroused much interest to these compounds, A wide range of studies has shown that the antioxidant properties of these compounds can offset atherosclerosis and coronary heart disease whilst showing selective cytotoxicity to breast cancer cells (Obrenovich et al. 2011; Walter et al.,2008; Xiang et al., 2014).

1.1.4 Maceration

Maceration is the winemaking process where the phenolic materials of the the grape are leached from the grape skins, seed and stems into the must. To macerate is to soften by soaking , and maceration is the process by which red wine receives its red color.

The main concern in maceration is to be as selective as possible. In addition to positive compounds like anthocyanins, polysaccharides, aromas and some minerals, unripe skins can also release harsh tannins, herbaceous notes, abnormal acidity and mouldy grapes can be source of oxidative enzymes, glucans and unpleasant aromas.

There are actually several different types of maceration processes. The three most common are the extended maceration, cold soak and carbonic maceration.

Maceration may take place either before or during fermentation. The process begins as soon as the grapes skins have been ruptured. At this time the juice is released from inside the grape and comes in contact with the exterior of the grape skins as well as the stems. To end maceration simply , the skins , seed and stems are removed from the must.

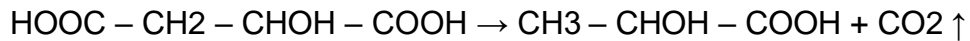
1.1.5 The Malolactic fermentation (MLF)

Malolactic fermentation is a secondary wine fermentation, carried out by malolactic bacteria, and is one of the main stages in the elaboration of red wines. . The word malolactic come from the conversion of L-malic acid in to L-lactic acid, the action occurs naturally or by adding bacteria.

During this conversion, CO₂ is also produced. Thos results in a natural decrease in total acidity and bacterial stability. Malolactic bacteria are capable of direct decarboxylation of malic acid to lactic acid by the enzyme malate carboxylase, which is present in various lactic acid bacteria, but particularly in three genera : *Lactobacillus*, *Leuconostoc* and *Pediococcus*. (Ugliano and Moio, 2005; du Toit et al., 2011) One of the main species identified during spontaneous malolactic fermentation is *Oenococcus oeni*

since it is the most tolerant to adverse wine conditions (Lonvaud-Funel, 1999).

So, the reduction in acidity is due to the fact that malic acid has two acid radicals (-COOH), while lactic acid only has one:



The main interest of winemakers is in the few species that have high tolerance to acidity and ethanol. In some cases it is regarded as a spoilage activity, but under proper circumstances, malolactic fermentation (Picture 3), either naturally or artificially encouraged, can be a normal part of good winemaking practice, something to be appreciated and desired. For high-quality wines, the fermentation brings positive effects, such as bacteriological stabilization (Moreno-Arribas, Gomez-Cordoves, & Martín-Alvarez, 2008).

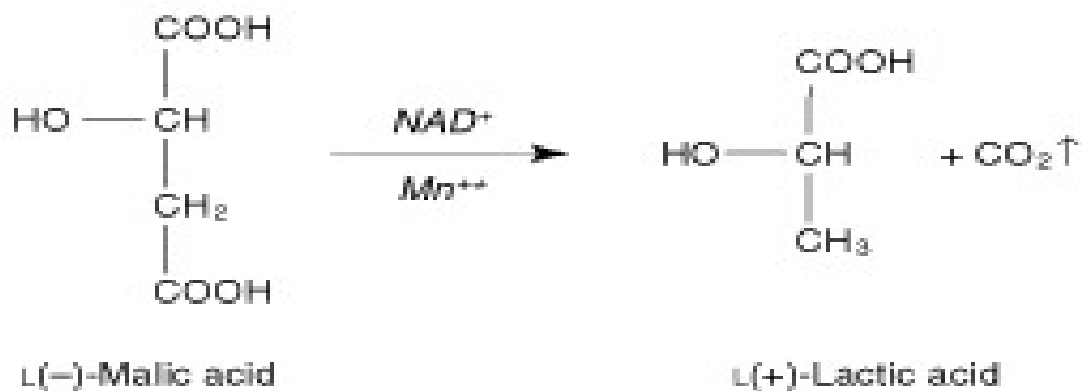


Figure 4 : The conversion of L-malic acid in to L-lactic acid

Although MLF is not technically a mandatory element of wine-making, and was historically described as a capricious and harmful phenomenon that

was difficult to understand, its contributions are vital to the development of the sensory characteristics of wine : except of the reduction of acidity, increases pH levels, adds microbiological stability and improves the organoleptic profile by producing a wide range of colors , flavors and aromas (E. Lerm, L. Engelbrecht, M. du Toit 2010). Thus, little by little this process has become practically indispensable to the winemaking industry

As a result of malolactic fermentation is that, volatile compound are formed during this process that enrich the wine's aromatic quality while also modifying color and phenolic composition (Izquierdo-Canas, García Gomez, & Palop, 2008; Martínez-Pinilla, Martínez-Lapuente, Guadalupe, & Ayestar_an, 2012). There is a number of reports showing changes in the volatile aroma profile of wines after MLF, summarised by Sumbly, Grbin, and Jiranek (Sumbly et al., 2010).

Some authors (Maicas et al., 1999; Bartowsky and Henschke, 2004) have studied the biosynthesis of aromatic compounds produced during MLF and their organoleptic consequences (Palacios et al., 2003). All agree that the resulting modifications are highly complex and often involve the reduction of vegetable and herbaceous aromas and the appearance of other fruity, floral, nutty or milky aromas. In contrast, Sauvageot and Vivier (1997) observed that changes occurring in MLF are not very important and reported only slight sensorial differences.

It is important to highlight the enormous influence of the lactic bacteria strains of strain participating in the process (Gambaro et al., 2001; Pozo-Bayon et al., 2005; Ugliano and Moio, 2005) and the type of elaboration process (industrial or laboratory) (Delaquies et al., 2000) on the aromatic complexity and composition of wine. In industrial processes, the development of malolactic fermentation starts immediately after alcoholic fermentation (over yeast lies) and the results can be different to those obtained in the laboratory when the MLF is carried out with clean and/or filtered wines.

Thus, with the regard to the oenological conditions, the success of MLF is not always guaranteed and the addition of a starter culture can improve its viability. Overall, two possibilities of lactic acid bacteria inoculation exist : traditional inoculation after alcoholic fermentation (sequential), or simultaneous inoculation in the must with yeast (co-inoculation).

Co-inoculation has several clear benefits compared to the sequential technique. The first advantage is that, by introducing lactic acid bacteria at the beginning of AF, it helps the bacteria to adapt to the medium better. Secondly, the contents of some compounds that are known to inhibit lactic acid bacteria growth, such as ethanol and SO₂, are lower and the medium is richer in nutritive elements during the first hours of AF than at the end.

Moreover, it is now well known that co-inoculation reduces the total fermentation time (Rosi et al., 2006; Jussier et al., 2006; Massera et al., 2009; Abrahamse & Bartowsky, 2012; Knoll et al., 2012; Pan et al., 2011). This reduction limits the risk of spoilage by other microorganisms, such as the *Brettanomyces* species, which are mainly responsible for 4-ethylphenol production (Jussier et al., 2006 ; Curtin et al., 2007 ; Gerbaux et al., 2009).

Currently, *Oenococcus oeni* is the main species used in MLF as lactic acid bacteria starters, but researches have demonstrated that some *Lactobacillus plantarum* species can also grow in wines and display the ability to survive the harsh conditions of wine (high ethanol concentration, low Ph and temperatures and sulphur dioxide) (G-Alegria et al., 2004; Lerm et al., 2011; Lee et al., 2012; Bravo-Ferrada et al., 2013), and simultaneously, possess many other favourable characteristics (du Toit et al., 2011; Lerm et al., 2011). For instance, the introduction of some *Lb.plantarum* strains to the fermenting must could significantly modify the wine aroma profile due to a more diverse enzymatic profile *Lb.plantarum* possess than that of *O.oeni* (Lerm et al., 2011).

When malolactic fermentation is complete, the wine is subjected to different clarification and stabilization treatments and/or is stored in oak

barrels for aging for a variable period of time. This practice modifies wine composition due to the compounds extracted from the wood and to the chemical reactions that take place when oxygen passes through wood pores or staves (Gomez García-Carpintero, Gomez Gallego, Sanchez-Palomo, & Gonzalez Vinas, 2012).

1.1.6 Aging, filtration and bottling

Aging in oak barrels is an oenological process which modifies the composition and sensory characteristics of the wine. Wood containers have been used for wine during centuries, and its presence has become part of the wine identity in many regions.

Nowadays the use of wood barrels is practiced for different reasons:

- i) Micro-aeration of wine
- ii) Tannin increase
- iii) Aroma contribution

As the three functions cannot be separated, the practice has to be limited to red and white wines of suitable original composition.

In traditional red wine production, malolactic fermentation is carried out in tanks and aging in barrels (Castro-Vazquez et al. 2011; Jarauta, Cacho, & Ferreira, 2005). It is widely known that, barrel MLF modifies the aromatic sensory profile of wine in varying degrees depending differences in the design of the studies performed (De Revel, Bloem, Augustin, Lonvaud-Funel, & Bertrand, 2005; Gomez García-Carpintero, Sanchez-Palomo, & Gonzalez Vinas, 2014), the general consensus showing a preference for barrel MLF wines over the tank MLF variety (Vivas, Lonvaud-Funel, & Glories, 1995).

However, the use of barrels involved a major financial commitment and entailed risks of microbial contamination, as well as the likelihood of communicating organoleptic faults to wine.

So, alternative use of wood has become popular in the last decades: chips, cubes or staves are added to wine for a limited time in order to replace the functions ii) and iii) and to add aroma and tannins typical of wood aging without oxidation and costs linked to barrel aging. Moreover, if these used together with micro-oxygenation, can partially replace even the function of the traditional use of wood.

Although filtration is not always carried out in red wine making, wines which have been aged for a long time in barrels or tanks usually have fewer problems of cloudiness and stability once in the bottle.

More attention in these final steps is focused on microbial contamination. This can be a problem in bottled wine even after several months and sometimes can occur randomly in some bottles of the same lot. The development of yeast and bacteria in bottle can lead to commercial problems.

After the filtration and addition of the preservatives the wines are ready to be closed. Once the bottle is filled, it is best to cork it promptly. Some wines are fermented in the bottle; others are bottled only after fermentation (Johnson & Hugh, 2004). Though cork has been the only option for hundreds of years, other options have recently seen a wide usage and an increasing acceptance by consumers. Synthetic closures are constituted by plastic polymers, and can have an appearance very similar to natural cork. Screw caps have seen a new life after having been used for decades on very short shelf-life products.

It is worth mentioning that wine ages are divided in two ways : aerobically, while it is fermenting, being pressed, racked and prepared for bottling , and anaerobically after bottling, when the myriad subtle chemical changes occur away from the air and produce true bouquet and complexities of flavor (MacNeil, Karen 2001).

1.2 Wine Aromas

Aroma is one of the most important quality factors of wine and is also the main determinants of consumer acceptance (Lockshin & Corsi, 2012; Rapp, 1998; Saénz-Navajas, Ballester, Pécher, Peyron, & Valentin, 2013). Wine aroma is a complex sensory characteristic that is determined by more than 1300 volatile compounds, including alcohols, esters, acids, aldehydes, isoprenoids, lactones and ketones, with a wide concentration range (Villamor & Ross, 2013). Differences in the aromatic profile of wines are determined by changes in the type, proportion and concentration of these volatile compounds (Atanasova et al., 2005).

