COFERMENTATION, POST-ALCOHOLIC, AND POST-MALOLACTIC FERMENTATION BLENDING OF MALBEC, MERLOT AND PETITE SIRAH WINES

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TITLE: Cofermentation, Post-Alcoholic, and Post-Malolactic Fermentation Blending of Malbec, Merlot and Petite Sirah Wines

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ABSTRACT

Cofermentation, Post-Alcoholic, and Post-Malolactic Fermentation

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Armando Arturo Vega-Osorno

A two-year study was conducted to assess the effects of cofermentation on red wine varietals. During the winemaking process, wines can be made from two or more varieties by picking, crushing and fermenting them together, a practice known as cofermentation. They can also be blended either after the completion of alcoholic fermentation or after malolactic fermentation. In the first year of the study, two grape varieties, Merlot (Mer), and Malbec (Mal) were cofermented. On the second year, a third varietal, Petite Sirah (PS) was also studied. Cofermented wines containing every possible binomial combination of the varietals was made and one trinomial on 2019. The cofermented wines were compared to monovarietal wines and also to wines that were produced by blending either after alcoholic fermentation or after malolactic fermentation. The phenolic profile of the wines was followed from the onset of fermentation up to 36 months of bottle aging for the 2018 vintage and in the case of the 2019 vintage, up until 250 days after crushing. In 2018, cofermented wines and wines that were blended after malolactic fermentation had an anthocyanin profile that was more similar to Malbec than to Merlot, while the tannin profile was more resemblant of Merlot. In 2019 cofermentation improved the anthocyanin content when compared to post alcoholic and post malolactic blend only when the three varietals were cofermented. A sensory analysis with 10 trained individuals was conducted on the
2018 vintage. It was demonstrated that Malbec wines had a higher amount of red fruit aromas while Merlot wines were perceived as being more astringent. Cofermented and post malolactic fermentation blended wines were indistinguishable to panelists and blending after alcoholic fermentation produced wines that highlighted the individual varietal character.

Keywords: Red wine, phenolics, blending, tannins, anthocyanins, sensory
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1. LITERATURE REVIEW

1.1 Introduction

Wine is a very important commodity in our modern world and of special relevance to the state of California. During the 2021 harvest, 3,878,000 tones of grapes were crushed, averaging a price of $861 per ton. California is responsible for 85% of the wine production in the United States (United States Department of Agriculture, 2022), which generates a multi-billion-dollar industry and employs thousands of people across different economic sectors. There is a tendency of consumers to gravitate to wines that are additive-free (Maykish et al., 2021). Winemakers are making use of diverse techniques to improve wine quality without the use of additives. Most of these techniques, for example cold soak, extended maceration and cofermentation, rely on physical processes that improve the extraction of phenolics (Casassa et al., 2019), rather than additions of exogenous chemicals. The perceived quality of red wine is closely related to its phenolic profile (Somer & Evans, 1974; Merkytė et al., 2020).

1.2 Wine Phenolics

Structurally, phenolics are aromatic compounds derived from a hydroxy-substituted benzene ring. Originating from this basic structural unit, a multitude of phenolic compounds have been identified in grapes and subsequently, in wine. Their occurrence in wine is related to the type of grape, their growth conditions, the vinification process, and extrinsic factors such as oxygen exposure and storage temperature (Singleton, 1969). Phenolic compounds play an important role influencing organoleptic characteristics of wine, such as color, taste, aroma, and mouthfeel. The phenolic content of red wines is also related to the health benefits derived from its moderate consumption (Renaud & de
Lorgeril, 1992). Due to their complex electron structure, polyphenols are highly reactive, and as such, they start reacting as soon as the grapes are removed from the vine and crushed. It is therefore essential for the winemaker to manage and improve phenolic content to achieve wine quality. Phenolic compounds that are relevant to wine can be sorted in two main classes: flavonoids and non-flavonoids (Boulton, 2001) (Figure 1). Phenolic compounds in red wines are present in ranges of 1100 to 3165 mg/L (Burns et al., 2000).

Figure 1. Structures of phenolic compounds.
Excerpted from Fulcrand et al., 2004.
1.2.1 Flavonoids

Flavonoids comprise most of the compounds that make up the polyphenolic profile of a wine and that are pertinent to assess wine quality. It is estimated that flavonoids in red wines represent from 80% to 90% of the total phenolic content (Stratil et al., 2008). Flavonoids are characterized by having the general structure of a 15-carbon skeleton, consisting of two phenyl rings and one heterocyclic pyran ring that has an embedded oxygen (Cheynier, et al., 1998). Since flavonoids are secondary metabolites in grapevines, their presence is modulated by environmental factors such as ripeness level in grapes, temperature, sun exposure, altitude, soil type, water and nutrient availability and growing region (Downey et al., 2006). Flavonoids in wine can be further divided into three main groups: Anthocyanins, flavonols and tannins (Figure 1).

1.2.1.1 Anthocyanins

Anthocyanins are water soluble blue-red pigments that are responsible for imparting color in grapes and eventually, in red wine. In most red grape cultivars, anthocyanins will be secluded in the vacuoles of the grape skin or exocarp (Fontes et al., 2011). In less common cases, namely in the varietals denominated as teinturier, anthocyanins will be also found in the grape pulp. At a normal wine pH range (3.5), anthocyanins will exist mostly in their flavylium form, which is the cationated form and will be associated with one or two sugars, and they can be acetylated. In grapes and wines, the anthocyanins present are derivatives of six main anthocyanidins: malvidin, cyanidin, petunidin, pelargonidin, peonidin and delphinidin (Monagas & Bartolomé, 2009) (Figure 2). Their hue depends strongly on pH, ranging from red in its basic range of 3.0 to purple in the 4.0 (Heredia et al., 1998). Traditional red winemaking entails the crushing of the
grape berries followed by alcoholic fermentation of the resulting must, which is composed of grape solids, skins, seeds and sometimes stems (Casassa et al., 2021). This process is known as maceration. During this procedure, anthocyanins from the skins will migrate into the fermenting liquid matrix following complex diffusion phenomena, in which temperature, increasing ethanol concentration and pH all play a crucial role. The extraction of anthocyanins into the must reaches a maximum in the first 4 days of fermentation and decreases thereafter (Kennedy, 2008). Absorption of pigments by yeast lees, precipitation in conjunction with tartaric acid crystals, along with the formation of anthocyanin-tannin adducts and anthocyanin oligomers account for the reported decrease in monomeric anthocyanins after the completion of alcoholic fermentation. Anthocyanin content can decrease up to 39% at this point (Mazza et al., 1999, Vasserot et al., 1997).

![Diagram of the basic structure of anthocyanins](image)

**Figure 2. Diagram of the basic structure of anthocyanins.**

Excerpted from García-Beneytez et al., 2003.
1.2.1.2 Flavonols

Flavonols are a class of flavonoids that have a 3-hydroxyflavone backbone (Cheynier et al., 1998). In grapes, these compounds are exclusively found in skins, where they have the role of protecting the berries from sunlight since they absorb strongly in the UV-A and UV-B spectra. Studies on Pinot noir showed that sun exposure increased the flavonol metabolic pathway (Price et al., 1995). Although flavonols are not as strongly colored as anthocyanins, they can act as co-pigments and stabilize wine color. Co-pigmentation is an important phenomenon where anthocyanins bind non-covalently to other molecules and increase chromatic intensity (Boulton, 2001). Flavonols also play a relevant role in wine astringency and bitterness (Ferrer-Gallego et al., 2016). Relevant wine flavonols include quercetin, myricetin and kaempferol (Figure 1). It has been demonstrated that flavonol profiles can be used to differentiate between grape cultivars as taxonomic markers (Castillo-Muñoz et al., 2007). Flavanols are also markers of quality, since high value wines can have up to four times the amounts of flavonols compared to low value wine (Ritchey & Waterhouse, 1999). The flavonol profile of every grape varietal is unique and thus can be used to discern between varietals in a finished wine. Flavonols have nutraceutical value and their beneficial effects on human health have been proven (Aherne & O’Brien, 2002). Flavanols protect the cardiac system, they have antidiabetic, anti-obesity and anticancer activity. These benefits are related to flavanol’s high antioxidant capacity (Ballard & Junior, 2019).

1.2.1.3 Tannins

Tannins are an important class of flavonoids that contribute to the sensory aspect of red wine by interacting with salivary proline-rich proteins, reducing oral lubrication, and
eliciting the sensation of astringency or puckering (Cheynier et al., 2006). Astringency is an important organoleptic feature and thus is considered a desirable trait in red wines. While astringency is known to be a tactile sensation, it can sometimes elicit cross-modal associations with bitterness, which if present in excess can be a detrimental attribute. Although tannins can be derived from oak in wines that have been put through barrel maturation, in grapes they can be found in the skin, the seeds, the stems and to a lesser extent in the flesh. Tannins can therefore be divided into hydrolysable tannins if their source is oak and non-hydrolysable or condensed tannins if they come from grapes. Hydrolysable tannins are composed of subunits of gallic acid, while grape tannins are composed of repeating units of catechin, epicatechin, epigallocatechin and epicatechin-3-gallate (Smith et al., 2015) (Figure 1). Tannins derived from grape skin are composed of primarily epigallocatechin polymers and trace amounts of gallicatechin and epigallocatechin-3-gallate, and their size can range from 3 to 83 subunits (Smith et al., 2015). Tannins originating from seeds are smaller, ranging from 2 to 26 subunits of catechin and epicatechin (Herderich & Smith, 2005). As other polyphenolic compounds, the amount and nature of tannins present in grapes are varietal-dependent and certain varietals such as Pinot noir are notable for their low amount of tannin content (Harbertson et al., 2008). The astringency perceived in a finished wine will also be affected by the vinification techniques implemented by the winemaker (Smith et al., 2015). In the case of wines made from high-tannic varietals such as Tannat, a technique known as micro-oxygenation was developed in order to soften the perceived astringency by promoting the polymerization of tannin-anthocyanin adducts (Sullivan, 2002). Apart from being an important element in wine mouthfeel, tannins are involved in polymerization reactions with
anthocyanins, which are responsible for the change in hue from purple to brick red as wine ages and the subsequent diminution in astringency (Boulton, 2001).

