ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

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IMPACT OF CARBONIC MACERATION AND OAK BARREL AGING ON AROMA AND PHENOLIC COMPOUNDS OF RED WINES FROM *VITIS VINIFERA* L.CV. ÖKÜZGÖZÜ

DEPARTMENT OF FOOD ENGINEERING

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ABSTRACT

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In the presentstudy, the effects of carbonic maceration (CM), oak barrel aging (OBA), and classic processing (as control wine) methods on aroma and phenolic compounds of Öküzgözü red wines were investigated. With respect to the relative aroma composition, CM wine possessed the highest content in higher alcohols acetates (HAAs) and ethyl esters of fatty acids (EEFAs), accounting respectively for 8.60% and 12.96% of total esters, whereas these values represented 4.34% and 6.83% in the control wine, respectively. Moreover, OBA wine was distinguished by the presence of oak aroma compounds, *cis*-oak lactone, and furfural. The results of aroma extract dilution analysis (AEDA), combined with aroma activity values revealed, ethyl hexanoate, isoamyl acetate, diacetyl and its alcohol derivatives *levo* and *meso*-2,3 butanediols as the main aroma-active compounds of CM wine, whereas *cis*-oak lactone, ethyl hexanoate, isoamyl acetate, and 4-vinyl guaiacol represented the main aroma-active compounds of OBA wine. The RP-HPLC analysis revealed that CM wine contained the highest concentration of anthocyanin monomers with an average of 756.65 mg/L, followed by control wine (657.15 mg/L) and OBA wine (578.80 mg/L). In contrast, CM led to red wine with less total tannins compared to OBA (2.11mg/L against 3.79 mg/L). The wines were further subjected to a Descriptive analysis (DA), from which all selected aroma attributes appeared to be positively correlated to the overall aroma quality. Moreover, the "*red fruits*" descriptor was assessed as the most pronounced of the aroma attributes in the three styles of wine, and its highest intensity was recorded in CM wine. Öküzgözü wines produced by CM are characterized by dark ruby red color, easy drinking, low tannins, intense red fruits and lacte aromas.

Key Words: Öküzgözü, Red Wines, Carbonic Maceration, Barrel Aging, Aroma, Phenolics

ÖZ

DOKTORA TEZİ

ÖKÜZGÖZÜ ÜZÜMÜNDEN ELDE EDİLEN KIRMIZI ŞARAPLARIN AROMA VE FENOLİK BİLEŞİKLERİ ÜZERİNE KARBONİK MAKERASYON VE MEŞE FIÇIDA OLGUNLAŞTIRMANIN ETKİSİ

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Bu çalışmada, kırmızı şarap üretiminde kullanılan karbonik maserasyon (CM), meşe fıçıda olgunlaştırma (OBA) ve klasik işleme (kontrol olarak) yöntemlerinin Öküzgözü kırmızı şaraplarının aroma ve fenolik bileşikleri üzerine etkileri araştırılmıştır. Aroma maddeleri bakımından, CM şarabı, toplam esterlerin sırasıyla %8.60 ve %12.96'sını oluşturarak yüksek alkollerin asetatlarını (HAA'lar) ve yağ asitlerinin etil esterlerini (EEFA'lar) en yüksek düzeyde içeren şarap olmuştur, kontol şarabında bu oranlar sırasıyla %4.34 ve % 6,83 olarak bulunmuştur. Bununla birlikte, OBA şarabı meşe fıçıdan gelen aroması bileşikleri olan *cis*-meşe laktonu ve furfural bulunması ile diğerlerinden ayrılmıştır. Aroma ekstrakt seyreltme analizi (AEDA) ve aroma aktiflik değerlerine göre CM şarabının temel aroma-aktif bileşiklerinin etil heksanoat, izoamil asetat, diasetil ve levo ve meso-2,3 bütandiol, OBA şarabının ise meşe laktonu, etil hekzanoat, izoamil asetat ve 4-vinil guaiakol olduğu belirlenmiştir. HPLC analiz sonuçlarına göre, CM şarabının ortalama 756,65 mg/L ile en yüksek antosiyanin miktarına sahip olduğu, bunu kontrol şarabı (657,15 mg/L) ve OBA şarabının (578,80 mg/L) takip ettiği saptanmıştır. Bunun karşın, CM şarabının, OBA şarabına göre daha az toplam tanen (3,79 mg/L'ye karşı 2,11mg/L) içerdiği belirlenmiştir. Şaraplara ayrıca duyusal açıdan tanımlayıcı analize (DA) tabi tutulmuş ve bu analizde seçilen tüm aroma tanımlayıcıları genel aroma kalitesiyle pozitif korelasyon göstermiştir. Ayrıca, kırmızı meyve tanımlayıcısı, üç yöntemle üretilen şarapta da en belirgin aroma tanımlayıcısı olarak değerlendirilmiş ve bu açıdan en yüksek aroma yoğunluğu CM şarabında belirlenmiştir. Karbonik maserasyonla üretilen Öküzgözü şarapları koyu yakut kırmızı renkli, kolay içimli, düşük tanenli, yoğun kırmızı meyve ve lakte aromaları ile karakterize edilmiştir.

Anahtar Kelimeler: Öküzgözü, Şarap, Karbonik Maserasyon, Fıçı, Aroma, Fenolik B.

EXTENDED ABSTRACT

In the food field, flavor (encompassing aroma and taste), color and texture are the most critical organoleptic properties of food or beverage related to consumer choice, and wine is not excluded from this rule. In addition to the choice of grape variety, the winemaking method dramatically impacts the flavor, color, and texture of the wine. Among various winemaking methods worldwide, there is the vinification by carbonic maceration (CM), leading mainly to produce young red wine, early-drinkable so-called “primeur.” This style of wine exhibits ruby color, soft and intensely fruity, floral characters. On the other hand, there is another style of red winemaking, consisting of aging wine in oak barrel for a certain period prior to bottling. The wine aged in an oak barrel possesses a medium to full body, less-intense red color, and complex aroma resulting from a mix of volatile wine compounds and those released by the oak wood.

In Turkey, many winemakers and wineries produce red wine of native or foreign grape varieties, aged in oak barrel. However, the “Kavaklıdere” winery is the only winery to produce red primeur wine with *Vitis vinifera* L.cv. Öküzgözü. In addition to primeur wine, this winery also produces with Öküzgözü, red wine aged in an oak barrel and classic red wine made by traditional vinification method.

With the goal to valorize the organoleptic potential of *Vitis vinifera* L.cv. Öküzgözü through winemaking discipline, we investigated the impact of CM and oak barrel aging on the aroma and phenolic compounds of red wines made with Öküzgözü grapes grown in Elazig province in this study. It was a comparative study between CM wine and the wine aged in an oak barrel wherein red wine made by classic vinification was used as control wine. The study was conducted for two successive vintages (2018 and 2019).

Regarding the analyses conducted, the general composition of wines were determined first, following the methods confirmed by the International Organization of Vine and Wine (OIV). Afterward, an analysis of volatile

compounds through a liquid-liquid extraction followed by an identification and quantification by gas chromatography coupled with mass spectrometry and flame ionization detection (GC-MS-FID). Moreover, the characterization of aroma-active compounds was performed by aroma extract dilution (AEDA) utilizing GC-olfactometry. Subsequently, color characteristics and phenolic compounds were determined by UV-Vis spectrometry. Furthermore, the analysis of anthocyanin monomers by reversed-phase HPLC with diode array detector (DAD) was done. Finally, the Descriptive Analysis (DA) was carried out by a panel of 9 assessors with great experience in the field of wine, to evaluate the impact of the three winemaking styles on the overall wine quality.

The winemaking method impacted general composition, since significant statistical differences were observed regarding the classic oenological parameters measured between the wine styles. In comparison with the control wine, parameters such as total and volatile acidity were lower at a significant level ($p < 0.05$) in CM wines, with average for both vintages of 4.31 g/L against 4.72 g/L, and 0.44 g/L against 0.47 g/L, respectively. In wine aged in oak barrel (OBA), the same parameters were recorded at higher concentrations with values of 5.01 g/L and 0.53 g/L, respectively. Among the three styles of Öküzgözü red wine, CM wine contained the highest concentration of ethanol (13.68%), whereas the lowest concentration was recorded in OBA wine (13.38 %).

The winemaking methods investigated in the present study showed impact on volatile composition of Öküzgözü red wine as well. Similar to the OBA wine (567.41mg/L), CM wine (464.25mg/L), also exhibited a higher content of total volatile compounds than the control wine (435.24 mg/L). While analyzing the relative compositions in detail, CM wine shared common features with other primeur red wines made from different grapes varieties, particularly esters. Indeed, compared to control wine, a higher concentration of higher alcohols of acetates (HAAs) and ethyl esters of fatty acids (EEFAs) were found in CM wine accounting for 8.60 % against 4.34 % and 12.96 % against 6.83 %, respectively, of total esters.

These two subclasses of esters contribute significantly to the fruity and floral character of young wines. Moreover, some other volatile compounds, such as diacetyl and 4-vinyl guaiacol, considered as markers of wine made by CM, were in higher concentration as well. On the other hand, OBA wine showed the highest content of ethyl esters of organic acids (EEOAs) due to its more extended period of aging, Additionally, volatile compounds released by oak such as *cis*-oak lactone and furfural were found to be present. Moreover, OBA wine was characterized by a low percentage of HAAs and EEFAs compared to control wine with values of 1.69% and 3.21%, respectively.

From aroma extract dilution analysis (AEDA) of the three styles of Öküzgözü red wines, a total of 40 aroma-active compounds consisted of 13 esters, eight alcohols, six acids, four lactones, four carbonyl compounds, two phenolic compounds, one sulfur compound and two unidentified compounds were determined. The butter-like diacetyl, the banana-like isoamyl acetate, the pineapple-like, and the honey-like ethyl vanillate displayed their highest intensity in CM wine with FD ≥ 729. Concerning OBA wine, three compounds, including *cis*-oak lactone, 4-ethoxycarbonyl-ɤ-butanolactone, and one unidentified compound, were detected up to the maximum FD of 2187.

Aroma activity values showed a positive correlation between FD of ethyl hexanoate, isoamyl acetate, and their relative AAVs accounting for 55.75% of total AAVs >1 in CM wine. In addition, a cluster of aroma including diacetyl and its alcohols derivatives (*levo* and *meso*-2,3 butanediols) imparting butter/cream and lacte aromas could be constituted in CM wine. That cluster represented 24.76% of the total AAV >1. In OBA wine, the results combined of AEDA and AAV allowed to propose a complex aroma profile characterized by fruity notes imparted by ethyl hexanoate/isoamyl acetate, oaky notes imparted by *cis*-oak lactone, and spicy/clove notes imparted by 4-vinyl guaiacol.

In comparison with control wine, CM wine contained the lowest total phenolic compounds with a concentration of 1711 mg/L, whereas OBA wine presented the highest concentration at 2146 mg/L. With respect to the total anthocyanin concentration determined by spectrometry, CM wine showed higher contents for both vintages at concentrations of 558.30 mg/L and 522.92 mg/L, respectively, than the control wine. In contrast, OBA wine displayed lower concentrations with values of 475.69 mg/L and 472.38 mg/L, respectively.

Moreover, by means of reversed-phase HPLC, a total of 13 monomeric anthocyanins, consisted of five free monoglucosides, five monoglucoside acetates, and three monoglucoside p-coumarates were determined across all Öküzgözü wines. The relative concentration of total anthocyanin monomers was similar to that observed while determined by bisulfite bleaching. Indeed, CM wine displayed the highest concentration of total anthocyanin monomers in both vintages (769.10 mg/L and 727.10 mg/L), whereas the lowest concentration was recorded in OBA wine (573.70 mg/L and 583.90 mg/L). Furthermore, regardless of the vintage, malvidin-3-glucoside was the most abundant anthocyanin monomer with average concentrations of 379.17 mg/L, 327.10 mg/L, and 286.60 mg/L, in CM, control, and OBA wines, respectively.

Descriptive Analysis allowed to establish for the three styles of Öküzgözü red wine, a positive correlation between all the selected aroma descriptors and the wine aroma quality, although only "*spicy,"* "*wood/vanilla*" and "*dry fruits* " aroma descriptors presented significant correlation coefficients regarding the wine aroma quality. Moreover, in three styles of wine, *"red fruit"* was the most pronounced aroma descriptor, both nasally and on the palate, and its highest intensity was recorded in CM wine. On the other hand, analysis of gustatory profile, CM wine distinguished by its greatest sweetness, whereas OBA wine displayed more astringency, body, and complexity. Finally, panelists preferred CM relatively to OBA wine.

GENİŞLETİLMİŞ ÖZET

Gıda alanında, lezzet (koku ve tat), renk ve yapı gıda veya içeceklerin tüketiciler tarafından tercihinde en önemli duyusal özelliklerdir ve bu şarap için de geçerlidir. Üzüm çeşidine ek olarak, şarap üretim yönteminin şarabın lezzeti (koku ve tat) rengi ve yapısı üzerinde büyük etkisi vardır. Dünyadaki çeşitli şarap yapım yöntemleri arasında, "primeur" olarak da adlandırılan, erken içilebilir kırmızı genç şarap üretmeyi sağlayan karbonik maserasyon yöntemi Bulunmaktadır. Bu yöntemle üretilen şarap yakut renkli, yumuşak, yoğun meyveli ve çiçeksi bir karakter sergiler. Öte yandan, şişelemeden önce şarabın belirli bir süre meşe fıçıda olgunlaştırılmasını kapsayan başka bir kırmızı şarap üretim yöntemi daha vardır. Meşe fıçıda olgunlaştırılan şarap, orta gövdeliden tam gövdeliye değişen dolgunluğa, daha düşük yoğunlukta kırmızı renge ve şaraptan ve fıçıdan gelen daha kompleks bir aromaya sahiptir.

Türkiye'de pek çok şarap üreticisi, yerli veya yabancı şaraplık üzüm çeşitlerinden meşe fıçıda olgunlaştırılmış kırmızı şaraplar üretmektedir. Ancak, Kavaklıdere Şarap İşletmesi, Karbonik Maserasyon yöntemiyle Öküzgözü üzümünden kırmızı primör şarap üreten tek işletme konumundadır. İşletmede bu çeşitten primör şarap yanında geleneksel yöntemle üretilen klasik kırmızı şarap ve meşe fıçıda yıllandırılmış kırmızı şarap da üretmektedir.

Bu çalışmada Elazığ ilinde yetiştirilen Öküzgözü üzümlerinden üretilen kırmızı şarapların uçucu ve fenolik bileşikleri üzerine karbonik maserasyon ve meşe fıçıda olgunlaştırma yöntemlerinin etkisi araştırılmıştır. Klasik metotla üretilen kırmızı şarap kontrol olarak alınmıştır. Karbonik maserasyonla üretilen şarap ile meşe fıçıda olgunlaştırılmış Öküzgözü şarabının bileşimleri ile karşılaştırılmıştır. Çalışma iki ardışık bağbozumu yılını (2018 ve 2019) kapsamaktadır. Öncelikle şarapların genel bileşimi Uluslararası Bağcılık ve Şarapçılık Organizasyonu (OIV) tarafından onaylanan yöntemlerle belirlenmiştir.

Daha sonra, aroma bileşiklerinin analizi sıvı-sıvı ekstraksiyonun ardından GC-MS-FID cihazıyla tanımlama ve miktar tayini yapılarak gerçekleştirilmiştir. Ayrıca,

aroma aktif bileşiklerin karakterizasyonu, GC-olfaktometri cihazıyla Aroma Ekstrakt Seyreltme Analizi (AEDA) yöntemi kullanılarak yapılmıştır. Şarapların renk ve fenolik bileşimleri spektrofotometrik yöntemlerle, monomer yapılı antosiyaninlerin analizleri ise HPLC-DAD yöntemiyle yapılmıştır. Şarap yapım yöntemlerinin Öküzgözü kırmızı şarapları üzerindeki etkisi, deneyimli 9 kişilik panelist grubu tarafından gerçekleştirilen Tanımlayıcı Duyusal Analiz ile de değerlendirilmiştir.

Şarap üretim yöntemi üretilen şarapların genel bileşimi üzerinde istatistiksel açıdan önemli farklılıklara neden olmuştur. Karbonik maserasyonlu şarapta toplam asitlik ve uçucu asitlik miktarları kontrol şarabına göre önemli düzeyde ($p < 0.05$) düşük bulunmuştur, karbonik maserasyona karşı kontrol şarabında sırasıyla toplam asitlik 4.31 g/L'ye karşı 4.72 g/L ve uçar asitlik 0.44 g/L'ye karşı 0.47 g/L bulunmuştur. Meşe fıçıda olgunlaştırılan şarapta (OBA) aynı parametreler sırasıyla 5.01 g/L ve 0.53 g/L değerleriyle daha yüksek konsantrasyonlarda tespit edilmiştir. Öküzgözü şarapları arasında karbonik maserasyon uygulanan şarap (%13.68) en yüksek oranda etil alkol içerirken, en düşük oran OBA şarabında (%13.38) tespit edilmiştir.

Bu çalışmada incelenen kırmızı şarap üretim yöntemleri, Öküzgözü kırmızı şarabının uçucu bileşimi üzerinde de etki göstermiştir. OBA şarabı 567.41 mg/L ile en yüksek toplam uçucu bileşik konsantrasyonuna sahip olurken, karbonik maserasyonla üretilen şarap 464.25 mg/L konsantrasyon ile OBA şarabını takip etmiş ve 435.24 mg/L ile kontrol şarabı en düşük konsantrasyona sahip şarap olmuştur. Uçucu bileşiklerin nispi oranları ayrıntılı olarak analiz edildiğinde, karbonik maserasyon yöntemiyle üretilen şarap, farklı üzüm çeşitlerinden üretilen diğer primör şaraplarla, özellikle esterler açısından, ortak özellikler göstermiştir. Gerçekten de, kontrol şarabı ile karşılaştırıldığında, karbonik maserasyon yöntemiyle üretilen şarabın daha yüksek konsantrasyonda yüksek alkollerin asetatlarını (HAAs) ve yağ asitlerinin etil esterlerini (EEFAs) içerdiği ve toplam esterlerin sırasıyla %4,34'üne karşı %8,60'ını ve %6,83'üne karşı %12,96'sını karbonik maserasyonlu şarapların sahip olduğu belirlenmiştir. Esterlerin bu iki alt sınıfı, genç şarapların meyvemsi ve çiçeksi karakterine büyük katkıda bulunur. Ayrıca, karbonik maserasyon ile yapılan şarabın belirteçleri olarak kabul edilen diasetil ve 4-vinil guaiacol gibi diğer bazı uçucu bileşikler de karbonik

maserasyon yöntemiyle üretilen şarapta daha yüksek konsantrasyonlarda tespit edilmiştir. Diğer yandan OBA şarabında, fıçıda uzun olgunlaşma süresi nedeniyle organik asitlerin etil esterleri en yüksek düzeyde belirlenmiş, ayrıca bu şarapta meşe fıçısından gelen meşe laktonu ve furfural gibi uçucu bileşikler dikkat çekmiştir. OBA şarabı, toplam esterler içerisinde yüksek alkollerin asetatlarını ve yağ asitlerinin esterlerini kontrol şarabına kıyasla daha düşük yüzde ile (sırasıyla %1,69 ve %3,21) içermiştir.

Öküzgözü kırmızı şaraplarında aroma ekstrakt seyreltme analizi ile 13 adet ester, 8 adet yüksek alkol, 6 adet uçucu asit, 4 adet lakton, 4 adet karbonil bileşiği, 2 adet uçucu fenol, 1 adet kükürtlü bileşik ve 2 adet tanımlanamayan bileşik olmak üzere toplam 40 adet aroma-aktif bileşik tanımlanmıştır. Karbonik maserasyon yöntemiyle üretilen şarapta FD ≥729 ile yüksek aroma yoğunluğu gösteren aktif bileşikler tereyağı/lakte kokusu ile diasetil, muz kokusu ile izoamil asetat, ananas ve bal benzeri koku ile etil vanilat olmuştur. OBA şarabında ise, cis-meşe lakton, 4-etoksikarbonil-ɤ-bütanolakton ve tanımlanamayan bir bileşik dahil olmak üzere üç bileşik 2187 FD'ye kadar tespit edilmiştir.

Alkol içeren matrislerde hesaplanan aroma aktivitesi değerleri, karbonik maserasyonla üretilen şarapta, etil hekzanoat ve izoamil asetatın lezzet seyreltme faktörü ve bunların nispi AAV'leri arasında pozitif korelasyon göstermiştir. Her iki bileşik %55.75 ve AAV>1 değerine sahiptir. Ayrıca, karbonik maserosyonlu şarapta tereyağı/krema/lakte kokuları veren diasetil ve bunun alkol türevleri (levo ve meso-2,3 bütandioller) bir aroma-aktif kümesi oluşturulabilir. Bu küme, toplam AAV ≥ 1'in %24,76'sını oluşturmaktadır. OBA şarabında AEDA ve AAV değerlerinden elde edilen sonuçlar, bu şarabın etil hekzanoat/izoamil asetattan gelen meyveli notalar, cis-meşe laktonundan gelen meşe aroması ve 4-vinil guaiakolden gelen "baharat/karanfil" aromaları ile kompleks bir aroma profiline sahip olduğunu göstermiştir.

Toplam fenolik bileşikleri bakımından, beklendiği gibi, kontrol şarabına kıyasla, karbonik maserasyon yöntemiyle üretilen şarap 1711 mg/L ile en düşük miktarda toplam fenolik bileşik içerirken, OBA şarabı 2146 mg/L ile en yüksek düzeyde toplam fenolik bileşik içeren şarap olmuştur. Spektrofotometrik yöntemle

belirlenen toplam antosiyanin miktarı açısından, karbonik maserasyon yöntemiyle üretilen şarap her iki yılda da 558.30 mg/L ve 522.92 mg/L değerleriyle en yüksek antosiyanin içeriğine sahip şarap olmuştur. OBA şarabı ise her iki yılda da 475.69 mg/L ve 472.38 mg/L değerleriyle en düşük toplam antosiyanin içeriğine sahip şarap olmuştur.

Öküzgözü şaraplarında HPLC ile 5 adet monoglikozit, 5 adet monoglikozit asetat ve 3 adet monoglikozit p-kumarat olmak üzere toplam 13 adet monomerik antosiyaninin belirlenmiştir. Şaraplarda saptanan toplam monomer yapılı antosiyaninlerin nispi konsantrasyonu ile ilgili eğilimin, bisülfit yöntemi ile elde edilenlere benzer olduğugörülmüştür. Gerçekten de, her iki yolda da (769.10 mg/L ve 727.10 mg/L) en yüksek toplam antosiyanin konsantrasyonunu karbonik maserasyonlu şarap gösterirken, en düşük konsantrasyon OBA şarabında (573.70 mg/L ve 583.90 mg/L) tespit edilmiştir. Ayrıca, yıldan bağımsız olarak, malvidin-3-glikozit, sırasıyla karbonik maserasyon, kontrol ve OBA şaraplarında ortalama 379.17 mg/L, 327.10 mg/L ve 286.60 mg/L konsantrasyonlarıyla en baskın bulunan antosiyanin monomeridir.

Tanımlayıcı duyusal analiz, üç farklı yöntemle üretilen Öküzgözü kırmızı şarapları arasında sadece *"baharat"*, "*meşe/vanilya*" ve "*kuru meyve*" aroma tanımlayıcılarının istatistiksel açıdan önemli düzeyde bir korelasyon vermesine karşın seçilen tüm aroma tanımlayıcıları ile şarabın aroma kalitesi arasında pozitif bir ilişki kurulmasını sağlamıştır. Ayrıca, her üç yöntemle üretilen şarapta en belirgin aroma tanımlayıcısı kırmızı meyve olmuş ve kırmızı meyve burunda ağızdan daha belirgin hissedilmiş ve en yüksek yoğunluk karbonik maserasyon yöntemiyle elde edilen şarapta tespit edilmiştir. Diğer yandan, tat profili bakımından karbonik maserasyon yöntemiyle elde edilen şarap kolay içimli, yumuşak ve tatlılıkla karakterize edilirken, OBA şarabı buruk ve gövdeli olarak karakterize edilmiştir. Son olarak panelistler, genel olarak karbonik maserasyonla üretilen şarabı, OBA şarabına tercih etmişlerdir.

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ABREVIATIONS

µg	: microgram
µmol	: micromole
AAB	: acetic acid bacteria
AEDA	: aroma extract dilution analysis
AF	: alcoholic fermentation
AM	: anaerobic metabolism
ANOVA	: analysis of variance
CM	: carbonic maceration
CO_2	: carbon dioxide
Cv	: cultivar
DAD	: diode array detection
DA	: Descriptive Analysis
DW	: distillated water
EEFAs	: ethyl esters of fatty acids
EEOAs	: ethyl esters of organic acids
FD	: factor dilution
GC	: gas chromatography
HAAs	: higher alcohols of acetates
HCl	: hydroxychloridric acid
HPLC	: high pressure liquid chromatography
ISO	: international organization of standardization
ITV	: Institut technique de la vigne et du vin
LAB	: lactic acid bacteria
MANOVA	: multivariate analysis of variance
MLF	: malolactic fermentation
MS	: mass spectrometry

nm	: nanometer
AAV	: aroma activity value
OD	: optical density
OIV	: international organization of vine and wine
SO_2	: sulfur dioxide
T.ant.	: total anthocyanins
TA	: total acidity
UV	: ultraviolet
UV-Vis	: UV-visible
VA	: volatile acidity

1 INTRODUCTION

Wine currently has the smallest segment in terms of market share among alcoholic beverages. However, it is experiencing growth that has been above average. In recent years, demand in emerging economies such as China has risen considerably, and nowadays, wine is among the most prized alcoholic beverages in the world. It has shown 4.4% of revenue growth for 2019, ahead of beer and spirit with 3.81% and 2.99%, respectively (Statista, 2020). Thanks to improvements in technology and strides in wine science, numerous and better ways to make wine of high quality are now available.

In general, vinification starts with the choice of grape and consists of converting the grape juice into wine through different steps according to the desired wine style. Thus the winemaker makes decisions at every step of the process to impact the organoleptic components such as volatile and phenolic compounds, which both widely contribute to wine quality. Among the stages leading to winemaking, both maceration and aging have a crucial effect on the final product.

Maceration is generally conducted before fermentation with white and rose wines, whereas, for red wine, it is performed together with fermentation and, in some cases later on. Maceration involves leaving the grape juice in contact with the seeds and skins (pomace) for several hours (white or rose wine) to several days or weeks (red wine). The duration depends on the desired intensity of flavors, tannins, and anthocyanins, the latter are the primary chemicals that distinguish red from white wine (Jackson, 2009). In winemaking, there are several types of maceration depending on the desired wine style; among them, there is so-called carbonic maceration (CM), invented by Michel FLANZY in 1934. The vinification by CM is a process allowing to take advantage of phenomena occurring spontaneously in intact grape clusters placed in carbon dioxide-rich atmosphere. The berries subsequently undergo an intracellular fermentation with ethanol production

between 1,5 - 2 % by utilization of the enzymes present within the grapes themselves without yeast intervention (Tesniere and Flanzy, 2011; Jackson, 2014). In addition, it is recorded, a diffusion of carbon dioxide and phenolic compounds, a significant fall of malic acid up to 50% of the total amount (Flanzy et al., 1987), and a release or evolution of likely precursors of wine aroma. All these changes occurring in the berries constitute the anaerobic metabolism (AM), and as such, the intensity depends on the grape variety, the vintage, the maceration temperature, and the given duration. CM leads mainly to young red wine characterized by its softness, low anthocyanin and tannin contents, unique floral, and intense fruity notes. Besides, young wine made by CM, is drinkable within few months after its production (Ribéreau-Gayon et al., 2006; Jackson, 2008; Tesniere and Flanzy, 2011).

After completion of fermentation, wine may taste rough and fairly unpleasant; thus a period of aging (maturation) is required in which a series of racking and other tasks are carried out for clarification and stabilization as needed. During aging, particularly in red wine, it is noticed, drop of acidity, softening of tannins, color and aroma evolution, and under certain conditions, the rise of the aging bouquet. The structure of tannins is modified mainly by self-polymerization of proanthocyanidins or polymerization of proanthocyanidins with other wine compounds such as anthocyanins, proteins, and polysaccharides. As a consequence, a decline of bitterness and astringency is observed conferring to the wine a more full-bodied and improved flavor (McRae et al., 2012). These reactions are partially responsible for color change during maturation. Indeed, red wine initially displays a cherry red and gradually moves towards ruby red and then brick red (Ribéreau-Gayon et al., 2006; Jackson, 2014). These shifts result from the disruption of self-association and co-pigment anthocyanin complexes (typical of young wines), progressive formation of new pigments, and the polymerization of proanthocyanidins with anthocyanins into anthocyanin–tannin complexes (Peng et al., 2002). Additionally, aroma, referred to as tertiary aroma, is formed during

maturation, slight oxidation of some existing components by dissolved oxygen present and absorbed by wine during storage. Thus during lengthy maturation, the young, fresh, and fruity bouquet, along with varietal aroma disappear and are replaced by an aged bouquet with subtle modifications in flavor, with more complexity when an oak barrel is used (Bakker and Clarke, 2011). Aging refers to changes that occur from the end of malolactic fermentation (MLF) to bottling. Hence, the conditions under which wine is stored and handled, as well as the types of vessels used, have a remarkable effect on these changes. There are many types of vessels used for aging, including stainless steel vats and wooden barrels. Stainless steel vats, by their features, are ideal storage vessels for some wines intended for early drinking, such as primeur wine which needs little or no aging. In oak barrel aging, the process depends on duration, oxygen, temperature, and oak characteristic, and complex events occur such as structural changes in the phenolic compounds and the release of oak aroma into the wine.

Among the indigenous Turkish Vitis vinifera, Öküzgözü is one of the most famous and the most produced red grape variety with around 11.830 tons per year (WinesofTurkey, 2020). Native to Mid-Eastern Anatolia and widely planted in Elazığ and Denizli provinces, it is known to have moderate acidity and soft tannins giving medium-bodied wines with lots of floral and ripe, red fruit flavors like cherry and pomegranate, along with a subtle spiciness and capable of medium-term aging. The phenolic and volatile compounds of red wine are affected by two major factors: the grape (e.g., variety, ripening, cultivation, location) and winemaking techniques (e.g., maceration time, temperature, yeast and enzyme used, SO2, fining agents, aging) (Sacchi et al., 2005; Karimali et al., 2020; Wang et al., 2020). Pertinent works have been carried out on Vitis vinifera L.cv Öküzgözü, notably on its derivative wines, and some were focused on their phenolic and volatile compounds (Cabaroglu et al., 2002; Kelebek et al., 2010; Yıldırım, 2013; Bayram, 2018; Tetik et al., 2018). Öküzgözü wine is produced in most Turkish wineries, as well as monovarietal wine or blended to Boğazkere or other grape varieties.

Previous researches about Öküzgözü wines allowed not only to get a wine with better quality but also opened the way for winemakers to produce different types of wines from a unique grape variety. Therefore, it would be pertinent to highlight organoleptic characters of these öküzgözü wines made by different vinification methods. Among Turkish wineries, Kavaklıdere is the only one to produce, through CM, primeur wine from Öküzgözü grape variety.

In the perspective to valorize the organoleptic potential of *Vitis vinifera* L.cv Öküzgözü through winemaking discipline, we investigated for two vintages (2018 and 2019), the impact of CM and oak barrel aging on volatile and phenolic compounds of red wines made with Öküzgözü grapes grown in Elazig province in this study.

2 LITERATURE REVIEW

2.1 Red winemaking overview

2.1.1 Classic red winemaking

Winemaking is one of the oldest human practices, starting from the early Neolithic period, as shown by some archeological excavations of pottery found in Georgia in the South Caucasus region dating back more than 7500 years (McGovern, 2003; McGovern et al., 2017).

Wine production encompasses two distinct stages: growing of grapes (viticulture) and transformation of grapes into wines of target style (vinification). In the path leading to obtain wine, alcoholic fermentation, involving the conversion of grape sugars into alcohol by yeast with carbon dioxide release, is the main step. Beyond this, the vinification is associated with other significant changes due to extraction and microbial metabolism of a multitude of other grape components. In addition to alcoholic fermentation, red wine is submitted to another major operation, malolactic fermentation. This operation is controlled by lactic acid bacteria (LAB) of grapes, and can occur sequentially or simultaneously with alcoholic fermentation.

However, many winemakers choose to inhibit natural yeasts and/or bacteria on grape skin, using cultured microflora to carry out a controlled fermentation. Although fermentations are the major steps in vinification, a range of additional operations under-appreciation of winemaker and targeted wine style is required to produce a high-quality wine (Figure 2.1 and Figure 2.2). Among these operations, we can cite maceration allowing extraction of matter from grapes and aging to promote mouthfeel changes and color stabilization.