Wine aroma depends on numerous factors, with special importance being given to the variety of grape, vinification, maturation, and aging (Schreier, 1976; Boulton 1995; Rapp, 1998). It is well-known that the secondary metabolites of grapes are responsible for the principal aroma compounds in grape must and provide the basis of varietal character (Rapp, 1991). Fermentation increases the chemical and aroma complexity of wine by assisting in the extraction of compounds from solids present in the grape must, modifying some grape-derived compounds, and producing a substantial amount of yeast metabolites (Lambrecht, 2000).

Surprisingly, even though 90% of world wine production is from non-aromatic grape varieties (Jurado, Pinilla, Ballesteros, Pérez-Coello, & Cabezudo, 2001), research has tended to concentrate primarily on wine produced by aromatic varieties.

A comparison of aroma compounds in different wines concluded that there were concentration differences of some volatiles among the wines. The most significant differences are quantitative rather than qualitative (Lopez et al., 1999; Ferreira et al., 2000).

Aroma characterization of wine is usually performed by gas chromatography-mass spectroscopy analyses, which enable the identification and quantification of volatile and non-volatile components (Francis & Newton, 2005). The type and concentration of these volatile

compounds are responsible for the characteristic aroma of wine. In particular, concentration usually explains variation in aroma between certain types of wine which contain the same volatile compounds (Boido et al., 2003).

Moreover, in the aroma of wines terpenes play an important role, being a group of flavor compounds characteristic for specific grapes used for the wine production. The dominating monoterpene alcohols, particularly from Muscat varieties, are linalool, geraniol, nerol, β -citronellol, α -terpineol, hotrienol and limonene.

A number of surveys have been made of monoterpene concentration in different grape varieties. However, since the reported quantitative data were obtained by different techniques and from samples of fruit from diverse areas, direct comparison on the analytical figures from different surveys is not feasible. Nevertheless, a general classification of those varieties which have been screened is possible allowing division into:

- 1) intensely flavoured muscats, in which total free monoterpene concentrations can be as high as 6 mg/l
- 2) non-muscat but aromatic varieties with total monoterpene concentration of 1-4mg/l
- 3) more neutral varieties not dependent upon monoterpenes for their flavor (Table 2)

Table 2: Classification of some grape varieties based on monoterpene content

(1) Muscat varieties	(2) Non-muscat aromatic varieties	(3) Neutral varieties
Canada Muscat	Traminer	Cabernet-Sauvignon

Gewurztraminer	Riesling	Cardigan
Moscato italiano	Schurebe	Trebbiano
Muscat of Alexandria	Sylvaner	Sauvignon Blanc
Muscat Hamburg	Wurzer	Merlot
Muscat Ottonel	Kerner	Syrah

1.3 Cabernet Franc

Cabernet Franc is a black-skinned French wine grape variety grown in most wine producing nations. The variety is most famously known as the third grape of Bordeaux and can be found in many of the world's top Bordeaux Blend wines. Cabernet Franc most commonly appears in blended red wines, where it adds herbaceous accents of tobacco and dark spice

In general, Cabernet Franc is very similar to Cabernet Sauvignon , but buds and ripens at least a week earlier. This trait allows the vine to thrive in slightly cooler climates than Cabernet Sauvignon, such as the Loire Valley.

Cabernet Franc can adapt to a wide variety of vineyard soil types but seems to thrive in sandy, chalk soil, producing heavier, more full bodied wines there. In the Loire Valley, terroir based differences can be perceived between wines made from grapes grown in gravel terraces versus tuffeau slopes. The grape is highly yield sensitive, with over-cropping producing wines with more green, vegetal note

1.4 Aim of the work

The present work is aimed to compare four different vinification protocols, connected with different fermentation management practices in order to evaluate possible alternative process to improve the organoleptic qualities of the variety Cabernet Franc from the area DOC Colli Orientali.

The type of yeast and lactic acid bacteria which are involved or are inoculated, were determine the profile of the aromatic wine and the stability of the final product.

UNIT 2: MATERIALS AND METHODS

2.1 Characteristics of the four samples are compared

Experiments were conducted on Cabernet Franc grapes (DOC Colli Orientali del Friuli, North-East Italy), during harvest 2015.

Four different vinification trials were compared, considering different combinations between spontaneous and piloted alcoholic and malolactic fermentation:

Trial I. Alcoholic fermentation using a commercial yeast starter culture (*S. cerevisiae* Anchor NT202, Oenobrand, Montferrier-sur-Lez, France), plus co-inoculation with a self-prepared *Oenococcus oeni* starter culture at the beginning of alcoholic fermentation (PC).

Trial II. Alcoholic fermentation with the same commercial strain (*S. cerevisiae* Anchor NT202), coupled with spontaneous malolactic fermentation (P).

Trial III. Spontaneous alcoholic fermentation plus co-inoculation, with the self-prepared *Oenococcus oeni* starter culture at the beginning of alcoholic fermentation (WC).

Trial IV. Spontaneous fermentation coupled with spontaneous malolactic fermentation (W).

2.2 Characteristics of yeasts and bacteria which are used as starter

In **table 3** it is possible to observe the characteristics of the commercial yeast used for the samples produced with piloted alcoholic fermentation.

Technical specifications	Anchor NT202
Species	Hybrid of <i>S. cerevisiae</i>
Fermentation kinetics	Fast and normal
Tolerance of low temperature	18 °C
Optimum temperature range	20-28 °C
Brix degrees	26° Brix
Alcohol	16% vol
Alcohol tolerance	16%
Foam production	limited
Production of glycerol	9-12 g/L
Production of volatile acidity	<0.3 g/L
Production of SO ₂	limited
Nitrogen requirements	medium
killer effect	positive

Activity of cinnamyl decarboxylase	negative (POF-)
Dosage	30 g/hL
Aromatic flavors	red berries, mint, blackberries, currants, tobacco
Notes favorite	It promotes malolactic fermentation, and indicated for high alcoholic musts

Table 3 : Characteristics of the yeast starter

Concerning the Lactic acid bacteria starter, it was a self-prepared culture of five different strains of *Oenococcus oeni*, previously isolated from the same winery where the fermentations were conducted. These specific strains were genetically and physiologically characterized at the laboratories of Food Microbiology of the University of Udine (Dipartimento di Scienze Agroalimentari, Ambientali ed Animali) and made available by kind permission of prof. Lucilla Iacumin.

The reasons of their selection are summarized in the following points: absent ability to produce biogenic amines, good tolerance to ethanol, good resistance to SO₂, and the presence of different genotypes.

The multi-strain inoculation option was chosen because it may ensure a certain degree of variability during fermentation and avoids possible crashes due to fermentative deviations.

2.3 Winemaking Protocol

Grapes were subjected to crushing-destemming and the mash was subdivided in four containers of 100 L capacity.

The inoculation with the commercial preparation of active dry yeast was immediately carried out in the piloted fermentation trials, while the remaining two samples were not inoculated (spontaneous fermentation). In the case of piloted fermentations, the yeasts have been rehydrated with water at a temperature of about 37 °C, and mixed to homogenize the solution, according to supplier's instructions. In all the trials, fermentation/maceration temperature was set at 18-20 °C.

After two days from the start of alcoholic fermentation Lactic Acid Bacteria (LAB) was coinoculated with the mix of strains previously described, at a concentration of 10^6 CFU / mL.

Concerning maceration management, the cap was punched down twice daily for the entire maceration length. After fifteen days, the samples were drained and pressed separately, keeping them in single 54 L glass containers for the whole duration of the alcoholic fermentation.

A few days before the end of the fermentation, the wine was decanted into smaller containers to make them all filled by prevent oxidation, eliminating the lees produced during fermentation.

Bottling was carried out approx.. twenty days after the end of alcoholic fermentation, and 50 mg/L of sulfur dioxide was also added before closing the bottles themselves.

2.4 Analytical determinations

The bottled wines were characterized for the parameters reported below

2.4.1 Determination of color using spectrophotometer

All the spectrophotometric analyses were carried out by using a UV–vis spectrophotometer, model V-530 (Jasco Co. Ltd., Tokyo, Japan).

To determine the color intensity and the hue of wine samples, spectrophotometric measurements were performed at the wavelengths of 420, 520 and 620 nm.

The absorbance at 420nm is an index of the yellow – orange pigment content.

The absorbance at 520nm is an index of the red pigment , linked to the presence of anthocyanins in flavilium form.

The absorbance at 620nm is an index of blue pigment , due to the fraction of anthocyanins present in the form of the anhydrous basis at the pH of the wine

Color intensity and hue are determined on the basis of the following calculations:

- Color intensity (Glories, 1984): it is given by the sum of the value of the three absorbances reported above (420 + 520 + 620 nm). A high value of color intensity represents a wine sample with intense color.
- Color hue (Sudraud,1958): it is given by the absorbance ratio 420 nm/520 nm. A high value of this ratio represents wines with an orange – yellow hue, whereas red purple colored wine samples show a low value of this parameters.

Measurements were carried out using 1 mm optical path length glass cuvettes (Hellma Analytics, Mülheim, Germany); readings were performed against distilled water.

2.4.2 Determination of phenolic compounds using Spectrophotometer

Wine phenolics belong two main groups of compounds: non-flavonoid and flavonoid. The former group, also called phenolic acids, notably includes hydroxycinnamic acids (e.g., caffeic acid, coumaric acid) and the latter group includes anthocyanins, flavonol and flavanol.

The content of total phenolic compounds was assessed by measuring the absorbance of the samples at 280 nm, using 10 mm optical path length quartz cuvettes (Hellma Analytics, Mülheim, Germany); readings were performed against distilled water. Wine samples were previously diluted fifty times and total phenolic index (TPI) was calculated multiplying by 50 the absorbance measured at 280 nm.

2.4.3 Determination of anthocyanins

Anthocyanins are the red pigment of the grape skins. Their concentration in the wine is linked to the type of winemaking and in particular to maceration, the contact between the must and the skins.

i) By Spectrophotometer

A usual method of reference is to Ribéreau-Gayon and Stonestreet (1965), based on the properties of anthocyanins to discolor in the presence of excess SO₂. The method provides the measurement of free anthocyanins and anthocyanins combined with the tannins and bleached by SO₂.

Reagents

- 95% v/v ethyl alcohol, acidified with 0.1 % v/v of 37% HCl
- 37 % hydrochloric acid diluted 1:50 in distilled water
- 15 % sodium bisulfite solution (NaHSO₃)

Method

- In a beaker (solution 1) 0.5 ml of wine, 0.5ml of acidified ethyl alcohol and 20 mL of diluted HCl solution were added

The mixture was then splitted in two test tubes:

- TEST TUBE A: introducing in it 2.5 ml of solution 1 + 1 ml of water;
- TEST TUBE B: introducing in it 2.5 ml of solution 1 + 1 ml of sodium bisulfite 15%.

Leave them to react for 30 minutes by placing the tubes in the dark, and then read the absorbance at 520 nm against water using cuvettes 1 cm thick. Readings were carried out against distilled water.