1.2.2 Non-Flavonoids

Although non-flavonoids only represent 10 to 20% of the phenolics in red wine, they contribute to important organoleptic characteristics by being involved in browning reactions due to oxidation (Garrido & Borges, 2013) and by eliciting particular taste and textural perceptions (Peleg & Noble, 1995). They are also precursors of relevant aromatic compounds (Gawel et al., 2018). Relevant non-flavonoids include phenolic acids (benzoic acids and hydroxycinnamic acids) and stilbenes (Figure 1). The majority of non-flavonoids are located in the flesh of grapes (Cheynier et al., 2006).

1.2.2.1 Benzoic Acids

Benzoic acids contain seven carbons and can be found as conjugated or free forms, the latter being the most abundant in wine (Rentzsch et al., 2009). The most relevant compounds are gentisic acid, \( p \)-hydroxibenzoic acid, protocatechuic acid, syringic acid, salicylic acid and gallic acid. Gallic acid is especially important (Figure 1) since it is both the precursor of all hydrolysable tannins and can also be the result of tannin hydrolysis (Gutiérrez-Escobar et al., 2021). It has been demonstrated that gallic acid has important antioxidant ability (Yilmaz & Toledo, 2004). Gallic acid has strong radical-scavenger capacities and has been proven to inhibit fungal and microbial activities. Its strong antioxidant capacity is related to the molecular conformation of its phenolic hydroxyl groups (Figure 1) (Badhani et al., 2015).
1.2.2.2 Hydroxycinnamic Acids

Hydroxycinnamic acids possess a C6-C3 common structure and are found mostly as esters of tartaric acid (Baranowski & Nagel, 1981) (Figure 1). They can appear in a cis- or trans- configuration, the most common being the -trans form which is more stable (Rentzsch et al., 2009). They are mostly derivatives of caffeic acid, coumaric acid, and ferulic acid (Ong & Nagel, 1978). When they exist as esters of tartaric acid they are referred to as caftaric acid, p-coutaric acid and ferraric acid, respectively (Waterhouse, 2002). These compounds are responsible for undesirable reactions when oxidized: browning reactions in fermenting juices and finished wines and for producing bitterness (Baranowski & Nagel, 1981). Levels of hydroxycinnamic acids in finished red wines are typically 60 mg/L and 130 mg/l in whites (Waterhouse, 2006).

1.2.2.3 Stilbenes

Stilbenes are structurally formed by two aromatic rings linked in their ethyl position and they can be found in a grape and vine tissues (Gutiérrez-Escobar et al., 2021) (Figure 1). The most important stilbene, resveratrol, is well-known for having therapeutic properties in human health and extensive research has been done on its effects as a cardioprotector, its antioxidant properties and its chemoprotective capacities against cancer (Jang et al., 1997). The presence of stilbenes in grapes has been associated to both biotic stress factors such as the presence of Botrytis cinerea, a prevalent fungal pathogen and abiotic sources of stress such as high UV radiation (Langcake, 1979). These compounds can form oligomers and polymers, which are referred to collectively as viniferins. Stilbenes can be glycosylated and can adopt cis and trans configurations in wine, although the trans form is the most abundant by far in grape skin. The isomerization of trans-resveratrol into its cis
counterpart occurs during the winemaking process and since resveratrol is extracted from grape skins, red wines tend to have a much higher content than white wines since they traditionally go through maceration, which implies a long contact time between skins, seeds and must (Jeandet et al., 1995). Vinification techniques will influence the extraction of stilbenes into wines and depending on the varietal, can cause fluctuations of up 35% percent in the extracted stilbenes (Favre et al., 2020).

1.2.3 Polymeric Pigments

One of the most evident characteristics of red wine as it ages, is its color drift from bright purple to brick reddish, accompanied by a noticeable reduction in astringency. These changes are due to polymerization reactions between anthocyanins and other polyphenols, namely tannins (Somers, 1971; Fulcrand et al., 2004). The compounds that form during wine aging are referred to as polymeric pigments. They confer a stable color to red wine, and they are resistant to sulfite bleaching. Sulfite in the form of potassium metabisulfite (KMBS) is a ubiquitous additive that protects the wine from microbial spoilage and oxidation. The pioneer work of Somers in 1968 demonstrated that while monomeric anthocyanins were responsible for the color of young Shiraz wine, most of the color in a 16-year-old wine was due to polymeric pigments. There are three pathways in which polymeric pigments are formed: first, direct anthocyanin-tannin reactions (Figure 3), in which a nucleophilic addition of the anthocyanin to the tannin, followed by dehydration results in a stable anthocyanin-tannin adduct (Fulcrand et al., 2004). Secondly, a reaction mediated by acetaldehyde (Figure 4). Acetaldehyde is the most abundant aldehyde in wine since it is a by-product of yeast metabolism and results from oxidation of ethanol (Romano et al., 1994). In this case, the aldehyde gets protonated and follows a nucleophilic addition
of either two tannin molecules, a pair of anthocyanins or a mixed set of anthocyanin-tannin. The third pathway (Figure 5) is when anthocyanins react to form pyranoanthocyanins by cycloaddition. In this case, a second pyran ring will be formed within the anthocyanin structure by the reaction of pyruvic acid, or in some cases, with vinyl phenols, vinyl flavanols or acetaldehyde. Pyruvic acid is an important yeast metabolite and different strains of yeast will produce variable levels of it (Morata et al., 2003). Pyranoanthocyanins derived from pyruvic acid are very stable pigments (Romero & Bakker, 2001) and they are sometimes referred to as vitisins A (Fulcrand et al., 1998). The various formation mechanisms of polymeric pigments have been demonstrated extensively both in wines and model solutions (Somers, 1971; Salas et al., 2004), yet many factors are still unknown. The dynamics of polymeric pigment formation depend on the initial amount and the type of anthocyanin and tannins, levels of acetaldehyde and pyruvic acid produced during fermentation and aging, pH of the wine, oxygen exposure and storage temperature. These factors rely on grape varietals, viticultural practices, choice of yeast, maceration time, fining techniques, extraction levels, temperature, and sulfite additions. It is in the best interest of the winemaker to modulate these dynamics in order to maximize phenolic extraction and hence produce wines that will develop polymeric pigments.
Figure 3. Products issued from direct reactions of anthocyanins and tannins.

Excerpted from Fulcrand et al., 2004.

Figure 4. Products issued from acetaldehyde-induced polymerization.

Excerpted from Fulcrand et al., 2004.
1.2.4 Vinification Techniques

Traditional red winemaking starts by either manually or mechanically harvesting the grapes at peak maturity (Boulton et al., 2013). The choice of when to harvest the grape material depends on the expertise and personal preference of the winemaker according to the style of wine; however, there are general guidelines that have been established to maximize quality, such as sugar content being in the range of 19º to 23º Brix, pH in the range of 3 to 3.4, and titratable acidity being 6 to 9 g/l tartaric acid equivalents. Harvesting before would produce wines with lower ethanol and unripe taste and doing so later would cause high ethanol content, lack of acid, and raisin-like character (Amerine & Winkler, 1944). If grapes are harvested manually, they will go through destemming and then crushed. Some clusters may remain intact in a practice known as whole cluster fermentation, which has been proven to increase aromatic complexity (Casassa et al., 2019). In the case of mechanically harvested grapes, they only need to be crushed since

Figure 5. Pigments issued from cycloadditions.

Excerpted from Fulcrand et al., 2004.
they will be detached from the stems in the vineyard. Fermentation from the grapes’ native yeast will occur if no sulfites are added, otherwise a commercial strain of *Saccharomyces cerevisiae* will be inoculated into the must. During fermentation, a floating cluster of grape solids or cap will be formed in the fermenting vessel, which needs to be homogenized at least daily with the fermenting liquid by either pump-overs or punch-downs. How often and how vigorously the cap is submerged into the fermenting must will affect phenolic extraction (Frost *et al.*, 2018). A wine is considered dry when fermentable sugars are depleted by yeast metabolism, although leaving residual sugars can be a stylistic choice. The period of contact between grape solids and dry wine can be prolonged in a technique referred to as extended maceration, the periods can range from several days up to several months (Francesca *et al.*, 2014). Extended maceration can result in wines with lower color and more astringency (Frost *et al.*, 2018). This can be explained by a re-absorption phenomena of anthocyanin monomers by yeast lees and continued extraction of tannins from seeds, which show an affinity for high ethanol content. After maceration, wine will be separated from the pomace, and in most cases, the solids will be pressed. Subsequently, malolactic fermentation will ensue, in which lactic acid bacteria will transform the malic acid present in wine into the softer, less tart lactic acid (Boulton *et al.*, 2013). During this stage, a small amount of color loss will occur, which has been attributed to the consumption of acetaldehyde by lactic acid bacteria *Oenococcus oeni* (Burns & Osborne, 2015) which will affect polymeric pigment formation. In order to increase wine’s microbial stability and improve clarity, some winemakers will resort to finning processes which can also deplete the wine color. Consequently, wine will be filtered, stabilized with SO$_2$ and bottled. During the entirety of the winemaking process, every step of the process will have an influence in
the phenolic content, therefore it is important to consider the consequences before each operation (Boulton et al., 2013). Wine additives such as pectolytic enzymes can ameliorate color content by facilitating anthocyanin extraction from the grape solid matrix (Muñoz et al., 2004). Oenological tannins can be added at the beginning of fermentation to increase polymerization. Concentrated grape juice can be used as a supplement to improve wine color. However adding dyes (Wilson, 2020) or exogenous tannins (Harbertson et al., 2012) can bring deleterious consequences to the taste of wine. There is a drive for winemakers to improve phenolic extraction and evolution by using simple techniques, such as cofermentation, cold soak, extended maceration, and stem addition (Casassa et al., 2019).