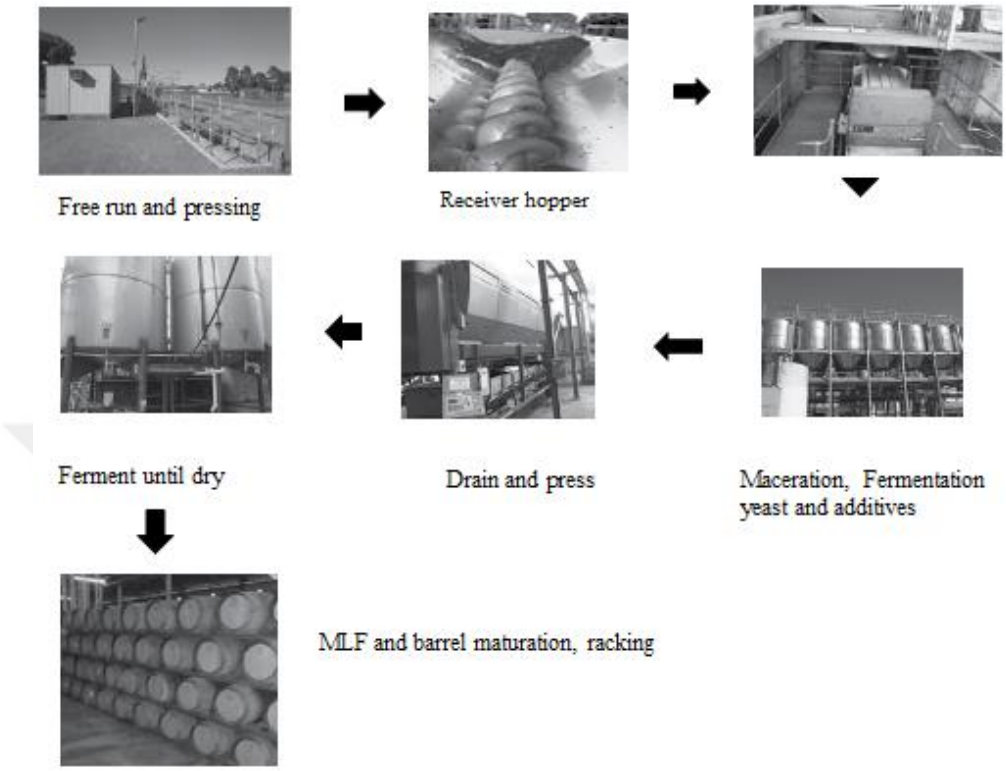


Figure 2.1. Indicative flowchart for commercial winery fermentation operations. İncorporation of oak can occur in a number of ways and at different stages of the process. The remainder of the operations is outlined in Figure 2.2

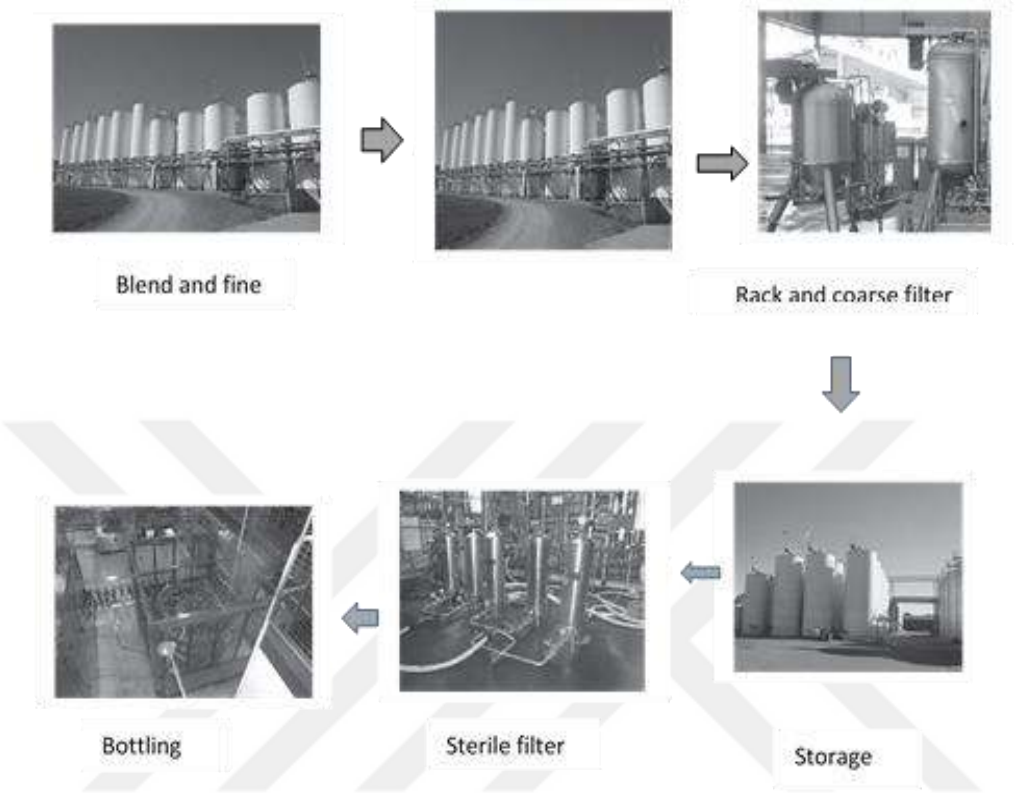


Figure 2.2. Indicative flowchart for commercial winery operations after fermentation is complete. Adjustments primarily involve the use of tartaric acid, carbonate salts, and SO2. Adapted from (Waterhouse et al., 2016).

The technology and the winemaking method depend on the style of wine produced. Thereby a wine classification is needed to guide consumers. Wines are grouped based on alcohol concentration and subdivided based on their stylistic features according to Waterhouse et al. (2016) (Table 2.1)

- Table wines encompassing still wines and sparkling wine with alcohol content ranging between 9 and 15% by volume.
- Fortified wines with alcohol range between 17 and 22%.

The table still wines present the largest subcategories because most of the wines belong to that category. The oldest division, based on color, separates table still wines into white, red, and rosé subgroups. This classification displays distinct differences in flavor, use, and production methods. For example, white wines often are more acidic, fragrant with some sweetness, while red wines are more flavorful, astringent, and drier. Rosés tend to be more astringent than white wines but lighter than reds and occasionally petulant and sweet

Table 2.1. Classification of major vinification or wine styles found throughout the world. From (Waterhouse et al., 2016)

Description		Color	Residual sugar(g/L)	Alcohol (% v/v)	Examples
White wines Still	Dry	Pale straw to gold	< 9	8 t0 14.5	Riesling, Chardonnay, Semillion, Sauvignon Blanc, Colombard, Grüner Veltliner, Trebbiano, Chenin Blanc
	Sweet	Light yellow to gold	9 to 30 (semi-sweet) 30 to 200 (sweet)		Riesling, Gerwurztraminer, Semillion
					Riesling, İce wines, Sauternes, Tokay
Sparkling	Dry to semi-sweet	Pale straw to amber (pink to light red for rosé wines)	0 to 50		Champagne, Chardonnay/pinot Noir/Pinot Meunier[a], Riesling, Sauvignon Blanc, Cava, Prosecco
Fortified	Dry	Pale straw to amber	0 to 30	15 to 20.5	Fino and Amontillado Sherries
	Sweet		100 to 300		Oloroso and Pedro Ximénez[b] Sherries, Topaque
Red wines Still	Dry	Dark red to red /brown	< 7.5	8 to 14.5	Grenache, Merlot, Cabernet Sauvignon, Tempranillo, Zinfandel, Sangiovese, Malbec, Pinot Noir, Shiraz
sparkling	Semi sweet		7.5 to 30		Pinot Noir, Shiraz, Cabernet Sauvignon
Fortified	Sweet	Red/gold to deep brown	100 to 300	10 to 22	Ruby, Tawny and Vintage Ports, Brown Muscat

[a] Of these classic Champagne grape varieties, the first two tend to be used more frequently in sparkling wines.
[b] Pedro Ximénez can be very sweet and may contain up to 450 g/L of residual sugar.

2.1.2 Carbonic maceration

CM is a winemaking technic taking profit of phenomena occurring spontaneously within intact and uncrushed grape berries when placed in a carbon dioxide-rich environment. The changes observed in the berry during CM referred to anaerobic metabolism (AM). Among all these modifications, intracellular fermentation, changes in phenolic compound and the metabolism of aroma precursors are crucial for the sensory attributes of red wine resulting from CM. The

quality of changes depends essentially on the temperature and duration of the procedure.

Under the control of grape enzymes, intracellular fermentation involves converting sugars into ethanol in proportions similar to those observed with yeast fermentation (17-18g of sugar for 1% v/v of ethanol) (Flanzy, 1978). The phenomenon is influenced by temperature and duration. Indeed, the rate of ethanol production increase with the rise of temperature, while, the maximum amount is high with lower temperature (Figure 2.3) (Flanzy et al., 1987).

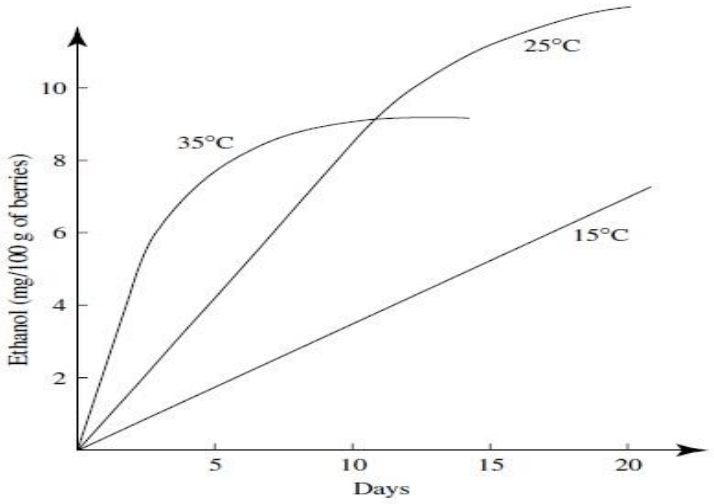


Figure 2.3. Temperature and ethanol production in intracellular fermentation, E = ethanol in g for 1000g of berries. Adapted from (Flanzy et al., 1987)

The transfer rate of phenolic compounds from skin towards pulp increases with temperature, moreover the diffusion of anthocyanins is prior to tannins (Bourzeix, 1971; Mourgues et al., 1984). The release of phenolic compounds is facilitated by the degradation of cell wall compounds (pectolytic and proteolytic phenomena) in anaerobic conditions (Mourgues et al., 1984).

During anaerobic metabolism, aroma precursors are released. The most characteristic fact is the metabolism of shikimic acid, which contributes to the

synthesis of some aroma precursors, later subjected to yeast and/or bacterial metabolism, as shown in figure 2.4 (Flanzy et al., 1981).

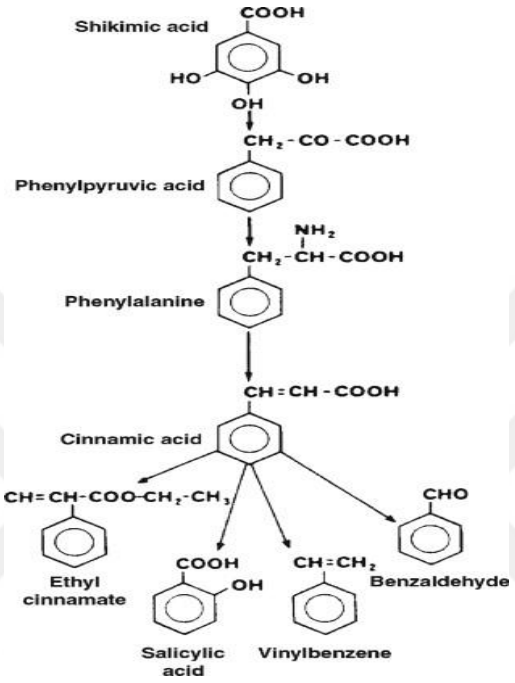


Figure 2.4. Shikimic acid as a precursor for aromatic compound. Adapted from (Jackson, 2014)

2.1.2.1 Management of CM

Red wine made by CM can be handled by several ways , giving a large panel of wines according to the wineries and winemakers. in Figure 2.5 below, the classic method designed by Michel Flanzy is summarized.

At first, bunches are manually and carefully harvested, transferred into vat previously filled up with CO_2 to set up an anaerobic environment afterward without crushing /destemming. Berries in vat are found in three batches:

- An upper batch of berries on the top of vat entirely in contact with an environment rich in CO_2.
- A batch of crushed berries on the bottom of vat due to either grapes loading during vatting or accumulated weight of the upper grape mass. These crushed berries release grape juice.
- An intermediate batch of intact bunches, between the two first batches, soaking in the grape juice released from the bottom of the vessel.

During this first step, 3 phenomena occur simultaneously: anaerobic metabolism for berries dived in CO_2 atmosphere or grape juice. A type of classic maceration where exchanges by diffusion are observed between berries, vat environment and grape juice. Finally, an alcoholic fermentation of grape juice on the bottom is triggered by indigenous yeast.

Once the CM is completed, partially fermented free-run juice is collected in another vessel to complete fermentation, either alone or with press-run juice. After fermentation, the wine undergoes a series of usual operations such as MLF (in some cases in parallel with alcoholic fermentation), racking, stabilization, and further bottling when it is destined for early consumption.

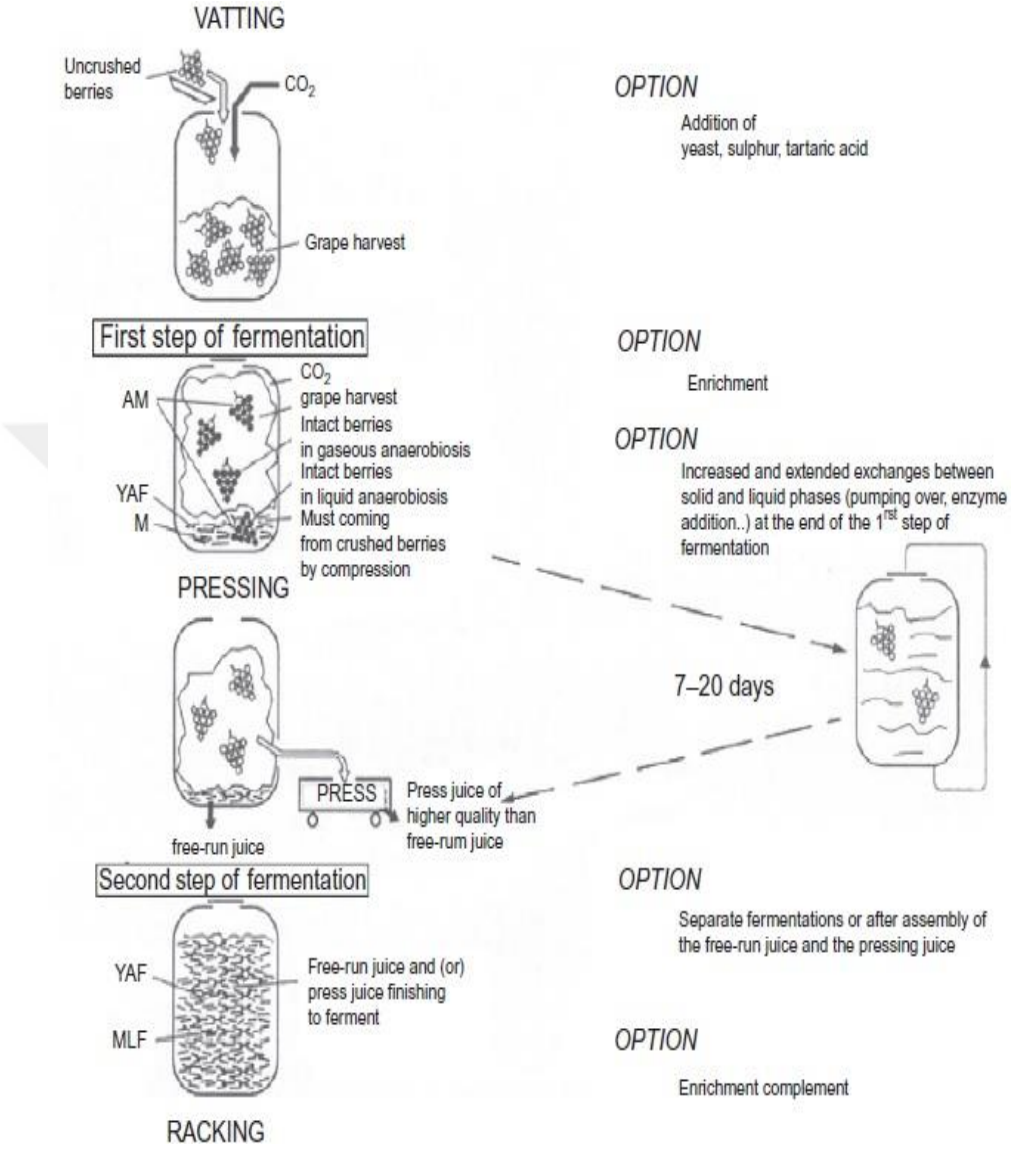


Figure 2.5. Scheme of CM winemaking. AM, anaerobic metabolism

of grape berries; YAF, yeast alcoholic fermentation; M, maceration. Adapted from (Tesniere and Flanzy, 2011)

The temperature and the duration for which CM is carried out greatly influence organoleptic characters of the obtained wine, hence, the choice of the pair " temperature-duration" is according to the desired type of wine (Flanzy et al., 1987). Indeed, CM offers endless variations, depending on what works the best for a specific grape variety and the style desired by the winemaker. Thus many winemakers introduce changes in the classic CM scheme to set up derivatives. For example, ordinarily classic CM scheme leads to produce young red wine so-called "vin primeur", with aromatic richness, softness, and harmonious balance nevertheless by extending CM duration followed by mechanic maceration, "Chateauneuf-du-pape" wineries have produced wine showing an excellent satisfaction while degusted after 20 years aging (Flanzy, 2014).

Another quite remarkable derivative of CM is semi-carbonic maceration. Generally associated to "Beaujolais nouveau" style, this winemaking method was developed in the Beaujolais region of France by Georges Duboeuf and allows to release every year on the third Thursday of November, a primeur red wine called " Beaujolais Nouveau". The used grape variety is Gamay, and the technic consists of loading in a sealable vat with CO_2-free (that was not previously filled with CO_2) whole cluster grapes. Once the vat is loaded, the berries at the bottom burst under the weight of clusters above and release juice which undergoes yeast fermentation. The alcoholic fermentation produces CO_2, which entails anaerobic metabolism and the relative changes into intact berries. The following steps are more or less similar to those occurring in classic CM, and the wine is released three months after harvest. "Beaujolais nouveau" wine has a light red color, low acidity, low tannins, and fresh fruity flavors such as strawberry or raspberry due to ethyl cinnamate and cherry aromas from benzaldehyde (Watrelot, 2020).

2.2 Red wine Aroma compounds

Aroma compounds are volatile organic compounds (VOCs) exhibiting pleasant olfactory proprieties. Because stages leading to red winemaking are more or less diverse according to the desired style, aroma compounds may arise from many sources during wine production. They can originate from grape, the maceration, both alcoholic and malolactic fermentation, and aging and storage conditions as well. Thus, as stated by Schreier and Drawert (1974), they may be classified following a technological approach, considering aroma compounds according to the stage of release throughout the winemaking process.

2.2.1 Varietal Aroma

The varietal aroma is grape-related VOCs, which take a source from the berry and are released directly as volatile odorants or odorless precursors. Usually characteristic of grape variety, only a few aroma compounds, such as rotundone in Shiraz cultivar, and 2-alkyl-3-methoxypyrazines in Cabernet-Sauvignon and related cultivars have been directly linked to specific varietal aroma (Bayonove et al., 1998; Polášková et al., 2008). Some of these compounds and their characteristics are listed in Table.2.2

Table 2.2. Impact odorants contributing to varietal aromas of selected wines adapted from Styger et al. (2011).

Odor characteristic	Impact compounds	Cultivars
Floral	Linalool	Muscat
Citrus, floral	Geraniol	Muscat
Citrus, floral	Nerol	Muscat
Kerosene	1,1,6-Trimethyl-1,2-dihydronaphthalene	Riesling
Bell pepper	3-Isobutyl-2-methoxypyrazines	Sauvignon Blanc
Coconut, woody, sweet	3,6-Dimethyl-3a,4,5,7a-tetrahydro-*3H*-1-benzofuran-2-one	Gewurztraminer
Black currant	4-Methyl-4-mercaptopentan-2-one	Sauvignon Blanc
Grapefruit, citrus peel	3-Mercapto-1-hexanol R-isomer	Sauvignon Blanc
Passion fruit	3-Mercapto-1-hexanol S-isomer	Semillon
Black pepper	Rotundone	Shiraz

However, the major part of grape-related aroma compounds occurs in berry as odorless precursors. That is why grapes of most *Vitis vinifera* cultivars used in winemaking have no characteristic varietal aroma. These non-volatile and odorless constituents are susceptible to transformation into volatile varietal aroma compounds by grape enzymes during maceration, yeast and bacteria during fermentation, and also aging and storage conditions (Figure.2.6). Most of these precursors belong to the following chemical families: unsaturated lipids, phenolic acids, carotenoids, S-cysteine conjugates and glycoconjugates.

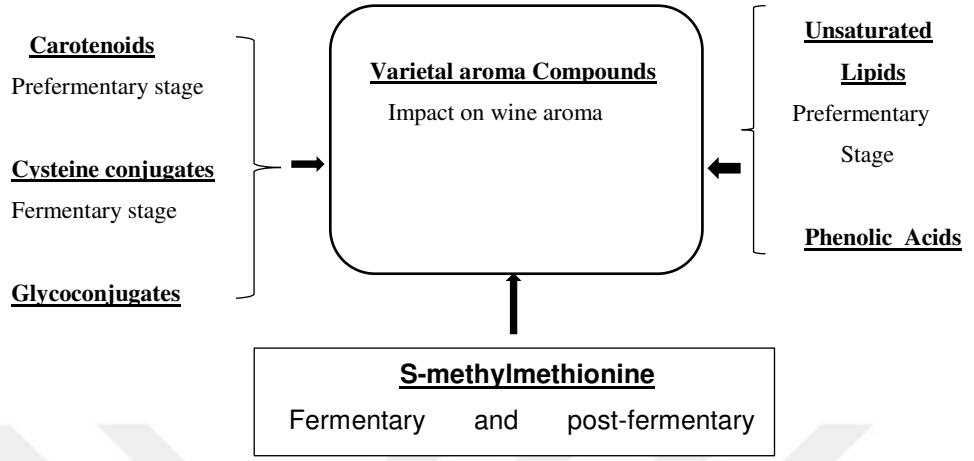


Figure 2.6. Wine aroma precursors, their main stages of degradation during the wine biotechnological sequence and general impact on wine aroma of the odorants generated. Adapted from (Moreno-Arribas and Polo, 2009).

Phenolic volatile precursors

Phenolic volatile precursors encompass the hydroxycinnamic acids located in the solid part of the berry and their relative esters formed with tartaric acid. Their contents are grape cultivars related and decrease with grape maturation (Boursiquot et al., 1986). Among them, coumaric and ferulic acid esters are precursors respectively of 4-vinyl phenol, 4-ethyl phenol, and 4-vinyl guaiacol, 4-ethylguaicol synthesized during FA by cinnamate carboxylase released by saccharomyces cerevisiae (Dugelay et al., 1992; Chatonnet, Barbe, et al., 1993; Chatonnet, Dubourdieu, et al., 1993). However, yeast carboxylase is inhibited by catechins, which are abundant in red wine. Thus red wine contains mainly, low content of vinyl phenols. In contrast, these phenolic aroma compounds are quite present in CM wines, wherein tannins are less available.

Carotenoids or C13-Norisoprenoids precursors

Among the carotenoids present in grapes,β-carotene and lutein are the most common and represent up to 85% of the total content. In grape, C13-norisoprenoids appear as traces under free and odorant form. They are essentially present bound to glycoside moiety in precursor form (Winterhalter, 1993; Baumes et al., 2002). The glycosylated C13-norisoprenoids result from degradation of carotenoids, particularly from β-carotene and lutein, representing up to 85% of the total carotenoid contents (Razungles et al., 1987; Bureau, 1998; Mendes-Pinto et al., 2004). The carotenoids degradation occurs from "véraison" to maturity under the control of sunshine and grape enzymatic pattern, whereby a carotenoid dioxygenase in releasing C13-norisoprenoidic carbonyl compounds is involved (Mathieu et al., 2005). Afterward, reductases and oxidases convert the obtain carbonyl compounds in C13-norisoprenoidic, and finally glycosylated C13-norisoprenoids are generated by grape glycosyltransferase (Ford and Høj, 1998; Wirth et al., 2002). These intermediary compounds released free odorant C13-norisoprenoids (figure 2.7) such as β-damascenone, α, and β-ionones, through an acid-catalyzed reaction, during vinification and maturation (Strauss et al., 1987; Schneider et al., 2001). The β-damascenone displays floral, fruity and spicy smells associated with stewed apple, honey, and Tobacco notes (Pineau, 2007). Its olfactory threshold varies from several nanograms per liter in a dilute alcohol solution to a few micrograms per liter in wine (Guth, 1997; Pineau, 2007). α et β-ionones compared to β-damascenone present more floral smells with nuances of fresh raspberry and violet aroma. According to the matrix, their olfactory threshold moves around tens and sometimes hundreds of nanograms per liter (Etievant and Bayonove, 1983; Buttery et al., 1997; Ferreira et al., 2000). Several studies have shown the importance of yeast (Segurel et al., 2009; Loscos et al., 2010) and bacteria metabolism (Boido et al., 2002; D'Incecco et al., 2004) on the release of these C13-norisoprenoids.

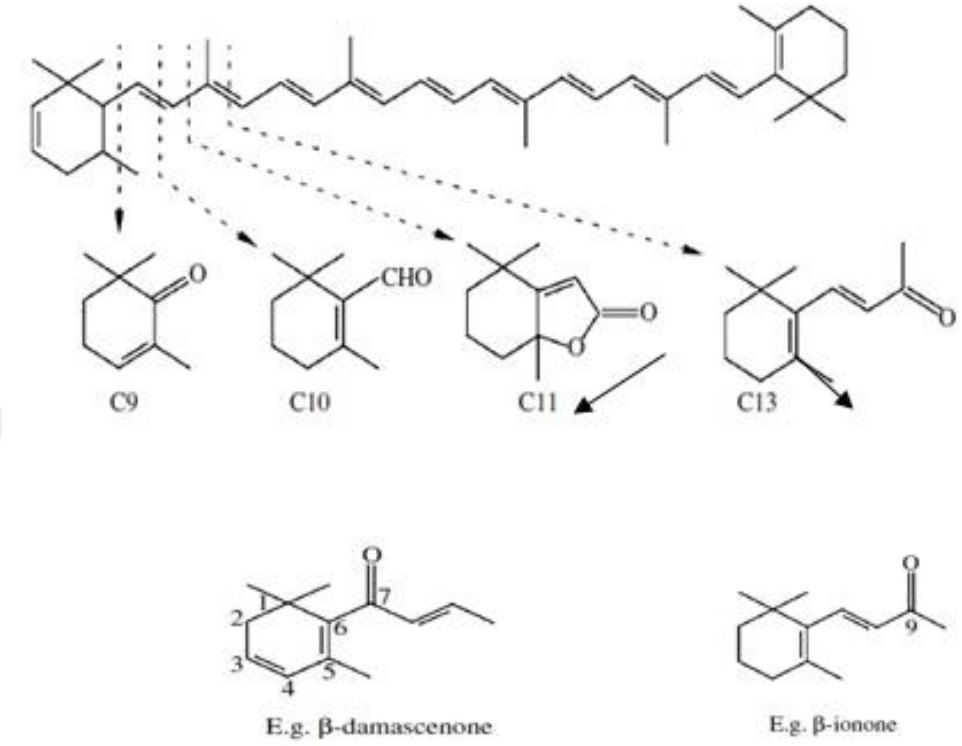


Figure 2.7. Breakdown of carotenoids leading to the formation of C9, C10, C11 and C13-norisoprenoids in grapes. Adapted from (Ribéreau-Gayon et al., 2006)

Volatile thiols

These potent aroma compounds were reported first in white wines. Indeed the contribution of 4-Mercapto-4-méthylpentan-2-one (4MMP) to guava note of South African sauvignon blanc wines has been early mentioned by Du Plessis and Augustyn (1981). Later, the 4MMP and other varietal volatile thiols were identified as key compounds of the characteristic aroma of Sauvignon Blanc (Darriet et al., 1995; Tominaga et al., 1998). Among volatile thiols, 3-Mercapto-1-hexanol(3MH), 3-Mercaptohexyl acetate(ac3MH), and 3-Mercapto-2-methyl propanol (3MMP) were also found in other white varieties such as Muscadet and Bacchus (Schneider et al., 2003). These compounds were also recorded in red wines of cabernet

sauvignon, merlot (Bouchilloux et al., 1998), and Grenache (Ferreira et al., 2002), at concentrations close to their perception thresholds (Tominaga et al., 1998).

The 3MH appear to be the most involved in the fruity aroma of red wine and are present in 2 enantiomer forms reflecting grapefruit zest for R form and passion fruit for the S form. Both likely reinforce the "red fruit" character in red wine (Ferreira et al., 2002; Rigou et al., 2014). These compounds are present in must as nonvolatile and odorless precursors because they are S-conjugate to cysteine and glutathione. They are released during AF through the yeast metabolism (Tominaga et al., 1998; des Gachons et al., 2002) (Figure. 2.8). A precursor of 3MH, S-3-(hexane-1-ol)-L-cysteine, identified in must by Bouchilloux et al. (1998), was separated from L-cysteine moiety with a yeast β-lyase (Thibon et al., 2008). Another biogenesis pathway through (E)-2-Hexen-1-al was suggested by Schneider et al. (2006).

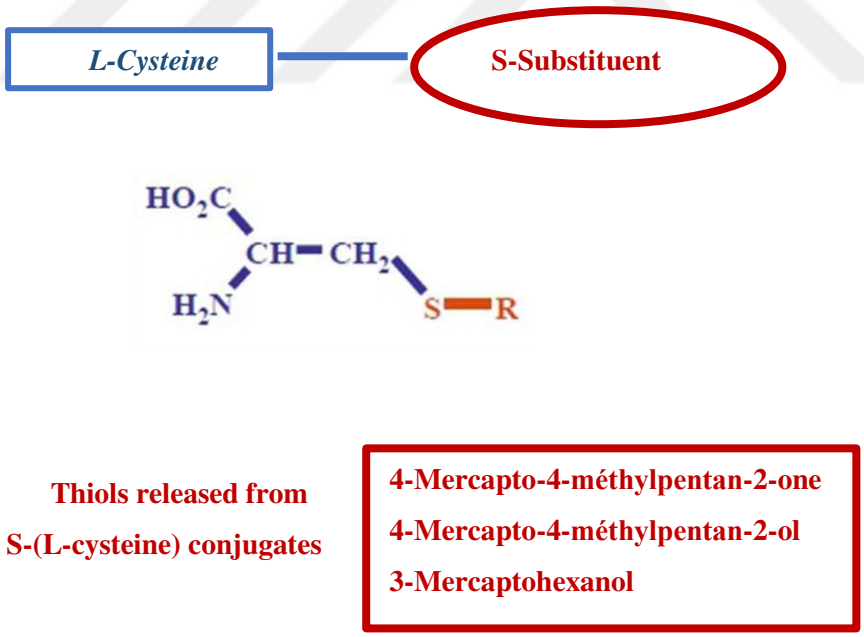
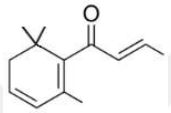
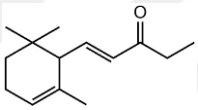
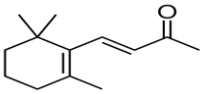
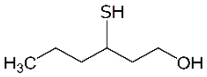
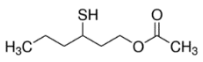


Figure 2.8. S-(L-Cysteine) conjugates occurring in grape, precursors of varietal thiols, adapted from (Moreno-Arribas and Polo, 2009)

The yeast strain involved has an essential role in the release of these volatile compounds. The capacity to hydrolyze their corresponding precursors varies regarding the species and even the strain used for fermentation (Dubourdieu et al., 2006).

Table 2.3. Some varietal-origin compounds identified in red wine. Adapted from (Gammacurta, 2014)

Compounds	**Formula**	**Odor threshold (µg/L)**	**Descriptors**
C13-Norisoprenoids			
β-damascenone		0.05 [1] ; 7 [2]	floral, fruity and spicy, stewed apple, honey, tobacco
α-ionone		0.4 [3] ; 2.6 [4]	floral, fresh raspberry
β-ionone		0.03 [3] ; 0.09 [5]	floral, violet
Thiols			
3-Mercapto-1-hexanol		0.012 - 0.015 [6]	grapefruit, passion fruits
3-Mercaptohexyl acetate		0.002 - 0.003 [6]	boxwood, passion fruits

[1] hydroalcoholic solution (10 % v/v ethanol) ; [2] red wine ; [3] water ; [4] wine; [5] synthetic wine (11 % v/v ethanol, 7 g/L glycerol, 5 g/L tartaric acid, pH 3,4), [6] water

2.2.2 Fermentative or Secondary Aroma compounds

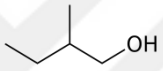
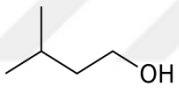
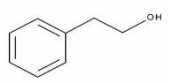
The fermentative aroma compounds are the most abundant in wine. They are referred to as secondary metabolites synthesized by microorganisms (yeast and LAB) involved in AF and FML. Certain of these compounds, such as esters, fusel alcohols, and volatile fatty acids, are responsible for fruity notes and the wine-like character of the wine. These aroma compounds derive from substrates such as sugars, nitrogen compounds, lipids, sulfur compounds, and many other molecules that are directly or indirectly involved in fermentation.

Higher Alcohols

Higher alcohols, also called "fusel alcohols," belong to the alcohol group with more than two carbon atoms, molecular weight, and boiling point higher than ethanol. In wine, they represent the most abundant group of VOCs generated by FA.

The main higher alcohols released during fermentation are essentially 1-propanol, 1-butanol, isobutyl alcohol, 2-Phenylethyl alcohol, and isoamyl alcohol (table 2.4.). The last one represents the most synthesized higher alcohol by yeast during AF and ranges between 40% and 70% of the total concentration of fusel alcohol in wine (Ribéreau-Gayon and Bertrand, 1971). At low concentrations (less than 300 mg/l), they positively impact aromatic wine complexity. In contrast, higher alcohols impart acrid and caustic notes at a high level (above 400 mg/l), giving a low-quality wine (Rapp and Mandery, 1986; Rapp and Versini, 1995).