Calculations:

$$\text{ANTHOCYANINS (mg / l)} = [\text{ABSORBANCE A} - \text{ABSORBANCE B}] \times 875$$

ii) By HPLC

HPLC analyses were performed on a LC-2010 AHT liquid chromatographic system (Shimadzu, Kyoto, Japan), equipped with an integrated autosampler and UV-Vis detector. Compounds were separated on a 5 μm packed, 150 x 4.6 mm Zorbax Eclipse Plus C18 column (Agilent Technologies, Santa Clara, CA, USA) thermostated at 25 °C. The elution was performed in gradient mode at a flow rate of 1.2 mL/min. The mobile phase was composed of 9 % (v/v) formic acid in Milli Q grade water (solvent A) and 9 % (v/v) formic acid in HPLC grade methanol (Solvent B). The gradient was set as follows: solvent B was held at 10 % for the first 3 min, increased to 50 % in the following 15 min and held at the 50 % for additional 2 min. Solvent B was then decreased in 1 min to the initial conditions (10 %) and equilibrated at 10 % for 2 min. The injection volume was 20 μL . Before injection, all samples were filtered on 0.20 μm nylon membranes (Albet-Hahnemühle, Barcelona, Spain). Detection was

performed at 525 nm. The absolute areas of the detected peaks were used in data elaboration.

Qualitative analysis was based on the order of elution reported in literature (Morata et al, 2007).

2.4.4 Determination of tannins using Spectrophotometer

Tannins in wine consist largely of condensed tannin polymers that are extracted from grapes and structurally altered during winemaking. Wine tannin is structurally quite different from preveraison grape tannin due to the incorporation of anthocyanins (Kennedy , 2002) and changes resulting from the chemical and enzymatic oxidation and rearrangement reaction that occur during the grape crushing and fermentation processes.

The tannins in red wine influence the in-mouth sensory properties including mouth feel, particularly with respect to astringency , (Gawel , 1998) and therefore the perceived quality of the wine.

The determination was carried out by the Bate-Smith assay (1954). Reagents: acidified butanol solution (500 mL of 37% HCl solution + 150 mg of $\text{Fe}_2(\text{SO}_4)_3$ + 500 mL of n-butanol). 2 mL of each sample (diluted 1:50 in distilled water), are mixed with 6 mL of acidified butanol solution (Tube A). Half of this solution is transferred into a second test tube (in PYREX glass) (Tube B), which is placed at 100 ° C for 30 minutes. After cooling of the tube B, the absorbance of both tubes is measured at 550 nm, reading against water (10 mm optical path length). Total tannins (procyanidins), are calculated by the following equation

$$\text{Total tannins (g/L)} = (\text{DO}_B - \text{DO}_A) * 0.01736 * 50$$

2.5 Sensory analysis

In addition to the chemical analyses a sensory test was also conducted. The samples were identified by a three- digit numerical code and were referred to a commission of twelve judges who were to bring them in order of intensity. The descriptors chosen were as follows:

- Intensity of color
- Orange hue
- Aromatic intensity
- Vegetal/ herbaceous aromas
- Body/ Structure
- Astringency
- General impressions

An example of the scorecard used is reported in Figure 2.

The statistical treatment of results was conducted using the Friedman test (Barillere and Benard, 1986), in order to identify the minimum significant difference between the ranks ($p < 0.05$). If there were significant differences between the sums of the ranks, samples for which a given attribute was perceived as more intense, were those with the lowest sum of the ranks.

$$F = \frac{12(R_1^2 + R_2^2 + \dots + R_k^2)}{nk(k+1)} - 3n(k+1)$$

	Ranking Test	
	RED WINES	

Campione _____ Judge Code _____

Analyze the wines, put them in order according to the intensity of each attribute
(Position 1: greater intensity; Position 5: lower intensity)

It is not permitted to position the code for more samples in the same cell

Position	1	2	3	4
-----------------	----------	----------	----------	----------

Visual attributes

Intensity of color				
Orange hue				

Olfactory attributes

aromatic intensity				
vegetal / herbaceous				

Gustatory attributes

body / structure				
Astringency				

Other Attributes

General impression				
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Note :

Fig.2 : Ordinary test

UNIT 3: RESULTS AND DISCUSSION

3.1 Chemical analysis of the results of the wines using Spectrophotometer

Three combinations of yeast/bacteria starter cultures were tested on Cabernet Franc wine; trials were carried out under winery conditions. The results of the chemical analysis from fermentation process (spontaneous and non spontaneous) are presented below.

In **Figure 3**, the differences between the concentration of total phenolic compounds (Abs 280 nm), anthocyanins and tannins in the wines are presented. Differences were found among all samples concerning total phenolics, while samples P and PC had a slightly higher concentration of anthocyanins (673 mg/L) and tannins (608 mg/L), with respect to W and WC. Anyway, the use of selected microorganisms led averagely to wines more rich in anthocyanins and tannins, probably, for a more rational behavior of alcoholic fermentation. The faster beginning of the process, could have played a positive role, in protecting color compounds from oxidation and promoting a higher extraction of polyphenols during maceration (Ribereau-Gayon, 2006).

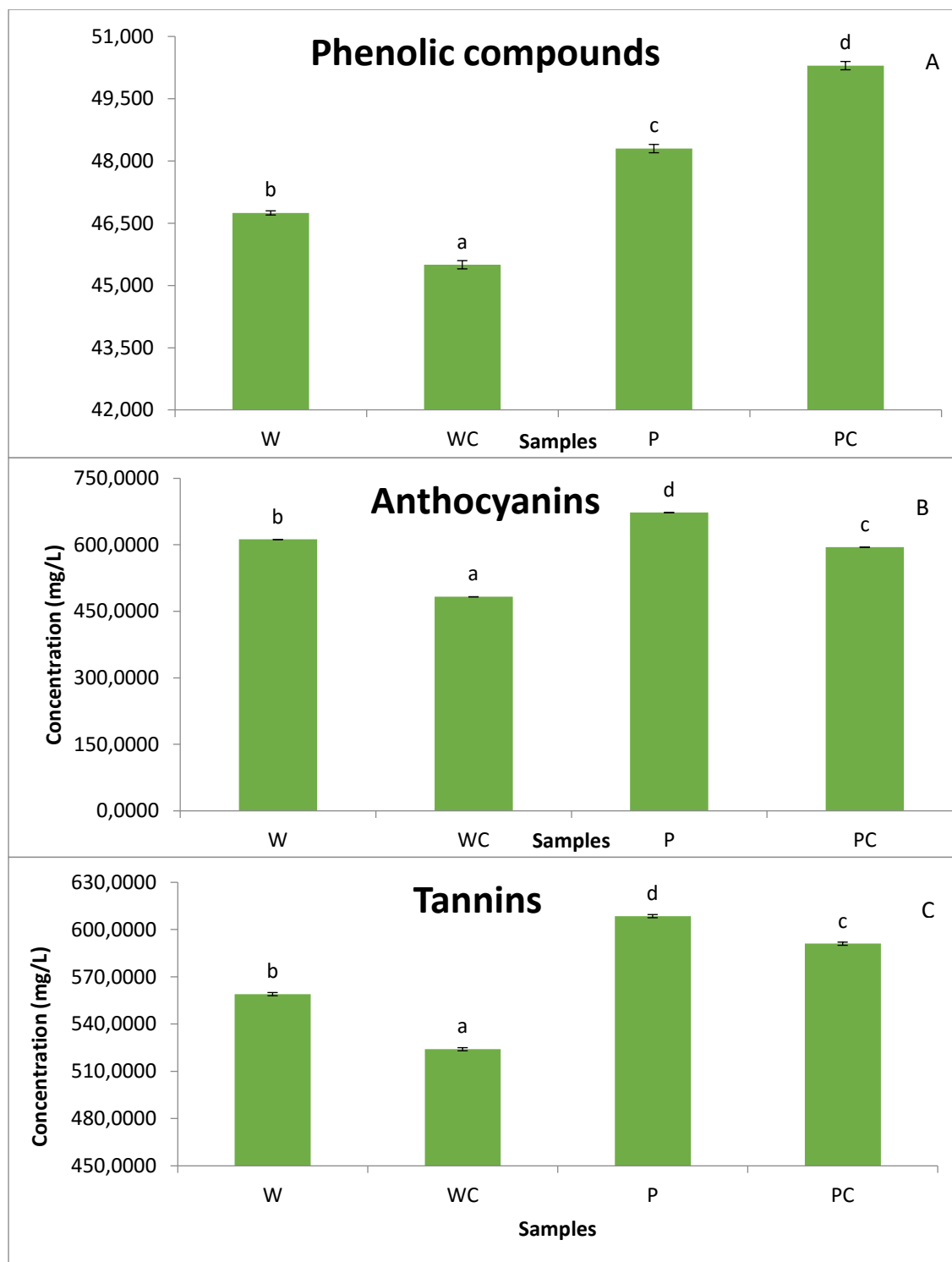


Figure 3 : Concentration of phenolic compounds, anthocyanins and tannins (means with the same letter do not differ significantly by Tukey's test, $p < 0.05$).

Sample P (**Figure 4**) had also higher color intensity, due to the higher concentration of anthocyanins found in this sample; intensity, was significantly different between samples W,PC,WC and P. Also, according to the color hue of the samples, no relevant differences were found (**Figure 5**).

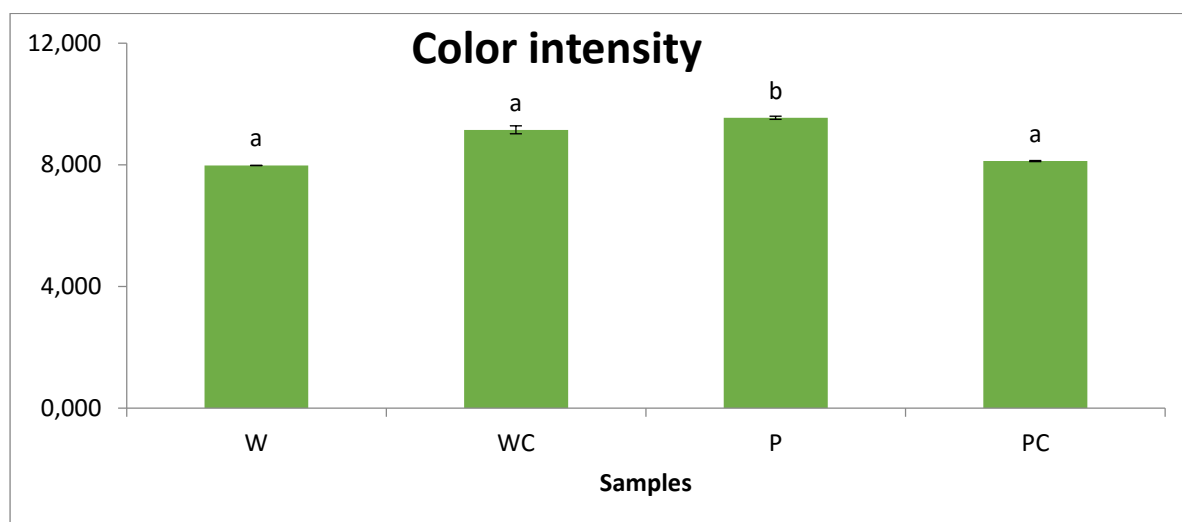


Figure 4 : Results of the chemical analysis of color intensity (means with the same letter do not differ significantly by Tukey's test, $p < 0.05$)