1.2.4.1 Cofermentation

Cofermentation is an ancient technique that entails blending two or more grape varietals during crushing. It is a traditional practice in the Old World, especially in the French Côte Rôtie AOC, where Syrah is traditionally cofermented with the white grape Viognier in ratios ranging from 5% to 20% (Casassa et al., 2020). The practice of cofermentation may have been a spontaneous result of the so-called field blends, in which several grape varieties were planted together and would get harvested and vinified simultaneously (Robinson & Harding, 2015). It represents a logistical challenge since both varieties must be picked concurrently. Viognier is a white aromatic grape, and it is anecdotally thought that cofermenting it with Syrah will improve complexity in aromatics and color evolution. Extensive research has been done on cofermenting Syrah with several white Rhône cultivars (Casassa et al., 2020). Results showed that even though wine’s chromatic characteristics are reduced by dilution, aromatic complexity was increased. Other studies have been done by cofermenting Viura (white grape) and Tempranillo (red
Wines containing Viura showed lower color and less tannins in respect to the ones made with only Tempranillo. Wines containing Viura were also perceived to have higher acidity than the monovarietals (Etaio et al., 2008). The aromatic effects of cofermenting the red varietals Cencibel, Bobal and Moravia Agria in different ratios were studied by gas chromatography (García-Carpintero et al., 2011). It was demonstrated that cofermented wines had a higher number of aromatic compounds detectable through gas chromatography. Wines made with the three varietals were the ones with the most diverse aromatic profile. In a parallel study (García-Carpintero et al., 2010), a trained sensory panel favored the cofermented wines over the monovarietal ones. It was demonstrated that cofermentation in the case of Merlot and Cabernet Sauvignon also promoted the extraction of co-pigments, which are important to establish long term color stability (Lorenzo et al., 2005). A study made by cofermenting warm climate Syrah with the pomace of a white Spanish variety (Pedro Ximenez) demonstrated that polymeric pigment formation was significantly higher in wines that were cofermented with 10% white pomace (Gordillo et al., 2014). This was due to a higher amount of catechins provided by the white varietal. Higher amounts of polymeric pigments will provide the wine with long lasting color (Boulton, 2001). The different dynamics seen in cofermented versus monovarietal blends can be explained by the variation in the ratios of anthocyanins to tannins (Lorenzo et al., 2005). While cofermentation may imply logistical challenges, the possible benefits derived from using it deserve further research to be developed.

1.2.4.2 Blending

Blending two or more different varieties of fermented wine is known as coupage. It allows winemakers to create wines that have a wider variety of aromas than their
individual counter parts (Hopfer et al., 2012). It also allows for the winemaker to modulate certain effects like astringency, acidity or ethanol levels by blending wines that differ in these characteristics (Rankine, 1988). Besides affecting aroma, blending also modifies the chromatic characteristics of wine and how they evolve (Dooley et al., 2012b). It has been demonstrated that blending a wine that is low in color with a wine that is highly saturated will produce a wine that has a higher consumer acceptance (Dooley et al., 2012a). Anthocyanin dynamics are affected by blending and different wines behave differently based on their content of anthocyanins and catechins. A study made with the varietals Cabernet Sauvignon, Tempranillo and Graciano showed that anthocyanins will decrease at a faster rate in wines made of blends of Tempranillo and Cabernet Sauvignon or Tempranillo and Graciano when compared to the Tempranillo monovarietal wine (Monagas et al., 2006). This can be explained by the fact that Cabernet Sauvignon and Graciano provide the blend with a higher level of tannins than Tempranillo, and the anthocyanins will aggregate to form polymeric pigments. This reaction is limited in the Tempranillo monovarietal wine by its lower levels of tannins. Blending will also have an effect on wine mouthfeel. A study made on the blend of Cabernet Sauvignon, Cabernet Franc, Carménère and Merlot showed a modulation in astringency and bitterness when Cabernet Sauvignon was blended with the other varietals in different proportions (Cáceres-Mella et al., 2014). While astringency is a desirable trait, excessive bitterness may be a deterrent for the consumer. Blending can have a variety of effects on the chromatic characteristics of the wine. A study that compared the post-malolactic blend of Syrah and white varieties versus cofermentation and post-alcoholic fermentation blending, showed that color was improved when Syrah was mixed with Viognier after the end of malolactic
fermentation (Casassa et al., 2020). The distinct color dynamics arisen from blending are related to the existence of different levels of various classes of anthocyanins in wine. Another study that focused on the anthocyanin composition of blends made from Tempranillo and Graciano showed the changes related to fluctuations in specific anthocyanins, notable derivatives of delphinidin and malvidin (Escudero-Gilete et al., 2010).

1.3 Grape Cultivars

1.3.1 Malbec

Malbec is a French red grape cultivar, also known as Côt or Auxerrois. It was once very popular in Bordeaux until a considerable frost in 1956 decimated many of the vines (Robinson & Harding, 2015). It is still oftentimes used in Bordeaux blends to provide color. It is still the norm in the French region of Cahors, where appellation wines must contain 70% of the varietal. It produces wines that are dark in color due to its high content of malvidin-3-glucoside followed by petunidin (Fanzone et al., 2010). Berries are spherical, medium sized, dark purple in color, and they tend to reach peak maturity during the middle of harvest season (Galet, 2000). Malbec was introduced to Argentina in 1868 by French agricultural engineer Michel Pouget and it is now considered the flagship varietal of Argentina. The phenolic profile of Malbec is heavily influenced by the growing region, as it was shown in a comparative study between Argentinian and Californian Malbec. It was demonstrated that Malbec from California was higher in anthocyanin content when compared to Malbec from Argentina: Californian wines had 475.2 mg/L malvidin-3-glucoside equivalents while Argentinian Malbec had 249.2 mg/L (Buscema & Boulton, 2015). It was stipulated that the difference in sun exposure due to the higher elevations of
the grapes grown in Argentina could have an influence on this factor (Fanzone et al., 2010). Common aromatic descriptors for Malbec include plum, dark fruit, tobacco and leather (Sánchez-Palomo et al., 2017).

1.3.2 Merlot

Merlot is a red French varietal that is extremely popular, and it is the second most planted varietal worldwide, after Cabernet Sauvignon (Robinson & Harding, 2015). It produces wines that are less chromatically saturated than those produced by Malbec grapes. Berries are spherical, medium-sized and they tend to ripe earlier in the season (Galet, 2000). Research has been done on Merlot’s anthocyanin profile, and it has been shown to be composed mainly of malvidin-3-glucoside followed by peonidin (Ignat et al., 2016). Common aromatic descriptors of Merlot include vegetal and red fruit aromas (Gürbuz et al., 2006).

1.3.3 Petite Sirah

Petite Sirah is a very dark grape of French origin also known as Durif. It is nowadays almost completely absent from its place of origin. Presently, Petite Sirah is mostly planted in California, where it is used to provide blends with color intensity and tannic structure. Berries are dark, small, and can range from spherical to ovoid in shape. They ripen mid-season (Galet, 2000). Petite Sirah produces wines that are dark, inky purple and heavily tannic (Patel & Shibamoto, 2002). Comparative research has demonstrated that Petite Sirah can contain up to twice the amount of extractable phenolics (3200 mg/L) when compared to Cabernet Sauvignon (1600 mg/L) (Kanner et al., 1994). As it ferments, Petite Sirah tends to produce considerable amounts of isoamyl acetate, which is the most abundant ester detected in wines made from this varietal and is responsible for a fruity,
banana-like aroma. Common aromatic descriptors for Petite Sirah include banana, plum, raisin, dark fruit, and aldehyde (Patel & Shibamoto, 2002).

1.4 Evaluating Cofermentation

The objective of this work is to evaluate the effects of cofermentation on phenolic content and sensory characteristics and compare them with wines that are either blended after alcoholic fermentation or after malolactic fermentation. To elucidate the possible effects of cofermenting varietals with different ratios of anthocyanins to tannins, a study was conducted over two consecutive vintages: 2018 and 2019. Monovarietal wines, cofermented wines and wines blended after alcoholic and malolactic fermentation were made from Merlot and Malbec in 2018 and from Merlot, Malbec and Petite Sirah in 2019. Cofermenting will imply certain logistical difficulties in the winery since the different grape varietals have to be picked on the same date. On another hand, it may reduce the usage of several tanks which will have a positive impact in the management of physical space. Other benefits of cofermentation include the increase in aromatic diversity (Lorenzo et al., 2008) and the enhancement of color stability due a phenomenon known as copigmentation, in which anthocyanins and non-colored molecules form non-covalent bonds and increase the chromatic intensity (Boulton, 2001). Cofermentation may also produce wines that have to spend less time in barrel for aging in the case of Merlot, Monastrell and Cabernet Sauvignon (Lorenzo et al., 2008). The extraction of non-colored molecules such as tannins will impact the intensity of color since tannins will bind to anthocyanins and will agglomerate into polymeric pigments. This dynamic will soften the astringency and will also provide the wines with strong pigmentation. A variety of tests (biochemical, spectroscopic, and sensorial) were performed on the different wines to
characterize them for a wide variety of traits. It is hypothesized that cofermented wines will show a distinct phenolic profile and thus will age differently in contrast to blended or monovarietal wines.
2. MATERIALS AND METHODS

2.1 Grapes

Grapes (Sunnybrook Vineyard, Paso Robles AVA, 35°57’29.1”N 120°59’41.3’’W) were manually harvested at maturity, transported in 0.5-ton bins and processed in the pilot winery of California Polytechnic University San Luis Obispo. Berry samples from each varietal were gathered randomly (n=250), kept in plastic bags and taken to the laboratory for analysis. In 2018 and 2019, the fruit was tested for Brix degrees, pH, titrable acidity (TA), yeast available nitrogen (YAN), malic and tartaric acids, glucose and fructose, ammonia, alpha-amino compounds, and potassium using the Y-15 enzymatic analyzer (Admeo Inc., Biosystems group, Hollister, CA, USA).