Table 2.4. Some main fusel alcohols identified in red wine. Adapted from (Gammacurta, 2014)

Names	Formula	Concentration in wine(mg/L)	Threshold perception (mg/L)	Descriptors
Propanol-1		9 - 68	306[1] 500[2]	Ripe fruits, solvent, Fusel oils
Butanol-1		0.5 - 8.5	150[1]	solvent, medicinal
Isobutyl alcohol		9 - 174	40[3] 500[2]	malted
Methyl-2-butanol-1		15 - 150	40[3]	Solvent ,malted, amylic
Isoamyl alcohol		60 - 490	30[3] 300[2]	malted, amylic,
Phenyl-2-ethanol		4 - 197	14[4]	floral, rose

[1] hydroalcoholic solution (10 % v/v ethanol, pH 3,5, tartaric acid); [2] wine ; [3] hydroalcoholic solution (10 % v/v ethanol) ; [4] synthetic wine (11 % v/v ethanol, 7 g/L glycerol, 5 g/L tartaric acid, pH 3,4)

These alcohols are synthesized mainly by yeast using grape amino acids through the Ehrlich pathway (Figure 2.9). The reaction involves a deamination followed by decarboxylation. The obtained fusel aldehyde undergoes a reduction to give an alcohol. An alternative way to obtain fusel alcohols is to integrate into the Ehrlich pathway α-keto acids from sugar metabolism. Several parameters influence alcohol synthesis, particularly the content of amino acids in must plays an essential

role (Schulthess and Ettlinger, 1978). Other parameters such as ethanol content, AF temperature, grape variety, and level of grape maturity have a considerable influence (Fleet, 1993). However, the yeast strain remains the crucial parameter for higher alcohol formation. Indeed Giudici et al. (1990) have shown in their works that fusel alcohols biosynthesis is an intraspecific characteristic of *Saccharomyces cerevisiae.*

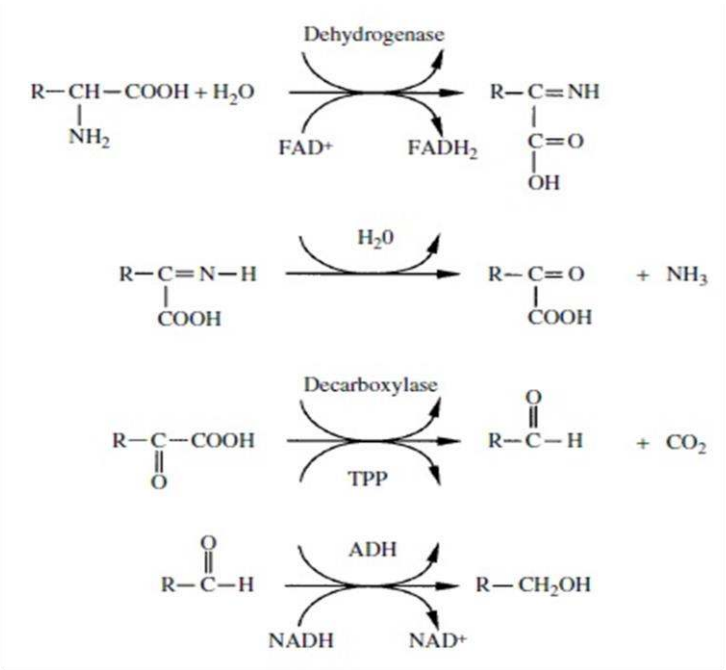


Figure 2.9. Biosynthesis of higher alcohols, according to Ehrlich (Waterhouse et al., 2016)

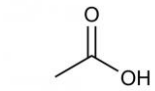
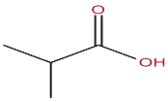
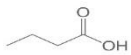
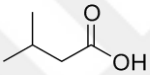
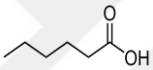
Volatile acids

Red wine contains numerous saturated and unsaturated acids generated from yeast metabolism during AF. The volatile acid concentration ranges between 500 and 1000 mg/L, accounting for 10-15% of wine total acids. Acetic acid represents around 90% of total volatile acids (Fowles, 1992; Fleet, 1993), the remaining 10% represent essentially short-chain (C2-C4) and medium-chain (C6-C10) fatty acids (Table 2.5).

Acetic acid plays a crucial role in wine aroma's modulation. Its content in wine is under legislation allowing concentration between 0,2 and 0,6g/L according to the style of wine. Above this level, acetic acid imparts a sourness/sharpness to the palate and a vinegary odor, often reducing fruity character (Dubois, 1994; Bartowsky et al., 2003). During fermentation, acetic acid is mainly synthesized by acetic acid bacteria (AAB) from ethanol essentially and glucose alternatively.

Other acids, more or less volatile with short or medium-chain, derive from fatty acids metabolism by yeast and bacteria. Their odor descriptors vary from vinegar-like to rancid, cheesy, and soapy as the length of the chain increases (Francis and Newton, 2005; Patrick and Etievant, 2017). However, these compounds display an aroma impact relatively low and are more considered as intermediary by-products than final products of acid catabolism. Nevertheless, they remain precursors of esters, one of the most important families of aroma compounds in wine.

Table 2.5. Some main volatile acids synthetized in wine during fermentation adapted from (Gammacurta, 2014).

Acids	formula	Concentration in red wine (ppm) [1]	Odor threshold value (mg/L)	Odor descriptor[2]
acetic		2.5-2.6	1	Vinegary
isobutyric		0.7-1.5	2.3	Fruity, buttery-cheesy.
butyric		0.4-4.8	0.173	Animal
isovaleric		0.3-1.3	0.003	Cheesy, herbaceous
hexanoic		0.7-2.6	0.42	Sweet-like odor
octanoic		0.7-3.7	0.5	Fatty odor, cheesy-sour flavor
decanoic		0-1.6	1	Rancid like

[1] (Patrick and Etievant, 2017); [2] (Bakker and Clarke, 2011)

Esters

Esters along with higher alcohols are the major volatile compounds in wine and represent almost the total fermentative aroma. The fermentation-derived esters are the main VOCs involved in fruity aroma, contributing to the overall aroma profile, particularly in young red wine, wherein they are found relatively at a high level. As shown in table 2.6, the major fermentation-derived esters may last up to 2 years above their threshold perception, therefore playing a role in the sensory composition of an aged wine (Moreno-Arribas and Polo, 2009).

Table 2.6. Changes in fatty acid ester concentrations (in μmol/L) depending on the aging time at 25°C and at two different pH values (Ribéreau-Gayon et al., 2006)

Compounds	pH = 3.00				pH = 3.50			
	0 months	2 months	5 months	29 months	0 months	2 months	5 months	29 months
Isobutyl acetate	0.70	0.40	0.00	0.00	0.70	0.60	0.00	0.00
Hexyl acetate	1.90	1.20	0.00	0.00	1.70	1.50	0.40	0.00
Isoamyl acetate	36.60	13.30	3.10	0.40	36.50	20.60	14.00	2.50
Phenyl-2-acetate	11.00	2.40	0.50	0.50	4.80	3.40	2.60	0.88
Ethyl hexanoate	12.20	8.70	6.40	4.30	11.00	8.80	8.40	4.60
Ethyl octanoate	9.30	9.00	7.40	6.40	5.70	5.50	5.50	3.69
Ethyl decanoate	2.70	3.40	3.10	2.00	1.20	1.20	1.40	0.79

The two major groups of esters arising during fermentation associated with fruitiness in wine, are higher alcohols of acetates (HAAs) and ethyl esters of fatty acids (EEFAs). HAAs are formed by condensing higher alcohol with acetyl-CoA, catalyzed by yeast alcohol acetyltransferase (Malcorps et al., 1991; Mason and Dufour, 2000), whereas EEFAs result from enzymatic esterification of the activated fatty acids (acyl-CoA), formed during the early stages of lipid biosynthesis.

In addition to these two main groups of esters, there are esters of organic acids (EOAs) formed with the acids such as tartaric, succinic, malic, citric, and glutaric acids. Although they display the highest amount of fermentation-derived esters, they contribute more to the wine body than the aroma. Nevertheless, ethyl cinnamate specific to some vinification processes such as CM exhibits sweet-balsamic and fruity-honey-like odors of excellent tenacity (Versini and Tomasi, 1983; Antalick et al., 2014).

Most fermentation-derived esters may contribute to wine aroma because their concentrations in wine are often much higher than their odor threshold values measured in the same medium. Even those occurring at the level below their threshold value contribute to the fruity character through possible additivity of their odors (Moreno-Arribas and Polo, 2009; Simpson, 2016). Moreover, Van der Merwe and Van Wyk (1981) established that HAAs were more important than EEFAs for the intensity and quality of wine odor. However, the latter improve these characteristics. Thus, the synergy-effect of odor existing between different esters determines the overall olfactory character of the mixture of the ester.

The qualitative composition of fermentation-derived esters and their relative concentrations were shown to be more dependent on the yeast strain used for fermentation than the other parameters. In most vinifications, yeasts used for fermentation are selected according to their ability to produce esters, the change in terms of ester quality and quantity vary both between and within species (Plata et al., 2003; Miller et al., 2007; Swiegers et al., 2009). This pattern of ester production by yeasts tends to preclude the use of many non-Saccharomyces strains as key yeasts in wine production; however, co-fermentation under mixed or sequential inoculation with *Saccharomyces cerevisiae* improves the production of esters and other compounds, rendering such wines with a greater diversity of acceptable flavors (Arslan et al., 2018; Dutraive et al., 2019). Besides, in wine underwent MLF by LAB, it is noticed an enhancement of fruity character due to an increase of ethyl esters such as ethyl lactate, ethyl acetate, ethyl hexanoate, and

ethyl octanoate (Gil-Sánchez et al., 2019). The effect of these bacteria is strain-specific and can vary greatly, as illustrated by Boido et al. (2009).

Table 2.7. Some major esters identified in red wine.

Compounds	Content in wine (red) mg/L	Odor threshold Value (mg/L)	Descriptors
Ethyl propanoate	tr-20[1]	1.84[1]	Strawberry, solvent[4]
Ethyl butyrate	1.08-1.8[1]	0.6[4]	Kiwi, strawberry, cheese
Ethyl hexanoate	tr-3.8[1]	0.8[1]	Green apple, fruity[3]
Ethyl octanoate	0.2-3.8[1]	0.58[1]	Fruity, peach [3]
Ethyl isobutyrate	0.03-0.08[2]	0.01–1[2]	Fruity(apple, banana, pineapple), herbaceous[2]
Ethyl 2-methylbutyrate	0.00-0.08[2]	0.1[2]	Fruity/apple, banana[2]
Ethyl isovalerate	0.00-0.09	0.1 – 0.4[2]	pineapple/herbaceous, fruity[2]
Ethyl 2- phenyl acetate	tr-4.8[1]	0.67-2.4[1]	Honey, rose[3]
Ethyl lactate	12-382[1]	150[1]	Fruity, late
Ethyl 3-hydroxybutyrate	0.1-1mg/L[5]	1.8[4]	Fruity, strawberry[3]
Diethyl succinate	-	200[4]	Fruity[4]
Ethyl acetate	9-257[1]	12.27[1]	Nail polish remover, Fruity[3]
Isobutyl acetate	-	2.1[4]	Fruity, solvent[4]
Butyl acetate	tr - 0.02[1]	1.83[1]	Solvent, fruity[4]
Isoamyl acetate	0.03 – 5.5[3]	0.16[3]	Banana[3]
Hexyl acetate	tr - 4.8[1]	0.67-2.4[1]	Pear, pineapple, fruity[4]
Ethyl cinamate	0-0.0012[2]	0.0011[2]	Honey, cinnamon[2]
Ethyl dihydrocinamate	0-0.00054[2]	0.0016[2]	flower[2]

[1](Patrick and Etievant, 2017); [2](Bakker and Clarke, 2011); [3] (Waterhouse et al., 2016) ; [4](Gammacurta, 2014); [5](Lytra et al., 2015)

2.2.3 Tertiary Aroma or aging aroma

Tertiary aroma refers to aroma arising while wine aging in a wood barrel, which later evolves during bottle storage (Bayonove et al., 1998; Taillandier and Bonnet, 2005). The phenomena encompass a loss or diminution of fruity character, transfer of wood aroma compounds to wine, and a progressive setting of a so-called "reductive bouquet" once in the bottle.

Changes during Oak barrel aging

During oak barrel aging, the most relevant phenomena are the transfer of the oak wood volatile compounds into the wine (Table 2.8) and oxygenation. Besides, a microbial action combined with adsorption of certain wine volatiles by the oak wood itself is noticed (Ramirez Ramirez et al., 2001; Moreno and Azpilicueta, 2007). These phenomena lead to a change in wine aroma profile from fruity to a more complex qualified "oaky" character.

Although there is a wide range of chemical families listed as oak wood-originated, they are mainly phenolic compounds, and only a few of them display a considerable sensory impact in wine. Thus, the main volatile compounds with organoleptic interest release by oak are γ-lactones, phenolic aldehydes and ketones, volatile phenols, and furans derivatives. The γ-lactones express various aromatic notes regarding the nature of their chemical structure (Guth, 1997). The β-methyl-γ-octalactone (also known as an oak lactone or whiskey lactone) is the main lactone release by oak, occurs in two isomer forms(*cis* and *trans*), by dehydration of specific fatty acid derivatives present in the wood (Wilkinson, Elsey, Prager, Tanaka, et al., 2004; Hayasaka et al., 2007). The *cis*-isomer with a lower detection threshold (0.092mg/L) is up to 5-fold more odoriferous than the trans-isomer with a concentration of 0.49 mg/L in white wine (Chatonnet et al., 1991). These two isomers are responsible for the coconut and Brazil nut aroma and they can also contribute to woody character at certain level (Chatonnet, 1995; Garde-Cerdán and Ancín-Azpilicueta, 2006).

Furan derivatives, including furfural, methyl furfural, and hydroxymethylfurfural, occur through the Maillard reaction from wood polysaccharides degradation during toasting (Ribéreau-Gayon et al., 2006; Morata, 2019). It is well known that they contribute to smoked and toasted nut notes (Perestrelo et al., 2011; Fernández de Simón et al., 2014; Navarro et al., 2018). However, in wine aged in oak barrel, they are found below their odor threshold perception, so they are less involved in the wine aroma (Pérez-Coello and Díaz-Maroto, 2009).

Among phenolic aldehydes and ketones, syringaldehyde, vanillin and their derivatives (coniferaldehyde, sinapaldehyde, acetovanillone, acetosyringone, and others) arise from lignin degradation during barrel toasting and are linked to the pleasant woody, spicy, smoky, and vanilla notes that the wood communicates to wine, perceptible after few months of barrel aging (Spillman et al., 2004; Díaz-Maroto et al., 2008). However, with an olfactory detection threshold of 0.32mg/L in red wine, vanillin remains the only one to contribute significantly to wine aroma (Prida and Chatonnet, 2010; Allamy, 2015).

Among the main volatile phenols released from the wood into the wine, eugenol, guaiacol, and its 4-vinyl and 4-ethyl derivatives contribute positively to the organoleptic character of red wine aged in a barrel. With its characteristic odors of cloves, Eugenol is the major volatile phenol, while the latter with spicy, toasted, and smoky hints are present in relatively insignificant quantities (Boidron et al., 1988; Prida and Chatonnet, 2010).

Table 2.8. Main volatile substances released from oak into wine. adapted from (Morata, 2019)

Origin	Family	Compounds	Indicative thresholds	Aroma
Polysaccharides	furans	furfural	3-20 mg/L	Toasted almonds
		Methyl furfural	45 mg/L	
		Hydroxymethyl furfural	45 mg/L	
		Furfurilic alcohol	50mg/L	
	Other volatile heterocycles	Maltol	35mg/L	Caramel
		Dimethyl pyrazines	0.2-35mg/L	Cocoa
	Acetic acid		0.6 - 1.0 g/L	vinegar
Lignin	Phenolic aldehydes	vanillin	20 - 200µg/L	Vanilla
		syringaldehyde	15 - 50mg/L	
		Sinapaldehyde	~ 50 mg/L	
		coniferaldehyde		
	Phenolic ketones	Acetophenone	65 µg/L	
		acetovanillone	1-9 mg/L	
		Propiovanillone	unknown	
		Butyrivanillone		

Table 2.8 (continued)

Origin	Family	Compounds	Indicative thresholds	Aroma
		Guaiacol	75 µg/L	
		Methyl Guaiacol	65 µg/L	Smoked toasted
		Ethyl Guaiacol	140 -150 µg/L	
	Volatile phenols	Vinyl Guaiacol	380 µg/L	
		Vinyl phenol	180 -725 µg/L	Medicinal
		Ethyl phenol	400-620 µg/L	Animal notes, horse
		Eugenol	100 -500 µg/L	Clove
		t-Isoeugenol		
Lipids		cis-β-methyl-γ-octalactone	46 - 83 µg/L	Coconut, Brazil nuts
	β-methyl-γ-octalactones			
		trans-β-methyl-γ-octalactone	390 - 460 µg/L	

Changes during bottle aging

During the storage of wine in the bottle, an aging bouquet is formed, which refers to a slow evolution of wine under reductive conditions. This phenomenon is associated with a loss of fermentative aroma with a variable diminution of fresh and fruity notes (Jackson, 2017). This type of aging allows the wine at the same time to keep its varietal aroma and to evolve towards a more complex and subtle aroma, reminiscent of a mixture of toasty, mint, and clove-like notes. The result is a wine with more organoleptic quality than it expressed before bottle aging (Tominaga et al., 2003; Ribéreau-Gayon et al., 2012). The chemical composition of

young, along with bottle aging conditions, allows for a reductive bouquet. The reductive bouquet occurs in the absence of oxygen and is associated with the development of sulfur dimethyl (DMS). During aging, the DMS increases with both the time and temperature of storage (Picard et al., 2015)

2.2.4 Incidence of CM on red wine aroma compounds

Initially CM is used to produce red young or CM wine with pronounced fruity and floral notes due to a higher level of esters and particularly ethyl cinamate which have been proposed as one of markers of wine made by CM (Versini and Tomasi, 1983). Nevertheless, the quality and the quantity of these aroma compounds depend on the grape variety and the chosen variant of CM. When applied to grape varieties with a varietal character such as shiraz, CM enhances its varietal aroma, in contrast, with varieties producing wine aromatically neutral such as Carignan, CM-originated aroma compounds are predominant such as isoamyl alcohol, ethyl cinnamate, and benzaldehyde giving to stewed wine fruits and floral notes (Flanzy et al., 1987). Compared to wine made by traditional winemaking, differences have been noticed about some intense odorant compounds presenting aromatic rings such as vinylbezene, 2-phenylethyl acetate, benzaldehyde ethylphenyl acetate, and ɤ-nonalactone (Ducruet, 1983; Crouzet, 1986). Moreover, Etievant (1981) in its works showed that some highly-odorant volatile phenols such as vinyl-4-guaiacol, vinyl-4-phenol, ethyl-4-gaiacol, ethyl-4-phenol, eugenol, methyl and ethyl vanillate, appear in higher levels in CM wines.

Table 2.9 shows aroma characteristic differences between wines elaborated by classic CM and Beaujolais method (semi CM).

Table 2.9. Comparative aroma characteristic between CM and semi CM. adapted from (Flanzy et al., 1987)

Olfactive attributes	**Wine elaborated by classic CM**	**Wine elaborated by Beaujolais method**
Apparent characteristic of aroma	kirsch	hyacinth
	coffee	coffee
	candy	vanilla
	Vanilla	cherry
	Toasted almond	raspberry
	Russian leather	Banana
	Resin	candy
Quality of aroma	Subtle (varietal and lactic)	Rich (vinous and phenolic)

Flanzy et al. (2001) published results of a study conducted at "Pech Rouge" (INRA) in Montpellier, in which the aim was to increase the aging potential of CM wines using different variants of CM. The trials were based on the production of wines with Carignan, Shiraz, Grenache, and Mourvedre varieties, following three derivatives of CM. The first method (T) used as control involved classic CM for 7-8 days at 30-32^{o}C. In the second method (P), CM was extended by a classic maceration with daily pumping over 7-10 days. Finally, the third method (E) was similar to (P), but, prior to first pumping over, a mixture of pectolytic enzymes was added. The sensory analysis of the three types of wines (T, P, E), assessed by well-experimented panelists after 16 months of aging (Figure 2.10), has shown remarkable results. Regarding aroma characteristics, among the wines, E wine displayed the most accurate CM-aroma typicity and the highest aroma intensity.

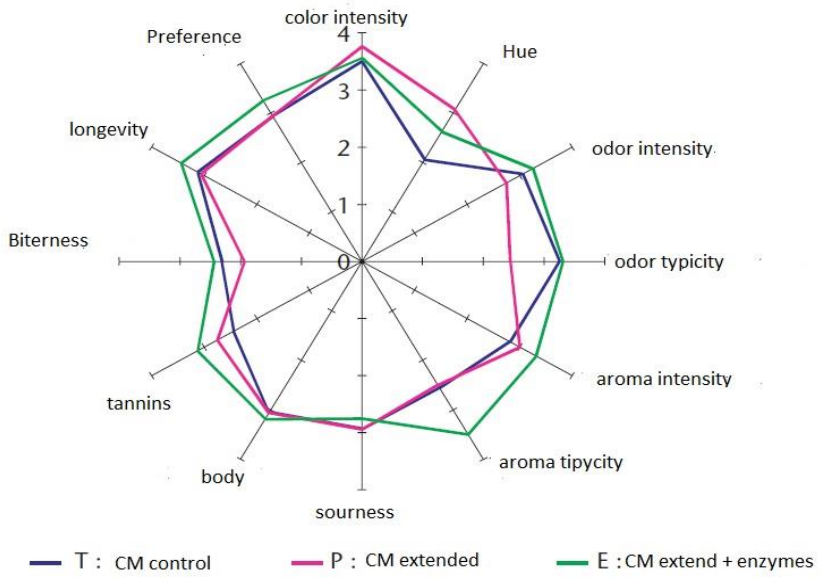


Figure 2.10. Sensorial analysis of 3 wines of shiraz 1995, made by different CM, aged of 16 months. Adapted from (Flanzy et al., 2001)

2.3 Red wine Phenolic compounds

Phenolic compounds, characterized by the presence of at least one phenyl group, are a wide family of secondary metabolites synthesized by the plant. Nowadays, over 8.000 of these compounds have been listed, and the diversity of structures makes their classification a bit complex. Some authors use their molecules' carbon skeleton to classify them into nine groups (C6-C1, C6-C2, C6-C3, C6-C4, C6-C1-C6, C6-C2-C6 C6-C3-C6), while others group them into four families (hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, and flavonoids). In enology, phenolic compounds are divided into two groups: non-flavonoids and flavonoids. They essentially take origin from grape berries, where flavonoids are mainly located into skin and seed, while non-flavonoids take place in pulp (Figure 2.11). They are extracted during vinification, and their structures evolve during storage and aging (Teissedre, 2008).

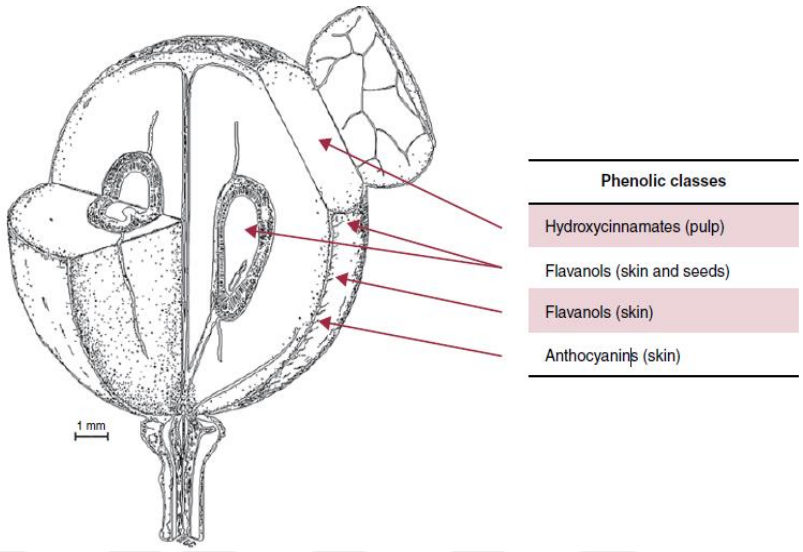


Figure 2.11. Tissue sources of grape phenolic compounds. From (Waterhouse et al., 2016).

These compounds are crucial, especially in red wine; they are responsible for its color, contribute to astringency and bitterness, take part in the stabilization of colloidal. Moreover, many works have shown the healthful benefit of wine phenolic compounds. They showed antioxidant proprieties (Da Silva, Darmon, et al., 1991; Aruoma et al., 1993; Spranger et al., 2008). There is a negative correlation between regular and moderate consumption of wine and the incidence of cardiovascular disease risks (Teissedre and Landrault, 2000), neurodegenerative diseases, and cancer (Damianaki et al., 2000; Strathearn et al., 2014; Amor et al., 2018), all these effects refer to “French paradox” concept.

2.3.1 Non-Flavonoid Compounds

Non-flavonoid compounds of grape and wine encompass two subclasses: phenolic acids, including hydroxybenzoic and hydroxycinnamic acid derivatives accounting for 100 to 200 mg/L in red wine, and on the other hand, the stilbenes ranging from 20 to 80 mg/L in red wine (Chira, 2009).

Table 2.10. Chemical structures of phenolic acids.

Benzoic acids					**Hydroxycinnamic acids**
	R2	R3	R4	R5	
p-hydroxybenzoic acid	H	H	OH	H	p-coumaric acid
Protocatechic acid	H	OH	OH	H	Cafeic acid
Vanillic acid	H	OCH_3	OH	H	Ferulic acid
Gallic acid	H	OH	OH	OH	
Syringic acid	H	OCH_3	OH	OCH_3	Sinapic acid
Salicylic acid	OH	H	H	H	
Gentistic acid	OH	H	H	OH	

2.3.1.1 Phenolic Acids

Hydroxybenzoic acids present a C6-C1 carbon skeleton. They take origin from grapes in which seven have been identified (Table 2.10), mainly combined to glycoside moiety or as an ester (Gallic and ellagic tannins). In red wine mainly, free forms of benzoic acids are quite present, resulting from the hydrolysis of their glycosylate and ester forms. They can result from the degradation of more complex molecules, especially anthocyanins (Galvin, 1993). Of these compounds, Gallic acid is the most representative in wine and grape , with a concentration between 100 and 230 mg/kg (Chira et al., 2008), in typical red table wine up to 70 mg/L (Teissedre et al., 1996). Its amount in wine increase while aged in the oak barrel through the extraction and hydrolysis of oak-derived tannins (figure 2.12), giving the release of gallic and ellagic acids.

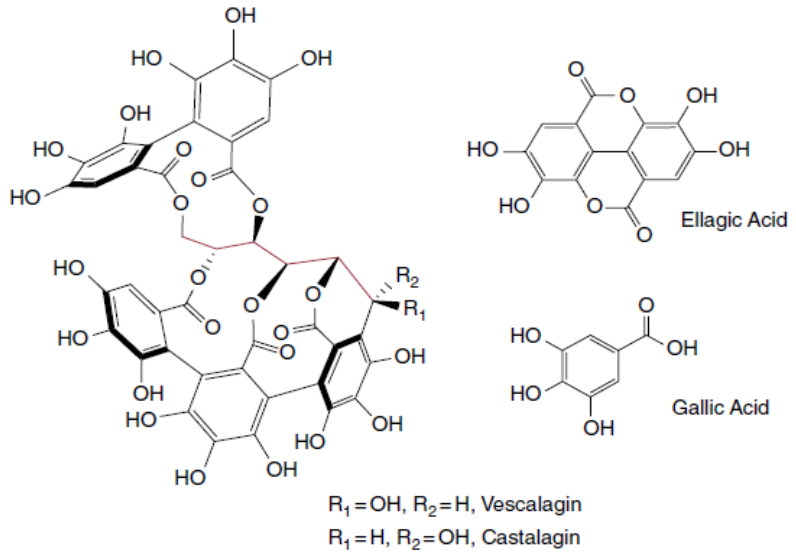
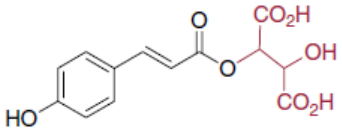
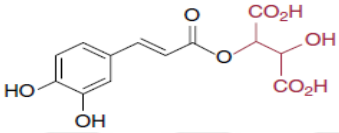
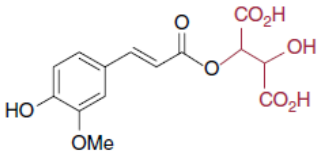


Figure 2.12. Oak tannins, vescalagin and castalagin, and ellagic acid and gallic acid. The sugar moiety is highlighted. From (Waterhouse et al., 2016)

Hydroxycinnamic acids have a C6-C3 structure and are located in the skin and pulp of berry as an ester of tartaric acid (Quaglieri, 2018), but also form other esters with some organic acids, sugars, polyamides, and polysaccharides (Weidner et al., 2000). Besides, they participate in the synthesis of acylated anthocyanins via esterification of acetic, *p*-coumaric, and cafeic acids with the glycoside moiety of anthocyanins. Moreover, the compounds resulting from *p*-coumaric and ferulic acids degradation are precursors of volatile phenols such as ethyl phenol and ethyl guaiacol, reminding "horse" malodor (Chatonnet, 1995). In wine, the grape-derived tartrate esters undergo hydrolysis by hydroxycinnamate ester hydrolase produced by LAB and other organisms. The action of these enzymes allows to release the free form of hydroxycinnamic acids (table 2.11), directly detectable in newly fermented wines (Somers et al., 1987).

Table 2.11. The hydroxycinnamic acids shown in their tartrate ester form as found in the grape. Enzymatic hydrolysis of the ester leads to the free acid form in wine, adapted from (Waterhouse et al., 2016).

Compounds	Structure	Representative levels (juice/wine, mg/L)
Courtaric acid, Coumaric acid, tartrate ester		20/15
Caftaric acid, caffeic acid, tartrate ester		170/40
Fertaric acid, ferulic acid, tartrate ester		5/4

2.3.1.2 Stilbenes

Stilbenes are phytoalexins with C6-C2-C3 structure produce by plants in response to stress, plant damages, or a microbial attack such as *Botrytis* infection (Langcake and Pryce, 1977; Joshi and Devi, 2009). Grapes and wine are some of the most abundant sources of these compounds (Goldberg, 1995; Mattivi et al., 1995). The main stilbenes found in grape are resveratrol and its glycoside derivative, the piceide (*trans*-resveratrol-3-glycoside). Are also found in grape some oligomer of stilbenes such as piceatannol, pallidal or viniferine, a dimer of resveratrol (Figure 2.13) which appear to be the actual antifungal compound (Waterhouse et al., 2016). The free forms are more abundant in wine due to the hydrolysis of glycosidic bonds during vinification and its levels are higher in red wine because resveratrol-3-glycosides are largely found in grape skin, with amount up to 7 mg/L (Lamuela-Raventos et al., 1995; Quaglieri, 2018). Furthermore,

several kinds of research highlighted stilbenes biologic properties, it has been reported that resveratrol has potent antimicrobial activity against wild yeast and *Acetobacter* in winemaking (Pastorkova et al., 2013), for human benefit, stilbenes possess antioxidant (Gris et al., 2011), anticarcinogen and anti-inflammatory properties (Smoliga et al., 2011; Calabriso et al., 2016; Santino et al., 2017). They possess cardioprotective virtues and inhibit platelet aggregation (Hung et al., 2000; Bijak et al., 2019).

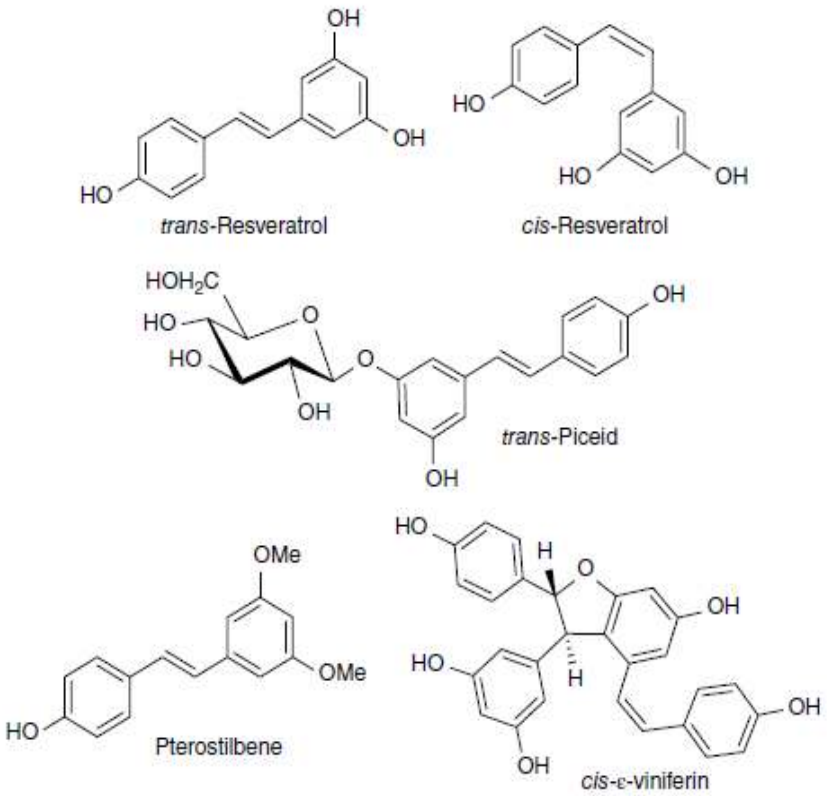


Figure 2.13. Selected stilbenes in wine, including the more abundant resveratrol isomers, along with an example of a resveratrol glucoside (trans-piceid), a dimethoxylated analog (pterostilbene), and a dimeric viniferin. From (Waterhouse et al., 2016)

2.3.2 Flavonoid Compounds

The flavonoids form a wide family of phenolic compounds with a common basic structure of the C15 skeleton organized through the C6-C3-C6 configuration (figure 2.14). Two aromatic rings are linked by a 3-carbon chain, giving a flavone unity. The A ring is phloroglucinol, B ring refers to catechol if there is an hydrogen in the C5'and pyrogallol ring while substituted by hydroxyl. C-ring is a pyrene.