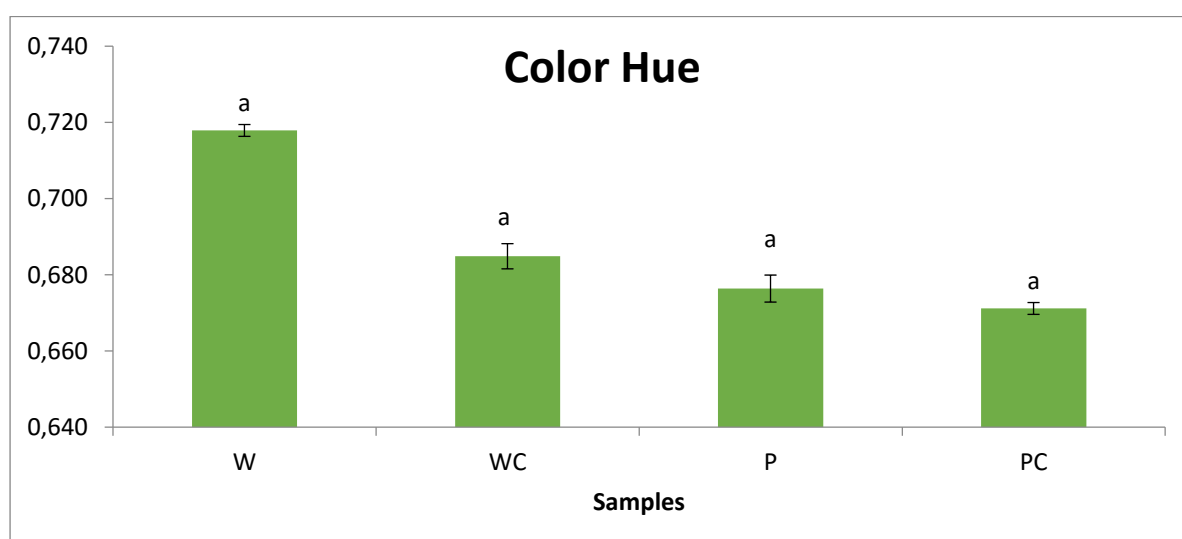


Figure 5 : Results of the chemical analysis of color hue (means with the same letter do not differ significantly by Tukey's test, $p < 0.05$)

3.2 Chemical analysis of the results of the wines using HPLC

Figure 6 illustrates the chromatographic separation of anthocyanin pigments using reverse-phase HPLC.

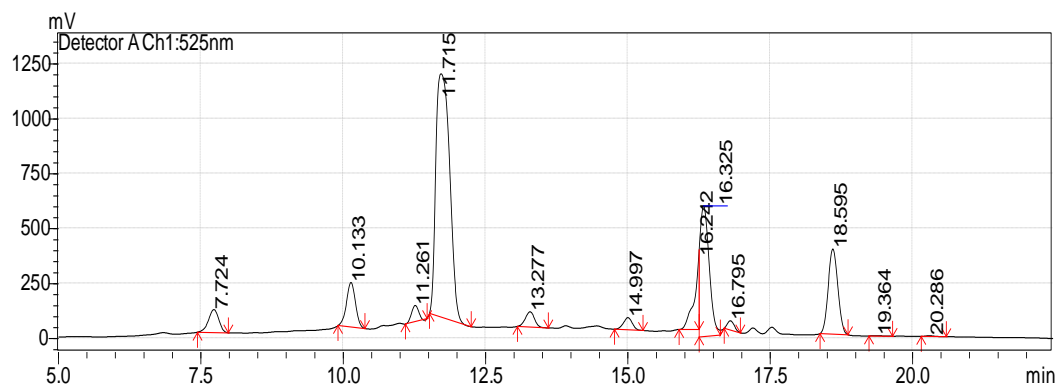


Figure 6 : Reverse-Phase HPLC analysis of anthocyanins, in sample P

The compounds detected by HPLC analysis are shown in **Table 4**.

Table 4 : Compounds detected by HPLC analysis

Compound	Rt
Delphinidin-3-monoglucoside	7.774
Petunidin-3-monoglucoside	10.189
Peonidin-3-monoglucoside	11.310
Malvidin-3-monoglucoside	11.767
Vitisin A	13.377
Petunidin-3-monoglucoside acetyltated	15.116
Peonidin-3-monoglucoside acetylated	16.250
Malvidin-3-monoglucoside acetylated	16.429
Delphinidin-3-monoglucoside p-coumarylated	16.897
Malvidin-3-monoglucoside p-coumarylated	18.686
Malvidin-3-monoglucoside vinylphenol	19.428
Malvidin-3-monoglucoside vinylphenol acetylated	20.391

In young red wines, free anthocyanins are the principal source of red color, though monomeric anthocyanins are not particularly stable. As red grapes are the exclusive source of these monomeric anthocyanins, their composition determines the composition of the anthocyanin profile of the corresponding red wines automatically and significantly. These monomeric or free anthocyanins are gradually incorporated into their derived pigments, including copigments and polymeric pigments involving other phenolic during wine aging, contributing to a progressive shift of the red-purple color of young red wine towards the more red-orange color of aged red wines (Jackson , 2008).

Normally, in the red wines which are made from *V. vinifera* grapes, the main monomeric anthocyanins are the 3-O-monoglucosides of the six free anthocyanidins, including pelargonidin-3-O-glucoside (callistephin), cyanidin-3-O-glucoside (kuromanin), delphinidin-3-O-glucoside (myrtillin), peonidin-3-O-glucoside (peonin), petunidin-3-O-glucoside (petunin) and malvidin-3-O-glucoside(oenin) (Jackson, 2008). Such anthocyanidins differ from each other by the number and position of the hydroxyl and methoxyl substituent groups in the B ring of the molecule. The hydroxylation pattern of the anthocyanins in the B ring can directly affect the hue and color stability due to the effect on the delocalized electrons path length in the molecule.

The vitisins are the most studied pyranoanthocyanin family, and they are formed in the reaction between the anthocyanins with some metabolites released during the yeast fermentation, such as pyruvic acid, or acetaldehyde (Bakker et al.,1997;Fulcrand et al.,1998; Hayasaka et al., 2002), the latter of which can also be found in the wine as a result of the oxidation of ethanol.

In the vitisin group, the most important are the carboxypyrananthocyanins or type A vitisins, formed upon the reaction between the enol form of the pyruvic acid and the anthocyanins. Due to the formation of pyruvic acid during alcoholic fermentation, it is likely that the formation of these derivatives begins at the stage of winemaking.

The vitisin formed from malvidin-3-O-glucoside was called vitisin A by Bakker et al.,1997. This vitisin has been found in the highest concentrations, due to that the malvidin-3-O- glucoside is the prevalent anthocyanin in *Vitis vinifera* (Morata et al.,2006). The vitisin A is the main anthocyanin derivative detected by HPLC in Port wines after a year of aging, which clearly shows its importance in wine color (Mateus et al, 2001, Romero et al.,2001).

However, other studies were found that vitisin A is only a minor contributor to the visually perceived color of red wines (color contribution ~ 5%). The major contributor is the polymeric fraction (color contribution~ 70-90%) (Scharz et al. 2008).

Also , in this study , vitisin A seems not to affect the color intensity. It has been found that the sample P has the higher amount of vitisin A and the lower concentration of vitisin A was in the sample W (**Figure 7**), confirming the color behavior reported above.

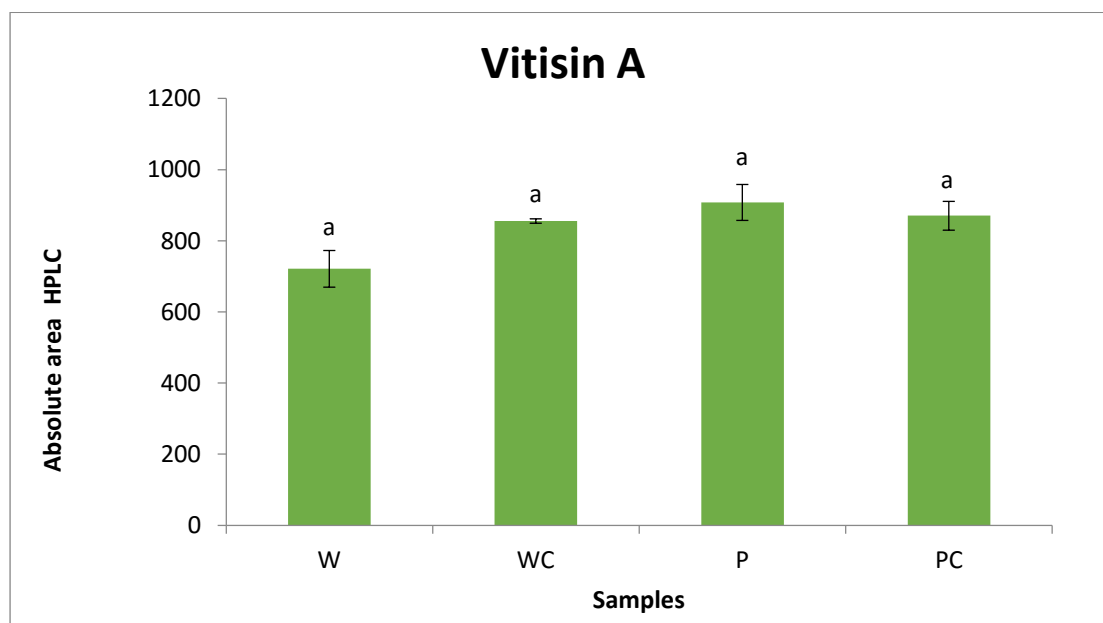


Figure 7 : Absolute Area detected by HPLC analysis for vitisin A (means with the same letter do not differ significantly by Tukey's test, $p < 0.05$)

In this study, the concentration of malvidin-3-monoglucoside was found to be higher in the sample P, and lower in the sample WC while W and PC are not significantly different from either one of the aforementioned samples (**Figure 8**), confirming the previous behaviors. Differences were detected for delphinidin-3-monoglucoside (**Figure 9**) between sample W and all the remaining samples, as well as in peonidin-3-monoglucoside, among samples WC, PC and both W and P (**Figure 10**).