2.2 Winemaking

Grapes were destemmed and crushed using a crusher-destemmer (IMMA, Emilia Romagna, Italy) and adjusted to 50 ppm of free SO$_2$ as they were being processed. Subsequently, the resulting must was transferred to 60-L food-grade fermenters (Spiedel Braumestier, Ofterdingen, Germany), filling them by mass (50 kg ±0.1 kg) with the assistance of a digital scale (Adam Equipment, Oxford, NY, USA). Five fermenters of each monovarietal (Merlot, Malbec in 2018; Merlot, Malbec and Petite Sirah in 2019) were set up along with five fermenters of a blend in a 50:50 ratio of the following coferments (Malbec-Merlot Coferm in 2018, Malbec-Merlot Coferm, Merlot-Petite Sirah Coferm, Malbec-Petite Sirah Coferm in 2019) and a 33:33:33 ratio for Malbec-Merlot-Petite Sirah Coferm in 2019, for a total of fifteen 60-liter vessels filled up with 50±0.1 kg of must in 2018 and 33 60-liter fermenters for 2019. Musts were inoculated with *Saccharomyces cerevisiae* yeast strain EC-1118 (Lallemand, Ontario, Canada) after 12 h crushing at a rate
of 20 g/hL, and cap management consisted of two gentle punch-downs of 1-min duration each day, administered regularly at 10:00 am and 5:00 pm. The musts were also supplemented with diammonium phosphate (BSG Wine, Napa, California, USA) to increase the yeast available nitrogen to 250 mg/L. In 2018, due to the possible infection by cross-contamination of malolactic bacteria prior to the achievement of alcoholic fermentation (AF), lysozyme (Sanovo Eiprodukte, Lower Saxony, Germany) was added at a rate of 50 g/hL on day 6 post-crushing in order to prevent the premature onset of malolactic fermentation (MLF) before AF was achieved. This incident occurred because another experiment was being inoculated with malolactic bacteria in close proximity. Wines were fermented to dryness (less than 0.2 g/l of sugar) and pressed off the skins manually in a pump over sump equipped with a grid, at day 12 after crushing. After this, wines were inoculated with *Oenococcus oeni* malolactic bacteria strain VP46 (Lallemand, Ontario, Canada) and transferred to 20-liter glass carboys (Vitro, Nuevo León, Mexico). During AF, Brix degrees and temperature were closely followed daily using a hand-held densimeter (DMA, Anton Paar, Graz, Austria). Malic acid status was surveyed by automated enzymatic analysis (Admeo Inc., Biosystems group, Hollister, CA, USA). Once MLF had finished, the PMLF wines were blended. The wines were racked and transferred to a cold room (12.7 ºC) for tartrate stabilization. After 2 months of cold storage, wines were filtered using a pad filtration system (BuonVino, Cambridge, Ontario, Canada) of 5 µm pore size. Wines were adjusted for SO₂ to 30 ppm, bottled in 750-ml dark green glass bottles with a DIAM 5 microagglomerated cork closure (G3 Enterprises, Modesto, CA, USA), and stored in cellar-like conditions (12ºC, humidity of 60% and darkness) until analysis.
2.3 Chemical Analysis of Wine

The wines were assessed for ethanol concentration using an Anton Paar wine Alcolyzer model M/ME (Anton Paar, Graz, Austria), for pH using a bench top pH meter (Thermo Fischer Scientific Waltham, MA, USA), and for TA with an autotitrator (Hanna Instruments, Woonsocket, RI, USA) set to an endpoint of 8.2 with 0.1 N NaOH as titrant. Wines were analyzed for total phenolics, anthocyanins, tannins, small polymeric pigments...
(SPP) and large polymeric pigments (LPP) using the Adams-Harbertson assay (Harbertson et al., 2003). This procedure was done on day 5 after crushing, after MLF was finished, 2-, 6- and 12-months post-bottling. An HPLC-array-diode detector (Agilent Technologies, Santa Clara, CA, USA) was utilized to measure specific anthocyanins, flavonols and anthocyanin-derived pigments. The system used for this purpose was an Agilent 1100 series HPLC coupled to a Diode Array Detector as established earlier by Downey and Rochfort (2008) with some minimal alterations. A Zorbax SB-C18 column (4.6 mm x 150 mm, 3.5 µm particle size, Agilent Technologies, Santa Clara, CA, USA) was set and held at 40 ºC while protected with a guard column of the same packing material. The eluent, a solution of methanol and acetonitrile, was transferred to the mass spectrometer operating in positive ionization mode and the compounds were detected by multiple reaction monitoring. Malvidin-3–glucoside chloride (Extrasynthèse, Lyon, France) and quercitin-3-glucoside (Sigma-Aldrich, St. Louis, MO, USA) were used as standards for the detection of monomeric anthocyanins and flavonols, respectively. In both cases, standard calibration curves with $R^2 = 0.99$ were used.

2.4 Sensory Analysis

Sensory analysis was solely performed on the 2018 wines. The sensory panel consisted of 10 individuals, none of them color blind according to Ishihara tests (Morrot et al., 2001). PROP (6-n-propylthiouracil) status is defined as the ability of a person to perceive bitterness and individuals can be classified in 3 categories: Super tasters, medium tasters and non-tasters. Since bitterness in wine is caused by the presence of flavonoid phenols, it is relevant to count on a diverse group of panelists. The panelists’ PROP and taster status was confirmed with the ingestion of 6-n-propylthiouracil –PROP- (Fluka
Chemical Company, Buchs, Switzerland) and further classified according to the following ratio: 4 super tasters, 5 tasters and 1 non-taster (Tepper et al., 2001). The panel was composed of experienced individuals with previous and extensive exposure to wine, a majority of whom were enology students and staff from the California Polytechnic State University in San Luis Obispo, CA (Cal Poly). The nature of the study was kept confidential at all times to avoid bias. All the procedures involved in this project were reviewed and approved by the Cal Poly Institutional Review Board for human subject participation. These 10 individuals were trained biweekly for a total of nine 90-minute sessions in order to evaluate the samples for 12 characteristics. In terms of color: ruby and purple, for aromas: red fruit, baked fruit, spice, vegetal, tobacco, herbal, and earthy. For mouthfeel: acidity, astringency and length measured in seconds (known as caudalies). During these sessions, the panelists were exposed to the experimental wines, deliberated, and agreed upon the characteristics that the wines displayed. Standards of color and aroma were presented to the panelists to calibrate and were adjusted accordingly by staff in order to reach a sensory consensus. For every training session, a total of 5 to 8 experimental wines were poured into clear ISO glasses (Ravenscroft Crystal, Easton, PA, US). For the evaluation sessions, panelists were located in a sensory cabinet and were monadically given 30 mL of wine poured in ISO glasses labeled with a randomly generated 3-digit code. The panelists’ responses were registered in ballots provided with a 10 cm unstructured scale. The scale represents the panelists’ perception of intensity, the leftmost side being counted as zero and the rightmost side being interpreted as 100. Every individual data point was measured manually and registered into a spreadsheet. Each panelist assessed every wine in 4 different occasions, according to a random block design.
2.5 Statistical Analysis

Chemical data obtained from grapes and wines were evaluated using a one-way ANOVA. Fisher’s least significant difference (LSD) test was used as a post-hoc comparison of means with a 5% level for rejection of the null hypothesis. A PCA (Principal Component Analysis) of sensory data was done with the help of XLSTAT version 2015.6 (Addinsoft, Paris, France).

2.6 Full Spectrum Analysis

Full spectrum scans of the wines were done in the range of 300 to 700 nm. Quartz cuvettes with 1-mm path length were utilized in the Cary 60 UV-Vis spectrophotometer equipped with an 18-sample cell auto-sampler under a D65 illuminant (Agilent Technologies, Santa Clara, CA, USA). Samples were processed with the Cary WinUV color software (version 6.0, Startek Technology, Boronia, Vic., Australia)
3. RESULTS AND DISCUSSION

The purpose of this study was to evaluate if cofermenting two or more grape varietals would produce wines with different characteristics than those produced by blending the individual varietals together either after alcoholic fermentation has been achieved or after the completion of malolactic fermentation. Monovarietal, blended and cofermented wines were made during two consecutive harvests (2018 and 2019), and a sensory evaluation was conducted on the wines made during the first vintage. The phenolic profile of the wines was measured at various times: for the 2018 harvest, at day 5, day 15, day 70, day 540 and day 1240; and for the 2019 harvest at day 30, day 90, day 130 and day 250.

3.1 Grape Fruit Chemistry

The logistics of cofermentation require that the varietals involved are to be picked on the same date. Table 1 shows a variety of different standard grape fruit chemistry measurements for the different varietals at harvest. Since different grape varieties ripen at different rates, cofermenting may result in using grapes that have different levels of sugars and acid. Fruit was harvested and processed on October 10th during the 2018 vintage and on October 14 in 2019. The growing degree days (GDD) for a weather station in Atascadero close to the vineyard in 2018 is 1635.8 and 1689.6 in 2019. These measurements are not significantly different which means that during both years the grapes received similar amounts of sunlight. This can also be observed in the fact that harvest dates for both years are only 4 days apart.