Among phenolic compounds, flavonoids are the most abundant, they are plant secondary metabolites involved in different roles such as pigmentation, atmospheric nitrogen trapping, they protect plants against UV and fungal attacks (Jourdes, 2003; Chira, 2009).

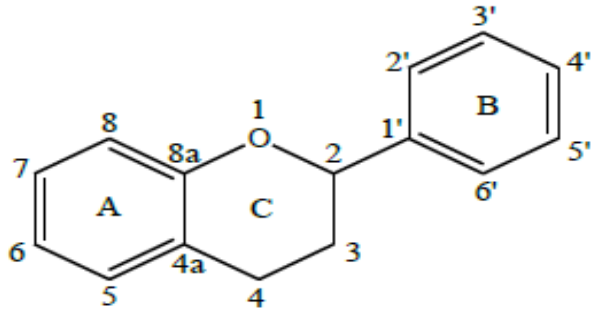


Figure 2.14 General structure of flavonoids

Flavonoids are grouped in different subclasses, according to the degree of oxidation on the C pyrene ring except for chalcones. They include different type of compounds such as flavones, flavononols, flavonols, flavan-3-ols, and anthocyanins (figure 2.15). Flavan-3-ols (proanthocyanidins) and anthocyanins are the major and the most important flavonoid subclasses in red wine. They deeply contributes organoleptic aspect of wine, providing color, astringency, and bitterness.

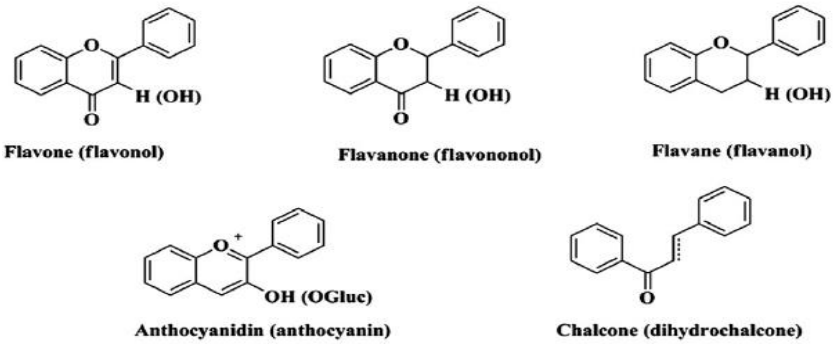


Figure 2.15 General structure of flavonoid subclasses (Garrido and Borges, 2013)

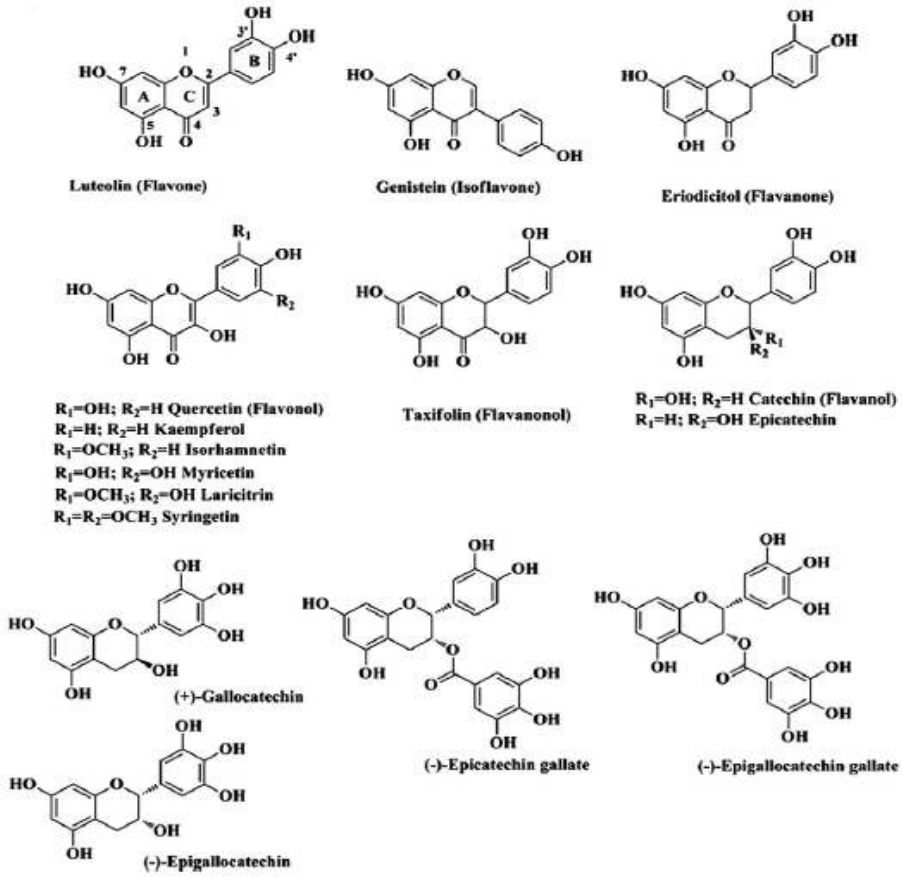


Figure 2.16. Some flavonoids present in grape and in wine from (Garrido and Borges, 2013).

2.3.2.1 Flavan-3-ols or Proanthocyanidins (condensed tannins)

Proanthocyanidins, also call condensed tannins are molecules able to combine and give stable complexes with polysaccharides (Riou et al., 2002), alkaloids and gelatin, and proteins as well (Bate-Smith and Swain, 1965). The combination tannin-protein provides the bitter taste and astringency mouthfeel while drinking red wine (Haslam, 1974; McManus et al., 1981; McManus et al., 1985).

Chemically proanthocyanidins are polymer of flavan-3-ols with C15 (C6-C3-C6) skeleton, divided into two subgroups: the procyanidins including (+)-catechin and (-)-epicatechin monomers and in another hand the prodelphinidins which include (+)-gallocatechin and (-)-epigallocatechin (figure 2.16). procyanidins and prodelphinidins release under heat and acid medium, cyanidin and delphinidin respectively (Da Silva, Rigaud, et al., 1991; Koponen et al., 2007) (figure 2.17). Proanthocyanidins may also be found in grapes and wine as well, esterified by Gallic acid giving compounds such as epicatechin gallate or epigallocatechin gallate (Garrido and Borges, 2013) .

Condensed tannins are located in stems and solid parts of grape (skin and seeds), although some traces have been reported to belong to flesh of berry (Ricardo-da-Silva et al., 1992). Skin tannins are present in free form into vacuole cells while they are bonded to protein or/and polysaccharides on the cell membrane and tonoplast (Geny et al., 2003; Gagné et al., 2006). In seeds, condensed tannins accumulate into endothelium of the seed coat. The number of monomer units constituting polymer refers to a mean degree of polymerization (mDP), this value may reach up to 18 in seeds and increase up to 30 for skins polymers (Prieur et al., 1994; Souquet et al., 1996). In contrast, quantitatively seeds contain more tannins than the skin and display a higher percentage of galloyted moieties. Hence, proanthocyanidins of skins distinguish from those in seeds by the fact that they have prodelphinidins with higher mDP and low galloyation percentage (Somers, 1971; Da Silva, Darmon, et al., 1991; Mattivi et al., 2009).

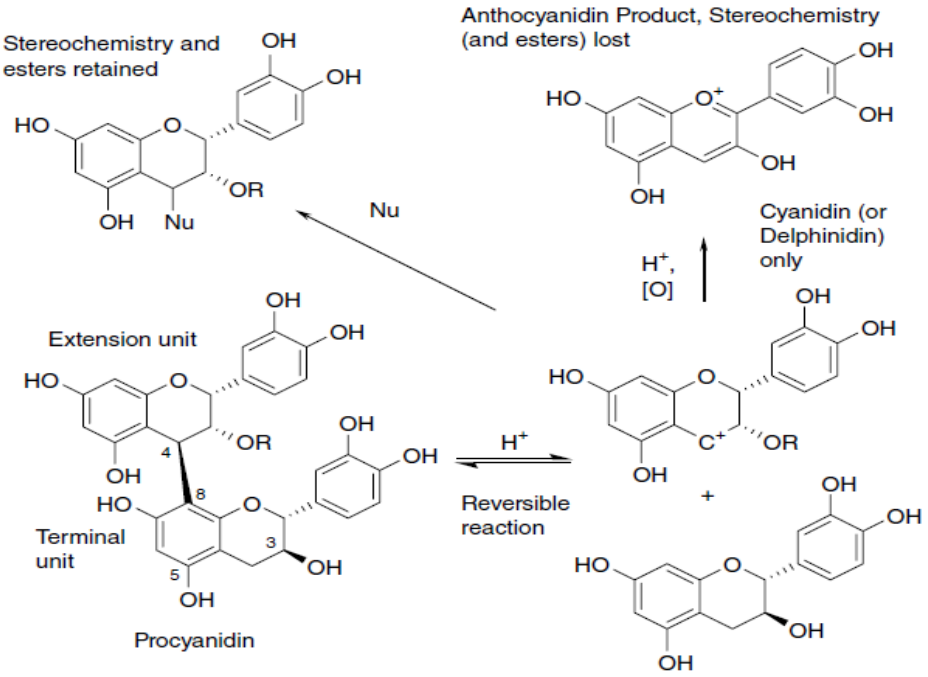


Figure 2.17. Reactions to analyse the proanthocyanidin structure. Strong acid product with oxidation shown on top right, nucleophile (thiolysis or phloroglucinolysis) product on top left. R = H or gallic acid. Adapted from (Waterhouse et al., 2016)

In red wine, proanthocyanidins are extracted during alcoholic fermentation and post-fermentation maceration. Their concentrations vary between 1 and 4g/L, depending on the grape variety and winemaking method (Ribéreau-Gayon et al., 2012). Catechin and epicatechin represent the main compounds among flavan-3-ols monomers, as have been reported in a study of Cabernet Sauvignon wine wherein their content was between 37-80mg/L with higher level for catechin (Ritchey and Waterhouse, 1999). Nevertheless, proportionally they are minor compare to oligomers and polymers of flavan-3-ols which represent 25-50% of phenolic compounds in red wine (Singleton, 1992). In Shiraz wine, flavan-3-ol polymers with up to 9 unities have been identified by the means of mass spectrometry (Delcambre and Saucier, 2013). The oligomers and polymers of proanthocyanidins

are able to build stable bounds with saliva proteins, to contribute to astringency mouthfeel. These interactions are proportional to mDP of the involved flavan-3-ols polymers, while in contrast bitterness increase with mDP decreasing (Peleg et al., 1999; Ma et al., 2016). As is it for high-concentration of phenolic compounds in red wine, proanthocyanidins can, accelerate the rate of oxygen consumption and react with products of oxidation such as quinones and aldehydes via the nucleophilicity of A ring, to scavenge and avoid their accumulation.

Table 2.12. Flavan-3-ol monomer wine composition. Adapted from (Waterhouse et al., 2016)

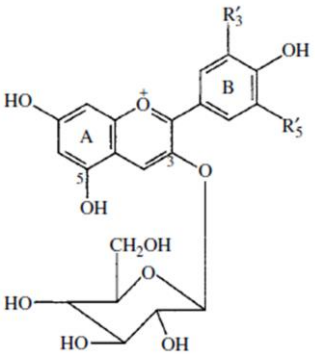
Wine grape variety	Catechin	Epicatechin	EGC	ECG	GC
Tempranillo	16	10	nr	nr	nr
Graciano	33	34	nr	nr	nr
Cabernet Sauvignon	42	20	nr	nr	nr
Merlot	27	19	nr	nr	nr
Cabernet Sauvignon	19	58	20	2	nr
Tannat	43	65	60	0	14
Nero D'Avola	25	32	nr	nr	nr
Cabernet Sauvignon	38	16	nr	nr	nr
Syrah	43	51	nr	nr	nr
Tempranillo	27	54	nr	nr	nr

EGC = epigallocatechin, ECG = epicatechin gallate, GC = gallocatechin, nr = not reported.

2.3.2.2 Anthocyanins

Anthocyanins are characterized by a core glycosylated flavylium in position C3 that combines two benzene rings A and B (figure 2.18). They are plant pigments that confer color, mainly to flowers and fruits. In *Vitis vinifera* , they are responsible for the color of grape berries and the resulting young red wine, wherein their concentration varies from 350 to 1500 mg/L (Michel, 2012). They are located

into grape skin, except for “teinturier” grapes such us Gamay de chaudenay and Gran negro, for which anthocyanins are also located within pulp (Figueiredo-González et al., 2012; Guan, 2014). The concentration of anthocyanins in grape depends on the degree of berry ripeness (Pérez-Magariño and González-San José, 2006), adding to exogenous parameters such us, climate (Mori et al., 2007), temperature (Mori et al., 2005), terroir, and grape variety (de Gaulejac et al., 2001).



R’3	R’5	
OH	OH	Delphinidin-3-O-glucoside
OH	H	Cyanidin-3-O-glucoside
OH	OCH_3	Petunidin-3-O-glucoside
OCH_3	H	Peonidin-3-O-glucoside
OCH_3	OCH_3	Malvidin-3-O-glucoside

Figure 2.18. Structure of the main anthocyanin 3-O-glucosides detected in grapes and red wines

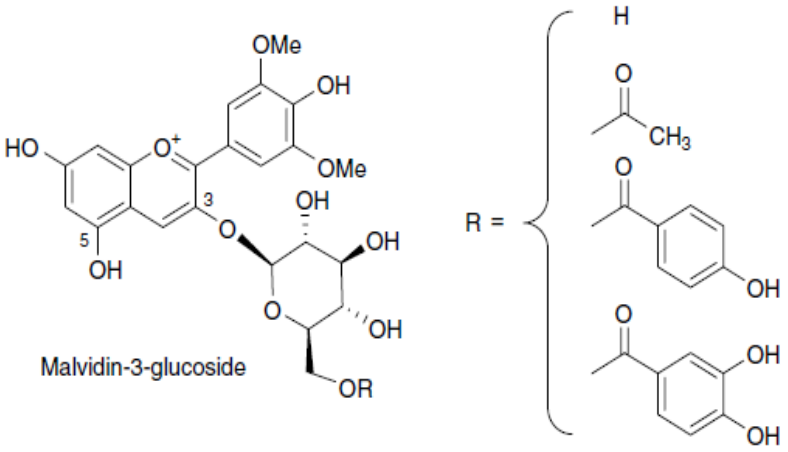


Figure 2.19. Malvidin-3-glucoside (M3G), the dominant anthocyanin in *Vitis vinifera* grapes. The simple sugar (R = H, malvidin-3-glucoside) is most abundant, but varying amounts of acetyl and coumaroyl substituents are found, with traces of caffeoyl, depending on grap

.

Anthocyanins are extracted during maceration and alcoholic fermentation, their level in wine is conditioned to, their concentration in grape, the winemaking conditions and the wall-cell conditions (Ortega-Regules et al., 2006). There are 9 main free anthocyanins in red wine: five forms 3-O-monoglucosides distinguishing by degree of hydroxylation and/or methoxylation on their B ring (figure 2.19), including malvidin-3-O-glucoside, peonidin-3-O-glucoside, petunidin-3-O-glucoside, delphinidin-3-O-glucoside, and cyanidin-3-O-glucoside. On another hand anthocyanins are also found in acylated glucoside forms including acetylglucosides, p-coumaroylglucosides, and in smaller amount caffeoylglucosides. In *Vitis vinifera* grapes malvidin-3-O-glucoside and its acylated forms, are the major anthocyanins accounting for 60% to 80% of total anthocyanins(Somers and Verette, 1988).

In wine, anthocyanins in their flavylium configuration are the main source of red color, though they are relatively unstable and under certain conditions, they

tend to convert toward new pigments which may contribute to color stability of red wine.

Effect of pH

In medium with low pH (pH 1), anthocyanins are present in their red flavylium cation form (A+), it is a stable cation because the charge is delocalized over the whole C-ring and stabilized by resonance. When the pH increase and reach wine pH (3.0-4.0), red flavylium cation (A+) undergoes two types of reactions, hydration and acid-base reaction (Brouillard and Dubois, 1977) (Figure 2.20). Both reactions lead to the formation of a purple quinoidal base (AOH), a colorless carbinol pseudobase (AO), and via a rearrangement of C ring a yellow pale chalcone (C). Therefore, at wine pH, colorless form AOH is predominant accounting for 65% to 85% while the red flavylium form represents 4 to 28% and the purple quinone from 2 to 5%, although the latter at pH 4 becomes the main form (Michel, 2012).

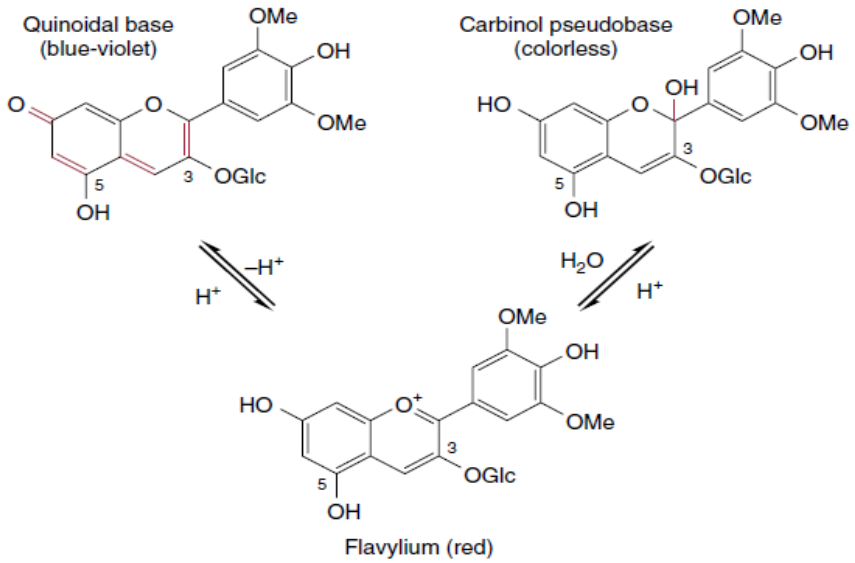


Figure 2.20 Equilibrating forms of anthocyanins

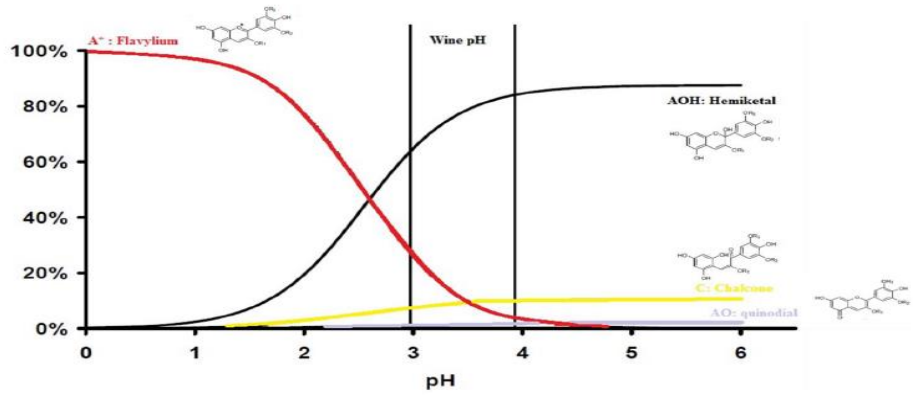


Figure 2.21. Anthocyanins chemical forms depending on wine pH. Adapted from Ghanem (2017)

Effect of SO_2

In presence of sulfur dioxide, free anthocyanin loses considerably their color. In an aqueous medium, SO_2 combines with water to produce bisulfite (HSO_3^-)giving an equilibrium by the following equation (Danilewicz, 2007) :

$$H_2O + SO_2 \overset{pKa_1}{\rightleftharpoons} HSO_3^- + H^+ \overset{pKa_2}{\rightleftharpoons} SO_3^{2-} + 2H^+$$

At pH 3,2 sulfuric acid (H_2SO_3) is found as bisulfite (HSO_3^-) (Barbe, 2000). Furthermore, the obtained bisulfite anion reacts with flavylium cation (A+) likely through C2 or C4 on the C ring, giving a colorless compound ($AHSO_3$) (Fig. 2.11). The formation of these colorless compounds depends on the concentration of free forms of anthocyanins. Thus the SO_2 displays less effect on polymerized and combined anthocyanins (Glories, 1978). At low pH (pH 1) the colorless adduct may dissociate and release the colored flavylium cation and SO_2.

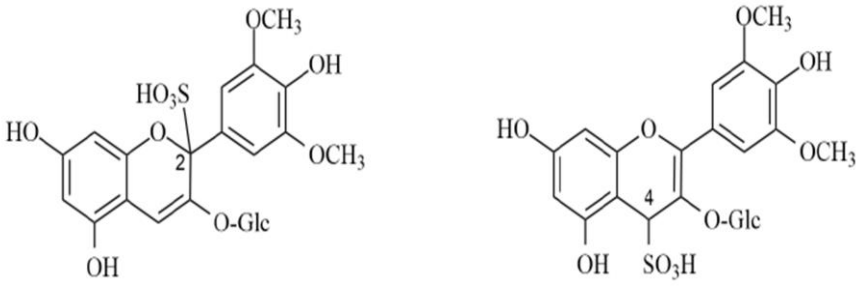


Figure 2.22. Two possible configuration of colorless anthocyanin (AHSO3) after reaction with HSO3-

Evolution of anthocyanins

In red wine, the concentration of free forms of anthocyanins decreases considerably during the first months of aging. That diminution is due as well as to the numerous associations with other wine compounds to give new adducts and anthocyanin derivative pigments, and in another hand to different reactions leading to their degradation. Several factors contribute to anthocyanin degradation, it can be cited temperature, oxygen, and presence of ketones in the medium.

Heating anthocyanins solution at 100°C produces a rapid and irreversible discoloration (Hillmann et al., 2011). Moreover, the percentage of discoloration is correlated to the rise of temperature and the concentration of anthocyanins in the solution (Galvin, 1993). This thermic degradation is likely due to a structural change toward chalcone and colorless forms (Ribéreau-Gayon et al., 2012). therefore the temperature is an important factor to consider for the maturation and aging of red wine.

Into acid-hydroalcoholic solution and light exposure as well, anthocyanins lose their color after few days (Laborde, 1987). Oxygen and light behave as a catalyzer and the increase of the phenomenon rate is positively correlated to pH (Abyari et al., 2006; Tseng et al., 2006; Bordignon-Luiz et al., 2007). Anthocyanins with two hydroxyls in "*ortho"* position on their B ring are

susceptible to oxidation and form *ortho*-quinones. Hence, due to the configuration of their B ring malvidin-3-O-glucosides and peonidin-3-O-glucosides are less susceptible to oxidation compare to cyanidin-3-O-glucosides (Cheynier et al., 1995; Kader et al., 1999).

Ketones under certain conditions, participate in anthocyanins degradation. In an aqueous and acidic medium containing acetone, anthocyanins turn toward orange color (Glories, 1984). The mechanism involves in this reaction may be either, via an opening of the C ring leading to benzoic acids or by condensation with acetone through the polarized double bond, forming orange adducts (Ribéreau-Gayon et al., 2012).

Copigmentation

Copigmentation is a reaction wherein, colored anthocyanin derivatives arise either, by an association between anthocyanin and another compound (usually colorless) in the medium, called copigment, or by an anthocyanin self-association. In red wine this reaction allows stabilization of colored form of anthocyanins where they supposed to be, for the major part, in their colorless form, due to wine pH. Thus, copigmentation contributes up to 50% of color in young red wine (Boulton, 2001; Cavalcanti et al., 2011). Although a diminishing of the phenomenon is observed during storage, meaning that anthocyanins are involve in other chemical reactions competing with copigmentation (Trouillas et al., 2016).

The reaction is qualified of intermolecular copigmentation when the anthocyanin and the copigment are different molecular unit. the resulting complex is stabilized by a non-covalent and hydrophobic bond or π-π stacking due to the planar structure of the involved anthocyanin and the planar portion of the copigment molecule. The main cofactors involved in these reactions are flavonoids and non-flavonoids, and proteins. Due to the conformation of its hydroxyl group, (-)-epicatechin appears to be most efficient cofactor to build an intermolecular association (Berké and de Freitas, 2005; Quaglieri, 2018). Another variant of

intermolecular copigmentation is self-association, where copigment is another molecule of anthocyanin, leading to formation of oligomers and polymers of anthocyanins. Compared to self-association, copigmentation involving colorless copigment has more effect on color changes in young red wine. This results in a positive displacement of the visible wavelength of maximum absorption from 5 to 20nm (bathochromic effect) and an increase in the absorbance at visible range (hyperchromic effect) (Boulton, 2001; Gauche et al., 2010).

Copigmentation is qualified as intramolecular when the copigment is a part of anthocyanin itself. This is noticeable with acylated anthocyanins, indeed it is observed in low acidic medium, an unusual color stability from acylated anthocyanins compare to those without acyl in their glycoside moieties, suggesting that the acyl acts as a copigment and the effect is greater when the glycoside moiety is acylated by phenolic acids (Escribano-Bailón et al., 2019).

2.3.2.3 Incidence of CM on red wine phenolic compounds

In red wine made by CM, color and tannins profile depend essentially on the management of CM temperature-duration. Flanzy et al. (1987) have reported, in case of *Grenache noir* variety, to obtain wine proportionally higher in anthocyanins than tannins, winemaker has choice to either, carry out CM at 32°C for 6 to 7 days, or to extend CM up to 10 or 12 days at temperature of 25°C. In another hand, if more tannic wine is desired, it is recommended to macerate grapes for 10 days at 32°C.

However, in some cases there is diminutions of phenolic indexes during CM related to the rise of temperature or extension of the process. The fall of level of phenolic compounds observed towards the end of anaerobic metabolism, is related to synthesis of non-soluble compounds via anthocyanins. The mechanism probably involves either, degradation of anthocyanins in anaerobic condition by enzymatic-related oxidation, or a polymerization of anthocyanins through an oxidation or an association with tannins. The rate of these phenomena increases with duration

rather than temperature. Under these conditions anaerobic metabolism limits diffusion of phenolic compounds towards grape juice, leading to less colored wines (Mourgues et al., 1984; Flanzy et al., 2001).

A recent work conducted by Pace et al. (2014) investigated the evolution of anthocyanins in the skin and pulp of Gamay berries subject to 12 days of CM. The results have shown that, maceration time influenced considerably not only the content and extraction yield, but also the profile of anthocyanins towards the predominance of malvidin derivatives. Additionally The study confirmed the finds previously reported by other authors (Mourgues et al., 1984; Flanzy et al., 2001) ,explaining that, extraction yield of anthocyanins is positively related with the ethanol formed and the skin softening throughout CM.

3 MATERIAL AND METHODS

3.1 Material

3.1.1 Grapes

The current research was carried out on grapes of *Vitis vinifera* L.cv Öküzgözü and its giving wines. The grapes was harvested at the end of September for the two vintages 2018 and 2019 respectively, from Koruk, a village of Elazığ province in Mid-Eastern Anatolia. Once pick up, grapes were convey to Kavklidere winery in Ankara, within 2 days following the harvest. From each harvest 2 kg of intact and healthy bunches were selected and kept in cold environment (10^{o}C) to avoid deterioration.

3.1.2 Wines

From each harvest, 3 types of red wine were made following different winemaking methods. Primeur wine made by CM, wine aged in French Oak barrel and normal wine made by classic winemaking method use as control wine. 6 bottles of each type of wine were supplied by Ankara Kavaklıdere Winery when the whole winemaking process was completed and prior to bottling, meaning that samples were taken directly from the vats after several racking in order to discard lees and avoid additional operation that could influenced the initial characteristics of wines. The different wines were codified as in the following Table 3.1

Table 3.1 Codification of Öküzgözü samples.

Sample name	vintage	supplier	quantity	code
Öküzgözü grapes	2018	Kavaklıdere	2 kg	OKG18
CM wine	2018	Kavaklıdere	6 bottles	OKWP18
Classic wine	2018	Kavaklıdere	6 bottles	OKWC18
Wine aged in oak barrel	2018	Kavaklıdere	6 bottles	OKWOBA18
Öküzgözü grapes	2019	Kavaklıdere	2kg	OKG19
CM wine	2019	Kavaklıdere	6 bottles	OKWP19
Classic wine	2019	Kavaklıdere	6 bottles	OKWC19
Wine aged in oak barrel	2019	Kavaklıdere	6 bottles	OKWOBA19

3.2 Methods.

3.2.1 Grape analysis

3.2.1.1 Brix, pH and total acid determination

One hundred berries were collected and squeezed in a nylon bag. From the released grape juice, Brix was measured with a refractometer and pH with a Mettler Toledo™ pH meter at 20°C. The pH meter was previously calibrated according to the manufacturer's instruction with a buffer solution at pH 47, and 10 at 20°C.

On the other hand, 10 mL of the grape juice was used for the determination of total acidity by potentiometric titration with sodium hydroxide (0.1N) as described by the method OIV-MA-AS313-01 from OIV (2016)

3.2.1.2 Phenolic compounds assessment

3.2.1.2.1 Grape sampling

The grape phenolic compounds were assessed using Glories and ITV (Institut technique de la vigne et du vin) methods as described by El Darra (2013)

with slight modifications. Before Glories and ITV treatments, 100 berries were selected for each case and crushed with a Warring blender for 2 min; 50 mg of the obtained mixture was placed in 250mL Erlenmeyer flask for ITV and 100g (50g+50g) in two different Erlenmeyer for Glories method (Figure.3.1)

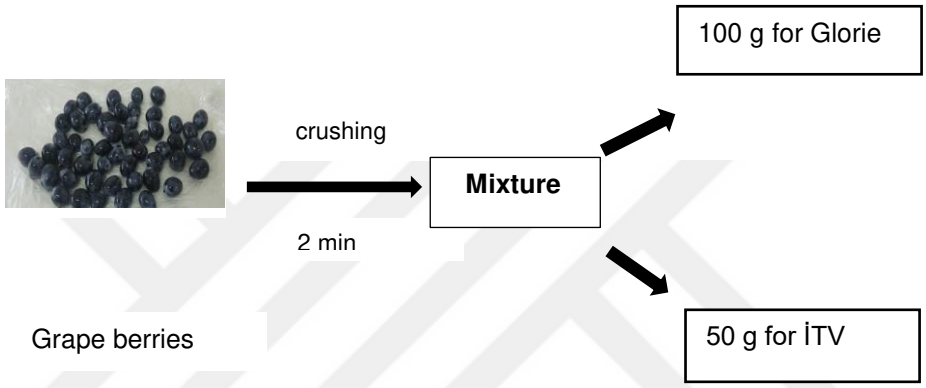


Figure 3.1. Sample preparation for Glories and ITV methods

3.2.1.2.2 Determination of total phenols and anthocyanins by ITV method

This method is based on micro-extraction of grape phenolic compounds in hydro-alcoholic and acid solution at the temperature room. In 250 mL Erlenmeyer flask containing 50 mg of the mixture (crushed gape berries), 15 mL of ethanol (95%) and 85 mL of 0.1% HCl were added, the whole mixture was held in maceration for one hour at 20^{o}C. Manual agitation was done every 15 min. after one hour, the mixture was filtered through cotton glass and the obtained filtrate underwent two different treatments:

Total phenol content (TPC): 1mL of the filtrate was sampled and diluted to 1/10 in distilled water, and 1mL of the obtained dilution was used to assess total phenolic content via the Folin-ciocalteu method. One mL of the diluted filtrate was transferred into a 100 mL flask and 50 mL of distilled water and 5 mL of Folin

reagent; it was mixed and left to sit for 8 min after 20 mL of sodium carbonate(20%) was added and completed to 100 mL with distilled water. The same procedure was repeated with another 100mL flask replacing the 1 mL of diluted filtrate with 1mL of distilled water, and this flask served as blank. The two flasks were stored both in a dark place for 30 min prior to reading. The total phenol content was measured using a UV-VIS spectrophotometer (Agilent Cary 60) at an OD (optical density) of 765 nm against the prepared blank in a 10 mm cell.

Concentration of anthocyanins: the second treatment aimed to assess grape anthocyanins concentration (ANT) as shown by Rajha et al. (2017). The procedure consisted of diluting 1/20 of filtrate in 1% HCl and measuring the OD at 520 nm against a blank of distilled water. ANT was calculated as follows:

$$\text{ANT (mg/L)} = \text{OD}_{520} \times 22.75 \times 20$$

3.2.1.2.3 Determination of total anthocyanin potential of easily extractable anthocyanins by Glories method

Into one of the 250 mL Erlenmeyer containing 50 mg of the mixture, 50 mL of aqueous solution at pH 3.2 were added. The pH 3.2 solution was prepared by adding 5 g of tartaric acid into 1L of distilled water with pH adjustment to 3.2 by NaOH. On the other hand, 50 mL of aqueous solution pH 1 (37% HCl in 1 L of distilled water with pH adjusted to 1) were added to the other 50 g of mixture. Samples were then macerated for 4 hours at room temperature and filtered through cotton glass. From the obtained filtrates, anthocyanin contents and potential were assessed using the treatment based on anthocyanins discoloration by bisulfite (SO_2) (Ribéreau-Gayon and Stonestreet, 1965). The analysis was carried out as follows: into 50 mL flask, 1mL of one of the filtrate (from S_{pH1} or $S_{pH3.2}$), 1 mL of ethanol 0,1%HCl, and 20 mL of 2%HCl. Afterward, 10 mL of the formed new mixture were transferred into a tube where 4 mL of distilled water were added, while into another tube, 10 mL of the mixture and 4 mL of sodium bisulfite (15%) were

transferred. The discoloration was practically instantaneous, especially in those with sodium bisulfite. The samples were held in a dark place for 20 min before measure optical density at 520 nm against distilled water for both tubes. Anthocyanin concentration (Ant) was calculated as follows:

$$Ant(mg/L) = 875^{*} \times (OD_{\text{tube 1 in water}} - OD_{\text{tube 2 in bisulfite}})$$

*: 875 is the slope of the calibration curve obtained from malvidin-3-glucoside.