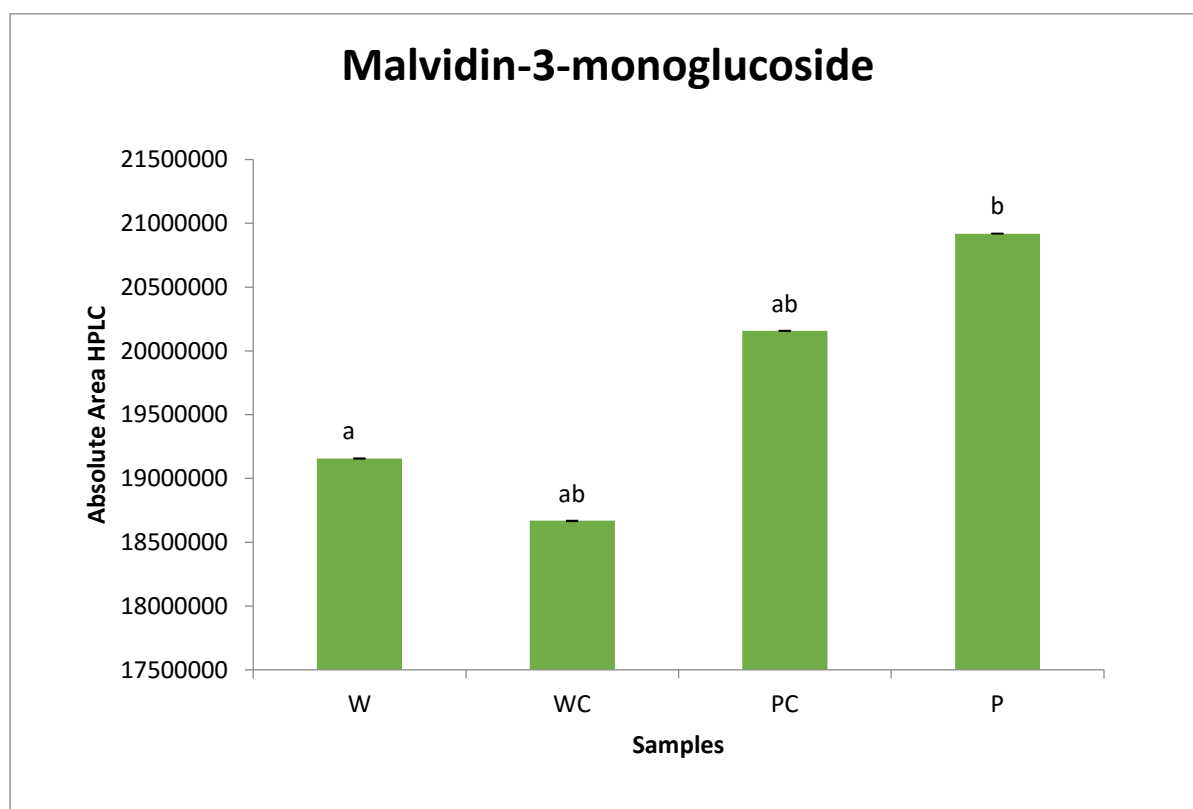


Figure 8 : Absolute Area detected by HPLC for malvidin-3-monoglucoside (means with the same letter do not differ significantly by Tukey's test, $p < 0.05$)

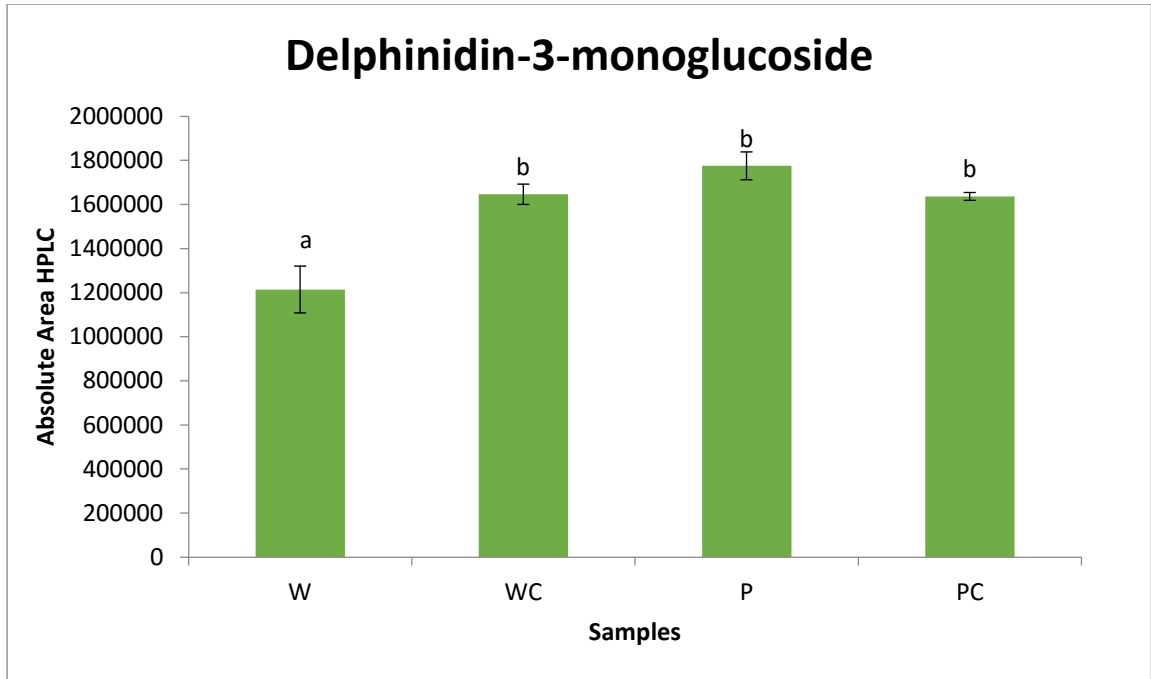


Figure 9 : Absolute Area detected by HPLC analysis for Delphinidin-3-monoglucoside (means with the same letter do not differ significantly by Tukey's test, $p < 0.05$)

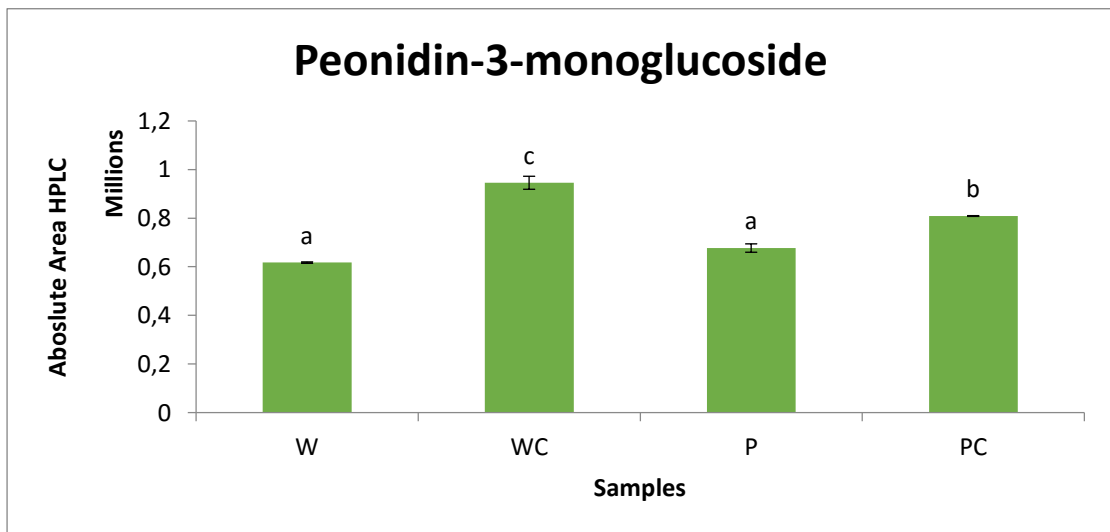


Figure 10: Absolute Area detected by HPLC for Peonidin-3-monoglucoside (means with the same letter do not differ significantly by Tukey's test, $p < 0.05$)

3.4 Correlation between the results

Also, the correlation of data has been investigated in order to detect if they are related and how properties interact with each other. The significance level was set at $p < 0.05$ (Table 5).

Table 5 : Correlation between analyzes ($p < 0,05$), (significant correlations are colored red)

Variable	Color intensity	Color hue	TPI	Anthocyanins (mg/L)	Tannins (mg/L)	Delphinidin-3-monoglucoside	Peonidin-3-monoglucoside	Malvidin-3-monoglucoside	Vitisin A
Color intensity	1,0000	-,5800	-,2454	,0716	,1424	,7578	,2143	,3462	,7107
	p= ---	p= ,420	p= ,755	p= ,928	p= ,858	p= ,242	p= ,786	p= ,654	p= ,289
Color hue	-,5800	1,0000	-,2131	,3176	-,0661	-,9278	-,7599	-,2427	-,9171
	p= ,420	p= ---	p= ,787	p= ,682	p= ,934	p= ,072	p= ,240	p= ,757	p= ,083
TPI	-,2454	-,2131	1,0000	,5645	,8128	,2988	-,2208	,7484	,3841
	p= ,755	p= ,787	p= ---	p= ,436	p= ,187	p= ,701	p= ,779	p= ,252	p= ,616
Anthocyanins (mg/L)	,0716	,3176	,5645	1,0000	,9045	,0206	-,8544	,8402	,0753
	p= ,928	p= ,682	p= ,436	p= ---	p= ,095	p= ,979	p= ,146	p= ,160	p= ,925
Tannins (mg/L)	,1424	-,0661	,8128	,9045	1,0000	,3509	-,5668	,9753	,4180
	p= ,858	p= ,934	p= ,187	p= ,095	p= ---	p= ,649	p= ,433	p= ,025	p= ,582
Delphinidin-3-monoglucoside	,7578	-,9278	,2988	,0206	,3509	1,0000	,4786	,5347	,9959
	p= ,242	p= ,072	p= ,701	p= ,979	p= ,649	p= ---	p= ,521	p= ,465	p= ,004
Peonidin-3-monoglucoside	,2143	-,7599	-,2208	-,8544	-,5668	,4786	1,0000	-,4361	,4404
	p= ,786	p= ,240	p= ,779	p= ,146	p= ,433	p= ,521	p= ---	p= ,564	p= ,560
Malvidin-3-monoglucoside	,3462	-,2427	,7484	,8402	,9753	,5347	-,4361	1,0000	,5897
	p= ,654	p= ,757	p= ,252	p= ,160	p= ,025	p= ,465	p= ,564	p= ---	p= ,410
Vitisin A	,7107	-,9171	,3841	,0753	,4180	,9959	,4404	,5897	1,0000
	p= ,289	p= ,083	p= ,616	p= ,925	p= ,582	p= ,004	p= ,560	p= ,410	p= ---

Early studies of copigmentation with grape pigments noted that tannin modified the color of malvidin 3- glucoside solutions. Overall the results indicate that tannins have a clear positive effect on the color because as their supplementation to a malvidine solution resulted in an increase of color and a decrease in lightness (Gombau et al.,2016).

Previous research in Shiraz wine samples, indicated that the concentration of delphinidin-3-monoglucoside is positively correlated to the concentration of Vitisin A, as well as that they had higher concentration than other anthocyanins (petunidin, peonidin, malvidin) (Birse, 2007).

No other significant correlations were observed between the rest of the parameters taken into consideration.

3.5 Statistical analysis of the wines

The results reported above, were also evident in a Principal Components Analysis (PCA), carried out on the absolute areas detected by HPLC analyses. Elaborations were performed by the Software Statistica for Windows (Version 8).

It was well clear that the higher amount of anthocyanins was concentrated in the samples PC and P (Figure 11).