For the 2018 harvest (Table 1), the sugar levels were 23.7 °Brix for Malbec and 22.3 °Brix for Merlot. These values were statistically different by 1.4 °Brix, Malbec having 6% more sugar than Merlot, which resulted in a higher alcohol level in mono-varietal Malbec
wines. Malbec fruit in 2018 showed a higher pH and lower level of titrable acidity at 3.58 and 5.3 g/l compared to Merlot, with values of 3.51 for pH and 6.1 g/l for titrable acidity. When Merlot is grown in colder regions such as Canada, TA can be as high as 10 g/l (Mazza et al., 1999). Malic acid levels in 2018 were higher for Malbec at 2.75 g/l while Merlot tested at 1.88 g/l. On the contrary, tartaric acid levels in 2018 were higher in Merlot at 7.3 g/l and 4.8 g/l for Malbec. Differences in acid levels and in pH were actually perceived by the trained panelists but contrary to what would be expected, Malbec monovarietal wines were deemed as more acidic than Merlot monovarietals. The pH measurements showed that Malbec had a pH of 4.01 and Merlot had a pH of 3.96 yet Malbec was perceived as statistically significantly more acidic than Merlot. It is possible that the perception of acidity is associated with the perception of fruitiness. While a difference in acidity was indeed perceived and reported, the difference in ethanol level between monovarietals was not noticeable for the panelists.

Table 1. Fruit chemistry at harvest in 2018.

<table>
<thead>
<tr>
<th>Harvest Year</th>
<th>Varietal</th>
<th>°Brix</th>
<th>pH</th>
<th>Titrable Acidity (g/l)</th>
<th>Malic Acid (g/l)</th>
<th>Tartaric Acid (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018</td>
<td>Merlot</td>
<td>22.3±0.08 b</td>
<td>3.51±0.01 b</td>
<td>6.1±0.2 a</td>
<td>1.88±0.11 b</td>
<td>7.3±0.03 a</td>
</tr>
<tr>
<td></td>
<td>Malbec</td>
<td>23.7±0.05 a</td>
<td>3.58±0.01 a</td>
<td>5.3±0.17 b</td>
<td>2.75±0.14 a</td>
<td>4.8±0.09 b</td>
</tr>
</tbody>
</table>

*p-value*  
<0.0001  
0.0213  
0.0064  
0.0008  
<0.0001

Averages followed by the standard error. (SEM)(n=3). Different letters within a column indicate significant differences for the Student t-Test and *p* < 0.05. Values represent the average of 3 (n=3) field replicates. Significant *p*-values are shown in bold fonts.
For the 2019 vintage, Malbec and Merlot grapes showed a similar level of sugar at 22.8 °Brix and 22.7 °Brix, respectively. In contrast, the sugar levels in Petite Sirah grapes were 24.3 °Brix, being significantly higher than the other two varietals by at least 1.5 °Brix. For the 2019 harvest, pH did not differ significantly among the three varietals: Merlot fruit had a pH of 3.56, while Malbec was at 3.59 and Petite Sirah’s was 3.54. Titrable acidity in 2019 varied significantly at levels of 5.4 g/l in Malbec, 5.1 g/l in Merlot and 5.8 g/l for Petite Sirah. During 2019, malic acid levels were much higher for Malbec at 2.44 g/l and lower for Merlot and Petite Sirah, having 0.97 g/l and 1.07 g/l, respectively. In terms of tartaric acid content, in 2019 the amounts varied significantly at 7.3 g/l for Petite Sirah, 7 g/l for Merlot and 5.6 g/l for Malbec. The differences in acidity and sugar levels among the three varietals are most likely related to genetic traits and their ripening physiology, since the three varietals were harvested in the same location and the same date (Galet, 1979). In general, the pH levels were in the higher spectrum of what is considered standard (Amerine & Winkler, 1944), but that can be explained by the geographic setting and the meteorological conditions that are characteristic of the Paso Robles AVA (Babin et al., 2022). Colder regions entail higher levels of acid that can cause the TA of Merlot to be twice the amount (Mazza et al., 1999) of what was observed in this study.
Table 2. Fruit chemistry at harvest in 2019.

<table>
<thead>
<tr>
<th>Harvest Year</th>
<th>Varietal</th>
<th>°Brix</th>
<th>pH</th>
<th>Titrable Acidity (g/l)</th>
<th>Malic Acid (g/l)</th>
<th>Tartaric Acid (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019</td>
<td>Merlot</td>
<td>22.7±0.05 b</td>
<td>3.56±0.03 a</td>
<td>5.1±0.03 c</td>
<td>0.97±0.06 b</td>
<td>7.0±0.06 b</td>
</tr>
<tr>
<td></td>
<td>Malbec</td>
<td>22.8±0.06 b</td>
<td>3.59±0.06 a</td>
<td>5.4±0.07 b</td>
<td>2.44±0.04 a</td>
<td>5.6±0.04 c</td>
</tr>
<tr>
<td></td>
<td>Petite Sirah</td>
<td>24.3±0.08 a</td>
<td>3.54±0.08 a</td>
<td>5.8±0.02 a</td>
<td>1.07±0.06 b</td>
<td>7.3±0.04 a</td>
</tr>
</tbody>
</table>

Averages followed by the standard error. (SEM)(n=3). Different letters within a column indicate significant differences for the Student t-Test and \( p < 0.05 \). Values represent the average of 3 (n=3) field replicates. Significant p-values are shown in bold fonts.

3.2 Wine Chemistry

The chemical composition of the wines for 2018 and 2019 is displayed in Table 3 and Table 4. During the 2018 vintage (Table 3), the highest difference in ethanol content occurred between monovarietal Merlot and Malbec wines. Merlot wines were lower in alcohol content by 1.2% v/v. Meanwhile, the difference in levels of ethanol content was less between cofermented and blended wines, cofermented treatments were significantly higher in ethanol than the blended wines, blended either post-alcoholic or post-malolactic fermentation. Blending produced no significant difference in ethanol content between the two treatments. Even though the difference in ethanol content between blended and cofermented treatments was statistically significant, it was of no practical relevance for organoleptic purposes, since differences of less than 1.8% v/v in ethanol content are not perceivable by the consumer (Longo et al., 2017, King & Heymann, 2014). For the 2018
wines, the monovarietal Malbec and the cofermented treatments showed the highest pH at 4.01 and 4.03, while the monovarietal Merlot treatment and the post-malolactic fermentation blend had the lowest pH, both at 3.96. The levels of lactic acid were the lowest in Merlot wines and the highest in Malbec wines.

Table 3. One-way analysis of variance (ANOVA) of the basic chemical composition of monovarietal, cofermented and blended Malbec and Merlot wines for 2018.

<table>
<thead>
<tr>
<th>Winemaking treatment</th>
<th>Ethanol (% v/v)</th>
<th>pH</th>
<th>Titratable acidity (g/L tartaric acid)</th>
<th>Glucose + Fructose (g/L)</th>
<th>Malic acid (g/L)</th>
<th>Lactic acid (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malbec</td>
<td>14.11 ± 0.08 a</td>
<td>4.01</td>
<td>4.61 ± 0.06 a</td>
<td>0.31 ± 0.01 b</td>
<td>0.10 ± 0.00 a</td>
<td>1.37 ± 0.02 a</td>
</tr>
<tr>
<td>Merlot</td>
<td>12.91 ± 0.05 d</td>
<td>3.96</td>
<td>4.63 ± 0.08 a</td>
<td>0.29 ± 0.01 d</td>
<td>0.09 ± 0.01 a</td>
<td>0.97 ± 0.01 c</td>
</tr>
<tr>
<td>MalMerCOF</td>
<td>13.92 ± 0.02 b</td>
<td>4.03</td>
<td>4.39 ± 0.03 b</td>
<td>0.30 ± 0.00 bc</td>
<td>0.09 ± 0.01 a</td>
<td>1.28 ± 0.01 b</td>
</tr>
<tr>
<td>MalMerPAF</td>
<td>13.66 ± 0.01 c</td>
<td>3.99</td>
<td>4.41 ± 0.01 b</td>
<td>0.34 ± 0.00 a</td>
<td>0.08 ± 0.01 a</td>
<td>1.22 ± 0.01 c</td>
</tr>
<tr>
<td>MalMerPMLF</td>
<td>13.56 ± 0.01 c</td>
<td>3.96</td>
<td>4.49 ± 0.02 ab</td>
<td>0.24 ± 0.00 cd</td>
<td>0.09 ± 0.00 a</td>
<td>1.17 ± 0.01 d</td>
</tr>
</tbody>
</table>

*p*-value <0.0001 0.0060 0.0150 <0.0001 0.6351 <0.0001

Values represent the mean of three replicates followed by the standard error of the mean (n = 3). Different letters within a column for each variety indicate significant differences for Fisher's LSD test and *p* < 0.05. Significant *p*-values are shown in bold fonts. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending.

In the 2019 vintage (Table 4), Petite Sirah wines had the highest levels of ethanol at 14.46 % v/v. The net difference between the highest amount and the lowest is 1.42 % v/v, again, not enough to impart any sensorial differences based on ethanol content. The significant difference in ethanol content can be attributed to the initial higher sugar content of the Petite Sirah fruit at crushing, which was 24.3º Brix. In the cofermented treatments, Merlot exerted a dilution effect, lowering the ethanol content by contributing a lower sugar level in all the treatments in which it was present (MalMer COF, MerPS COF, MalMerPS COF), and mixtures containing Malbec and Petite Sirah only had a significantly higher content of ethanol than other cofermented treatments. When Malbec and Petite Sirah were blended either PAF or PMLF, ethanol content was significantly higher compared to blends.
that included Merlot. A similar tendency happened in a study that blended the red varieties Cencibel, Bobal and Moravia Agria (García-Carpintero et al., 2010).