Following this calculation, two values were calculated as Ant pH1 and AntpH3.2. From these values, two results were provided:

- The potential of easily extractable anthocyanins (PEEA).

$$PEEA (mg/L) = \text{factor dilution} \times Ant\ pH3.2$$

- The total anthocyanin potential (TAP).

$$TAP (mg/L) = \text{factor dilution} \times AntpH1$$

Note: factor dilution is two according to the 50 mL of an aqueous solution.

3.2.2 Wine making process

For industrial purposes, the details of the entire winemaking process were kept at discretion of “Kavaklıdere” winery. Therefore, in our study, the three winemaking styles described in an overall manner.

Control wine: Control wine was made by the traditional winemaking method. After destemming-crushing, the resulting must was transferred in stainless steel vat for fermentation. The must was subject to yeast addition (20 g/hL of Zymoflore FX10, LAFFORT, France) to entail alcoholic fermentation (AF), and the operation was carried out at a temperature of 25 ± 2°C for six days. Along with

fermentation, the must were subject to pectolytic enzymes (4g/100kg of LAFASE® FRUIT, LAFFORT, France) addition and mechanical maceration with pumping-over two times daily. Once the fermentation was completed, free-run juice was drawn off, and the must was transferred into a pneumatic press to release press run juice. Further, free and press run juice was assembled and underwent malolactic fermentation (MLF) at 18 ± 2°C. Once the MLF was achieved, the wine was stored and matured in stainless steel vat for four months. Throughout that period, the wine underwent several rackings in order to remove lees. Later, additional operations including fining, filtration and cold stabilization. At the term of this stage, samples for our study were collected and bottled. The analysis of control wine was performed six months after malolactic fermentation was completed

CM wine: The process started by manual sorting to select only intact and healthy bunches. Afterward the bunches were transferred into a sealed stainless steel vat. Once in the vat, 4g/100 kg pectolytic enzymes (LAFASE® FRUIT, LAFFORT ,France) were added. The vat was cooling by a flow of CO_2 up to 13 ± 1°C to set up AM conditions, and CM was performed under these conditions for four days. Afterward, the free-escape juice was collected in another vessel, and the grapes were transferred into a pneumatic press to obtain press juice. Free-run juice and press juice were assembled and underwent alcoholic fermentation at 25°C for six days. Once fermentation was completed, the wine underwent MLF at 18 ± 2°C . later, wine underwent racking, a fining with egg white (8g/hL), a filtration through a diatomaceous earth and cold stabilization prior to sampling. CM wine was analyzed two months after the MLF was completed.

OBA wine: Here, the whole procedure was similar to control wine, excepted for one major modification: once the MLF was completed, wine was stored and aged into a light toasted French oak barrel with a capacity of 225 L. The oak barrel was manufactured in France and has been used for four years (55%). The wine was stored between 16°C and 18°C, and the aging lasted for 6 months with regular racking. Before sampling wine was subject to a fining, a filtration and

a cold stabilization. The analysis of OBA wine was performed eight months after MLF.

3.2.3 General analysis of Öküzgözü wines

Alcohol strength, dry matter and density were determined at 20°C using an Alcolyzer ME coupled to a DMA™ 4500 M by Anton Paar technology.

3.2.3.1 Total acidity analysis

Total acidity of Wine was determined after diluting 10 mL of sample in 1/ 1 of distillated water, titrating to pH 7 against 0.1N of sodium hydroxide as described by (OIV-MA-AS313-01) (OIV, 2016).

3.2.3.2 pH measurement

The pH of wine samples were measured using a pH meter (Mettler Toledo) at 20 ± 2°C

3.2.3.3 Volatile acidity

Volatile acids were separated from 20 mL of the sample by steam distillation and titrated with 0.1N sodium hydroxide and iodine solution (0.005M) respectively once 250 mL of distillate were collected,(OIV-MA-AS313-02) (OIV, 2016).

3.2.3.4 SO_2 analysis

Sulfur dioxide was determined using a device wherein free or total sulfur dioxide containing 50 mL of sample was dragged by nitrogen, fixed, and oxidized by a neutral solution of hydrogen peroxide (0.3%). The formed sulfuric acid was titrated with 0.01N sodium hydrogen solution. For free SO_2, the conical flask containing the sample was immersed in cold water (10 °C) while for total SO_2 it

was under heat as described by the OIV method (OIV-MA-AS323-04A) (OIV, 2016).

3.2.4 Analysis of volatile compounds of Öküzgözü wines

3.2.4.1 Macro volatile compounds analysis

Macro **volatile** compounds were determined through wine distillation followed by a direct analysis by gas chromatography. Briefly, 100 mL of the sample and 30 mL of distilled water (DW) were transferred into a 500 mL conical flask for distillation. The procedure was continued until 100 mL of distillate was collected. From the obtained distillate, 900 µL were collected in a vial and spiked 100 µL of 3-pentanol (28.081 mg/100 mL) as internal standard. The extract was injected and analyzed with an Agilent 7890A GC single FID with 7683B autosampler and tray.

The identification of volatiles was determined by injection of reference compounds and comparing the linear retention indices (LRI) with those of macro compounds from previous literature. Furthermore, the reference standards were confirmed by the injection into the GC–MS system.

The concentration of each compound was determined concerning the internal standard from the relative response factors (RF), which were obtained during calibration under the same chromatographic conditions as those of the analysis of the wine as described by Darıcı et al. (2019). The concentration of each compound was calculated using the following equation:

$$C_c = A_c \times A_{is} \times C_{is} \times RF \times 100 / D$$

C_{is}: Concentration of internal standard (mg/100 mL), A_c: Peak area or height of compound, A_{is}: Peak area or height of internal standard, D: Alcoholic strength of the sample.

3.2.4.2 Micro volatile compounds analysis

Liquid-liquid-extraction

Micro volatile compounds were isolated following a liquid-liquid extraction method, reported by Selli et al. (2004) with slight modifications. 100 mL of the samples were placed in a 500 mL Erlenmeyer flask, completed up to 40 mL of dichloromethane, spiked of 5µL of 4-nonanol (0.446 mg/L) as the internal standard for quantification. The mixture was then stirred at 700 rpm with a magnetic stirrer at 4°C for 60 minutes, followed by centrifugation at 9000 rpm for 15 minutes at 4°C. The obtained dichloromethane phase (supernatant) was dried over anhydrous sodium sulphate. The resulting extract evaporated up to 5mL through a Kuderna Danish concentrator equipped with a Snyder column (Supelco, St. Quentin, France). Furthermore, additional evaporation was done with a gentle flow of nitrogen to bring the extract up to 200µL and collected in a glass vial. This sample extraction was performed in triplicate, and the extracts were stored at -20 °C until analysis.

GC-FID-MS parameters

For gas chromatography (GC), an Agilent 6890 chromatograph equipped with an FID (Wilmington, DE, USA), coupled to an Agilent 5973-Network mass selective detector was used. Volatile compounds were separated with a DB-WAX capillary column with dimensions 30 m length × 0.25 mm id. × 0.5µm film thickness (J&W Scientific Folsom, CA, USA). 1µL sample of the extract was injected in pulsed splitless (40 psi, 0.5 min) mode. The injector and FID detectors were set at 270 and 280°C, respectively. The flow rate of carrier gas (helium) was 1.5 mL/min. The initial oven temperature was settled at 50°C, increased to 200°C at a rate of 5°C/min, and then to 260°C for 8 min with a final hold at 260°C for 5 min (Selli et al., 2004; Celik et al., 2019).

The MS (electronic impact ionization) were carried out as follow: ionization energy, 70 eV; mass range, m/z 30–300 a.m.u.; scan rate, 2.0 scans/s;

interface temperature, 250°C; and source temperature, 180°C (Celik et al., 2019). The volatile compounds were identified by comparing the retention indices and their mass spectra from the DB-WAX column with a commercial spectra database (W9 N11.L, NIST98, flavor 2) and the internal library created from previous laboratory studies. The linear retention index (LRI) of compounds were determined using an n-alkane series (Celik et al., 2019).

After identifying peaks using MS and FID detectors, the concentration of each compound was calculated using the equation below. The response factor of each compound was taken as 1.

$C_i = (A_i / A_{st}) \times C_{st} \times RF \times CF$

C_i : concentration of the compound
A_i : peak area of the compound
A_{st} : peak area of internal standard
C_{st} : concentration of internal standard
RF : response factor
CF : calculation factor (factor used to complete sample up to 1 liter)

3.2.5 Characterization of aroma-active compounds of Öküzgözü red wines by GC-MS-Olfactometry

Wine aroma-active compounds were assessed by olfactometry analyses conducted using a compilation of previous studies carried out by Lyu et al. (2019), Tetik et al. (2018) and Selli et al. (2014)

3.2.5.1 Sample preparation

The aroma extract was prepared similar to those in section 3.2.5.2, although here the extract was more concentrated, bringing the amount of extract,

during the solvent evaporation up to 200 µl, afterward the collected extract was transferred in a vial and conserved at - 4°C before analysis

3.2.5.2 GC-Olfactometry parameters

The GC-Olfactometry analysis was performed with the same GC-FID-MS system used for volatile compound assessment (section 3.2.5.2). However, here, the system was completed with a Gerstel ODP-2 (Baltimore, Maryland, USA) olfactometry sniffing device coupled with humidified air at 40°C using deactivated fused silica capillary (30 cm × 0.3 mm) used for aroma-active analysis. The effluent was split within MS, FID, and sniffing port into 3 equal rates (1:1:1) to simultaneously identify and quantify aroma and aroma-active compounds.

The aroma compounds were separated with a DB-Wax capillary column (30m x 0.25mm x 0.5µm). The amount of extract injected was 1µL, Helium was used as carrier gas at a constant flow rate of 3.5mL/min. The injector was configured in pulsed splitless mode. Injector and detector temperatures were set at 250°C, while the oven temperature was programed as follows: held at 40°C for 5 min, increased to 100°C at the rate of 4° C/min, increased to 220°C at the rate of 6°C/min, and finally held at 220°C for 20 minutes, the run time was set at 80 minutes (Darıcı, 2017). Aroma compounds were determined similar to previous analysis of aroma compounds in section 3.2.5.2 by comparing their retention indexes and mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 9, N11.L, NIST98, flavor 2) and the internal library created from previous laboratory studies. The linear retention index was determined using a series of n-alkanes under the same chromatographic conditions.

3.2.5.3 Sniffing procedure

The sniffing step was conducted by four panelists (two females and two males with ages between 28 and 55 years old) experimented in GC-olfactometry analysis from the Food Engineering laboratory (Cukurova University). During the

analysis, panelists recorded the retention index (calculated according to the time which de odor was detected) and descriptors of the odor peak for each compound. To avoid decreasing alertness of panelists, which could be due to length of run time (80 min the sniffing period was divided into two parts (40 minutes each), each panelist sniffed for 40 min interchangeably. The procedure was performed in duplicate for each extract**.** After discussion and checking the aroma descriptors with chemical standards and literature, a lexicon for descriptors was generated. Furthermore, the aroma extracts underwent an Aroma extract dilution analysis (AEDA).

3.2.5.4 Aroma extract dilution analysis (AEDA)

The aroma extract dilution analysis was carried out to identify the most potent aroma-active compounds. From the initial aroma extracts (200µL), a series of dilutions with dichloromethane as solvent (1:3/ v:v) was prepared to yield dilutions of 3, 9, 27, 81,etc., and 2187 (Lyu et al., 2019). GC-O analyzed each dilution under the same conditions until no more odorant appeared. Each compound's flavor dilution (FD) factor was defined as the highest dilution at which the odorant could still be perceived by at least three of four panelists.

Additionally, to emphasize key aroma-active compounds, aroma activity value (AAV) was calculated for each compound with FD ≥ 9. The AAV consisted on the ratio between the concentration of the aroma-active compound (obtained from the first sniffing before any dilution) and its olfactometry threshold value in alcoholic matrix, obtained from literature (Ferreira et al., 2002; Rocha et al., 2004; Escudero et al., 2007; van Gemert, 2011; Miller, 2019).

3.2.6 Analysis of Phenolic compounds of Öküzgözü wines

3.2.6.1 Spectrometric analysis

Similar to grapes, the spectrophotometric analysis of wine were performed using an Agilent Cary 60 UV-Vis Spectrophotometer.

Total phenolic contents:

Total phenolic compounds were calculated according to the Folin-Ciocalteu method (Ribéreau-Gayon et al., 1972), and the results were expressed as gallic acid equivalent .

Total anthocyanins:

Total anthocyanins were evaluated using the bisulfite bleaching method followed by measurement of the absorbance at 520nm using a UV-Vis spectrophotometer. Total anthocyanin concentration was expressed as malvidin-3-glucoside equivalent as described by Ribéreau-Gayon et al. (2006).

Total tannins:

Total tannins were calculated according to the Bate-Smith method involving acid hydrolysis. Total tannins were determined by the measurement of the absorbance at 550 nm. Total tannins concentration was expressed in mg/l as described by Ribéreau-Gayon and Stonestreet (1966).

Chromatic characteristics :

Color intensity defined as sum of absorbencies (A_{420}, A_{520}, A_{620}) and color tonality expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm (OIV-MA-AS2-07B : R2009)(OIV, 2016)

3.2.6.2 Analysis of anthocyanins monomers by RP-HPLC

The samples were analyzed by direct injection without any pretreatment, excepted a passage through a 0.45µm Nylon filter. Each sample was analyzed in triplicate. The analysis was performed with an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, California, USA), equipped with an autosampler and consisted of a quaternary pump, a diode-array detector (DAD), an automatic injector, and advanced software (ChemStation). The instrumental parameters and

the elution design are summarized in Table 3.2 and Table 3.3. A binary gradient of the mobile phase and the elution system was elaborated according to the OIV method (OIV-MA-AS315-11: R2007) (OIV, 2016). The separation of anthocyanin compounds was performed on reversed-phase HiChrom Ultrasphere C18 ODS (250 x 4.6 mm x 5µ) coupled with a pre-column having identical granulometry. Identification was processed concerning retention time and spectrums of normal standard (Table 3.4) and current literature data. Quantification was performed as indicated by Kelebek et al. (2006), with respect to peak areas measured at 520 nm

Table 3.2 HPLC instrumental parameters for anthocyanins analysis

Apparatus :	Agilent 1100
Column :	HiChrom Ultrasphere C18 ODS (205 x 4.6 mm x 5µ)
Column temperature:	40°C
Mobile phase:	Solvent A: water/formic acid / acetonitrile (87:10:3/ v:v:v)
	Solvent B: water/formic acid/ acetonitrile (40:10:50 / v:v:v)
Flow rate:	0.8 mL/min
Wave length:	520 nm
Detector type:	Diode array detector

Table 3.3 HPLC elution system

Time(min)	0	15	30	35	41	50	55	60
%A	94	70	50	40	6	0	100	100
%B	6	30	50	60	94	100	0	0

Table 3.4. Retention time and maximum absorption wave length of anthocyanin standards

Standard names	**Retention time (min)**	**Maximum absorption wave length (nm)**
Delphinidin-3-glucoside	10.298	520
Cyanidin-3-glucoside	12.327	519
Peonidin-3-glucoside	12.927	517
Pelargonidin-3-glucoside	13.646	525
Petunidin-3-glucoside	15.792	518
Malvidin-3-glucoside	16.778	530
Delphinidin-3-glycoside acetate	18.632	529
Cyanidin-3-glycoside acetate	20.124	517
Petunidin-3-glycoside acetate	22.514	529
Peonidin-3-glucoside acetate	26.521	517
Malvidin-3glucoside acetate	27.412	529
Petunidin-3-glycoside *p*-coumarate	28.217	527
Peonidin-3-glycoside *p*-coumarate	29.894	525
Malvidin-3-glycoside *p*-coumarate	30.361	530

3.2.7 Sensory analysis by descriptive analysis (DA)

Sensory analysis was conducted to assess the overall quality of the three styles of Öküzgözü red wine. The analysis was based on previous work carried out by Darıcı (2017) on sensory descriptors of Turkish premium red wines. In our study, the term overall quality consisted of three characteristics: color, aroma, and gustatory perception. The analysis was performed by nine panelists, including the winemaker of Kavaklıdere winery (Ankara) and eight members of food engineering department (Çukurova University), all with great experience on wine

sensory analysis. The panelists were composed of four females and five males with ages between 28 and 58 years old. Analysis was held for seventh sessions lasting 2 hours each.

In the first session, panelists were requested to determine and recognize the aroma attributes of Öküzgözü red wines. During the session, standards aroma referring to aroma attributes were submitted to the panel, and they were invited to familiarize themselves with them. In the second session, the panel discussed general gustatory characteristics associated with red wine. Afterward, their sensitivity to these characteristics was evaluated. In the third session, Aroma standards at different concentrations were presented to the panel for rating the lowest and highest concentration values. In the fourth session, a series of concentrations of standard references linked to gustatory attributes were submitted to the panel to rank the lowest and the highest concentration for each attribute. In the fifth session, all chosen aroma attributes and their standard references (compiled in Table 3.5), along with color and gustatory attributes, were listed in order to perform sensory analysis of Öküzgözü red wines.

In the last two sessions, the panelists were requested to score the intensity of each selected attribute on a fifteenth-point scale for the proposed wines. In both sessions, samples (20mL at 20^{o}C) were coded and served in a wine glass in random order to eliminate first-order carry-over effects. All samples were evaluated twice in separate sensory booths according to the standards ISO 8589:2007.

For on-palate evaluation, the panelists sipped the samples and were required not to swallow them after determining the attribute intensities. Water was provided for mouth rinsing between samples. The form used for sensory analysis is shown in the appendices part.

Table 3.5 Selected aroma attributes and their relative reference standards

Aroma attributes	**Standard references***
Red fruits	2 g of cherry, red plum, strawberry and raspberry mixture
Black fruits	2 g of mulberry, black plum, currant and blueberry mixture
Dry fruits	2 g of dry fig, raisin and dry plum mixture
Ripe fruits	5 grape berries(Öküzgözü), 5 mL grape juice (Öküzgözü) and 5 mL of pear juice
spices	A mixture of 0,5g of cinnamon, 0,5g of coconut and 0,5 of clove
Lollipop/candy	A mixture of 0.5g of red, green and yellow jelly beans
Chocolate/mocha	1g of dark chocolate and 0,5g of mocha coffee
Vanilla/woody	1 drop of vanilla essence and 2 small pieces of oak wood

*the standards were added into neutral red wine.

3.2.8 Statistical analysis

Quantitation of data was calculated as mean value with standard deviation from replicate determination (Microsoft Excel 2011). One way analysis of variance (ANOVA) was carried out for analytical results determined for the three styles of Öküzgözü red wine and significant differences in their means were established using Tukey's post-hoc test at $p<0,05$ level. Additionally, data from the general composition of wines, analysis of volatile compounds and anthocyanin monomers were submitted to a multivariate analysis of variance (MANOVA) to test the effect of two studied factors (style and vintage). Moreover, Pearson correlation coefficients were calculated for each style to evaluate the correlation between aroma attributes and wine aroma quality. Analysis of variance and Pearson correlation coefficients were performed using IBM ®SPSS® Statistics version 26 (Armonk, New York, USA).

4 RESULTS AND DISCUSSION

4.1 General composition of Ökügözü grapes and wines

4.1.1 Grapes

Table 4.1 shows the results of grape chemical analysis and the assessment of the phenolic potential of grapes for the two years of harvest (2018 and 2019).

A statistically significant difference ($p<0.01$) was recorded in terms of total acidity with a concentration higher for the 2018 harvest than for 2019 at values of 4.36 and 4.21 g /L, respectively. However, from one harvest to the other significant differences were found concerning Brix and pH measures, though these values were higher for the 2019 harvest (23.9 and 3.60 against 23.5 and 3.55).

The grapes harvested in 2018 showed a higher phenolic potential, as indicated by all the considered parameters using the ITV-Folin Ciocalteu and ITV methods to calculate total phenolic compounds and total anthocyanins, respectively. The 2018 harvest yields 2206 mg/L of total phenolic compound and 259.71 mg/L of total anthocyanins, whereas the 2019 harvest displays a total phenolic content of 2102 mg/L and total anthocyanins of 212.28 mg/L. The mean differences were statistically significant ($p < 0.01$) for all measured parameters. Among them, total anthocyanins content displays the higher mean difference, representing 14.21% against 3.41% for total phenolic compounds.

The differences observed between the two years under consideration may stem from the possible change in climatic factors from 2018 to 2019. Indeed many authors (Rodríguez Montealegre et al., 2006; Mira de Orduña, 2010; Nicholas et al., 2011; Xu et al., 2011) have opined that the climate has a considerable influence on grape quality. In concordance with our findings, Kelebek et al. (2010) have carried out a study where the phenolic profile of Öküzgözü grapes grown in the Elazig region was investigated for two consecutive years (2005 and 2006). The study showed a significant change among grape phenolic compounds when the climatic parameters were more or less different from 2005 to 2006. Elsewhere,

with different grape varieties, similar studies (Ferrer-Gallego et al., 2012; Blank et al., 2019) were conducted and led to the same conclusions

Table 4.1. General composition of Ökügözü grapes

Year of Harvest	**2018**	**2019**	**Sig.**
Brix	23.5	23.9	ns
Total Acidity[a](g/L)	4.36 g/L	4.21g/L	*
pH	3.55	3.60	ns
Total phenol[b] (mg/L)	2206 ±0.05	2102 ±0.09	*
Anthocyanin[c] (mg/L)	259.71 ±0.02	212.28 ±0.03	*
Potential easily extractable anthocyanin (PEEA)(mg/L)	525. 5	483	*
Total anthocyanin potential (TAP)(mg/L)	741.82	686.75	*

Results are the means ± SD of three repetitions. [a] : expressed as tartaric acid equivalent; [b]: expressed as gallic acid equivalent
[c] : expressed as Maldivin-3-glucoside. *: Significance at p<0.01.

4.1.2 Wines

The results of classic oenological parameters of the three different wine styles investigated for two vintages (2018 and 2019) are reported in Table 4.2. The results show for the overall evaluated parameters, significant differences between different wine styles and between vintages as well. Among the vintages, the same trend observed for grape harvests is noticed. The general composition of 2018 vintage wines presents higher values compared to those from 2019 vintage wines. The climate conditions likely did not only influence the quality of the grapes but also the resulting wines - such an observation had already been mentioned in the literature (Kelebek et al., 2010, 2011; Blank et al., 2019).

For the 2018 vintage, wine made by CM shows the highest ethyl alcohol level with a mean of 13.64%, followed by wine made by classic vinification at 13.63%, and the lowest ethyl alcohol volume of 13.33% is attributed to wine aged in oak barrels. These values are similar in the 2019 vintage, where the highest ethyl alcohol content is measured in CM wine at 13.72% and the lowest in OBA wine at 13.44%. A previous study carried out by Darıcı (2017) on Turkish premium red wines made with Öküzgözü grapes grown in Elazig province reported a range of ethyl alcohol levels from 12.5 to 14.8%. Likewise, our results agree with the Turkish food codex, which recommends that alcohol levels in wine range from 9 up to 15% (Anonymous, 2009). Statistical analysis did not show significant differences between vintages concerning ethyl alcohol content; however, within each vintage, a significant difference ($p<0.05$) was noted between OBA wine and both classic and CM wines (Table 4.2). Based on this - as reported by other authors (Geffroy et al., 2013; Ozturk and Anli, 2014) – the winemaking style has also effect on the ethyl alcohol content of wine. Low alcohol content observed in OBA wines was probably linked to loss of ethyl alcohol by evaporation when the wine is aged in oak barrels (García and González-Mendoza, 2001; Morata, 2019).

Table 4.2 General composition of Öküzgözü wines.

	2018			2019			S	V	V×S
	CM	Control	OBA	CM	Control	OBA			
Density (g/L)	0.9984a	0.9987a	0.9989a	0.9895a	0.9920a	0.9930a	ns	ns	ns
Ethyl alcohol (v/v)	13.64 ±0.04b	13.63±0.03b	13.33 ±0.02a	13.72 ±0.04b	13.55 ±0.17a	13.44 ±0.03a	**	ns	*
Dry matter (g/L)	23.9a	25.5b	26b	23.6a	25.1b	25.8b	ns	*	ns
pH	3.68c	3.48b	3.38a	3.72a	3.55b	3.45b	ns	**	**
Ethyl alcohol %(v/v)	13.64 ±0.04b	13.63 ±0.03b	13.33 ±0.02a	13.72 ±0.04b	13.55 ±0.17a	13.44 ±0.03a	**	ns	*
TA (g/L)	4.35 ±0.057a	4.81 ±0.085b	5.12 ±0.081c	4.27 ±0.139a	4.63 ±0.18b	4.90 ±0.09c	**	**	*
VA (g/L)	0.44±0.03a	0.48 ±0.02a	0.54 ±0.03c	0.43 ±0.02a	0.45 ±0.01b	0.51 ±0.03b	**	**	ns
Total so_2 (mg/l)	51.61 ±0.46a	60.92 ±0.89b	78.14 ±0.14c	46.09 ±1a	55.56 ±3.98b	72.20 ±0.58c	**	**	**

TA: total acidity expressed sd tartaric acid equivalent; VA: volatyle acidity expressed as acetic acid equivalent; T. Ant.: expressed as malvidin-3-glucoside; TP: expressed as gallic acid equivalent. S: style, V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by analysis of variance: ns: not significant; *: Significant at p<0,05; **Significant at p<0,01. For each row, values with different letters are significantly different between the styles ($p<0.05$).

Results of total acidity were similar in the two vintages, all mean values obtained were significantly different between the styles. In each vintage, OBA wine presented the highest total acidity with levels of 5.12 g/L and 4.90 g/L respectively for vintages 2018 and 2019. In contrast, CM wines appear to have the lowest total acidity, 4.35 g/L for vintage 2018 and 4.27 g/L for vintage 2019. Darıcı (2017) reported a total acidity range of 4.3 to 5.6 g/L in Öküzgözü red wine from Elazig. However, in the same region, earlier studies (Cabaroglu et al., 2002; Kelebek et al., 2010) reported higher values, reaching up to 6.56 g/L in Öküzgözü red wines. Turkish Food Codex has set the minimum content of total acidity in wine at 3.5 g/L (Anonymous, 2009). It is have been noted by many authors (Etaio et al., 2008; Harmon, 2016) that in CM wines, the total acidity is lower than in wines made by the conventional classic method. The fall in total acidity is thought to be attributed to the catabolism of malic acid without simultaneous production of lactic acid during anaerobic metabolism (Flanzy et al., 1987). Low content of total acidity along with other factors contribute to confer roundness to CM wines, thus allowing for these wines to be early drinkable (Tesniere and Flanzy, 2011; González-Arenzana et al., 2020). The results of OBA wines are in congruence with previous findings (García and González-Mendoza, 2001) showing an increase of total acidity in wine aged in an oak barrel compared to those made without oak barrel storage. According to Aiken and Noble (2016) the rise of total acidity observed during oak barrel aging was due to extraction of carboxylic, phenolic and volatile acids from oak wood.

Volatile acidity calculated as acetic acid equivalent follows the same trend as total acidity. Its contents are lower in CM wines (0.44 g/L in 2018 vintage and 0.43 g/L in 2019 vintage), while the higher volatile acidity contents are measured in OBA wines with mean values of 0.54 and 0.51 g/L respectively for 2018 and 2019 vintages. Kelebek et al. (2007) and later Darıcı (2017) reported average volatile acidities of 0.43 g/L and 0.4 g/L respectively, in Öküzgözü red wines from Elazig region. Furthermore, the maximum legal content of volatile acidity in red

wine has been set at 1.2 g/L by the Turkish codex (Anonymous, 2009). As with total acidity, a low level of volatile acidity contributes to the roundness and early drinkable features of CM wines (Tesniere and Flanzy, 2011). The high volatile acidity noted in OBA wine has an oak-wood origin. Indeed as reported Marín (2003), during toasting of wood staves, free acetic acids are released by hydrolysis of xylose moieties. Moreover, an increase in volatile acidity around 0.1- 0.15g/L was recorded in wine aged in these oak barrels (Zamora, 2019).

4.2 Volatile compounds of Öküzgözü red Wines

The volatile composition of the three styles of Öküzgözü red wine were analyzed for two vintages (2018 and 2019). The volatile compounds of each style of wine were identified, quantified, and further grouped according to their chemical classes (Table 4.3, 4.4, 4.6, 4.7). A complete and more detailed table of wine volatile composition is available in appendix 2.

For the 2018 vintage, the inventory of volatile compounds in different wine styles is as follows: for CM wine, 51 volatile compounds among which there are 10 acids, 15 esters, 13 alcohols, 2 ketones, 4 lactones, 2 phenolic compounds, 2 sulfur compounds, and 1 pyrazine for a total concentration of 450.11 mg/L. In control wine, a total of 54 volatile compounds was listed including 10 acids, 13 alcohols, 17 esters, 3 ketones, 3 lactones, 2 aldehydes, 2 phenolic compounds, 3 sulfur compounds, and 1 pyrazine, all accounting for a total concentration of 448.99 mg/L. The OBA wine showed the highest concentration (588.73 mg/L) without necessarily containing more volatile compounds . In OBA wine, 53 volatile compounds were identified and quantified, including 9 acids, 12 alcohols, 16 esters, 3 ketones, 4 lactones, 3 aldehydes, 2 phenolic compounds, 3 sulfur compounds, and 1 pyrazine.

For the 2019 vintage, the results were quite similar to those observed in the 2018 vintage. Compared to both CM and control wines, OBA wine again displayed the highest concentration (546.70 mg/L), but in contrast, possessed the least

volatile compounds with a total of 50 components including 10 acids, 10 alcohols, 15 esters, 2 ketones, 4 lactones, 3 aldehydes, 2 phenolic compounds, 3 sulfur compounds, and 1 pyrazine. On the other hand, CM and control wines contained each, 53 volatile compounds with concentrations of 478.42 mg/L and 422.02 mg/L respectively. Volatile compounds of CM wine consisted of 8 acids, 13 alcohols, 16 esters, 3 ketones, 4 lactones, 2 aldehydes, 4 phenolic compounds, 3 sulfur compounds, and 1 pyrazine, whereas volatile compounds of control wine included 12 acids, 12 alcohols, 16 esters, 3 lactones, 3 ketones, 2 phenolic compounds, 1 aldehyde, 3 sulfur compounds, and 1 pyrazine. From the results, we deduced that regardless of the vintage, OBA wine possessed in a significant manner ($p<0.01$) a higher concentration of volatile compounds than CM and control wines. Besides, considering the fact that all wines were made with the same grape variety harvested in the same location, these observations led us to suggest that the use of oak barrels resulted at least, in the increase of the quantity of wine volatile compounds.

Moreover, it is well known that throughout oak barrel aging, various phenomena contribute to increasing the concentration of the total volatile compounds of the wine. These phenomena include the conversion of non-volatile varietal precursors into volatile compounds (Baumes, 2009), the accumulation of fermentation-originated volatile compounds (Jackson, 2014), and the volatile compounds released by oak, generated during toasting (Chira and Teissedre, 2013). A previous study carried out by Cabaroglu et al. (2002) on Öküzgözü red wine from the Elazig region highlighted 37 volatile compounds with a total concentration of 197.9 mg/L. More recently, Darıcı's findings have shown an average concentration of 168.1 mg/L for a total of 77 volatile compounds in Öküzgözü premium red wines from Elazig region (Darıcı, 2017). In our study, the data of essential major volatile compounds were reported in the same table with minor volatile compounds. However, a more detailed table of these compounds is found in appendix 1. These compounds represented for both vintages an average of 39.37% of the total concentration in CM wines, 40.46% in control wines, and

50.10% in OBA wines. Thus, the presence of these major volatile compounds may explain the difference with the findings of the aforementioned authors.

Alcohols: These compounds are listed in Table 4.3 and constitute the most abundant chemical class among others, accounting for an average of 69.46% in CM wines, 66.15% in control wines, and 56.67% in OBA wines. In CM wines, for both vintages, an average of 322.63 mg/L and a total of 13 components in each vintage were recorded, whereas, in control wines, 13 and 12 compounds were detected in 2018 and 2019 vintages, respectively, with an average content of 287.92 mg/L. OBA wines displayed a level relatively close to those of CM, with an average concentration of 318.998 mg/L for a total of 12 components in the 2018 vintage and 10 in the 2019 vintage.