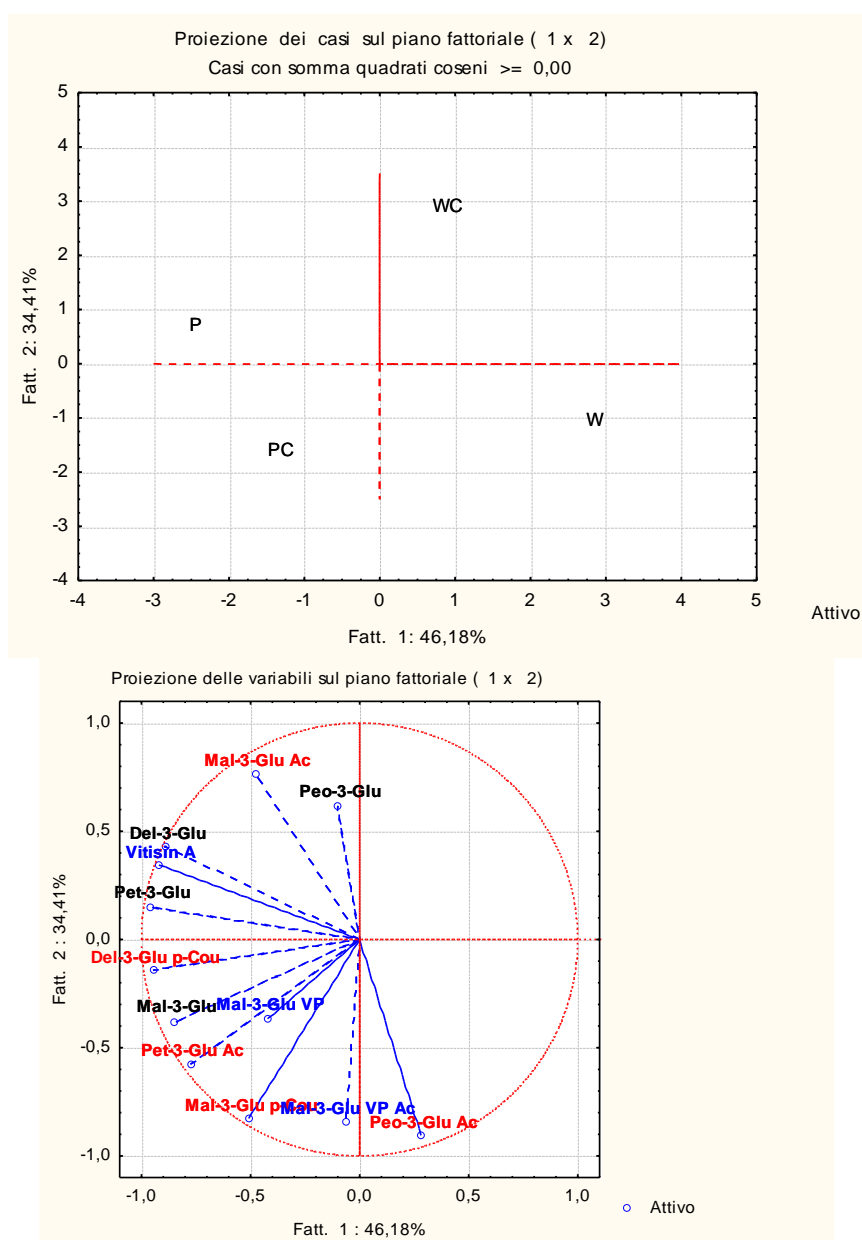


Figure 11: Results of PCA carried out on the absolute areas detected for anthocyanins in HPLC

3.6 Sensory analysis

Sensory test results are shown in **Table 6**.

Contrary with respect to what observed for anthocyanins, no significant differences were found concerning color, and the only attribute which seemed to be affected by the use of the different fermentation starters was “Fruity aromas”. In this case, the PC wine, which was inoculated with the starter NT202 and co-inoculated with *O. oeni*, was the most characterized and intense, and this could be due to the fact that commercial yeasts have better ability to produce aromas, as well as the presence of a higher percentage of *O.oeni* strains with β - glucosidase activity. This activity is also confirmed by the intensity of color in the thesis PC, inoculated with these strains, which is lower than in the other samples. Other studies had showed that co-inoculation treatments were rated highly for *fresh citrus* and low in *cooked vegetal* and *bruised apple* (Molina et al., 2009).

Table 6 : Sensory results. The values shown correspond to the sum of the calculated ranks. The samples perceived as more intense for a given attribute, are those with the lower sum of the ranks (minimum significant difference between the ranks 17-p <0.05).

Sam ples	Color intensity		Orange Hue		Fruity aromas		Vegetable aromas		Body- Structure		Astringency		General impression	
W	33	a	32	a	37	a	27	a	30	a	34	a	27	a
WC	28	a	27	a	32	ab	27	a	28	a	27	a	30	a
P	24	a	35	a	31	ab	35	a	37	a	29	a	35	a
PC	35	a	26	a	20	b	31	a	25	a	30	a	28	a

UNIT 4: CONCLUSION

A few years ago there was debate if yeast and bacteria strains used to conduct wine fermentation had an effect on wine composition and aroma (Thorngate,1999). Many studies have now shown this to be the case. The results of this study have demonstrated that the choice of yeast and bacteria affects the wine composition and sensory properties. The study highlights certain differences between spontaneous and non spontaneous fermentation.

The use of commercial strains gave the best results, concerning both the concentration of color compounds and tannins and the intensity of fruity aroma. Differences in color, were perceived from the analytical point of view, rather than from the sensory one, confirming that also spontaneous fermentation gave good results in the experimental conditions tested. This is an interesting fact, considering that all fermentation trials were carried out without sulfur dioxide supplementation, and only 50 mg/L were added to the wines at bottling. Further experiments, shall keep into account how wines will undergo chemical and sensory modifications during storage and ageing.

To sum up, the use of selected microorganisms led to wines richer in anthocyanins and tannins, probably due to more rational behavior by the alcoholic fermentation .It was observed that the hue of wines of spontaneous alcoholic fermentation was higher, indicating the oxidation of phenolic compounds of wines. In the sample P was observed higher concentration in anthocyanins. VitisinA, maldivin and delphinidin, while the W sample had an average of the less anthocyanins absorbance in HPLC. There were positive correlations between malvidin and tannins and VitisinA- delphinidin while the only sensory attribute affected was the "fruity" aroma, which was more pronounced in the sample PC

References

1. Abrahamse, C.E. & Bartowsky, E.J., 2012. *Timing of malolactic fermentation inoculation in Shiraz grape must and wine: Influence on chemical composition*. World J. Microb. Biot. 28, 255-265
2. Alcalde-Eon, C., Escribano-Bailon, M. T., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2006). *Changes in the detailed pigment composition of red wine during maturity and ageing: A comprehensive study*. Analytica Chimica Acta, 563,238–254
3. Atanasova, B., Thomas-Danguin, T., Chabanet, C., Langlois, D., Nicklaus, S., & Etiévant, P. (2005). *Perceptual interactions in odour mixtures: odour quality in binary mixtures of woody and fruity wine odorants*. Chemical Senses, 30, 209–217.
4. Atanasova, B., Thomas-Danguin, T., Chabanet, C., Langlois, D., Nicklaus, S., & Etiévant, P. (2005). *Perceptual interactions in odour mixtures: odour quality in binary mixtures of woody and fruity wine odorants*. Chemical Senses, 30, 209–217
5. B.W. Zoecklein, C.H. Hackney, S.E. Duncan, J.E. Marcy, J. Ind. Microbiol. Biotechnol. 22 (1999) 100
6. B.W. Zoecklein, J.E. Marcy, J.M. Williams, Y. Jasinski, J. Food Comp. Anal. 10 (1997) 55.
7. Bakker and Timberlake, “*Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines,*” Journal of Agricultural and Food Chemistry, vol. 45, no. 1, pp. 35–43, 1997.
8. Bakker, Bridle, Honda et al., “*Identification of an anthocyanin occurring in some red wines,*” Phytochemistry, vol. 44, no. 7, pp. 1375–1382, 1997.
9. Barillere J.M., Benard P. (1986). *Exemples d'interpretation de resultats de degustation*.Connaissance de la Vigne et du Vin 20: 137-154

10. Bartolome, B., Numez, V., Monagas, M., & Gomez-Cordovis, C. (2004). *In vitro antioxidant activity of red grape skins*. European Food Research and Technology, 218, 173–177.
11. Bartowsky, E., & Henschke, P. A. (2004). *The “buttery” attribute of wine-diacetyledesirability, spoilage and beyond*. International Journal of Food Microbiology, 96, 325e352.
12. Blateyron, L., & Sablayrolles, J. M. (2001). *Stuck and slow fermentations in enology: Statistical study of causes and effectiveness of combined additions of oxygen and diammonium phosphate*. Journal of Bioscience and Bioengineering, 91(2), 184–189.
13. Boido, E., Lloret, A., Medina, K., Fariña, L., Carrau, F., Versini, G., et al. (2003). *Aroma composition of Vitis vinífera cv. Tannat: the typical red wine from Uruguay*. Journal of Agricultural and Food Chemistry, 51, 5408–5413.
14. Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E. *Principles and Practices of Winemaking*; Chapman and Hall: New York, 1995.
15. Bravo-Ferrada, B., Hollmann, A., Delfederico, L., Valdés La Hens, D., Caballero, A., Semorile, L., 2013. *Patagonian red wines: selection of Lactobacillus plantarum isolates as potential starter cultures for malolactic fermentation*. World J. Microbiol. Biotechnol. 29 (9), 1537e1549.
16. Castro-Vazquez, L., Alanon, M. E., Calvo, E., Cejudo, M. C., Díaz-Maroto, M. C., & Perez-Coello, M. S. (2011). *Volatile compounds as markers of ageing in Tempranillo red wines from La Mancha D.O. stored in oak wood barrels*. Journal of Chromatography A, 1218, 4910e4917.
17. Chimi, H.; Cillard, J.; Cillard, P.; Rahmani, M. Peroxyl and Hydroxyl radical scavenging activity of some natural phenolic antioxidant. *J. Am. Oil Chem. Soc.* **1991**, 68, 307-312.