In the 2019 trials, the Merlot monovarietal had the lowest pH of all treatments at 3.80, while both Malbec and Petite Sirah had a significantly higher pH of 3.97. The blend of Malbec and Petite Sirah resulted in an increase in pH, being significantly higher than the the cofermented and post-alcoholic fermentation blending treatments as well as the monovarietals. When cofermenting Malbec and Petite Sirah, pH was higher than either of the monovarietals by itself but blending after malolactic fermentation produced wines with a similar pH to both monovarietals. An analogous tendency occurred in a study made by cofermenting Monastrell with Cabernet Sauvignon, where the cofermented treatment had a higher pH than any of the monovarietals (Lorenzo et al., 2005). Also, wines that contained Merlot showed a statistically significant higher pH compared to the monovarietal and hence closer to either Malbec or Petite Sirah. Besides some significantly different ethanol content between the monovarietal wines, no evident effects of cofermentation on the general wine chemistry were observed. As a general recommendation to winemakers, the advantage of blending after alcoholic fermentation has been completed (instead of cofermenting) is that the levels of ethanol are predictable.
Table 4. One-way analysis of variance (ANOVA) of the basic chemical composition of monovarietal, cofermented and blended Malbec, Merlot and Petite Sirah wines for 2019.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethanol (v/v)</th>
<th>pH</th>
<th>Titratable Acidity (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merlot</td>
<td>13.05±0.36 f</td>
<td>3.80±0.01 g</td>
<td>4.83±0.03 a</td>
</tr>
<tr>
<td>Malbec</td>
<td>13.84±0.17 bc</td>
<td>3.97±0.01 bcd</td>
<td>4.76±0.03 ab</td>
</tr>
<tr>
<td>Petite Sirah</td>
<td>14.46±0.07 a</td>
<td>3.97±0.04 bcd</td>
<td>4.53±0.08 ef</td>
</tr>
<tr>
<td>MalMer Coferm</td>
<td>13.04±0.08 def</td>
<td>3.92±0.08 def</td>
<td>4.43±0.03 abc</td>
</tr>
<tr>
<td>MerPS Coferm</td>
<td>13.66±0.03 cd</td>
<td>3.94±0.03 cde</td>
<td>4.73±0.02 ab</td>
</tr>
<tr>
<td>MalPS Coferm</td>
<td>14.07±0.09 b</td>
<td>4.04±0.04 a</td>
<td>4.41±0.04 g</td>
</tr>
<tr>
<td>MalMerPS Coferm</td>
<td>13.69±0.07 cd</td>
<td>4.01±0.01 abc</td>
<td>4.65±0.08 bcd</td>
</tr>
<tr>
<td>MalMer PAF</td>
<td>13.22±0.14 ef</td>
<td>3.92±0.01 def</td>
<td>4.72±0.01 bcd</td>
</tr>
<tr>
<td>MerPS PAF</td>
<td>13.58±0.06 cde</td>
<td>3.89±0.02 ef</td>
<td>4.69±0.01 bcd</td>
</tr>
<tr>
<td>MalPS PAF</td>
<td>14.12±0.01 ab</td>
<td>4.01±0.01 ab</td>
<td>4.61±0.01 de</td>
</tr>
<tr>
<td>MalMerPS PAF</td>
<td>13.78±0.03 bc</td>
<td>3.92±0.01 def</td>
<td>4.71±0.04 bcd</td>
</tr>
<tr>
<td>MalMer PMF</td>
<td>13.61±0.05 cd</td>
<td>3.87±0.01 f</td>
<td>4.67±0.02 bcd</td>
</tr>
<tr>
<td>MalPS PMF</td>
<td>14.07±0.04 b</td>
<td>3.98±0.01 abcd</td>
<td>4.41±0.01 fg</td>
</tr>
<tr>
<td>MalMerPS PMF</td>
<td>13.79±0.03 bc</td>
<td>3.92±0.01 def</td>
<td>4.71±0.01 bcd</td>
</tr>
</tbody>
</table>

Values represent the mean of three replicates followed by the standard error of the mean (n = 3). Different letters within a column for each variety indicate significant differences for Fisher's LSD test and $p < 0.05$. Significant p-values are shown in bold fonts. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending.
3.3 Wine Phenolics

Wine phenolics were analyzed following the Adams-Harbertson protocol (Harbertson et al., 2013) in both years at different points in time: for the 2018 harvest, at day 5, day 15, day 70, day 540 and day 1240; and for the 2019 harvest at day 30, day 90, day 130 and day 250. These phenolic classes included anthocyanins, tannins, total and non-tannin phenolics and small and large polymeric pigments.

In 2018, while alcoholic fermentation was occurring, anthocyanins were the lowest in Merlot wines and the highest in Malbec wines, with intermediate values observed in cofermented wines and blended wines (Figure 7). When wines were analyzed on day 70 post-crush, which was when the malolactic fermentation has ended, the anthocyanin levels were the same in Malbec monovarietals and the cofermented treatment, while the post-alcoholic and post malolactic blends had similar levels and the Merlot monovarietals were the lowest. At this point in time, the cofermented treatments did not show a dilution effect from Merlot having fewer anthocyanins, which suggested that Merlot may have aided in stabilizing the anthocyanins. On the last date of analysis, day 1360, Malbec wines were significantly higher in anthocyanins compared to Merlot monovarietals, cofermented and post-fermentation blends. Wines produced by cofermentation were statistically significantly higher in anthocyanins than their post-alcoholic or post-malolactic blending counterparts by at least 29%. The effect of cofermenting thus can be assessed as increasing and stabilizing anthocyanin content when compared to blending after alcoholic fermentation or after malolactic fermentation. Previous studies (Bautista-Ortín et al., 2005) made with exogenous tannin additions in Mourvèdre proved that these added tannins may help stabilize anthocyanin content in the first stages of aging, although the effect was not
relevant after more than a year. The influence of Malbec in anthocyanin content was more relevant than the influence of Merlot. The cofermented and blended wines exhibit an anthocyanin profile withy values that were closer to Malbec than to Merlot. On day 1260 Malbec had 261 mg/l of anthocyanins, the cofermented treatment had 205 mg/l and Merlot had 113 mg/l. On a study made with Moravia Agria, Cencibel and Bobal, the cofermented blends of Moravia Agria and Cencibel and Moravia Agria with Bobal resemble more the phenolic profile of Moravia Agria (García-Carpintero et al., 2010). This is relevant because in certain cases one of the varietals will exhibit a dominant influence on the phenolic profile of the resulting wines.

All the treatments exhibited a similar tannin profile during the first stage of alcoholic fermentation. When the wines were fermented dry and pressed, the tannin content in the cofermented and Merlot treatments had increased by at least 20%. In Merlot it went from 283 mg/l to 445 mg/l. Tannins in Malbec did not increase. A longer period of tannin extraction in both Merlot and the cofermented treatment can be attributed to the fact that Merlot is high in seed-derived tannins (Ortega-Regules et al., 2008). From pressing to the end of malolactic fermentation, Malbec lost 70% of its tannins, while Merlot and the cofermented treatment dropped only 34% and 42% respectively. At the last date of analysis, the tannin levels of Merlot, cofermented and post-alcoholic fermentation blended wines were similar. The Malbec wines only had 30% of the tannins that Merlot wines had. Cofermentation has the same effect on tannins as blending after alcoholic fermentation, and these wines display the tannic structure of Merlot. A similar phenomenon occurred in a work made on Merlot, Monastrell and Cabernet Sauvignon, (Lorenzo et al., 2005) where the tannin structure of the cofermented blends would resemble more Cabernet Sauvignon.
While Cabernet Sauvignon and Merlot have different levels of color and a distinct anthocyanin composition they do have a shared tannin conformation and a similar aromatic profile (González-Neves *et al*., 2007). The abundance of catechins in both Cabernet Sauvignon and Merlot could explain why their presence promoted the formation of polymeric pigments.

During the vinification process of the 2018 vintage, the wines were supplemented with lysozyme in order to prevent the premature onset of malolactic fermentation caused by a cross-contamination event. Lysozyme has a positive net charge and can therefore bind with the negatively charged tannins (Liburdi *et al*., 2014). It will have a noticeable effect on the wine (Bartowsky *et al*., 2004). Eventually, a precipitate in the form of lees was formed. This had an impact on the tannin and phenolic content as well on the formation of polymeric pigments. Lysozyme has been proven to bind with non-anthocyan flavonoids and form a precipitate (Tirelli *et al*., 2007). Since all the wines were treated, the effect of tannin precipitation and a reduced load of phenolic and polymeric pigments was observed across the board in all the experiment.

The measurement of total phenolics and non-tannin phenolics provided a detailed profile of the content of flavan-3-ols (the monomers than polymerize to form tannins), flavonols and tannins. It is an assessment of the phenolic characteristics without considering anthocyanins. At the beginning of vinification of the 2018 vintage, cofermented and Malbec wines had the highest levels of both non-tannin phenolics and total phenolics, while the Merlot wines were the lowest. There was an abrupt drop of both classes of phenolics in Malbec, which lost 46% percent of its total phenolics and 44% of its non-tannin phenolics from 5 days post-crush until the last data point. The phenolic
decline was less pronounced in all the other treatments, with the cofermented treatment decreasing the most after Malbec with only 26% of phenolic reduction. In the final reported analysis, Merlot wines had significantly more total phenolics and non-tannin phenolics. In this case, cofermenting did not exert noticeable effect from blending at a later point in time. Similar declining trends have been reported in the literature.