Table 4.3Alcohol composition of the three styles of Öküzgözü wines (µg/L)

	2018			2019			MANOVA		
	CM	**Classic**	**OBA**	**CM**	**Classic**	**OBA**	**S**	**V**	**S×V**
Alcohols									
methanol[α]	110224a	110856a	136584b	102020a	104300a	128110b	**	**	ns
1-propanol	2474a	2273a	2787a	3659a	2406ab	1030b	*	ns	*
isobutyl alcohol	9512a	9023a	11307a	12429a	9016a	7462a	ns	ns	ns
1-butanol	528	557a	3790a	660a	355a	0	ns	ns	*
isoamyl alcohol	147427a	135290a	141040a	176144a	132793a	121358a	ns	ns	ns
1-hexanol	252ab	220a	313b	177a	175a	0	ns	**	**
diacetone alcohol	239a	307a	0	446	0	0	**	ns	**
3-ethoxy 1-propanol,	210a	160a	220a	217a	128a	306b	*	ns	ns
2,3-butanediol meso	1678a	3250a	3157a	3467a	2132a	2385a	ns	ns	ns
2,3-butanediol levo	431a	617a	583a	709a	466a	585a	ns	ns	ns
benzyl acohol	118a	103a	143b	111a	75a	57a	ns	*	ns
2-phenylethyl alcohol	33562a	32997a	32610a	36163a	26108a	41667a	ns	ns	ns
tyrosol	1203a	1353a	1100a	1205a	893a	1402a	ns	ns	ns
Total	**307858a**	**297006a**	**333634a**	**337407a**	**278847a**	**304362a**	**ns**	**ns**	**ns**

Values listed are the mean of three replicates per wine style. Values on the same column followed by a different letter are significantly different according to Tukey's least significant difference test ($p < 0,05$). V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by analysis of variance: ns: not significant; *: Significant at p<0,05; **Significant at p<0,01. [α]: obtained by wine distillation.

Except for methanol, all the listed alcohols belong to the higher alcohols subclass. Among these compounds, isoamyl alcohol followed by methanol and 2-phenylethyl alcohol were the most abundant in all wines. By means of Tukey post-hoc test at level $p < 0.05$, significant differences were found across the wines in at least one vintage regarding methanol, 1-propanol, benzyl alcohol, and 3-ethoxy-1-propanol.

Higher alcohols are the byproduct of yeast amino acid metabolism released during alcoholic fermentation through the Ehrlich pathway. Isoamyl alcohol, the most synthetized of higher alcohols (Ribéreau-Gayon and Bertrand, 1971) and 2-phenylethyl alcohol were found above their odor threshold value in all Öküzgözü wines. Although the difference was not significant (Figure 4.1), the highest levels were quantified in CM wines, except in the 2019 vintage, where the highest content of 2-phenylethyl alcohol was measured in OBA wine. Above its threshold odor value, Isoamyl alcohol exhibits a fruity-winey odor (Bakker and Clarke, 2011), whereas, above 14 mg/L in red wine, 2-phenylethyl alcohol imparts a pleasant odor of rose and honey (Waterhouse et al., 2016).

Unlike higher alcohols, methanol is not a yeast product but is found in wine with enzymatic degradation of grape's skin pectin (Waterhouse et al., 2016). A survey reported methanol value ranged from 21 to 194 mg/L in red table wine (Gnekow and Ough, 1976). The relatively high values in our findings (total average of 115.34 mg/L) may be due to the use of pectolytic enzymes, which contribute to increased methanol content in wine (Cabaroglu, 2005). On the other hand, the methanol content was found to be significantly higher in OBA wines (fig. 4.1), and the increase may result from cellulosic ethanolysis of oak wood during aging, as was suggested by Dumitriu et al. (2017) while noticing an increase of methanol content in red wine subject to aging in oak cask.

Excluding methanol which does not contribute to sensory properties of wine, the total content of alcohol, regardless of the wine style, dropped below 300 mg/L. Below that level, alcohols contribute to the complexity of wine aroma and add to the desirable aspect of its flavor (Bakker and Clarke, 2011).

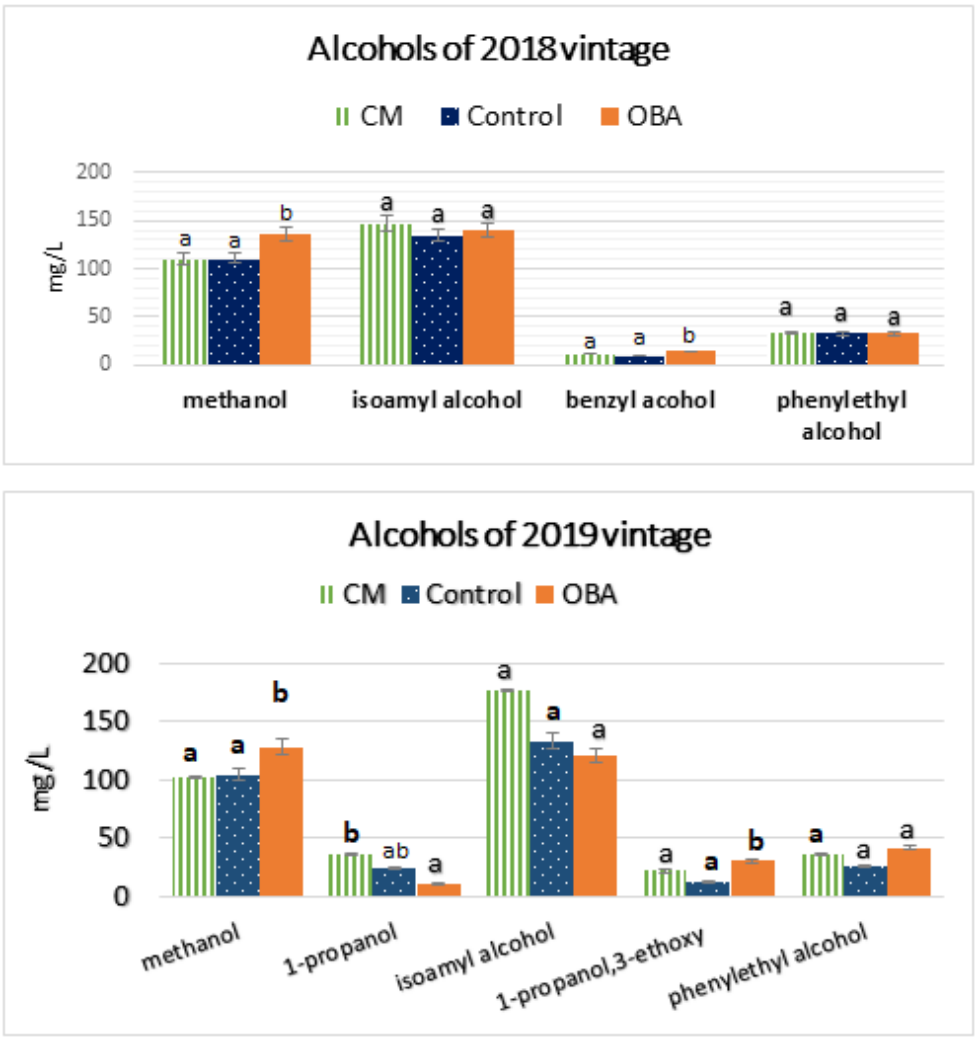


Figure 4.1. Alcohol compounds above their OTV and alcohol compounds with significant difference in Öküzgözü wines. 1-propanolx10;1-propanol,3-ethoxyx100; benzyl alcohol x100. Different letters indicate significant differences between the samples ($p<0.05$)

Esters: in terms of quality, esters are the major and the most important constituents of wine (Patrick and Etievant, 2017). They are qualified as secondary while synthetized during fermentation through yeast enzymes intervention and tertiary while they arise and accumulate throughout post-fermentation stages via acid-catalyzed reactions (Waterhouse et al., 2016). Esters contribute to the floral and ripe fruits aromas of wine and are essentially grouped in higher alcohols of acetates (HAAs), ethyl esters of fatty acids (EEFAs), and ester of organic acids (EOAs).

The composition in esters of Öküzgözü wines in this study were reported in Table 4.4. OBA wines showed the highest concentration at a significant level ($p<0.05$) for both vintages, with an average of 222.71 mg/L, followed by control and CM wines, at values of 127.69 mg/L and 121.57 mg/L respectively. In contrast, the relative quality of the esters did not follow the trends of ester concentrations across the wines. In the 2018 vintage, 16 esters were identified in OBA wine, 17 esters in control wine, and 15 components in CM wine. Comparatively, in the 2019 vintage, CM and control wines shared the same number of esters (16 components), whereas 15 esters were identified in OBA wine.

Excluding ethyl acetate, which was obtained from wine distillation and analyzed separately, the listed esters were grouped and discussed according to their subclasses, involving: higher alcohol acetates (HAAs), the ethyl ester of fatty acids (EEFAs), and esters of organic acids (EOAs) (Figure.4.2).

Table 4.4 Esters composition in the three styles of Öküzgözü red wine

	2018			2019			MANOVA		
	CM	**classic**	**OBA**	**CM**	**classic**	**OBA**	S	V	S×V
Ethyl acetate[α]	80020b	71140a	149600c	72240a	63200a	145800b	**	**	ns
İsoamyl acetate	3999c	2853b	917a	2773b	1781ab	1211a	*	**	*
Ethyl hexanoate	1226b	1070ab	920a	974a	513a	528a	*	**	ns
Hexyl acetate	443b	300a	0	0	0	0	**	**	**
Ethyl lactate	6457a	7800a	17193b	6317a	11931b	14753b	**	ns	*
Ethyl octanoate	875b	727b	490a	908a	653a	665a	ns	ns	ns
Ethyl-3-hydroxybutyrate	241a	263a	267a	420a	347a	247a	ns	ns	ns
Ethyl-2-hydroxy hexanoate	553b	557b	383a	319a	220a	0	**	**	ns
Diethyl succinate	2308a	4677b	10637c	849a	4497b	10742c	**	*	*

(Continued)

Table 4.4 (continued)

	2018			2019			MANOVA		
	CM	**classic**	**OBA**	**CM**	**classic**	**OBA**	**S**	**V**	**S×V**
Ethyl decanoate	392b	383b	237a	641a	372a	134a	*	ns	ns
Ethyl-4-hydroxybutanoate	2551b	2197b	630a	2671a	999ab	348b	**	ns	ns
2-phenylethyl acetate	526c	127a	177b	320a	219a	211a	**	ns	*
Diethyl malate	116a	103a	170a	131a	208a	261a	ns	*	ns
Diethyl 2-hydroxyglutarate	0	467b	273a	261a	726b	663b	**	**	ns
Ethyl hydrogen succinate	23810a	39197b	43610b	30291a	36279ab	41961b	**	ns	ns
2-ethoxycarbonyl-5-oxopyrolidine	0	377a	710b	301a	292a	476a	**	ns	*
Ethyl vanillate	371a	653ab	813b	177a	257a	393b	**	**	ns
Total	**123886a**	**132890b**	**227027c**	**119260a**	**122495a**	**218394b**			

Values listed are the mean of three replicates per wine style. Values on the same column followed by a different letter are significantly different according to Tukey's least significant difference test ($p < 0,05$). V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by analysis of variance: ns: not significant; *: Significant at p<0,05; **Significant at p<0,01. [α]: obtained by wine distillation

HAAs in the 2018 vintage showed significant differences ($p<0.05$) between the different styles. As expected the highest concentration was registered in CM wine with a value of 4.97 mg/L and the second highest concentration was quantified in control wine (3.28 mg/L) whereas OBA wine contained the lowest content of HAAs (1.09 mg/L). The trend was similar for the 2019 vintage with the following concentrations: 2.76 mg/L, 2 mg/L, and 1.42 mg/L attributed to CM, control , and OBA wine respectively. The trend of HAAs displayed in Öküzgözü wines was in accordance with the results from a previous study carried out by Antalick et al. (2014). They reported a higher concentration of HAAs in CM wine (red Beaujolais nouveau) than in control red wine and the lowest concentration in aged red wine. Furthermore, our results underscored that significant variations of HAAs contents were related to the winemaking method. In our study, control wine underwent maturation in a stainless steel vessel for less than six months before the sampling and was considered young red wine. However, the concentration of HAAs in this young red wine was almost 2-times less than those from CM wine. Hence, it can be considered that CM induces a significant increase in HAAs, contributing strongly to the fruity and floral aroma of the wine. Moreover, it is well known that the highest concentration of HAAs is produced in anaerobic conditions by oxygen alcohol acetyl-transferase activities that regulate the yeast HAAs synthesis (Sumby et al., 2010), and CM wines undergo four days of CM favoring these esters production.

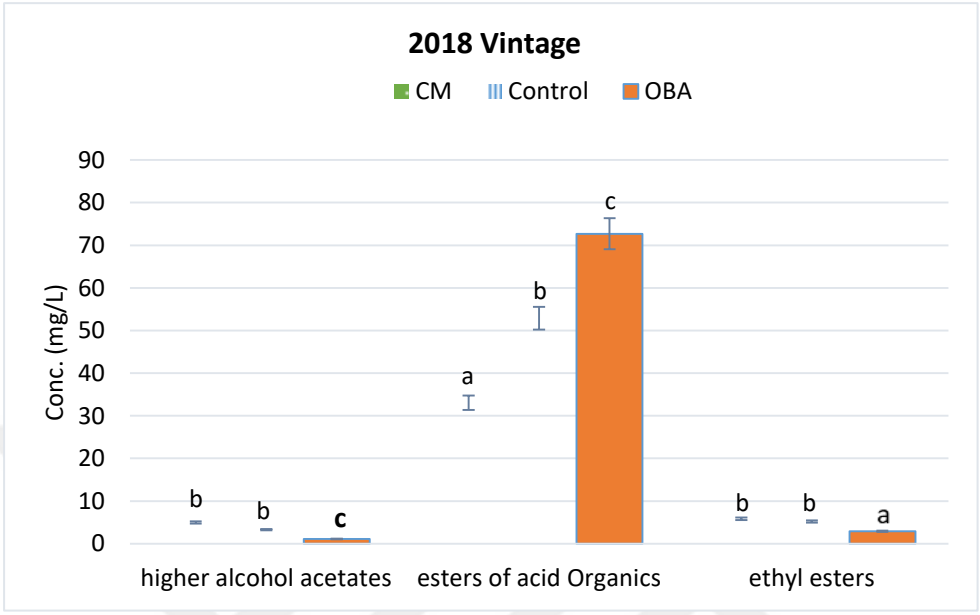
On the other hand, the low amount of HAAs observed in OBA may be related to the decrease in HAAs concentration caused by acid hydrolysis while wine is aged in an oak barrel (Bakker and Clarke, 2011; Patrick and Etievant, 2017). Consequently, the HAAs in OBA wines might contribute less to wine aroma than CM and control wines. When investigated in detail, isoamyl acetate represented the major HAA in the three styles of wines. Its concentration was significantly different across the wines and regardless of the vintage. The highest concentration was quantified in CM wines and the lowest in OBA wines (Table

4.4). However, in all wines, its concentration was well above its odor threshold value (0.03 mg/L). Above its odor threshold, isoamyl acetate exhibits fruity/bananas and pears odor (Bakker and Clarke, 2011) and may impart individually fruity aroma to young wines (Ferreira et al., 2002; Escudero et al., 2004). The second major ester in this subclass was 2-phenylethyl acetate. Its contents in CM wines for both vintages were significantly higher ($p<0.05$) compared to control and OBA wines, wherein no significant difference was found (Table 4.4). It was suggested that the concentration difference was exclusively related to the use of CM. Likewise, above its threshold value (0.25mg/L), 2-phenylethyl acetate exhibits the odor of a rose and floral (Escudero et al., 2007) and, among the studied wines, only CM wines showed a concentration of 2-phenylethyl acetate higher than 0.25 mg/L at values of 0.526 mg/L and 0.320 mg/L in 2018 and 2019 vintages respectively. Whereas in control and OBA wines, these concentrations were quite below the threshold for both vintages with average values of 0.173 mg/L in control wines and 0.194 mg/L in OBA wines. These results highlighted the fruity and floral features attributed to red wine made by CM by previous authors (Dubois et al., 1977; Tesniere and Flanzy, 2011).

EEFAs in wine result from an esterification between ethanol and fatty acid, either through yeast enzymatic activity during fermentation or from acid-catalyzed esterification during post-fermentation stages (Waterhouse et al., 2016). In all Oküzgözü wines, the EEFAs identified were exclusively esterified by mid-chain fatty acids (Table 4.4). With respect to their relative concentrations, there were no significant differences between CM and control wines. However, the level was higher in CM wines, particularly in the 2019 vintage with a concentration of 5.933 mg/L against 3.105 mg/L in control wine. EEFAs of OBA wines showed lower concentration at significant levels ($p<0.05$) in comparison to both others and regardless of the vintage (Figure 4.4). Due to their greater volatility and solubility, mid-chain fatty-acids esters can have a significant flavor impact on the wine and

are known as key contributors to its fruity aroma (Waterhouse et al., 2016). With this in mind, it could be reasonable to expect a higher amount of these compounds in CM and control wines (considered as young wine), to which fruity character is emphasized to describe them.

Moreover, Dufour et al. (2003) demonstrated that anaerobic conditions contribute to greater mid-chain fatty-acid esters. Hence the extension of the anaerobic period during CM winemaking likely involved higher production of EEFAs. On the other hand, lower concentrations in OBA wines may be due to the EEFAs hydrolysis occurring in the wine during storage to reach equilibrium (Marais, 1980; Ramey and Ough, 1980), and the degree of the phenomenon was likely related to oak barrel features (Dumitriu et al., 2017). Concerning relative composition, three EEFAs were identified and quantified above their odor threshold value in the three styles of wine. These compounds include ethyl hexanoate, ethyl octanoate, and ethyl decanoate. Among them, ethyl hexanoate was the major compound with its highest levels recorded in CM wines (Table 4.4), at a significant level ($p<0.05$) in the 2018 vintage, whereas no significant difference was found in the 2019 vintage. Ethyl hexanoate, at a concentration above 0.014mg/L, gives out fruity, pineapple, strawberry, and anise odors (Escudero et al., 2007). Besides, the ratio of mean concentration about ethyl hexanoate (Table 4.5) measured in OBA wines compared to control wines was close to 1 (0.86) in the 2018 vintage, and even above 1 (1.03) in the 2019 vintage. These data suggest that ethyl hexanoate did not alter after 6 months of oak barrel aging. Similar findings were reported by Cerdán et al. (2004) in the monitoring of volatile compounds of Cabernet-Sauvignon and merlot wines aged in an oak barrel, ethyl octanoate and ethyl decanoate showed the same trends as ethyl hexanoate across the wines, and for both compounds, a significant difference was found only in the 2018 vintage.



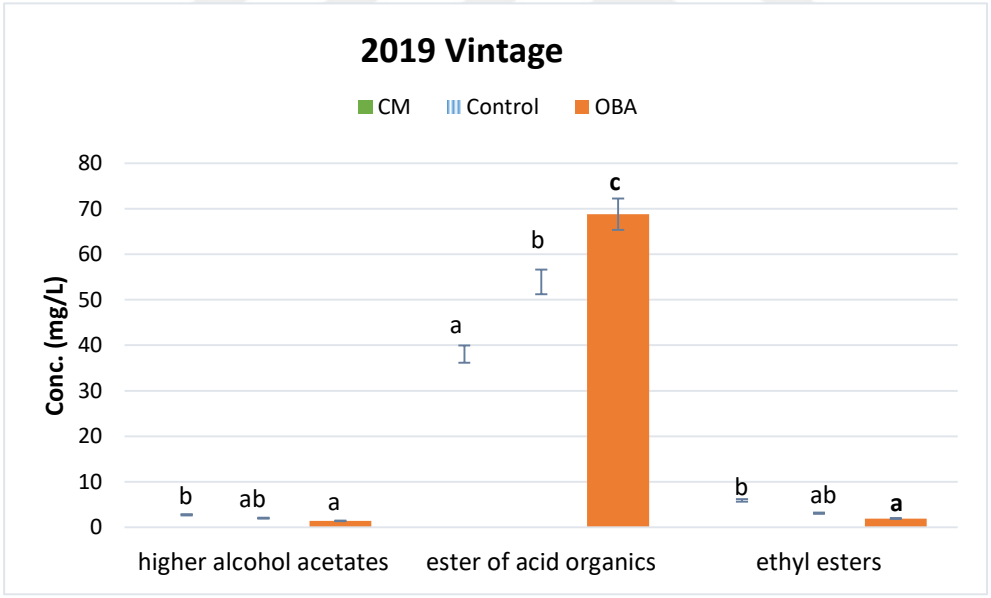


Figure 4.2. Comparison of relative esters composition in Öküzgözü red wine grouped according to their subclasses. Different letters indicate significant differences between the styles ($p < 0,05$).

In terms of concentration, it is well known that ethyl esters of organic acids (EEOAs) are the most abundant subclass esters in wine (Waterhouse et al., 2016; Patrick and Etievant, 2017). The same trend was observed in the Öküzgözü wines. However, significant differences ($p<0.05$) were found between the styles in both vintages. As predicted, the lowest contents were measured in CM wines with an average concentration of 35.54 mg/L for both vintages. In contrast, in OBA wines, these contents were two times higher (70.73 mg/L), accounting for the highest average concentration (Figure 4.2). Considering these results, it could be suggested that, overall, EEOAs accumulate with the length of wine maturation, and oak barrel aging likely increases the rate of their formation. When EEOAs were considered individually, the same trend was observed with the highest contents quantified in OBA wines except for diethyl 2-hydroxyglutarate, whose content was higher in control wine for both vintages (Table 4.4). Ethyl hydrogen succinate, followed by ethyl lactate, was listed as the major compound across the wines. However, the formation of ethyl lactate is chemical hence abundant during aging. However, the existence of bacterial esterase during malolactic fermentation is not ruled out (Dumitriu et al., 2017). It may explain the presence of ethyl lactate even at a low level in CM wine made with a short period of maturation. EEOAs in wine contribute more to the wine body than to its aroma (Shinohara et al., 1979). In this study, all identified and quantified EEOAs were found well below their sensory threshold; however, they could contribute effectively to wine aroma by the possible additivity of their odor or by a synergic effect (Simpson and RF, 1978; Lytra et al., 2013). Considering this hypothesis, the phenomenon is thought to be more critical in OBA wine.

Concerning ethyl acetate, it is the most prevalent volatile compound in wine (Bakker and Clarke, 2011), and the same trend was noted in Öküzgözü wines. Contrary to other ester acetates listed in these wines, ethyl acetate displayed its highest concentrations at a significant level ($p < 0.05$) in OBA wines with a ratio of

mean concentration 2-fold higher than control wines (Table 4.5). On the other hand, CM wines showed the second-highest ethyl acetate with an average concentration of 76.13 mg/L against 67.17 mg/L in control wines. These results suggest that ethyl acetate was produced at a high level through an enzymatic activity during yeast fermentation, after which it decreased at the early stages of storage before further increasing over aging due to the non-enzymatic reaction between acetic acid and ethanol. This phenomenon was more important while the wine was stored in oak barrel due to the availability of acetic acid from wood. Although high levels (over 200 mg/L) of ethyl acetate are considered to contribute to the defects in wine (Patrick and Etievant, 2017), at a concentration below or equal to 80 mg/L, it may contribute to the pleasantness of the wine odor (Ribereau-Gayon, 1978). In Öküzgözü wines, contents of ethyl acetate in CM and control wines showed acceptable concentrations at 80.02 mg/L and 71.14 mg/L respectively in the 2018 vintage, and 72,24 mg/L and 63.2 mg/L for the 2019 vintage. Hence, this compound may contribute to the eventual desirability of these wines. In contrast, the ethyl acetate levels in OBA wines (149.6 mg/L and 145.8 mg/L) had high limits to contribute in a positive manner to wine aroma..

Table 4.5. Ratio of mean concentrations of ethyl esters of fatty acids, ethyl acetates and ethyl esters of organic acids measured in CM and OBA wines over the mean concentration measured in control wines

	2018		2019	
Compounds	**P/C**	**OBA/C**	**P/C**	**OBA/C**
Ester acetates				
Ethyl acetate	1.12	2.10	1.14	2.31
Isoamyl acetate	1.40	0.32	1.37	0.68
Hexyl acetate	1.48	0.00	xx	xx
2-phenylethyl acetate	4.15	1.39	1.46	0.96
Ethyl esters of fatty acids				
Ethyl hexanoate	1.15	0.86	1.90	1.03
Ethyl octanoate	1.20	0.67	1.39	1.02
Ethyl-3-hydroxybutyrate	0.91	1.01	1.21	0.71
Ethyl-2-hydroxy hexanoate	0.99	0.69	1.45	0.00
Ethyl decanoate	1.02	0.62	1.72	0.36
Ethyl-4-hydroxybutanoate	1.16	0.29	2.67	0.35
Ethyl esters of organic acids				
Ethyl lactate	0.83	2.20	0.53	1.24
Diethyl succinate	0.49	2.27	0.19	2.39
Diethyl dl-malate	1.12	1.65	0.63	1.25
Diethyl 2-hydroxyglutarate	0.00	0.59	0.36	0.91
Ethyl hydrogen succinate	0.61	1.11	0.83	1.16
Ethyl vanillate	0.57	1.24	0.69	1.53

P: CM wine; C: control wine.

Lactones: are cyclic esters generated by the intramolecular condensation of carboxylic acid and alcohol groups. In wine, the most important group of lactones are ɤ-lactones with a thermodynamically stable ring of five atoms. They are known to arise either from yeast's metabolism of amino acids (Tressl et al., 1978; Wilkinson, Elsey, Prager, Pollnitz, et al., 2004) or from precursors extracted from wood during wine aging (Van Straten et al., 1978).

The three styles of Öküzgözü wine shared three common lactones including, ɤ-butanolactone, pantolactone, and 4-ethoxycarbonyl-ɤ-butanolactone (Table 4.6). *Cis*-oak lactone was identified and quantified exclusively in OBA wines. Likewise, ɤ-nonalactone was uniquely detected in CM. The common lactones cited above were all quantified at their highest concentration in OBA wines regardless of the vintage. However, it is pertinent to note that these compounds were significantly higher in the 2018 vintage, whereas no significant difference was found in the 2019 vintage.

ɤ-butanolactone was the most abundant lactone in all the wines. Together with 4-ethoxycarbonyl-ɤ-butanolactone and pantolactone, they are formed during fermentation through a yeast enzymatic activity (Fagan et al., 1981). Besides, higher concentrations of these compounds were measured in OBA wines which could be linked to an eventual acid-catalyzed reaction as an additional source of these lactones. These compounds have similar “cherry, red fruits“ odors, which may have a minor effect on the wine aroma even at subthreshold concentration through additive effects (Ferreira, 2010).

ɤ-nonalactone, with a characteristic peach odor (Waterhouse et al., 2016) has been reported to be one of the marker volatile compounds of wine made by CM (Flanzy et al., 1987). This may explain why it is exclusively present in CM wine at a concentration above its threshold level.

The *cis*-oak lactone is a lactone that originated from oak wood; therefore, it is a marker of wine that has undergone oak barrel aging or is treated with an oak alternative (Waterhouse et al., 2016). Its concentration in both vintages was above

its threshold detection (67 µg/L) with concentrations of 270 µg/L and 176 µg/L, respectively. Thus at these levels, *cis*-oak lactone imparted to OBA wines an oaky to coconut-like odor (Jackson, 2014). This lactone is formed by an acid-catalyzed reaction (dehydration) of a non-volatile precursor (a wood acid) extracted from oak wood (Waterhouse et al., 2016). Moreover, the extraction of this precursor and further its conversion in *cis*-oak lactone depend on the wine composition (Garde-Cerdán and Ancín-Azpilicueta, 2006). Therefore, the difference in this lactone concentration in OBA wines was likely linked to a vintage effect.

Volatile phenols: responsible for distinct aroma in red wine, volatile phenol arise mainly either from a yeast enzymatic decarboxylation of grape precursors during fermentation (Cabaroğlu, 1995) or by extraction from toasted oak wood during aging (Waterhouse et al., 2016).

The concentration of volatile phenols in Öküzgözü wine was different from one vintage to the other. In the 2018 vintage, CM and control wine had very close concentrations (0.252 mg/L and 0.257 mg/L respectively), whereas, in OBA wine, this concentration was two times higher (0.590 mg/L). The relative composition accounted for two components in all styles of wine - syringol and 4-vinyl guaiacol - with the latter quantified at its highest levels in OBA wines (Table 4.6). Conversely, in the 2019 vintage, the relative total concentration was significantly higher in CM wine ($p<0.05$), with levels about 3-fold and 2-fold higher than those measured in control and OBA wine, respectively. An increase in CM wine concentration was linked to the identification of two exclusive additional compounds (guaiacol and 4-vinyl phenol), which were not detected earlier in the CM wine of the 2018 vintage.

4-vinyl guaiacol is formed in wine through the hydroxycinnamic acid (feluric acid) decarboxylation by yeast enzyme; nonetheless, a small additional amount is also obtained from lignin pyrolysis during oak toasting (Waterhouse et al., 2016). This assertion may explain the higher concentration of OBA wines,

especially in the 2018 vintage, where its value was significantly higher ($p < 0,05$) (table 4.6). On the other hand, it has been reported by Flanzy et al. (1987) that wine made by CM contains more concentrations of 4-vinyl guaiacol than wine made by classic vinification, and this has been confirmed in Öküzgözü wines where for both vintages, 4-vinyl guaiacol contents were superior in CM wine compared to control wines (Table 4.6).

4-vinyl phenol is formed in the same way as 4-vinyl guaiacol; however, for this compound, the involved hydroxycinnamic acid is *p*-coumaric. Furthermore, 4-vinyl phenol is regarded as a marker compound of wine made by CM, and it mainly occurs at a higher level compared to wine made by classic vinification (Flanzy et al., 1987). This may explain its unique presence in CM wine. Concerning the sensory attributes, at a high level (> 0.8 mg/L), vinyl phenols confer unpleasant barn and horse sweat odors; conversely, at a low level, reports have shown that they impart clove and spice-like odors (Ugliano and Henschke, 2009). In Öküzgözü CM wine, this compound was quantified well below that limit, at 0.148 mg/L.

Table 4.6. Lactones and volatile phenols composition in three styles of Öküzgözü red wines

	2018			2019			MANOVA		
	CM	**Classic**	**OBA**	**CM**	**Classic**	**OBA**	**S**	**V**	**S×V**
lactones									
ɣ-butanolactone	2068a	2610a	3990b	3035a	2588a	3332a	*	ns	ns
(cis) oak lactone	0	0	270	0	0	176a	**	*	**
pantolactone	66a	53a	107b	64a	45a	80a	**	ns	ns
ɣ-nonalactone	60a	0	0	73a	0	0	*	*	*
4-ethoxycarbonyl γ-butanolactone	435a	593b	1130c	801a	1154a	1126a	**	*	ns
Total	**2629a**	**3257a**	**5497b**	**3973a**	**3787a**	**4713a**	*	ns	ns
Volatile phenols									
guaiacol	0	0	0	199	0	0	**	**	**
4-vinyl guaiacol	155a	83a	427b	100a	84a	144a	**	**	**
syringol	96a	173a	163a	356a	163a	279a	ns	*	ns
4-vinyl phenol	0	0	0	148	0	0	ns	ns	ns
Total	**252a**	**257a**	**590b**	**803a**	**246a**	**423a**	ns	ns	ns

Values listed are the mean of three replicates per wine style. Values on the same column followed by a different letter are significantly different according to Tukey's least significant difference test ($p < 0,05$). V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by analysis

Among other volatile compounds analyzed in the three styles of Öküzgözü red wine, some showed pertinent results. These are listed as follows:

Diacetyl is a diketone that arises from LAB involved in malolactic fermentation and is associated with the buttery, milky and creamy-like aroma of wine (Waterhouse et al., 2016). Regarding the flavor threshold, its content in wine is low within a 0.2-2.5 mg/L range, and it is considered an important tool for determining wine style (Bartowsky and Henschke, 2004). In Öküzgözü wines, the highest concentration of diacetyl was measured in CM wines with an average of 2.09 mg/L. In both vintages, the difference in concentration between CM and the other two wine styles was statistically significant ($p<0.05$) (Table 4.7). In contrast, no significant difference was noted between control and OBA wines. After MLF, diacetyl, since its carbonyl groups are highly reactive, can be involved in chemical reactions with other wine compounds leading to a decrease in its concentration throughout the post-malolactic fermentation stages. Castagnino and Vercauteren (1996) observed an eventual reaction of diacetyl with malvidin-3-glucoside leading to the formation of castavinol derivatives. Also, Bartowsky and Henschke (2004) took cognizance of a reaction of diacetyl with an amino acid such as cysteine, which resulted in the formation of new odorant compounds in wine. The low contents of diacetyl in control and OBA wine may be attributed to these assertions since they are allowed to age for a longer time than CM wine. Hence, it is thought that the short period of aging for CM wine favors the production of wine with higher diacetyl content. Likewise, the same trends were reported by López et al. (2014) when diacetyl concentrations were compared in wines made by CM against control vinification for three different grape varieties. Diacetyl was quantified well above its threshold sensory (0.1 mg/L) in CM wine. Hence, it may contribute largely to the aroma profile of this wine.

Table 4.7. Other volatile compounds analysed in the three types of Öküzgözü red wines

Compounds	**2018**			**2019**			**MONAVA**		
	CM	**classic**	**OBA**	**CM**	**Classic**	**OBA**	**S**	**V**	**S×V**
Ketones									
Diacetyl	1889b	937a	1163a	2294b	982a	708a	**	ns	*
2,3 petanedione	0	363a	307a	660b	111a	0	ns	ns	**
Acetoin	1131a	1223a	3400b	956a	958a	1567b	**	**	**
Total	**3021a**	**2523a**	**4870b**	**3910b**	**1512a**	**1775a**	*	*	**
Aldehydes									
Acetaldehyde[1]	834a	2100b	5430c	432a	1215b	3430c	**	**	**
Furfural	0	0	170	0	0	44	**	**	**
Phenylacetaldehyde	99a	133a	147a	54a	0	68a	*	**	*
Total	**99a**	**133a**	**317b**	**486a**	**1215b**	**3542a**	**	**	**
Other compounds									
3-ethoxypyrazole	293ab	263a	310b	283a	181a	210a	*	**	ns
Methionol	762a	803a	693a	725a	619a	954a	ns	ns	ns
vinyl sulfide	0	213a	220a	154a	166a	64a	*	ns	**
Dihydrothiophene	250b	150a	283b	0	164a	242a	*	*	*
Total	**1305a**	**1430a**	**1507a**	**1162a**	**1130a**	**1470a**	ns	ns	ns

Values listed are the mean of three replicates per wine style. Values on the same column followed by a different letter are significantly different according to Tukey's least significant difference test ($p < 0,05$). V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by analysis of variance: ns: not

4.3 Aroma-active compounds of Öküzgözü red wines

Due to the period given for the olfactometric analysis, the Aroma Extract Dilution Analysis (AEDA) of the 2018 vintage, particularly CM, showed weak results with fewer odorants and low flavor dilution factor; therefore, only the results of the 2019 vintage with better features were reported in this document.