18. Cliff, M. A., King, M. C., & Schlosser, J. (2007). *Anthocyanin, phenolic composition, color measurement and sensory analysis of BC commercial red wines*. Food Research International, 40, 92–100.
19. Colombie, S., Malherbe, S., & Sablayrolles, J. M. (2005). *Modelling alcoholic fermentation in enological conditions: Feasibility and interest*. American Journal of Enology and Viticulture, 56(3), 238–245.
20. Curtin, C.D., Bellon, J.R., Henschke, P.A., Godden, P. & De Barros Lopes, M., 2007. *Genetic diversity of Dekkera bruxellensis yeasts isolated from Australian wineries*. FEMS Yeast Res. 7, 471-481.
21. Czyzowska, A., & Pogorzelski, E. (2002). *Changes to polyphenols in the process of production of must and wines from blackcurrants and cherries. Part I. Total polyphenols and phenolic acids*. European Food Research and Technology, 214, 148–154.
22. De Revel, G., Bloem, A., Augustin, M., Lonvaud-Funel, A., & Bertrand, A. (2005). *Interaction of Oenococcus oeni and oak wood compounds*. Food Microbiology, 22, 569e575
23. Delaquies, P., Cliff, M., King, M., Girad, B., Hall, J., Reynolds, A.J., 2000. *Effect of two commercial malolactic cultures on the chemical and sensory properties of Chancellor wines vinified with different yeast and fermentation temperatures*. American Journal of Enology and Viticulture 51, 42–48.
24. Du Toit, M., Engelbrecht, L., Lerm, E., Krieger-Weber, S., 2011. *Lactobacillus: the next generation of malolactic fermentation starter cultures dan overview*. Food Bioprocess Technol. 4 (6), 876e906.
25. Dubourdieu, D., Tominaga, T., Masneuf, I., Peyrot des Gachons, C., & Murat, M. L. (2006). *Role of yeasts in grape flavour development during fermentation: The example of Sauvignon Blanc*. American Journal of Enology and Viticulture, 57(1), 81–88.
26. Eglinton, J. M., McWilliam, S. J., Fogarty, M. W., Francis, I. L., Kwiatkowski, M. J., Hoj, P., et al. (2000). *The effect of Saccharomyces bayanus – mediated fermentation on the chemical*

- composition and aroma profile of Chardonnay wine*. Australian Journal of Grape and Wine Research, 6(3), 190–196.
27. Eugenio Revilla, Manuel M. Losada and Encina Gutiérrez. *Phenolic Composition and Color of Single Cultivar Young Red Wines Made with Mencia and Alicante-Bouschet Grapes in AOC Valdeorras* (Galicia, NW Spain. Departamento de Química Agrícola y Bromatología, Universidad Autónoma de Madrid, Spain; Published: 25 July 2016
 28. Ferreira, V., Lopez, R. & Cacho, J.F., 2000. *Quantitative determination of the odorants of young red wines from different grape varieties*. J. Sci. Food Agr. 80, 1659-1667
 29. Francis, I. L., & Newton, J. L. (2005). *Determining wine aroma from compositional data*. Australian Journal of Grape and Wine Research, 11(2), 114–126.
 30. Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. *Inhibition of human LDL oxidation by phenolic substances in red wine*. *Lancet* **1993**, 341, 454-457.
 31. Fulcrand, Benabdeljalil, Rigaud, Cheynier, and Moutounet, “A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins,” *Phytochemistry*, vol. 47, no. 7, pp. 1401–1407, 1998.
 32. G-Alegria, E., Lopez, I., Ruiz, J.I., Saenz, J., Fernandez, E., Zarazaga, M., Dizey, M., Torres, C., Ruiz-Larrea, F., 2004. *High tolerance of wild Lactobacillus plantarum and Oenococcus oeni strains to lyophilisation and stress environmental conditions of acid pH and ethanol*. FEMS Microbiol. Lett. 230 (1), 53e61.
 33. Gambaro, A., Boido, E., Zlotajblko, A., Meidna, K., Lloret, A., Dellacassa, E., Carrau, F.M., 2001. *Effect of malolactic fermentation on the aroma properties of Tannat wines*. Australian Journal of Grape and Wine Research 7, 27–32.
 34. Gawel, R. Red wine astringency; a review. Aust.J. Grape Wine Res. 1998, 4, 74–95.

35. Gerbaux, V., Briffox, C., Dumont, A. & Krieger, S., 2009. *Influence of inoculation with malolactic bacteria on volatile phenols*. Am. J. Enol. Vitic. 60, 233-235.
36. Jordi Gombau¹, Adeline Vignault², Olga Pascual¹, Joan Miquel Canals¹, Pierre-Louis Teissedre², and Fernando Zamora¹, 2016 *Influence of supplementation with different oenological tannins on malvidin-3-monoglucoside copigmentation*. BIO Web of Conferences 7, 02033 (2016)
37. Gomez García-Carpintero, E., Gomez Gallego, M. A., Sanchez-Palomo, E., & Gonzalez Vinas, M. A. (2012). *Impact of alternative technique to ageing using oak chips in alcoholic or in malolactic fermentation on volatile and sensory composition of red wines*. Food Chemistry, 134, 851e863.
38. Gomez García-Carpintero, E., Sanchez-Palomo, E., & Gonzalez Vinas, M. A. (2014). *Volatile composition of Bobal red wines subjected to alcoholic/malolactic fermentation with oak chips*. LWT- Food Science and Technology, 55, 586e594
39. Harborne, J. B., & Baxter, H. (1999). *The handbook of natural flavonoids* (Vol. I and II). Canada: Wiley & Sons.
40. Hayasaka and Asenstorfer, "Screening for potential pigments derived from anthocyanins in red wine using nanoelectrospray tandem mass spectrometry," Journal of Agricultural and Food Chemistry, vol. 50, no. 4, pp. 756–761, 2002. View at Publisher
41. Hidalgo Togores, J. (2003). Tratado de enología (Vol. I and II). Madrid: Ediciones MundiPrensa
42. Hong and Wrolstad. *Characterization of anthocyanin-containing colorants and fruit juices by hplc/photodiode array detection*. J. Agric. Food Chem. 38: 698-708 (1990)
43. Ivanova, V., Dornyei, A., Mark, L., Vojnoski, B., Stafilov, T., Stefova, M., et al. (2011). *Polyphenolic content of Vranec wines produced by different vinification conditions*. Food Chemistry, 124, 316–325.
44. Iwahashi, H.; Ishii, T.; Sugata, R.; Kido, R. *The effects of caffeic acid and its related catechols on hydroxyl radical formation by 3-*

- hydroxyanthranilic acid, ferric chloride, hydrogen peroxide. Arch. Biochem. Biophys.* **1990**, 276, 242-247
45. Izquierdo-Caracas, P. M., García, E., Gómez, S., & Palop, M. L. L. (2008). *Changes in the aromatic composition of Tempranillo wines during spontaneous malolactic fermentation.* Journal of Food Composition and Analysis, 21, 724e730.
46. Jaitz, L., Siegl, K., Eder, R., Rak, G., Abranko, L., Koellensperger, G., et al. (2010). *LC- MS/MS analysis of phenols for classification of red wine according to geographic origin grape variety and vintage.* Food Chemistry, 122, 366–372.
47. Jarauta, I., Cacho, J., & Ferreira, V. (2005). *Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: an analytical study.* Journal of Agricultural and Food Chemistry, 53, 4166e4177.
48. Jackson, R.S. Wine Science: Principle and Applications, 3rd ed.; Elsevier-Academic Press: Oxford, UK, 2008; pp. 287–295.
49. Johnson, Hugh (2004). *The Story of Wine.* Sterling Publishing. ISBN 1-84000-972-1.
50. Jurado, J. F., Pinilla, M. J., Ballesteros, M. A., Pérez-Coello, M. S., & Cabezudo, M. D. (2001). *Características varietales de los vinos Moscatel de grano menudo y Albillo en comparación con los Moscatel de Alejandría y Chardonnay.* Tecnología del vino, Septiembre–Octubre. (pp. 57–64).
51. Jussier, D., Dube Morneau, A. & Mira de Orduna, R., 2006. *Effect of simultaneous inoculation with yeast and bacteria on fermentation kinetics and key wine parameters of cool-climate Chardonnay.* Appl. Environ. Microbiol. 72, 221-227.
52. Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. *Effect of maturity and vine water status on grape skin and wine flavonoids.* Am.J. Enol. Vitic. 2002,53, 268–274.
53. King, E. S., Swiegers, J. H., Travis, B., Francis, I. L., Bastian, S. E. P., & Pretorius, I. S. (2008). *Coinoculated fermentations using Saccharomyces yeasts affect the volatile composition and sensory*

- properties of Vitis vinifera L. cv. Sauvignon Blanc wines*. Journal of Agricultural and Food Chemistry, 56(22), 10829–10837.
54. Knoll, C., Fritsch, S., Schnell, S., Grossmann, M., Krieger-Weber, S., Du Toit, M. & Rauhut, D., 2012. *Impact of different malolactic fermentation inoculation scenarios on Riesling wine aroma*. World J. Microb. Biot. 28, 1143-1153.
 55. Lambrecht, M. G.; Pretorius, I. S. *Yeast and its importance to wine aromas A review*. S. Afr. J. Enol. Vitic. 2000, 21, 97- 129.
 56. Lambrechts, M. G., & Pretorius, I. S. (2000). *Yeast and its importance to wine aroma review*. South Africa Journal Enology and Viticulture, 21, 97e129.
 57. Lee, K. W., Hur, H. J., Lee, H. J., & Lee, C. Y. (2005). *Antiproliferative effects of dietary phenolic substances and hydrogen peroxide*. Journal of Agricultural and Food Chemistry, 53, 1990–1995.
 58. Lee, S.G., Lee, K.W., Park, T.H., Park, J.Y., Han, N.S., Kim, J.H., 2012. *Proteomic analysis of proteins increased or reduced by ethanol of Lactobacillus plantarum ST4 isolated from Makgeolli, traditional Korean rice wine*. J. Microbiol. Biotechnol. 22 (4), 516e525.
 59. Lerm E., Engelbrecht, M. du Toit, *Malolactic fermentation: the ABC's of MLF*, S. Afr. J. Enol. Viticulture 31 (2010) 186–212.
 60. Lerm, E., Engelbrecht, L., du Toit, M., 2011. *Selection and characterisation of Oenococcus oeni and Lactobacillus plantarum South African wine isolates for use as malolactic fermentation starter cultures*. S. Afr. J. Enol. Vitic. 32 (2), 280e295
 61. Lockshin, L., & Corsi, A.M. (2012). *Consumer behaviour for wine 2.0: A review since 2003 and future directions*. Wine Economics and Policy.
 62. Lopez, R., Ferreira, V., Hernamde, Z.P. & Cacho, J.F., 1999. *Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study*. J. Sci. Food Agr. 79, 1461-1467.