Polymeric pigments were also assessed and distinguished in small (SPP) and large (LPP). All the treatments displayed a progressive increase in SPP. The post-malolactic fermentation blend along with the Malbec monovarietal had statistically significant higher amount of SPP. The cofermented and the post-alcoholic blended wines were significantly lower followed by the Merlot monovarietals with the lowest amount of SPP in the whole experiment. The production of LPP was on the rise when alcoholic fermentation was achieved, then it dropped after malolactic fermentation and then it increased gradually. This type of curve is common and expected in LPP formation (He et al., 2012). The presence of Merlot, being either cofermented or blended at any other point in time increased the proliferation of LPP, which is expectable since Merlot had higher tannins and these molecules play an essential role in the development of LPP. A study made on blends based on Cabernet Sauvignon, Merlot and Zinfandel demonstrated that the formation of polymeric pigments is responsible for the fluctuations in total phenolics (Dooley et al., 2012).
Figure 7. Evolution of anthocyanins, tannins, non-tannin phenolics, total phenolics, and polymeric pigments throughout winemaking and bottle aging of the monovarietal, cofermented, and blended Malbec and Merlot wines, 2018 harvest. Different letters at day 5 (alcoholic fermentation, capital fonts), day 70 (completion of malolactic fermentation, lower fonts), and day 1260 (36 months of bottle aging, apostrophe fonts), indicate significant differences for Fisher’s LSD test and \( p < 0.05 \). EQ: equivalents; CE: catechin equivalents; AU: absorbance units. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending. SPP: Small polymeric pigments; LPP: large polymeric pigments; TPP: total polymeric pigments.
During the second vintage, Petite Sirah was introduced as a third varietal. Petite Sirah monovarietal wines had the highest number of anthocyanins during the whole experiment. In the beginning of aging, the anthocyanin content of Petite Sirah was the highest followed by the triple confection (MalMerPS COF), the cofermented treatment that contained Malbec and Petite Sirah (MalbecPS COF), the Malbec monovarietal, and the post-alcoholic blend of Malbec and Petite Sirah (MalPS PAF). Wines containing at least 50% Merlot were placed in the lower tier, descending from MerlotPs PAF, MalMerPS PAF, the cofermented blend of Merlot and Petite Sirah (MerlotPS COF), the mixtures containing Malbec and Merlot (MalMer COF and MalMer PM LF) and lastly, the Merlot monovarietal. Merlot acted as a dilution agent because of its lower concentration of anthocyanins. When Merlot was cofermented with Malbec, it did not produce a wine that was substantially different compared to its post alcoholic or post malolactic fermentation blend counterparts. This differs from what was observed on the first year of data. A plausible explanation for this observation could be effect on lysozyme during the first vintage. The depletion of non-anthocyanin compounds will hinder the formation of polymeric pigments (Tirelli et al., 2007). In a similar way, when Merlot was cofermented with Petite Sirah, it produced wines that were statistically no different in their anthocyanin profile to those blended at a later point in time. In the case of the wines containing the three varietals, cofermentation did produce a wine that in the long run was significantly higher in anthocyanins than the post alcoholic or the post-malolactic blends. Petite Sirah had a 51% decrease in its anthocyanin content from post crushing to day 250 and Merlot decreased at a similar rate of 52%. As practical advice to winemakers, in the case of anthocyanin content and the effect of cofermentation, cofermenting the three varietals
together will enhance anthocyanin content when compared to their post alcoholic or post malolactic blended counterparts. The extraction and retention of more anthocyanins in the triple cofermented wine could be attributed to the initial presence of seed-derived tannins from Merlot (Ortega-Regules et al., 2008) and a higher proportion of monomeric anthocyanins provided by Petite Sirah and Malbec. The synergistic tendency of cofermenting 3 grape varietals has been proven before in the case of Merlot, Cabernet Sauvignon and Moravia Agria (García-Carpintero et al., 2011).
Figure 8. Evolution of anthocyanins, tannins, non-tannin phenolics, total phenolics, and polymeric pigments throughout winemaking and bottle aging of the monovarietal, cofermented, and blended Malbec and Merlot wines, 2019 harvest. Different letters at day 30 (capital fonts), day 130 (lower fonts), and day 250 (apostrophe fonts), indicate significant differences for Fisher’s LSD test and p < 0.05. EQ: equivalents; CE: catechin equivalents; AU: absorbance units; COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending; SPP: Small polymeric pigments; LPP: large polymeric pigments; TPP: total polymeric pigments.
Figure 9. Evolution of anthocyanins, tannins, non-tannin phenolics, total phenolics, and polymeric pigments throughout winemaking and bottle aging of the monovarietal Petite Sirah, the Merlot Petite Sirah cofermented, Malbec Petite Sirah cofermented, Malbec Merlot Petite Sirah cofermented and the Malbec Merlot Petite Sirah post alcoholic fermentation, 2019 harvest. Different letters at day 30 (capital fonts), day 130 (lower fonts), and day 250 (apostrophe fonts), indicate significant differences for Fisher’s LSD test and $p < 0.05$. EQ: equivalents; CE: catechin equivalents; AU: absorbance units. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending. SPP: Small polymeric pigments; LPP: large polymeric pigments; TPP: total polymeric pigments.
Figure 10. Evolution of anthocyanins, tannins, non-tannin phenolics, total phenolics, and polymeric pigments throughout winemaking and bottle aging of Merlot Petite Sirah post alcoholic fermentation, Malbec Petite Sirah post alcoholic fermentation, Merlot Petite Sirah post malolactic fermentation, Malbec Petite Sirah post malolactic fermentation, Malbec Merlot Petite Sirah post malolactic fermentation, harvest 2019.

Different letters at day 30 (capital fonts), day 130 (lower fonts), and day 250 (apostrophe fonts), indicate significant differences for Fisher’s LSD test and $p < 0.05$. EQ: equivalents; CE: catechin equivalents; AU: absorbance units. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending. SPP: Small polymeric pigments; LPP: large polymeric pigments; TPP: total polymeric pigments.
In regards to tannins, the Malbec monovarietals had the lowest amount in the beginning. Blending Malbec with Merlot either by cofermenting them or mixing them after alcoholic or post-malolactic fermentation significantly improved their tannin content. This trend is also observed in the triple cofermentation. In the case of blends based on Merlot and Petite Sirah, cofermenting produced no differences in the tannin profile from wines that are blended at later points in time. The same occurs with the interactions of Malbec/Petite Sirah and Merlot/Malbec. Overall, blending in any form seems to improve the tannin-deficient profile of Malbec. The MerlotPS PAF blend had a tannin drop of 22% and the rest of treatments dropped less than 20% of their tannins. Tannins will agglomerate with anthocyanins and will form polymeric pigments. The reduction in tannins will occur as the formation of polymeric pigments is regulated by the availability of free anthocyanins (Cheynier et al., 2006).

The total phenolics in the beginning of vinification were the lowest in Malbec monovarietals. Cofermenting Malbec with Merlot provided with a higher amount of phenolics than any of the varietals by themselves at this point, however this effect did not last throughout bottle aging. The cofermented wines did not evolve to show a phenolic profile that is statistically different from the wines that were blended after alcoholic or after malolactic fermentation. This tendency has been reported in previous experiments that blended Syrah and white varietals (Casassa et al., 2019). In general, the wines that contained Malbec and Petite Sirah, either being cofermented or blended, were lower in total phenolics that the ones containing Merlot and Petite Sirah. Cofermenting the three varietals produced a wine that had a statistically significant higher content of phenolics compared to the wine that was blended after malolactic fermentation, while blending after
alcoholic fermentation provided with a similar profile to cofermenting. The same tendency was observed during the first year of the experiment.

There were some marked differences in the content of non-tannin phenolics at the beginning of the 2019 experiment: The MalMer COF, MerlotPS COF and MalPS COF treatments were statistically significantly higher than the rest of the wines. As they evolved, the difference became less marked and in the last point of analysis the MalMer COF, MerlotPS COF and MalMerPS COF treatments had statistically significant higher amounts of non-tannin phenolics than MalPS PAF and MalMerPS PMLF. The rest of the treatments pooled together and did not differ in terms of non-tannin phenolics.

Small polymeric pigments had the tendency to increase gradually in all treatments except in MalMer PMLF, in which they decreased 25% from day 130 to day 250. Petite Sirah had the highest amount of SPP throughout the whole evolution, which can be attributed to its high anthocyanin content. Cofermenting or blending Merlot with Malbec resulted in wines having statistically significant lower SPP than cofermenting Merlot and Petite Sirah or Malbec and Petite Sirah. Cofermenting did not provide with a statistically substantial difference in SPP content when compared to blending after alcoholic fermentation or after malolactic fermentation.

The production of LPP increased in all treatments as they aged. At day 30, the cofermented treatment MalPS COF had a statistically significant higher amount of LPP compared to the triple conferment MalMerPS COF, which only had 33% of the LPP that MalPS COF had. At day 250 the amount of LPP was similar in these two treatments. There was a very low level of variance across the treatments and cofermenting or blending seemed to have little to no effect on LPP content.
The amount of total polymeric pigments displayed a similarity to the trend seen in the SPP, in which these grew over time and in which the abundance of anthocyanins was a drive for the formation of the oligomers. Blends and cofermented experiments which were based on Malbec and Merlot were in the lower tier, while the presence of Petite Sirah, either by cofermenting it or by blending it in at a posterior time, boosted the TPP content. As with the SPP, the Petite Sirah monovarietal wine had the highest amount of TPP and the MalMer PMLF had the lowest amount. Cofermentating either the pair Merlot and Petite Sirah, Malbec and Petite Sirah or the three varietals altogether did not provide with a different content of TPP when compared to their post alcoholic blending or post malolactic blending counterparts.