4.3.1 Aroma extract dilution analysis (AEDA).

The results obtained from the olfactometric analysis using AEDA are provided in Table 4.8. A comparative evaluation was conducted to investigate the potent aroma differences between the three styles of Öküzgözü wine. Moreover, this procedure was helpful to determine the major aroma-active compounds contributing to the aroma profile of each wine style.

A total of 40 aroma-active compounds (odorants) consisting of 13 esters, eight higher alcohols, six acids, four lactones, four carbonyl compounds, two volatile phenols, one sulfur compounds, and two unidentified compounds were detected among the three styles of Öküzgözü red wines (Table 4.8).

In CM wine, 35 aroma-active compounds were detected. Among them, ethyl vanillate showed the greatest FD with the value of 2187, followed by five other odorants (diacetyl, isoamyl acetate, isoamyl alcohol, ethyl hexanoate, and diethyl malate) with FD at 729. Likewise, 34 aroma-active compounds were listed with FD ≥ 9 in CM wine.

Only 27 out of 40 active compounds were detected in control wine, and among these compounds, three (ethyl-3-hydroxybutyrate, 2-phenyl ethyl alcohol, and ethyl hydrogen succinate) displayed an FD of 2187. Ethyl lactate was the only compound with FD of 729 in control wine, and a total of aroma-active compounds with FD ≥ 9 were of 22 compounds (table 4.8).

On the other hand, in OBA wine, identical to CM wine, 35 odorants were listed, among which there were two unidentified compounds. Three aroma-active compounds, including *cis*-oak lactone, 4-ethoxycarbonyl-ɤ-butanolactone, and one of the unidentified compounds showed FD of 2187. In addition, five compounds displayed FD of 729. Finally, in OBA wine, 34 aroma-active compounds were recorded with FD ≥ 9.

Table 4.8. Aroma-active compounds in three styles of Öküzgözü red wines determined by GC-Olfactometry-MS and Aroma extract dilution analysis (AEDA) values.

No	LRI[a]	Odor description[b]	Compounds[c]	FD[d]		
				CM	control	OBA
1	974	Butter, cream	diacetyl	729	9	81
2	1030	Pungent, harsh	1-propanol	nd	nd	81
3	1055	Butter, cream	2,3 pentanedione	81	nd	nd
4	1087	wine, solvent, Bitter	isobutyl alcohol	3	1	243
5	1125	Banana, pear	isoamyl acetate	729	243	81
6	1227	Fusel	isoamyl alcohol	729	243	81
7	1237	Fruity, pineapple, anise	ethyl hexanoate	729	81	243
8	1248	Fatty, toasted bread	acetoin	81	27	729
9	1327	Fruity, butter	ethyl lactate	27	729	27
10	1339	sweet, minty	diacetone alcohol	9	nd	nd
11	1401	Sour, vinegar	acetic acid	243	81	729
12	1422	Toasted bread, almond	furfural	nd	nd	243
13	1437	Fruity, banana ,sweet	ethyl octanoate	243	81	81
14	1487	Strawberry, fruity	ethyl-3-hydroxybutyrate	81	2187	27
15	1518	Butter, creamy	2,3-butanediol(*levo*)	81	27	3
16	1533	acid, fatty	isobutanoic acid	9	9	9
17	1554	Butter, creamy	2,3-butanediol (*meso*)	243	nd	243
18	1582	sweet, caramel	gamma butyrolactone	243	81	729
19	1601	Cheese	butanoic acid	9	1	nd
20	1641	Rancid, cheese	isovaleric acid	27	9	nd
21	1662	fruity, wine-like,	diethyl succinate	9	243	81
22	1694	Raw potato, garlic	methionol	81	nd	nd
23	1705	sweet, fruity	ethyl decanoate	81	9	27

Table 4.8 (continued)

No	LRI[a]	Odor description[b]	Compounds[c]	FD [d]		
				CM	control	OBA
24	1779	caramel	ethyl-4hydroxybutanoate	81	3	9
25	1791	Rose, floral	2-phenyl acetate	81	9	9
26	1874	Flowery, sweet	benzyl acohol	81	nd	9
27	2147	Rose	2-phenylethyl alcohol	81	2187	27
28	2186	coconut, oak wood	*cis*-oak lactone	nd	nd	2187
29	2271	Spicy, caramel	pantolactone	nd	nd	81
30	2317	Fruity	diethyl malate	729	3	81
31	2353	Sweet, cheese	octanoic acid	243	3	81
32	2415	phenolic	Unknown*	nd	nd	81
33	2494	caramel cotton candy	diethyl 2-hydroxyglutarate	729	nd	81
34	2518	Clove, curry	4-vinyl guaiacol	243	243	81
35	2559	Red fruits, cherry	4-ethoxycarbonyl-γ-butanolactone	243	243	2187
36	2596	smoky, woody	syringol	243	3	729
37	2626	Rancid, fat	decanoic acid	243	nd	243
38	2738	Dry and overripe fruit	ethyl hydrogen succinate	243	2187	729
39	2864	ripe fruits, vinous	Unknown*	729	nd	2187
40	2874	Vanilla, Honey	ethyl vanillate	2187	9	243

[a]: Linear retention index calculated on DB-WAX capillary column. [b]: Odor description as perceived by panelists during olfactometry sessions. [c]: The odorants were identified by comparing their retention indices (RIs), mass spectra, and aroma attributes with those of pure standards. [d]:Flavor factor dilution: The highest dilution of the extract at which an odorant is determined by aroma extract dilution analysis. *: the odorant could not be identified.

In literature, some volatile compounds such as ethyl hexanoate, diacetyl, 2-phenyl acetate, isoamyl acetate, ethyl vanillate, and benzyl alcohol are referred to as marker compounds in young wine made by CM (Flanzy et al., 1987; López et al., 2014; Zhang et al., 2019). All these compounds displayed great odorant features in Öküzgözü CM wine, with FD higher than those recorded for control and OBA wines (Table 4.8). These observations emphasized the capacity of CM to improve fruity, floral, and sweet notes in the wine, since the aroma-active compounds mentioned above-exhibited notes of pineapple/strawberry, butter, rose (intense), ripe banana, honey, and rose, respectively. With respect to *cis*-oak lactone, as expected, it was specific to OBA wine. This aroma compound occurs in wine through extraction from toasted oak wood (Garde-Cerdán and Ancín-Azpilicueta, 2006). In OBA wine, its FD reached 2187 and exhibited oaky notes at the beginning and gradually evolved towards coconut notes with dilution. Both furfural and pantolactone were other active compounds specific to OBA wine, with FD of 243 and 81 respectively, with a toasted-bread and almond odor description for furfural and a pleasant spicy and caramel-like odor for pantolactone (Selli et al., 2004; Zamora, 2019). This compound, like *cis*-oak lactone, arises from oak wood treatment.

Moreover, the presence of 4-ethoxycarbonyl-ɤ-butabolactone could be noticed in OBA wine, with a cherry-like odor and an FD up to 2137. Whereas in CM and control wines, its FD was lower (FD of 243 in both). On the other hand, one of the unknown odorants with ripe fruits and vinous-like odors displayed an FD of 2187 in OBA wine, 729 in CM and not detected in control wine (Table 4.8). Finally, it is important to note that the strawberry-like ethyl-3-hydroxybutyrate, the rose-like 2-phenylethyl alcohol, and the ripe fruit-like ethyl hydrogen succinate displayed their maximum FD (2187 for each odorant) in control wine, suggesting that these compounds were the greatest contributors to the overall aroma of control wine

4.3.2 Aroma activity value

The screening of aroma-active compounds in Öküzgözü red wines via AEDA gave insights into the contribution of these odorants to the overall aroma of the relative wines. Therefore, to determine their real contribution to the overall aroma profile, AAV of the key aroma active compounds needed to be determined in alcoholic matrices. In Öküzgözü wines, only aroma-active compounds with FD ≥ 9 were considered as key aroma-active compounds. The results of aroma activity values were reported in Table 4.9.

From 34 key aroma-active compounds selected with FD ≥ 9, a total of 18 aroma compounds were quantified above their threshold odor detection in the relative alcoholic matrix (Table 4.9). Obvious differences could be noticed between the AAV and its intensity according to the style of wine. However, isoamyl acetate with ripe banana-like odor showed the greatest AAV in all wines, with its highest intensity measured in CM wine, accounting for about 81-fold its threshold odor detection.

Based on the similarity of their sensory attributes and the cumulative intensity of their AAVs, two clusters of aroma-active compounds responsible for the overall aroma of CM wine could be suggested. The first one includes the ripe banana-like isoamyl acetate (AAV 81.33) and the pineapple/strawberry-like ethyl hexanoate (AAV 69.57), with a cumulative AAV accounting for 55.75% of the total AAV > 1. Therefore, together, these aroma-active compounds are likely the main contributors to the fruity character in CM wine. The second group of odorants constituted of diacetyl and its alcohol derivatives *levo* and *meso*-2,3 butanediols. All these compounds, during AEDA, exhibited sweet odors of butter and fresh cream. Their AAVs accounted for 24.76% of total AAV >1. Similar to isoamyl acetate and ethyl hexanoate for fruitiness, the creamy butter character of the overall aroma of CM wine may be attributed to diacetyl and its alcohol derivatives.

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As observed in CM wine, the fruitiness-imparting isoamyl acetate followed by ethyl hexanoate were the aroma-active compounds with the highest AAVs in control wine with values of 59.36 and 36.64, respectively (Table 4.9). It is imperative to note that the ethyl ester of organic acids, which showed high FD in AEDA, such as ethyl lactate, ethyl 3-hydroxybutyrate, and ethyl hydrogen succinate, did not display any AAV > 1. This could be attributed to the fact that the relative threshold detection of these compounds in the considered matrix is much higher than in air. However, they are being quantified in a range of milligram per liter.

The fruity aroma-active compounds, particularly isoamyl acetate, were still abundant in OBA wine, with the highest AAV (40.36). OBA wine could be considered the most complex wine among the three styles in terms of AAVs quality and relative values. With the predominance of the fruitiness imparted by esters isoamyl acetate/ethyl hexanoate, the oaky character by the *cis*-oak lactone, and the spicy/clove notes conferred by 4-vinyl guaiacol. This aroma profile may result from a short oak-aging period. An extension of aging time could involve a change of equilibrium towards a fall in fruity character, mainly imparted by isoamyl acetate and other ester acetates (Cerdán et al., 2004; Antalick et al., 2014). Likewise, it would also lead to an increase in *cis*-oak lactone content and other oak-related aroma compounds.

Table 4.9 Odor threshold, mean concentration and aroma activity values(AAVs) of key aroma-active compounds in the three styles of Öküzgözü red wines(All Data are expressed as Micrograms per Liter

			CM		**Control**		**OBA**	
Compounds	Threshold	M*	Conc.	AAV	Conc.	AAV	Conc.	AAV
Diacetyl	0.1[a]	1	2.29	22.94	0.98	9.82	0.71	7.1
2,3 petanedione	1[c]	7	0.66	< 1	xx	xx	xx	xx
Isobutyl alcohol	40[d]	1	xx	xx	xx	xx	7.46	< 1
Isoamyl acetate	0.03[a]	2	2.44	81.33	1.78	59.38	1.21	40.37
Isoamyl alcohol	30[a]	1	176.14	5.87	132.79	4.43	121.36	4.05
Ethyl hexanoate	0.014[a]	2	0.97	69.57	0.51	36.64	0.53	37.71
Acetoin	150[a]	3	0.95	< 1	0.96	< 1	1.57	< 1
Ethyl lactate	154[a]	3	6.317	< 1	11.93	< 1	14.75	< 1
Acetic acid	200[d]	1	2.06	< 1	2.04	< 1	2.55	< 1
Furfural	14.1[a]	2	xx	xx	xx	xx	0.04	< 1
Ethyl octanoate	0.58[a]	3	0.90	1.57	0.65	1.13	0.67	1.15
Ethyl-3-hydroxybutyrate	20[a]	15	0.42	< 1	0.35	< 1	0.247	< 1
2,3-butanediol (*levo*)	0.0951[c]	4	3.46	36.46	2.13	22.42	xx	xx
Isobutanoic acid	0.05[a]	4	0.305	6.09	0.37	7.45	0.30	5.91
2,3-butanediol	0.0951[c]	4	0.70	7.46	xx	xx	0.59	6.15
ɣ-butyrolactone	35[a]	15	3.03	< 1	2.59	< 1	3.33	< 1
Butanoic acid	0.173[a]	2	0.318	1.83	xx	xx	xx	xx
Isovaleric acid	0.0334[d]	8	0.530	15.87	0.39	11.51	xx	xx
Diethyl succinate	200[a]	3	0.849	< 1	4.50	< 1	10.74	< 1
Methionol	1[a]	2	0.726	< 1	xx	xx	0.95	xx
Ethyl decanoate	0.2[a]	2	0.641	3.21	0.37	1.74	0.134	< 1
Ethyl-4-hydroxybutanoate	45[c]	9	2.671	< 1	xx	xx	0.35	< 1

(continued)

Table 4.9 (continued)

Compounds	Threshold	M*	CM		Control		OBA	
			Conc.	AAV	Conc.	AAV	Conc.	AAV
2-phenylethyl acetate	0.25[a]	1	0.320	1.28	0.219	< 1	0.21	< 1
Benzyl acohol	200[a]	14	0.111	< 1	xx	xx	0.06	< 1
Phenylethyl alcohol	14[e]	14	36.163	2.58	26.11	1.87	41.67	2.98
(*cis*) oak lactone	0.067[a]	12	xx	xx	xx	xx	0.18	2.63
Diethyl dl-malate	10[c]	13	0.13	< 1	xx	xx	0.26	< 1
Octanoic acid	0.5[a]	2	4.37	8.75	xx	xx	3.66	7.31
4-Vinyl guaiacol	0.04[a]	1	0.10	2.51	0.08	2.09	0.14	3.60
4-Ethoxycarbonyl γ-butanolactone	0.4[e]	ns	0.80	2.00	1.15	2.89	1.13	2.82
Syringol	2[b]	11	0.35	< 1	xx	xx	0.28	< 1
Decanoic acid	1[d]	8	1.32	1.32	xx	xx	0.39	< 1
Ethyl hydrogen succinate	1200[b]	10	30.29	< 1	36.28	< 1	41.96	< 1
Ethyl vanillate	3[a]	12	0.176	< 1	0.26	< 1	0.39	< 1

Aroma activity values have been calculated for the odorants with at least FD≥ 9 in one of the wine style, in matrix threshold odor detection available in literature (M*). [1]: mixture with 10% in ethanol , [2]: matrix with 11% water/ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH, [3]: wine, [4]: water, [6]:White wine at 10%, [7]: 14% v/v ethanol/water, [8]: the matrix was a 10% water/ethanol solution containing 7 g/L glycerin at pH 3.2, [9]:15% ethanol, [10]: beer, [11]: red wine, [12]:the matrix was a 10% water/ethanol solution at pH 3.2, [13] :14%(v/v) ethanol, tartaric acid to pH 3,5, [14]:the matrix was a 11% water/ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid, with the pH adjusted to 3.4 with 1 M NaOH [15]: assessed in lab.[ns]: not specified and provided by Rocha et al. (2004). [a]: (Escudero et al., 2007); [b]: (Miller, 2019); [c]:(van Gemert, 2011); [d]:(Ferreira et al., 2002) ; [e] :(Rocha et al., 2004)

4.4 Phenolic compounds and color coordinates of Öküğözü wines

The level of total phenolic compounds increases from CM wines to reach its maximum values in OBA wines, and different means between the styles are significant ($p<0.01$) regardless of vintage. The average of CM wines for the two vintages was 1711 mg/L, while the value was 2146 mg/L for OBA wines. The literature reports disparate results about the total phenolic compounds measured in red wines made with Öküzgözü grapes from Elazig. Indeed, Darıcı (2017) reported a range of total phenolic compounds from 1891 to 3237 mg/L, whereas Cabaroglu et al. (2002) reported a lower content with an average of 1090 mg/L. These differences in the statistical analysis may be credited to both wine style and vintage effect. Besides numerous other phenolic compounds, oak wood confers to wine additional gallic acid resulting from hydrolysis of oak-derived tannins such as vescalagin and castalagin (Zhang et al., 2015). As a result, the wine matured in the oak barrel contains higher total phenolic compounds than wine made without oak barrel storage. The importance of wood compounds transfer depends on many factors such as storage duration, degree of toasting, and the number of times the barrel has been used (Zamora, 2019).

The results of total anthocyanin expressed as malvidin-3-glucoside displayed the same trend from one vintage to another. CM wines showed the highest concentration, at a significant level ($p<0.05$), with an average of 540.61 mg/L, followed by control wines at 526.11 mg/L, and the lowest content was recorded in OBA wines. In previous studies, various contents of anthocyanins were listed in Öküzgözü red wine from Elazig, and none of them was made by CM. Wine with the lowest total anthocyanins contained 144.6 mg/L (Darıcı, 2017), and the highest contained 598.3 mg/L (Kelebek et al., 2010). Generally, a red wine made by CM contains less anthocyanin compared to wine made by classic vinification (Tesniere and Flanzy, 2011; Jackson, 2014). However, the results may differ when the CM process is subject to modifications (González-Arenzana et al.,

2020). In our study, CM involved a kind of cold maceration with carbon dioxide and pectolytic enzymes. This procedure resulted in red wine with high anthocyanin contents, even higher when compared to control wines (Table 4.10). Kelebek et al. (2007) showed that the use of pectolytic enzymes enhances the yield of anthocyanins in Öküzgözü red wine. Furthermore, Bayram (2018) showed that for Öküzgözü red wines, the use of cold maceration along with pectolytic enzymes yields more anthocyanins content than the classic maceration with pectolytic enzymes. Likewise, it has been reported that low temperature during maceration prevents the start of yeast fermentation and enhances the extraction of anthocyanins, involving an increase in its concentration into the resulting wine (Aleixandre-Tudo and du Toit, 2018). As a result, the high content of total anthocyanins showed in CM wine may be linked to the effect of cold CM associated with enzyme treatment. It is well known that red wine aged in the oak barrel contains fewer anthocyanins than those aged in other vessels. A certain amount of free anthocyanins initially present in wine will form with compounds released from oak wood, new complexes which contribute to wine taste and texture sensations (Zamora, 2019). Pomar and Gonzalez Mendoza (2001) showed that during the maturation of wine in the oak barrel, free anthocyanins decreases to benefit from the rise and accumulation of anthocyanins-tannins polymers.

Tannins in red wine particularly are of great importance; they contribute to wine sensory and by building new complexes with other compounds, they improve wine color stability. In red wine, tannins originate from grapes (skins and seeds) and oak wood, while wine undergoes oak barrel aging (Jordão and Ricardo-da-Silva, 2019). With respect to our study, the highest total tannins contents were measured in OBA wines for both vintages, with values of 3.86 g/L and 3.71g/L, respectively. In contrast, CM wines contain the lowest total tannins, 2.14 g/L and 2.09 g/L, respectively, for 2018 and 2019 vintages. The higher content of total tannins in OBA compared to both control and CM wines may be explained by the combination of extension of maceration and the use of oak barrel aging. Besides,

the average total tannins for both vintages was 3.78 g/L. A close average of 3.50 g/L was found by Darıcı (2017) in premium red wines of Öküzgözü aged in oak barrels. The low total tannins content in CM wines was predictable with regard to the winemaking process. Alcoholic medium favored tannins extraction; however, the use of cold CM prevents alcohol production by yeast (Aleixandre-Tudo and du Toit, 2018). Thus, the tannins yield observed in CM wines likely occurs essentially when the process shifts to mechanical maceration with enzymes addition.

Regarding the color intensity and color tonality, regardless of the vintage, a progressive decrease of color intensity from CM wine to OBA wine was noted with average values of 0.88 and 0.64, respectively. In contrast, color tonality values were higher in OBA wine with an average of 0.67 and lower in CM wine at 0.51. The mean differences between the styles were statistically significant at $p < 0.05$ level. In red wine, color intensity depends on monomeric anthocyanins' content and their polymeric forms, especially red oligomeric anthocyanins (Escribano-Bailón et al., 2019). However, during aging and after bottling, the content of anthocyanin monomers gradually declines while that of polymeric anthocyanins increases, and a part of evolves toward more complex forms (Pomar and Gonzalez Mendoza, 2001). This phenomenon induces the diminution of color intensity to the profit of the increase of color tonality involving a change in wine color, from purple-red hue, characteristic of young red wine to a brick-red color, more related to aged wine (He et al., 2012b). In addition, since CM wine aged for a short time and was analyzed earlier compared to OBA wine contributed to the significant differences in color characteristics between these two styles of red wine.

Table 4.10 Phenolic compounds and color coordinates of Öküzgözü wines

	2018			2019			S	V	V×S
	CM	**Classic**	**OBA**	**CM**	**Classic**	**OBA**			
TT (g/L)	2.14 ±0.06a	2.72 ±0.01b	3.86 ±0.01c	2.09 ±0.01a	2.81 ±0.05b	3.71 ±0.06c	**	**	**
T. Ant. (mg/L)	558.30 ±0.04c	550.09b	475.69 ±0.0a	522.92c	502.12 ±0.4b	472.38a	**	**	**
TP(mg/L)	1727 ±0.06a	1840 ±0.08b	2163c	1695 ±0.57a	1738b	2129 ±0.08c	**	**	**
Color intensity	0.90c	0.86b	0.61a	0.87c	0.75b	0.68a	**	**	**
Color tonality	0.54a	0.64b	0.70c	0.48a	0.56b	0.67c	**	**	**

TT.: total tannins; T.Ant.: expressed as malvidin-3-glucoside; TP: expressed as gallic acid equivalent. Color intensity: A420+A520+A620, color tonality: A420/A450. S: style, V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by analysis of variance: ns: not significant; *: Significant at p<0,05; **Significant at p<0,01. For each row, values with different letters are significantly different between the styles (p<0.05).

4.4.1 Anthocyanins monomers of Öküzgözü wines

Anthocyanins are wine components of great importance, contributing to organoleptic quality of wine. Associated to the red color in wine, anthocyanins are extracted from the solid parts of grapes during maceration and their contents increase with pressing when it is operated (Tetik et al., 2018; Escribano-Bailón et al., 2019). Therefore, their content in wine differs, among others, from the winemaking method (Kelebek et al., 2007; Bayram, 2018). In red wine, anthocyanins are found in two main forms at different degrees. In one hand, they are present as free monomer glycosides or acylated by acetic or coumaric acid; in the other hand, they form polymeric pigments with other anthocyanin adducts or other wine compounds.

In the three styles of Öküzgözü red wine investigated in the present study, only the monomers of anthocyanins and their acylated forms (acetyl glucosides and p-coumaroyl glucosides) were analyzed. Table 4.11 listed the relative composition of anthocyanins and their individual concentrations across the three styles, for 2018 and 2019 vintages.

The trend of relative concentrations in the Table 4.11 is similar to the results of the total anthocyanins evaluated by spectrometry through the bisulfite bleaching method. Indeed, the sum of total concentration of monomeric anthocyanins in the 2018 vintage was higher than that in the 2019 vintage. This observation may be attributed to the vintage effect as suggested with spectrometric analysis and confirmed by multivariate analysis of variance (MANOVA).

The RP-HPLC analysis revealed, a total of 13 monomeric anthocyanins consisting of 5 free monoglucosides, 5 monoglucoside acetates, and 3 monoglucoside *p*-coumarates were identified and quantified across all Öküzgözü wines, except for the control wine of the 2018 vintage wherein two monoglucoside *p*-coumarates were not detected (Table 4.11).

Table 4.11. Relative compositions and concentrations of anthocyanins monomers in Öküzgözü wines. all data are expressed in mg/L.

Compounds	RT	2018			2019			MANOVA		
		CM	classic	OBA	CM	Classic	OBA	S	V	SxV
Dp-3-glc	7.78	88.50 ±0.37c	80.40 ±0.92b	50.4 ±1.50a	74.3 ±0.05b	73.4 ±0.7b	42.4 ±0.2a	**	***	**
Cy-3-glc	9.31	15.40 ±0.22b	8.9 ±0.31a	7.1 ±1.41a	6.9 a	6.3 ±0.46a	5.3 ±0.1a	*	**	ns
Pt-3-glc	10.82	61.40 ±0.61b	62.2 ±1.68b	53 ±2.34a	65.8 ±0.5b	61 ±0.10a	59 ±0.12a	ns	ns	ns
Pn-3-glc	12.69	36.70 ±0.36b	26.7 ±0.58a	23 ±0.39a	26.5 ±0.02c	20.5 ±0.13a	23.7 ±0.07b	***	***	***
Mv-3-glc	14.07	392.34 ±2.47c	339.1 ±3.58b	297.6±3.57a	366 ±0.02c	315.1 ±0.6b	275.6 ±0.3a	**	**	**
Dp-3-glc-A	18.07	18.40 ±0.61b	16 ±0.53b	8.3 ±0.12a	14 ±0.01c	9 ±0.19b	3.1 ±0.01a	***	***	***
Cy-3-glc-A	19.14	4.9 ±0.14a	2.8 ±0.09a	3.9 ±0.28a	2.5 a	3.1 ±0.16a	2.1 ±0.09a	ns	ns	ns
Pt-3-glc-A	20.24	14.80 ±0.91a	16.1 ±0.47a	14.9 ±0.98b	7.7 ±0.1a	7.8 ±0.08a	12.6 ±0.1b	*	**	**
Pn-3-glc-A	21.34	16.60 ±0.39a	15.2 ±0.07a	13.2 ±1.32b	11.3 ±0.5b	9.7 ±0.09a	11.6 ±0.5b	ns	ns	ns
Mv-3-glc-A	22.86	96.10 ±1.56b	82 ±0.81a	85.4±1.93ab	82.4 ±0.05c	74.2 ±0.29b	72.7 ±0.1a	***	***	***
Pt-3-glc-*p*C	24.84	11.70 ±0.43b	9.5 ±0.61ab	9.3 ±0.17a	8.2 b	5 ±0.10a	8.5 ±0.15b	***	***	***
Pn-3-glc-*p*C	26.42	2.7 ±0.08a	nd	2.4 ±0.02a	8.6 ±0.9b	7.8 ±0.11b	6.01a	**	***	***
Mv-3-glc-*p*C	28.25	9.7 ±0.64a	nd	5.2 ±0.05a	5.29 ±0.2b	4.98 ±0.10a	6.13 ±0.9c	***	***	**
Total		**769.10±6.75c**	**658.9 ± 6.2b**	**573.7±3.9a**	**727.1 ±2.69c**	**642.7 ±1.85b**	**583.9 ±0.96a**	*	*	*

Mean (n=3)+SD. Different letters in the row significant difference at *p<0,05* level according to Tukey test. V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by multivariate analysis of variance: ns: not significant; *: Significant at *p<0,05*; **Significant at *p<0,01*; *** Significant at *p <0,001*. Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; A: acetate; *p*C: p-

For both vintages, CM wine contained the highest concentration of total anthocyanin monomers with values of 769.10 mg/L and 727.10 mg/L, respectively. It was followed by control wine with a concentration average for both vintage at 657.20 mg/L. Finally, OBA wine with an average content of 578.80 mg/L presented the lowest level of total anthocyanin monomers. The mean differences between the three styles concerning total anthocyanin monomers, were significant at $p< 0.05$ level (table 4.11). Considering monomer anthocyanins individually, malvidin-3-glucoside was the major anthocyanin in all the three styles, representing in average 50.12%, 49.76%, and 49.55% of the total content of anthocyanin monomers in CM, control, and OBA wines, respectively. Moreover, its highest content was recorded in CM wine and the lowest in OBA wine. The preponderance of malvidin-3-glucoside in Öküzgözü red wine was reported by several other authors (Kelebek et al., 2010; Tetik et al., 2018). By extension, cyanidin-3-glucoside was the minor non-acylated anthocyanin monomer detected in the three styles of wine. Its highest concentrations were recorded in CM wine (11.20 mg/L), whereas OBA wine displayed the lowest content (6.2 mg/L) (Table 4.11).

Generally, a young red wine made by CM, along with red wine aged in the oak barrel, has a low concentration of anthocyanin monomers compared to young red wine made by classic vinification (Tesniere and Flanzy, 2011; Escribano-Bailón et al., 2019). However, the content of anthocyanin monomer may increase in young red wine made by CM through an eventual modification of the vinification procedure (Flanzy et al., 1987). Carried out CM at low temperature prevents yeast alcohol production and consequently enhances extraction of anthocyanin monomers due to its water-soluble properties (Gómez-Míguez et al., 2007). Likewise, the transfer is favored by the softening of grape skins occurring in AM facilitating the diffusion of these components (Flanzy et al., 1987). Previous research conducted by Bayram (2018) on Öküzgözü red wines made with different types of maceration showed that cold maceration combined with pectolytic

enzymes resulted in a wine with the highest level of both total anthocyanin monomers and malvidin-3-glucoside. Moreover, throughout aging and bottle storage, anthocyanin monomers undergo numerous reactions with other wine components over time, leading to a decrease in their amount in wine (Escribano-Bailón et al., 2019). This phenomenon likely contributes to the higher content in CM wine than control wine, since CM wine underwent a short period of aging and its analysis was performed 2 months after production, whereas control wine aged for longer and its analysis was performed four months after production.

Throughout the oak aging period, the level of anthocyanin monomers, due to their weak stability, falls. Their degradation causes this due to micro-oxygenation (Shenoy, 1993; He et al., 2012a) or complexation of anthocyanins with other wine compounds leading to anthocyanin derivative pigments (Cheynier et al., 2006; de Freitas and Mateus, 2006). These two phenomena are the basis of color evolution in red wine throughout the aging period (He et al., 2012b). Hence, these findings may justify the significant difference in the level of anthocyanin monomers between control and OBA wine (Table 4.11). İn addition, analysis of OBA wine was performed eight months after its production, leading to a fall of anthocyanin monomers more pronounced than for control wine.

It is pertinent to note that cyanidin-3-glucoside and its acetate derivative were the least abundant in the three styles of Öküzgözü red wine, regardless of the vintage. Such observations have been reported elsewhere, not only in Öküzgözü red wine but also in red wine made with other grape varieties (Kelebek et al., 2011; Ghanem, 2017; Tetik et al., 2018). Cyanidin-3-glucoside is the precursor of all other anthocyanin monomers (Núñez et al., 2004), and this fact could explain its low content in Öküzgözü red wines.

4.5 Sensory characteristics of Öküzgözü red wines

The sensory attributes of the three styles of Öküzgözü red wine were evaluated by means of descriptive analysis. Each style of Öküzgözü red wine was analyzed by a panel of 9 assessors who were asked to describe the wine according to a set of 16 descriptors scored on a structural scale from 0 to 15. The data of the result of the descriptive analysis are reported in Tables 4.12 and 4.14.

4.5.1 Aroma profile evaluation of the three styles of Öküzgözü red wine

The eight aroma attributes evaluated by the panelist by smelling and retro nasally (with the palate) as well, were used for aroma profile characterization of three styles of Öküzgözü red wine (Figure 4.3). According to their relative mean scores, four descriptors, including "*chocolate/mocha,"* "*vanilla/wood,"* "*black fruits,"* and "*sugar candy/lollipop"* presented significant statistical differences across the wine (Table 4.12). However, "*chocolate/mocha"* and "*vanilla/woody"* descriptors showed significant differences for both smelling and on the palate, whereas "*black fruits"* displayed significant difference uniquely with smelling and "*sugar candy/lollipops"* on the palate.

As expected, the "*vanilla/woody"* attribute showed its highest score in OBA wine and smelling than retro nasally with a difference at $p < 0.0001$ level. However, it is useful to note that a slight perception of this aroma attribute (exclusively the vanilla-like note) was recorded in CM wine. In contrast, none of this attribute could be detected in control wine. For the "*sugar candy/lollipop"* descriptor, its score was highly significant in CM wine compared to the other two styles when it was evaluated on the palate. However, no significant differences were shown by smelling, though CM wine still presented the highest score. Concerning the "*red fruits"* attribute, the scores were positively correlated to the trend of ethyl hexanoate across the wines, as observed in the analysis of volatile compounds (section 4.2) and aroma activity values (section 4.3.2). The red fruits

received high scores in all wines, with the highest intensity recorded in CM wine, followed by control wine, and the lowest score was reported in OBA wine.