63. MacNeil, Karen (2001). *The Wine Bible*. Workman. ISBN 1-56305-434-5
64. Maicas, S., Gil, J.V., Pardo, I., Ferrer, S., 1999. *Improvement of volatile composition of wines by controlled addition of malolactic bacteria*. Food Research International 32, 491–496.
65. Marquez, A., Serratos, M. P., Lopez-Toledano, A., & Merida, J. (2012). *Color and phenolic compounds in sweet red wines from Merlot and Tempranillo grapes chamber-dried under controlled conditions*. Food Chemistry, 130, 111–120.
66. Martínez-Pinilla, O., Martínez-Lapuente, L., Guadalupe, Z., & Ayestarán, B. (2012). *Sensory profiling and changes in colour and phenolic composition produced by malolactic fermentation in red minority varieties*. Food Research International, 46, 286e293
67. Massera, M., Soria, A., Catania, C., Krieger, S. & Combina, M., 2009. *Simultaneous inoculation of Malbec (Vitis vinifera) musts with yeast and bacteria: Effects on fermentation performance, sensory and sanitary attributes of wines*. Food Technol. and Biotech. 47, 192-201.
68. Mateus and De Freitas, “*Evolution and stability of anthocyanin-derived pigments during port wine aging*,” Journal of Agricultural and Food Chemistry, vol. 49, no. 11, pp. 5217–5222, 2001
69. Mateus, N., Pascual-Teresa, S., Rivas-Gonzalo, J. C., Santos-Buelga, C., & Freitas, V. (2002). *Structural diversity of anthocyanin-derived pigments in port wines*. Food Chemistry, 76, 335–342
70. Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., & Ewert, B. (1999). *Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia*. Journal of Agricultural and Food Chemistry, 47, 4009–4017.
71. Medina, K., Boido, E., Dellacassa, E., & Carrau, F. (2005). *Yeast interactions with anthocyanins during red wine fermentation*. American Journal of Enology and Viticulture, 56(2), 104–109.
72. Michael Schwarz, Peter Quast, Dietrich von Baer and Peter Winterhalter, *Vitisin A Content in Chilean Wines from Vitis*

- vinifera* Cv. *Cabernet Sauvignon* and Contribution to the Color of Aged Red Wines (2008). Institute of Food Chemistry, *J. Agric. Food Chem.*, 51 (21), pp 6261–6267
73. Moine-Ledoux, V., & Dubourdieu, D. (2002). *Role yeast mannoproteins with regard to tartaric stabilisation of wines*. *Bulletin de l'O.I.V.*, 75(857/858), 471–482.
74. Molina, A. M., Guadalupe, V., Varela, C., Swiegers, J. H., Pretorius, I. S., & Agosin, E. (2009). *Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains*. *Food Chemistry*, 117(2), 189–195
75. Morata A, et al. (2007) *Formation of vinylphenolic pyranoanthocyanins by selected yeasts fermenting red grape musts supplemented with hydroxycinnamic acids*. *Int J Food Microbiol* 116(1):144-52
76. Morata, M. C. Gómez-Cordovés, F. Calderón, and J. A. Suárez, “*Effects of pH, temperature and SO₂ on the formation of pyranoanthocyanins during red wine fermentation with two species of Saccharomyces*,” *International Journal of Food Microbiology*, vol. 106, no. 2, pp. 123–129, 2006
77. Morel-Salmi, C., Souquet, J. M., Bes, M., & Cheynier, V. (2006). *Effect of flash release treatment on phenolic extraction and wine composition*. *Journal of Agricultural and Food Chemistry*, 54, 4270–4276.
78. Moreno-Arribas, M. V., Gómez-Cordovés, C., & Martín-Alvarez, P. J. (2008). *Evolution of red wine anthocyanins during malolactic fermentation, post fermentative treatments and ageing with lees*. *Food Chemistry*, 109, 149e158.
79. Nagel, C. W., & Wulf, L. W. (1979). *Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon*. *American Journal of Enology and Viticulture*, 30, 111–116.
80. Nardini, M.; D’Aquino, M.; Tomassi, G.; Gentili, V.; DiFelice, M.; Scaccini, C. *Inhibition of human low density lipoprotein oxidation by*

- caffeic acid and other hydroxycinnamic acid derivatives. Free Radical Biol. Med.* **1995**, *19*, 541-552.
81. Nikolaou, E., Soufleros, E. H., Bouloumpasi, E., & Tzanetakis, N. (2006). *Selection of indigenous Saccharomyces cerevisiae strains according to their oenological characteristics and vinification results.* Food Microbiology, *23*, 205e211.
82. Nikolaou, E., Soufleros, E. H., Bouloumpasi, E., & Tzanetakis, N. (2006). *Selection of indigenous Saccharomyces cerevisiae strains according to their oenological characteristics and vinification results.* Food Microbiology, *23*, 205–211
83. Obrenovich, M. E., Li, Y., Parvathaneni, K., Yendluri, B. B., Palacios, H. H., Leszek, J., et al. (2011). *Antioxidants in health, disease and aging.* CNS and Neurological Disorders-Drugs Targets, *10*, 192–207
84. Palacios, A., Navascués, E., Santiago, L., Macías, C., Rasines, G., Fuster, A., Krieger, S., Julien, A., 2003. Control Biológico de la Fermentación Maloláctica. Viticultura/Enología Profesional *84*, 45–53.
85. Pan, W., Jussier, D., Terrade, N., Yada, R.Y. & Mira de Orduna, R., 2011. *Kinetics of sugars, organic acids and acetaldehyde during simultaneous yeast-bacterial fermentations of white wine at different pH values.* Food Res. Int. *44*, 660-666.
86. Pozo-Bayon, M.A., G-Alegriá, E., Polo, M.C., Tenorio, C., Martín-Alvarez, P.J., Calvo de la Banda, M.T., Ruiz-Larrea, F., Moreno-Arribas, M.V., 2005. *Wine volatile and amino acid composition after malolactic fermentation: effect of Oenococcus oeni and Lactobacillus plantarum starter cultures.* Journal of Agricultural and Food Chemistry *53*, 8729–8735
87. Rapp, A.; Versini, G. *Influence of nitrogen compounds in grapes on aroma compounds of wine. In Proceedings of the International Symposium on Nitrogen in Grapes and Wines; Rantz, Ed.; American Society of Enology and Viticulture: Davis, CA, 1991; pp 156-164.*
88. Rapp, Linskens, Jackson (Eds.), *Wine and trihydroxylated terpene and norisoprenoid al- Analysis*, Springer-Verlag, Berlin, 1988, p. 28.

89. Rastija, V., Srećnik, G., & Medic-Šarić, M. (2009). *Polyphenolic composition of Croatian wines with different geographical origins*. Food Chemistry, 115, 54–60.
90. Rauhut, D. (1993). *Sulfur compounds and their influence on wine quality*. Wein- Wissenschaft, 48, 214–218.
91. Ribereau-Gayon P., Glories Y., Maujean A. and Dubourdieu D.; 2006. Handbook of Enology (Volume 2). The Chemistry of Wine and Stabilization and Treatment. John Wiley & Sons, Ltd, New York
92. Robinson (ed) The Oxford Companion to Wine Third Edition, Oxford University Press (2006), pg. 746: "*Vine varieties*",
93. Robinson Jancis Robinson's Wine Course Third Edition pg 142–143 Abbeville Press (2003)
94. Robinson Vines, Grapes & Wines pg 91–94 Mitchell Beazley (1986)
95. Robinson, J. Harding and J. Vouillamoz Wine Grapes - *A complete guide to 1,368 vine varieties, including their origins and flavours* pgs 630-634, Allen Lane (2012)
96. Romero and Bakker, "*Anthocyanin and colour evolution during maturation of four port wines: effect of pyruvic acid addition*," Journal of the Science of Food and Agriculture, vol. 81, no. 2, pp. 252–260, 2001
97. Rosi, I., Fia, G. & Canuti, V., 2006. *Influence of different pH values and inoculation time on the growth and malolactic activity of a strain of Oenococcus oeni*. Aust. J. Grape Wine R. 9, 194-199.
98. Saénz-Navajas, M.P., Ballester, J., Pêcher, C., Peyron, D., & Valentin, D. (2013). *Sensory drivers of intrinsic quality of red wines: Effect of culture and level of expertise*. Food Research International, 54, 1506–1518.
99. Sauvageot, F., Vivier, P., 1997. *Effects of malolactic fermentation on sensory properties of four Burgundy wines*. American Journal of Enology and Viticulture 48, 187–192.
100. Scanes, K. T., Hohmann, S., & Prior, B. A. (1998). *Glycerol production by the yeast Saccharomyces cerevisiae and its relevance*

- to wine: A review*. South African Journal for Enology and Viticulture, 19(1), 17–24.
101. Schreier, P. Flavour composition of wines: a review. CRC Crit.nReV. Food Sci. Nutr. 1979, 12, 59-111.
 102. Schwarz M1, Picazo-Bacete JJ, Winterhalter P, Hermosín-Gutiérrez I. *Effect of copigments and grape cultivar on the color of red wines fermented after the addition of copigments*. J Agric Food Chem. 2005 Oct 19;53(21):8372-81.
 103. Sellappan, S., Akoh, C. C., & Krewer, G. (2002). *Phenolic compounds and antioxidant capacity of Georgia-Grown blueberries and blackberries*. Journal of Agriculture and Food Chemistry, 50, 2432–2438.
 104. Shinohara, T., Kubodera, S., & Yanagida, F. (2000). *Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation*. Journal of Bioscience and Bioengineering, 90(1), 90–97.
 105. Subramani, S., Casimir, C. A., & Krewer, G. (2002). *Phenolic compounds and antioxidant capacity of Georgia-Grown blueberries and blackberries*. Journal of Agricultural and Food Chemistry, 50, 2432–2438
 106. Sumbly, K.M., Grbin, P.R., Jiranek, V., 2010. *Microbial modulation of aromatic esters in wine: current knowledge and future prospects*. Food Chem. 121 (1), 1e16.
 107. Thorngate, J. H. III, (1999). *Yeast strain and wine flavour: nature or nurture?* In A. L. Waterhouse & S. E. Ebeler (Eds.), Chemistry of wine flavour. Washington, DC: American Chemical Society
 108. Tominaga, T., & Dubourdieu, D. (2000). *Studies on varietal aroma of wines from Vitis vinifera L. cv. Sauvignon blanc and their formation from non-aromatic precursors in grapes*. Revue des Oenologues et des Techniques Vitivinicoles et enologiques, 97, 22–28.

109. Ugliano, M., & Moio, L. (2005). *Changes in the concentration of yeast-derived volatile compounds of red wine during malolactic fermentation with four commercial starter cultures of Oenococcus oeni*. *Journal of Agricultural and Food Chemistry*, 53, 10134e10139.
110. Ugliano, M., Bartowsky, E. J., McCarthy, J., Moio, L., & Henshke, P. A. (2006). *Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three Saccharomyces yeast strains*. *Journal of Agricultural and Food Chemistry*, 54(17), 6322–6331.
111. Villamor, R., & Ross, C. (2013). *Winematrix compounds affect perception of wine aromas*. *Annual Review of Food Science and Technology*, 4, 1–20.
112. Vivas, N., Lonvaud-Funel, A., & Glories, Y. (1995). *Observations sur l'augmentation de l'acidité volatile dans les vins rouges aux cours de leur élevage en barriques*. *Journal des sciences et techniques de la Tonnellerie*, 1, 81e104.
113. Walter, A., Etienne-Selloum, N., Brasse, D., Khallouf, H., Beretz, A., & Schini-Kerth, V. B. (2008). *Intake of red wine polyphenols prevents tumor growth and neovascularization in a syngenic model of colon cancer*. *Anticancer Research*, 28 (3534–3534).
114. Waterhouse, A. L. (2002). *Wine phenolics*. *Annals of New York Academy of Sciences*, 957, 21–36.
115. Wrolstad, R. E., Durst, R. W., & Lee, J. M. (2005). *Tracking color and pigment changes in anthocyanin products*. *Trends in Food Science and Technology*, 16, 423–428.
116. Wulf, L. W., & Nagel, C. W. (1980). *Identification and changes of flavonoids in Merlot and Cabernet Sauvignon wines*. *Journal of Food Science*, 45, 479–484.
117. Xiang, L. M., Xiao, L. Y., Wang, Y. H., Li, H. F., Huang, Z. B., & He, X. J. (2014). *Health benefits of wine: Don't expect resveratrol too much*. *Food Chemistry*, 156, 258–263

118. Yoo, K. M., Al-Farsi, M., Lee, H., Yoon, H., & Lee, C. Y. (2010). *Antiproliferative effects of cherry juice and wine in Chinese hamster lung fibroblast cells and their phenolic constituents and antioxidant activities*. *Food Chemistry*, 123, 734–740.