**Table 5: Phenolic values in cofermented and blending treatments.**

<table>
<thead>
<tr>
<th>Cofermented pair</th>
<th>Year</th>
<th>Phenolic class significantly different from PAF</th>
<th>Phenolic class significantly different from PMLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malbec-Merlot</td>
<td>2018</td>
<td>Anthocyanins</td>
<td>Anthocyanins, tannins, total phenolics, SPP</td>
</tr>
<tr>
<td>Malbec-Merlot</td>
<td>2019</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Merlot-Petite Sirah</td>
<td>2019</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malbec-Petite Sirah</td>
<td>2019</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malbec-Merlot-Petite Sirah</td>
<td>2019</td>
<td>Anthocyanins</td>
<td>Anthocyanins, non-tannin phenolics</td>
</tr>
</tbody>
</table>

When both years were compared in terms of phenolic profile (Figure 5), it was evident that the addition of lysozyme during 2018 had an impact on the tannin content, the phenolics and the formation of polymeric pigment. The analyses in 2018 were done at day 70 and the ones on 2019 were performed on day 90, however the differences in every phenolic class studied (besides anthocyanins) was statistically significant. Tannins during 2018 were statistically significantly lower than in 2019, ranging from being 22% to 10% of the tannins in 2019. The differences between harvests can also be found in the content of non-tannin phenolics, total phenolics and polymeric pigments, both small and large. It
is very relevant to be able to assess the addition of a wine finning agent, and the fact that it has such significant effects on the phenolic profile of the wines.
Figure 11. Comparison of anthocyanins, tannins, non-tannin phenolics, total phenolics, and polymeric pigments of harvest 2018 and 2019. Different letters indicate significant differences for Fisher’s LSD test and $p < 0.05$. EQ: equivalents; CE: catechin equivalents; AU: absorbance units. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending. SPP: Small polymeric pigments; LPP: large polymeric pigments; TPP: total polymeric pigments.
3.4 Wine chromatic composition

During both vintages, a full visible spectrum of every wine was taken at certain points during vinification and bottle aging. Every repetition was averaged, and the curves are displayed in Figures 12 and 13. The peak of absorbance for anthocyanins is in the range of 520-540 nm (Tamura & Yamagami, 1994).

![Figure 12](image12.png)

**Figure 12.** Full visible absorption spectrum scans recorded throughout winemaking and up to 36 months of bottle aging (day 1260 post-crush) of monovarietal, cofermented and blended Malbec and Merlot wines, 2018 harvest. Lines represent the average of all treatment replicates (n=3). COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending.

In the 2019 vintage, full spectrum scans were taken on day 30, day 130 and day 250 post-crush.

![Figure 13](image13.png)

**Figure 13.** Full visible absorption spectrum scans recorded on day 30, day 130 and day 250, of monovarietal, cofermented and blended Malbec, Merlot and Petite Sirah wines made in 2019. Lines represent the average of all treatment replicates (n=3). COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending.
3.5 Sensory Analysis

Panelists (n=10) were trained and exposed extensively to the 2018 vintage wines and to aroma standards. They evaluated the experimental wines in a randomized block design. The attributes analyzed were color, aroma, taste, mouthfeel, and perceived remaining length of taste after expectoration, measured in seconds and referred to as caudalies. The sensory data was gathered and analyzed in both a 3-way ANOVA (Table 5) and a Principal Component Analysis (PCA, Fig. 14) with confidence ellipses, which were constructed with 95% of confidence according to the Hotelling’s test (Husson et al, 2005). These ellipses are graphical descriptions of the variance of the sensory data, and if they do not overlap, it can be interpreted as the wines being significantly distinct from a sensory standpoint. The PCA can explain 80% of the variability of the data with its two components. Principal Component 1, which accounted for 65% of the variability, clearly distinguishes monovarietal Merlot wines from monovarietal Malbec wines. Merlot wines had higher amounts of ruby coloration, earthy, tobacco and spice notes and also were perceived as more astringent. In contrast, Malbec monovarietals had a purple hue and were high in baked fruit and red fruit aromas. The blended wines and the cofermented treatments populate the middle of the PCA plot, in an intermediate position between Malbec and Merlot. The confidence ellipses of the cofermented treatments and the post-malolactic blend were completely overlaid on each other, suggesting that to the panelists assessment, these wines were indistinguishable. The ellipse representing the post-alcoholic fermentation blends was closer to the monovarietals’ areas, suggesting that when blending after alcoholic fermentation, the unique varietal character prevailed more than when cofermenting or blending after post-malolactic fermentation. This is a contrary tendency
to what has been proven in previous work, where Syrah was cofermented with white varieties (Casassa et al., 2019). This could be explained by the fact that Viognier has a higher amount of aromatic isoprenoids that are extracted during fermentation and that may not be retained (Pisaniello et al., 2022), therefore reducing the Viognier typicity in cofermented wine. Malbec had the highest amount of purple color, baked fruit and acid and was also the wine that had the longest presence after expectoration. The Merlot monovarietal was the highest scoring wine for the attributes of ruby color, spice, vegetal and tobacco aromas, and shared the astringency levels with the cofermented and the post alcoholic blend. The post alcoholic fermentation blend was reported as being high in tobacco and earthy aromas and astringency. The post malolactic fermentation blend was perceived as having the highest amount of baked fruit and a high perception of spice aromas. As a general recommendation for winemakers, if vegetal or earthy notes want to be modulated, cofermenting or blending after malolactic fermentation may be advisable than blending after alcoholic fermentation (Casassa et al., 2019).

Table 6. One-way analysis of variance (ANOVA) of the sensory descriptors of monovarietal, cofermented and blended Malbec and Merlot wines. Values represent the mean of three replicates followed by the standard error of the mean (n = 3)*

<table>
<thead>
<tr>
<th>Wine</th>
<th>Ruby</th>
<th>Purple</th>
<th>Red fruit</th>
<th>Baked fruit</th>
<th>Spice</th>
<th>Vegetal</th>
<th>Tobacco</th>
<th>Herbal</th>
<th>Earthy</th>
<th>Acidity</th>
<th>Astringency</th>
<th>Length (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malbec</td>
<td>38.421 d</td>
<td>58.317 a</td>
<td>55.970 a</td>
<td>44.796 b</td>
<td>24.332 b</td>
<td>24.458 b</td>
<td>22.887 b</td>
<td>26.917 b</td>
<td>20.166 b</td>
<td>17.100 a</td>
<td>23.326 b</td>
<td>6.361 a</td>
</tr>
<tr>
<td>Merlot</td>
<td>54.206 a</td>
<td>30.088 d</td>
<td>37.116 c</td>
<td>42.542 b c</td>
<td>30.102 a</td>
<td>30.032 a</td>
<td>27.944 a</td>
<td>28.787 a</td>
<td>27.935 a</td>
<td>34.685 b</td>
<td>28.162 a</td>
<td>5.366 b</td>
</tr>
<tr>
<td>Cofem</td>
<td>46.199 bc</td>
<td>45.667 bc</td>
<td>42.986 b</td>
<td>41.074 c</td>
<td>29.241 a</td>
<td>23.009 b</td>
<td>22.912 b</td>
<td>27.370 a</td>
<td>26.218 a</td>
<td>38.370 a</td>
<td>28.965 a</td>
<td>5.000 b</td>
</tr>
<tr>
<td>Post AF</td>
<td>44.919 c</td>
<td>48.333 b</td>
<td>45.625 b</td>
<td>45.569 b</td>
<td>26.477 b</td>
<td>27.494 a</td>
<td>27.159 a</td>
<td>26.685 ab</td>
<td>25.813 a</td>
<td>36.600 ab</td>
<td>28.650 a</td>
<td>5.551 b</td>
</tr>
<tr>
<td>Post MLF</td>
<td>48.722 b</td>
<td>44.366 c</td>
<td>45.218 b</td>
<td>49.877 a</td>
<td>30.338 a</td>
<td>23.958 b</td>
<td>22.299 b</td>
<td>26.813 ab</td>
<td>26.044 a</td>
<td>37.201 a</td>
<td>25.032 b</td>
<td>5.674 b</td>
</tr>
</tbody>
</table>

*Different letters within a column for each variety indicate significant differences for Fisher’s LSD test and p < 0.05. Significant p-values are shown in bold fonts. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending.
Figure 14. Principal component analysis of descriptive sensory data of monovarietal, cofermented, and blended Malbec and Merlot wines evaluated by a trained sensory panel (n=10). Confidence ellipses indicate 95% confidence intervals. COF: cofermentation; PAF: post-alcoholic fermentation blending; PMLF: post-malolactic fermentation blending.
4. CONCLUSION

The present work reported the effects of cofermentation of varietals with different levels of anthocyanins and tannins for two consecutive years: 2018 and 2019. The cofermented wines were contrasted with wines that were made in the same varietal proportion but blended after alcoholic fermentation or after the completion of malolactic fermentation. During the first year, the interactions between Malbec and Merlot were studied, and in the second year, Petite Sirah was added to the experiment. Monovarietal, cofermented and blended wines were evaluated in terms of phenolic and chromatic profile. In 2018, cofermented wines were statistically significantly higher in anthocyanins than their blended counterparts. The tannin content of the cofermented and blended wines was more similar to the profile of Merlot. While the anthocyanin profile resembled the profile of Malbec. Sensory analysis was performed on the 2018 vintage. Malbec wines were higher in red fruit aroma and perception of length while Merlot wines were the most astringent. Cofermented wines were practically indistinguishable from their blended counterparts. Wines blended after malolactic fermentation retained more of the varietal character than when cofermented. On the second vintage, cofermentation significantly improved anthocyanin content only when the three varietals were cofermented. As practical advice to winemakers, cofermentation should only be done if the varietals involved are ripe at the same time. Cofermentation is a tool to promote the formation of polymeric pigments. As possible research opportunities in the future, the interaction that arises from other varietals can be of interest. Future research should be done on the phenolic profile of the wines and on their sensory aspects.
REFERENCES


Waterhouse, A., and Ebeler, S.