Table 4.12. Average intensities (scores) of the aroma attributes and identification of statistically significant differences among the three styles of Öküzgözü red wines, by one-way ANOVA and Ducan test analyses

Descriptors	CM	Control	OBA	Sig.	Pr>f(model)
Red fruits	10.67a	9.80a	8.33a	ns	0.248
Black fruits	7.20b	7.20b	4.25a	**	0.008
Dry fruits	2.22a	2.56a	4.00a	ns	0.245
Ripe fruit	0.92a	0.40a	0.67a	ns	0.533
Lollipop	4.83a	4.60a	2.17a	ns	0.103
Vanilla/woody	2.52b	0.00a	9.17c	***	<0.0001
Chocolate/mocha	0.00a	0.00a	6.83b	***	<0.0001
Spicy	6.18a	4.80a	6.67a	ns	0.423
P-red fruits	10.73a	9.50a	8.67a	ns	0.184
P-black fruits	6.17a	5.90a	5.75a	ns	0.916
P-dry fruits	3.00a	3.00a	2.92a	ns	0.998
P-ripe fruit	1.63a	0.40a	0.83a	ns	0.106
P-sugar candy/lollipop	8.17b	4.90a	3.17a	**	0.001
P-vanilla/woody	1.58b	0.00a	9.50c	***	<0.0001
P-chocolate/mocha	0.83a	0.60a	4.00b	**	0.001
P-spicy	3.75a	3.90a	5.42a	ns	0.120

Sig.: significance at which means differ as shown by analysis of variance; ns.: not significant, significant at the* P <0.05 level, **p <0.01, ***p <0.0001. Different letters in the same row indicate statistical differences at the p<0.05 level according to Duncan multiple test. P: sensory attribute evaluated retronasally

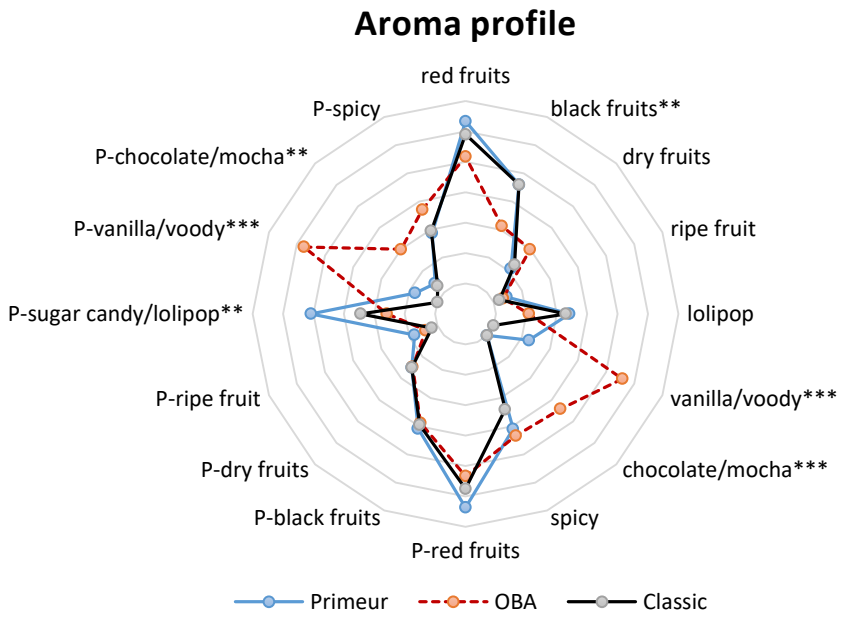

Figure 4.3. Spider web plot showing aroma profiles of the three styles of Öküzgözü red wine wines. Average scores of attribute intensity are shown as distance from the center. * P <0.05 level, **p <0.01, ***p <0.0001

4.5.1.1 Correlation between aroma attributes and wine aroma quality

The aroma quality is the score that combines the whole aroma attributes evaluated by each panelist. For analysis of the correlation, the scores of the aroma attribute perceived by smelling and that perceived retronasally were grouped. Therefore, in order to understand the relationship between the aroma attributes and the wine aroma quality, the linear correlations between the scores of the attributes averaged across tasters, and the aroma quality of all Öküzgözü red wines was calculated.

Table 4.13. Linear correlation coefficients (Pearson correlation) between the attribute scores and the aroma quality of Öküzgözü wines (data set used for calculation: scores from all wines averaged per wine across panelists, n=3 Öküzgözü red wines).

Sensory attributes	**Correlation coefficient**	**Significance (2-tailed)**
Red fruits	0,227	0,380
Black fruits	0,032	0,904
Dry fruits	0,605*	0,010
Ripe fruit	0,241	0,351
Lollipop	0,006	0,983
Chocolate/mocha	0,672**	0,003
Vanilla/woody	0,665**	0,004
Spicy	0,685**	0,002

*Correlation is significant at $p < 0,05$ level. ** Correlation is significant at $p < 0,01$ level

The Pearson correlations showed that all aroma descriptors were positively correlated with aroma quality of the wines at different levels. "*Spicy"*, "*chocolate/mocha,"* "*vanilla/woody"* ($p<0.01$), and "*dry fruits"* ($p< 0.05$) aroma descriptors displayed significant correlation coefficients with the aroma quality of wines (Table 4.13). whereas non-significant correlation coefficients were showed for "*sugar candy/lollipop,"* "*black fruits"* and "*red fruits"* descriptors.

4.5.2 Gustatory profile Evaluation of three styles of Öküzgözü red wines

To generate a more global plot of the gustatory profile of Öküzgözü wines, "*color*" and "*wine overall quality*" criteria were taken into consideration by panelists during the evaluation sessions. Therefore, table 4.14 showed mean score of the selected gustatory attributes, the relative color, and the overall quality of each style of Öküzgözü red wine. 3 out of 7 attributes, including "*astringency", "complex"* and "*body,* " showed across the wines, significant differences at p<

0,001 and p<0,05 levels, whereas for the rest of the attributes with "*color*" and "*overall quality*" included, no significant differences were underlined.

Table 4.14. Average scores of the gustatory attributes and identification of statistically significant differences among the three styles of Öküzgözü red wines, by one-way ANOVA and Ducan test analyses.

Attributes	**CM**	**Control**	**OBA**	**Sig.**	**Pr > F(Model)**
Color	12,50 a	11,50a	11,08a	ns	0,150
Sweetness	7,433 a	5,80a	5,50a	ns	0,376
Acidity	5,833 a	5,40a	5,58a	ns	0,947
Bitterness	2,03a	1,10a	1,75a	ns	0,629
Astringency	5,58a	5,90a	9,50b	**	0,002
Body	4,13a	6,40a	9,75b	**	0,001
Complex	6,24a	7,70a	9,25a	*	0,013
Aftertaste	7,83a	6,90a	8,42 a	ns	0,338
Overall quality	10,72a	9,10a	10,33a	ns	0,341

Sig.: significance at which means differ as shown by analysis of variance; ns.: not significant, significant at the* P <0.05 level, **p <0.01,. Different letters in the same row indicate statistical differences at the p<0.05 level according to Duncan multiple test. P: sensory attribute evaluated retronasally

The score of the "*astringency"* attribute in OBA wine (9.50) was highly significant (*p<0.01*) in comparison to the other two styles (Table 4.14). The relatively high astringency in OBA wine is linked to the winemaking method. Indeed, wines aged in oak barrels have more astringency due essentially to additional tannins extracted from oak wood(Garde-Cerdán and Ancín-Azpilicueta, 2006). The comparable astringency levels between CM and control wines, with scores of 5.58 and 5.90 respectively, cannot be overlooked. Usually, wine made by CM is characterized by low astringency compared to that made by classic vinification (Flanzy et al., 2001). Hence, the moderately high level shown in

Öküzgözü CM wine might result from the use of pectolytic enzymes during the winemaking process (Bayram, 2018).

Concerning body attribute, since a great "body" level is a feature of the wine aged in an oak barrel (Garde-Cerdán and Ancín-Azpilicueta, 2006), thus as expected, this attribute presented a higher score with significant difference ($p<0.01$) compared to control and CM wines (Table 4.14).

With respect to the *"color,"* although no significant difference was noted, CM wine was recognized as the wine with the most intense color. In contrast, the scores of control and OBA wines appeared to be close to each other (11.50 and 11.08, respectively). The color in red wine aged in oak barrels is correlated to the duration of storage; hence, a longer aging period leads to the progressive change from intense red to brick red color (Glories, 1984).

Finally, in terms of the overall quality of the wine, although no statistically significant differences were observed across the three styles of Öküzgözü red wine, panelists concluded that CM wine had the most outstanding overall quality, followed by OBA wine and control wine, respectively. Figure 4.4 illustrates the gustatory profile of the three styles of Öküzgözü red wine.

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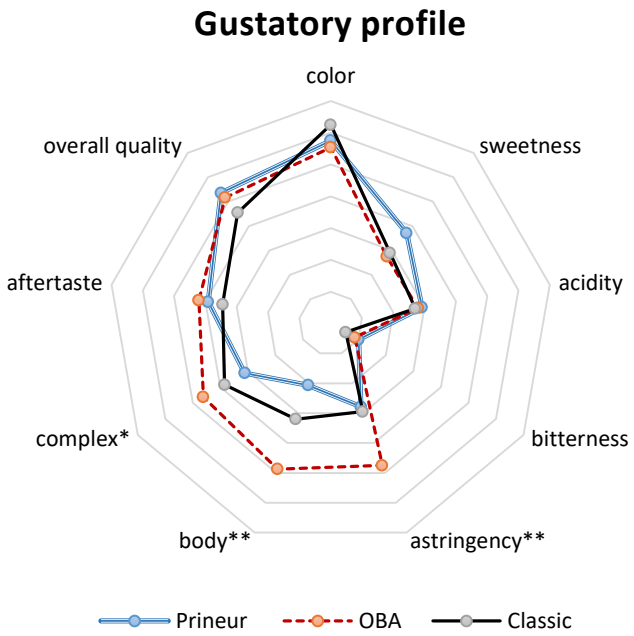

Figure 4.4. Spider web plot showing gustatory profiles of the three styles of Öküzgözü red wine wines. Average scores of attribute intensity are shown as distance from the center. * P <0.05 level, **p <0.01, ***p <0.0001

5 CONCLUSION AND RECOMANDATIONS

Intending to highlight organoleptic properties of *Vitis vinifera* L.cv Öküzgözü through the winemaking discipline, we have evaluated throughout this study the impact of CM and oak barrel aging in red wines made from Öküzgözü grape variety harvested from Elazig province. The evaluation was carried out for two vintages (2018 and 2019), and from each harvest, Öküzgözü red wine made by control vinification was used as control wine. The evaluation focused on the analysis of volatile compounds and the characterization of aroma profile by AEDA performed with GC-olfactometry. Afterward, we analyzed by means of spectrometry, general phenolic compounds, and further through RP-HPLC, free and acylated anthocyanin monomers across the three styles of wine. To complete this study, an descriptive analysis (DA) of each wine was performed to evaluate its overall quality. Thus this section presents the conclusions drawn from the results of this study, and on the other hand, areas for further researches have been proposed.

With respect to classic oenological parameters, the significant differences observed and between the wines style and the vintages have been associated with vinification method and a vintage effect, respectively. Wine made by CM contained a higher ethyl alcohol concentration compared to OBA wine. It was suggested, as mentioned elsewhere (Gómez-Plaza and Bautista-Ortín, 2019), that low ethanol content in OBA wines resulted in its evaporation through oak wood staves during aging. In comparison to control wine, volatile and total acidity were average for both vintages ,inferior in CM wine and superior in OBA wines at a significant level (Table 4.2). A low amount in total acidity into CM wine was attributed, at least partially, to degradation of malic acid without lactic acid formation as a response occurring during anaerobic metabolism in wine made by CM (Flanzy et al., 1987). On the other hand, the increase of volatile acidity in OBA was likely linked to additional acetic acid released from the hydrolysis of oak wood components (Aiken and Noble, 2016).

An effective impact on volatile compounds linked to winemaking methods has been noted in Öküzgözü red wines. Due partially to a short period of aging, CM wine is distinguished from control wine particularly, by its higher contents in HAAs, EEFAs, and high content of diacetyl and inversely lower content of EEOAs. Indeed, HAAs and EEFAs are synthesized during yeast fermentation and tend to decrease during post-fermentation stages (Antalick et al., 2014). Similarly, diacetyl is mainly formed during malolactic fermentation, undergoes further reactions during storage to form derivatives such as 2,3 butanediol, acetoin, and other more complex compounds when aging is longer (Bartowsky and Henschke, 2004; Çelik et al., 2019). This feature displayed by CM wine contributes to enhancing fruity, floral, and sweet notes characteristic of young red wine made by CM (Tesniere and Flanzy, 2011). Due to a more extended period of aging and the exogenous contribution of oak wood volatile compounds such as *cis*-oak lactone and furfural, OBA wines were the most abundant in terms of quality and quantity, regarding volatile composition, among the three styles of Öküzgözü red wine.

The identification of key aroma-active compounds using AEDA performed on gas chromatography and analysis of aroma activity values allowed to underline that:

- CM wine was oriented towards a fruity/floral and sweet aroma character since key odorants affiliated to these aroma families such as isoamyl acetate (banana), ethyl hexanoate (pineapple/strawberry), 2-phenylethyl acetate (rose), diacetyl (butter, lacte/creamy), 2,3 butanediol (butter/creamy), have displayed FD much greater than those recorded in control wine. This trend was confirmed referring to the results of AAVs of these key active-aroma compounds, cumulating 69,55% of total AAVs >1.
- Concerning OBA wine, a more complex aroma has been highlighted involving a quite pronounced fruity character related to high AAVs of isoamyl acetate (46,37) and ethyl hexanoate (36,71), an oaky character imparted by *cis*-oak lactone, and finally a spicy character attributed to 4-vinyl guaiacol.

The difference in the total phenolic compounds was significant at $p<0,05$ across the wine styles. As expected, Öküzgözü CM wine contained fewer total phenolic compounds than control wine made by classic vinification. In contrast, OBA wine presented the highest content of total phenolic compounds due to the extended maceration period and additional phenolic compounds extracted from oak wood. According to findings collected over both vintages, the use of cold CM and pectolytic enzymes treatment enhanced anthocyanins yield at a significant level in CM wines and performed analysis two months after its production contributed to keep the level of malvidin-3-glucoside high. It is well known that red wine aged in an oak barrel contains less total anthocyanins than those aged in other vessels due to the degradation reactions and the formation of new complexes with oak wood compounds, leading to a fall of anthocyanins level (Escribano-Bailón et al., 2019). Hence, the impact of oak barrel aging on anthocyanins content in OBA wine was effective, since for both 2018 and 2019 vintages, OBA wine displayed the lowest concentration of anthocyanins.

Concerning anthocyanin monomers analyzed by RL-HPLC, the same conclusion was drawn with total anthocyanins determined by spectrometry. Indeed, for CM wine, since grapes were treated at low temperature for three days delayed yeast alcoholic fermentation, allowing an enhancement of extraction of water-soluble anthocyanins (Aleixandre-Tudo and du Toit, 2018). Likewise, the use of oak barrel aging led to obtaining red wine with less content of anthocyanin monomers. Moreover, since the analysis of CM wine was carried out earlier than OBA wine, it contributed to the difference in their level of anthocyanin monomers.

DA of the three styles of Öküzgözü red wines was in accordance with the finding of AEDA along with AAVs and anthocyanins analysis. Indeed, the results of descriptive analysis of aroma profile of the three styles of wine showed great intensity of "*red fruits*" aroma descriptor in all Öküzgözü red wines, with the highest intensity recorded in CM wine. Moreover, overall aroma quality was highly correlated positively to *"woody/vanilla"* and *"spicy"* descriptors, for which the

highest intensities were recorded in OBA wine. On the other hand, in addition to gustatory evaluation, panelists preferred CM wine more than OBA wine.

The results of this study and their highlighted implications can be related to the broader framework of the red winemaking field and the red winemaking industry as a whole. Therefore the findings along with limitations underlined in this study can serve as support for further researches on Öküzgözü red wine quality.

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AUTOBIOGRAPHY

Marvin KINDZONZI-MBENZA was born on 6th May, 1983 in Brazzaville, Congo. He attended his primary, middle and secondary school respectively at Niamankessi, Angola libre and Lumumba located in Brazzaville. In 2006, he completed his Bachelor's degree in cellular and molecular biology from Marien Ngouabi University, at faculty of sciences and technology. In 2011, he obtained his Master's degree in Food engineering from Marien Ngouabi University and continued working as a lecturer in the same university at department of cellular and molecular biology until 2014. At the same time, from 2013 to 2014, he has worked as lab's staff members in BRALICO brewery at Pointe noire, Congo. He began his PhD study in Department of food engineering at Çukurova University after winning an international scholarship. During his studies and thesis work, he worked on the impact of carbonic maceration and oak barrel aging, on aroma and phenolic compounds of red wines made with *Vitis vinifera* L.cv. Öküzgözü, under the supervision of Prof. Dr. Turgut CABAROĞLU. His particular area of research interests are Flavors, Phenolic compounds, gas chromatography, HPLC, alcoholic beverages, fermented foods and food engineering.

APPENDICES

Appendix 1 Macro and high volatile compounds in three styles of Öküzgözü red wine (all data are expressed as mg per 100 mL).

Compounds	LRİ	2018			2019			MANOVA		
		Primeur	Classic	OBA	Primeur	Classic	OBA	S	V	VxS
Acetaldehyde	500	0.83 ±0.05a	2.10 ±0.02b	5.43±0.05c	0.43 ± 0.03c	1.22 ± 0.11b	3.43 ± 0.23a	**	**	**
Ethyl acetate	898	8 ±0.01 a	7.11 ±0.01 a	14.96 ±0.03b	7.22 ±0.35b	6.32 ± 0.47b	14.58 ± 0.39a	**	**	ns
Methanol	930	11.02 ±0.23a	11.08 ±0.35a	13.65 ±0.52b	10.20 ±0.26a	10.43 ± 0.32a	12.81 ± 0.49b	**	**	ns
N-propanol	1047	4.74 ±0.02b	4.80 ±0.04b	3.70 ±0.01 a	4.47 ±0.07 a	4.73 ± 0.48a	3.64 ± 0.01 b	**	ns	ns
2-methyl-1-propanol	1074	3.81 ±0.2a	3.94 ±0.14ab	4.11±0.15b	3.56 ±0.18a	3.75 ±0.13ab	4.06 ± 0.01 b	**	*	ns
2-methyl-1-butanol	1204	3.87 ±0.02a	3.89 ±0.06a	4.75±0.03b	3.619 ±0.12a	3.76 ± 0.14a	4.62 ± 0.05b	**	**	ns
3-methyl-1-butanol	1204	19.07 ±0.11 a	19.03 ±0.12a	19.77±0.21b	18.46 ±0.33a	18.87±0.47ab	19.69 ± 0.25b	**	ns	ns
Total		**51.34 ±0.64a**	**51.95 ±0.74a**	**66.37±1b**	**47.98 ±0.37a**	**49.08 ± 1.99a**	**62.83 ± 1.01b**	**	ns	ns

Values listed are the mean of three replicates per wine style. Values on the same column followed by a different letter are significantly different according to Tukey's least significant difference test ($p < 0,05$). V: vintage, VxS: vintage and style interaction. Significance at which means differ as shown by analysis of variance: ns: not significant; *: Significant at p<0,05; **Significant at p<0,01.

Appendix 2. Volatile composition of the three styles of öküzgözü red wine from 2018 and 2019 vintages (All data are expressed as miligrams per liter)

Compounds	LRI[1]	2018			2019			MANOVA		
		primeur	classic	OBA	primeur	classic	OBA	V	S	VS
Acids										
Acetic acid	1399	1.18 ±0.16a	2.09 ±0.46b	3.997 ±0.19c	2.061 ±0.37a	2.035 ±0.04a	2.548 ±0.61 a	**	ns	**
Propanoic acid	1499	0.06 ±0.01 ab	0.053 ±0.01 b	0.087 ±0.02b	0.087 ±0.05a	0.054 ±0.01 a	0.060 ±0.01 a	ns	ns	ns
Isobutanoic acid	1533	0.3 ±0.02a	0.293 ±0.07a	0.330 ±0.02a	nd	0.372 ±0.02a	0.295 ±0.05b	**	**	**
Butanoic acid	1590	0.19a	0.197 ±0.042a	0.177 ±0.03a	0.318 ±0.18a	0.174 ±0.02a	0.222 ±0.04a	ns	ns	ns
Isovaleric acid	1633	2.14 ±0.02b	1.887 ±0.33ab	1.540 ±0.1 a	0.530 ±0.32a	0.385 ±0.05a	0.471 ±0.05a	ns	**	**
Hexanoic acid	1820	3.69 ±0.05b	3.393 ±0.50b	2.617 ±0.15a	2.513 ±1.65a	1.838 ±0.08a	2.087 ±0.03a	ns	ns	ns
Octanoic acid	2342	0.37 ±0.02b	0.127 ±0.01 a	0.677 ±0.05c	4.375 ±2.66a	3.582 ±0.17a	3.656 ±0.02a	ns	ns	ns
Nonanoic acid	2487	0.76 ±0.01 b	0.913 ±0.19b	0.440 ±0.11 a	0.296 ±0.13a	0.677 ±0.28a	1.289 ±0.02b	**	**	**
Decanoic acid	2612	0.45 ±0.10b	0.203 ±0.08a	nd	1.317 ±0.83a	1.165 ±0.09a	0.318a ±0.24a	*	ns	ns
Tetradecanoic acid	2911	1.10 ±0.15b	0.233 ±0.05a	nd	1.374 ±1.25a	0.367 ±0.18a	nd	**	*	**
Linoleic acid	2945	1.18 ±0.16a	2.09 ±0.46b	3.997 ±0.20c	0.087 ±0.05a	1.059 ±1.61 a	0.577 ±0.22a	ns	ns	ns
Hexadecanoic acid	3019	0.06 ±0.01 ab	0.053 ±0.01 b	0.087 ±0.02b	nd	0.549 ±0.63a	2.548 ±0.61 a	*	ns	**
		10.25 ±0.53a	**9.39 ±1.81a**	**9.86 ±0.66a**	**11.496±4.554a**	**12.255 ±3.23a**	**11.523 ±1.28a**	ns	ns	ns

Appendix 2 (continued)

		Primeur	Classic	OBA	Primeur	Classic	OBA	S	V	VS
Alcohols										
1-propanol	1035	2.47 ±0.13	2.27 ±0.46	2.79 ±0.3	3.66 ±1.56	2.41 ±0.41	1.03 ±0.34	*	ns	*
Isobutyl alcohol	1092	9.51 ±0.07	9.02 ±1.77	11.31 ±0.87	12.43 ±6.19	9.02 ±1.21	7.46 ±1.38	ns	ns	ns
1-butanol	1138	0.53 ±0.01	0.56 ±0.1	3.79 ±3.2	0.66 ±0.31	0.36 ±0.04	nd	ns	ns	*
Isoamyl alcohol	1224	147.43 ±2.22	135.29 ±24.03	141.04 ±9.37	176.14 ±9.56	132.79 ±1.7	121.36 ±2.92	ns	ns	ns
3-hexanol	1315	0.25 ±0.02	0.22 ±0.04	0.31 ±0.02	0.18 ±0.11	0.18 ±0.06	nd	ns	**	**
2-propanol	1334	0.25 ±0.01	0.21 ±0.02	0.27 ±0.04	0.14 ±0.08	0.18 ±0.04	nd	*	**	**
Diacetone alcohol	1339	0.24 ±0.11	0.31 ±0.06	nd	0.45 ±0.21	nd	nd	**	ns	**
1-hexanol	1347	0.86 ±0.06	1.03 ±0.17	1.08 ±0.08	1.3 ±0.75	0.82 ±0.17	0.68 ±0.03	ns	ns	ns
1-propanol.3-ethoxy	1359	0.21 ±0.01	0.16 ±0.03	0.22 ±0.03	0.22 ±0.13	0.13 ±0.04	0.31 ±0.03	*	ns	ns
2.3-butanediol	1521	1.68 ±1.21	3.25 ±1.03	3.16 ±0.01	3.47 ±1.95	2.13 ±0.72	2.39 ±0.25	ns	ns	ns
2.3-butanediol	1558	0.43 ±0.01	0.62 ±0.18	0.58 ±0.01	0.71 ±0.41	0.47 ±0.16	0.59 ±0.04	ns	ns	ns
Benzyl acohol	1891	0.12 ±0.01	0.1 ±0.01	0.14 ±0.01	0.11 ±0.07	0.07 ±0.01	0.06 ±0	ns	*	ns
2-phenylethyl alcohol	2106	33.56 ±0.33	33 ±5.44	32.61 ±1.91	36.16 ±2.13	26.11 ±0.32	41.67 ±0.22	ns	ns	ns
Tyrosol	3082	1.2 ±0.27	1.35 ±0.12	1.1 ±0.31	1.21 ±0.76	0.89 ±0.09	1.4 ±0.14	ns	ns	ns
		198.74 ±3.79	**187.39 ±33.16**	**198.4 ±15.42**	**236.83 ±24.22**	**175.55 ±4.97**	**176.93 ±5.34**	ns	ns	ns

Appendix 2 (continued)

		Primeur	Classic	OBA	Primeur	Classic	OBA	S	V	VxS
Esters										
isoamyl acetate	1126	4 ±0.02	2.85 ±0.44	0.92 ±0.08	2.44 ±1.27	1.78 ±0.25	1.21 ±0.1	**	*	*
ethyl hexanoate	1237	1.23 ±0.04	1.07 ±0.1	0.92 ±0.06	0.97 ±0.51	0.51 ±0.04	0.53 ±0.02	*	**	ns
hexyl acetate	1290	0.44 ±0.02	0.3 ±0.06	nd	nd	nd	nd	**	**	**
ethyl lactate	1327	6.46 ±0.08	7.8 ±1.35	17.19 ±1.03	6.32 ±0.36	11.93 ±1.71	14.75 ±0.91	**	ns	*
ethyl octanoate	1440	0.87 ±0.01	0.73 ±0.11	0.49 ±0.03	0.91 ±0.53	0.65 ±0.02	0.67 ±0.01	ns	ns	ns
ethyl-3-hydroxybutyrate	1496	0.24 ±0	0.26 ±0.04	0.27 ±0.03	0.42 ±0.25	0.35 ±0.02	0.25 ±0	ns	ns	ns
Ethyl-2-hydroxy hexanoate	1652	0.55 ±0.01	0.56 ±0.11	0.38 ±0.01	0.32 ±0.15	0.22 ±0.01	nd	**	**	ns
diethyl succinate	1664	2.31 ±0.03	4.68 ±0.73	10.64 ±0.7	0.85 ±0.5	4.5 ±0.06	10.74 ±0.01	**	*	*
ethyl decanoate	1715	0.39 ±0.03	0.38 ±0.06	0.24 ±0.03	0.64 ±0.38	0.37 ±0.01	0.13 ±0	*	ns	ns
ethyl-4-hydroxybutanoate	1790	2.55 ±0.06	2.2 ±0.49	0.63 ±0	2.67 ±1.54	1 ±0.03	0.35 ±0.03	**	ns	ns
2-phenylethyl acetate	1802	0.53 ±0.01	0.13 ±0.02	0.18 ±0.02	0.32 ±0.19	0.22 ±0	0.21 ±0.01	**	ns	*
diethyl dl-malate	2339	0.12 ±0.02	0.1 ±0.02	0.17 ±0.04	0.13 ±0.13	0.21 ±0.03	0.26 ±0	ns	*	ns
diethyle-2-hydroxypentanedioate	2511	nd	0.47 ±0.09	0.27 ±0.02	0.26 ±0.16	0.73 ±0.07	0.66 ±0.18	**	**	**
ethyl hydrogen succinate	2724	23.81±0.71	39.2 ±6.97	43.61 ±1.13	30.29 ±0.72	36.28 ±2.52	41.96 ±1.81	**	**	ns
2-ethoxycarbonyl-5-oxopyrolidine	2877	nd	0.38 ±0.05	0.71 ±0	0.3 ±0.32	0.29 ±0.09	0.48 ±0.06	**	ns	ns
ethyl vanillate	2893	0.37 ±0.21	0.65 ±0.09	0.81 ±0.03	0.18 ±0.15	0.26 ±0.09	0.39 ±0.04	**	ns	*
		43.87±1.16	**61.75±10.68**	**77.43 ±2.9**	**47.02±7.16**	**59.29 ±4.94**	**72.59 ±3.16**	**	**	ns

Appendix 2 (continued)

		Primeur	Classic	OBA	Primeur	Classic	OBA	S	V	VxS
Ketones										
Diacetyl	981	1.89 ±0.05	0.94 ±0.15	1.16 ±0.15	2.29 ±0.89	0.98 ±0.1	0.71 ±0.03	**	ns	*
2,3 petanedione	1055	nd	0.36 ±0.08	0.31 ±0.04	0.66 ±0.29	0.11 ±0.06	nd	ns	ns	**
Acetoin	1261	1.13 ±0.06	1.22 ±0.23	3.4 ±0.08	0.96 ±0.54	0.96 ±0.23	1.57 ±0.13	**	**	**
Total		**3.02 ±0.11**	**2.52 ±0.43**	**4.87 ±0.26**	**3.91 ±1.72**	**2.05 ±0.39**	**2.26 ±0.16**	*	*	**
Lactones										
γ-butyrolactone	1588	2.07 ±0.05	2.61 ±0.35	3.99 ±0.21	3.04 ±0.17	2.59 ±0.04	3.33 ±0.04	*	ns	ns
(Cis) oak lactone	2186	nd	nd	0.27 ±0.11	nd	nd	0.18	**	*	**
Pantolactone	2304	0.07 ±0.01	0.05 ±0.01	0.11 ±0.01	0.06 ±0.04	0.05	0.08	**	ns	ns
γ-nonalactone	2346	0.06	nd	nd	0.07 ±0.05	nd	nd	*	*	*
4-ethoxy γ-butanolactone	2581	0.43 ±0	0.59 ±0.09	1.13 ±0.06	0.8 ±0.48	1.15 ±0.16	1.13 ±0.01	**	*	ns
Total		**2.63 ±0.06**	**3.26 ±0.44**	**5.5 ±0.38**	**3.97 ±0.74**	**3.79 ±0.2**	**4.71 ±0.05**	*	ns	ns
Aldehydes										
Furfural	1422	nd	nd	0.17 ±0.01	nd	nd	0.04	**	**	**
Phenyl acetaldehyde	2632	0.1	0.13 ±0.03	0.15 ±0.04	0.05 ±0.04	nd	0.07	*	**	*
Total		**0,1**	**0,13 ±0,03**	**0,32 ±0,03**	**0,05 ±0,04**		**0,11**	**	**	**

Appendix 2 (continued)

		Primeur	Classic	OBA	Primeur	Classic	OBA	S	V	SxV
Volatile phenols										
Guaiacol	1858	nd	nd	nd	0.2 ±0.1	nd	nd	**	**	**
4-vinyl guaiacol	2543	0.16 ±0.05	0.08 ±0.01	0.43 ±0.14	0.1 ±0.05	0.08 ±0.02	0.14 ±0	**	**	**
Syringol	2619	0.1 ±0.05	0.17 ±0.01	0.16 ±0.03	0.36 ±0.02	0.16 ±0.04	0.28 ±0.01	ns	*	ns
4-vinyl phenol	3043	nd	nd	nd	0.15 ±0.02	nd	nd	ns	ns	ns
Total		**0.25 ±0.09**	**0.26 ±0.01**	**0.59 ±0.11**	**0.8 ±0.19**	**0.25 ±0.05**	**0.42 ±0.01**	ns	ns	ns
Other compounds										
3-ethoxypyrazole	2248	0.29 ±0.01	0.26 ±0.02	0.31 ±0.02	0.21 ±0.01	0.18 ±0.03	0.21 ±0.01	*	**	ns
Methionol	1694	0.76 ±0.03	0.8 ±0.14	0.69 ±0.01	0.73 ±0.42	0.62 ±0.01	0.95 ±0.04	ns	ns	ns
Vinyl sulfide	2672	nd	0.21 ±0.06	0.22 ±0.09	0.15 ±0.1	0.17 ±0.05	0.06 ±0.02	*	ns	**
Dihydrothiophene	2660	0.25 ±0.02	0.15 ±0.04	0.28 ±0.01	nd	0.16 ±0.07	0.24 ±0.02	*	*	*
Total		**1.3 ±0.03**	**1.42 ±0.12**	**1.5 ±0.08**	**1.09 ±0.52**	**1.13 ±0.13**	**1.46 ±0.07**	ns	ns	ns

Values are listed as mean ± standard deviation of three replicates per wine style. S: style, V: vintage, VxS: vintage and style interaction
Significance at which means differ as shown by analysis of variance: ns: not significant; *: Significant at p<0,05; **Significant at p<0,01
Values on the same line followed by different letter are significantly different according to Tukey's least significant difference test (p <
0.05). [1]: linear retention index was calculated using standard alkane series

Appendix 3. Form of Sensorial analysis of Öküzgözü red wine

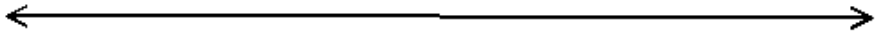
Name-Surname: **Sample No :**

Please, it is requested first to assess the color of sample, followed by a nasally and on-palate evaluation .

Visual

Color

Orange red Garnet Rubis Purple red



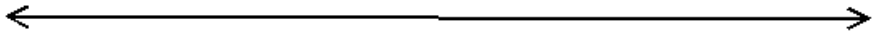
Appendix 3 (continued)

Nasally

After smelling, evaluate the wine according to below descriptors.

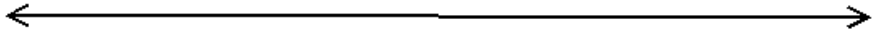
Red fruits

light pronounced



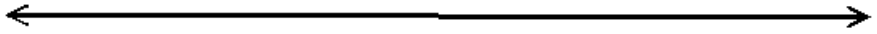
Dark fruits

light pronounced



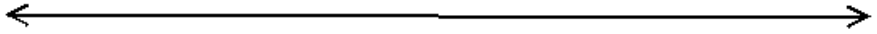
Dry fruits

Light pronounced



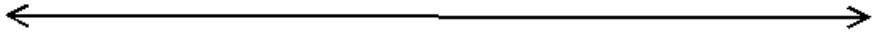
Ripe fruits

light pronounced



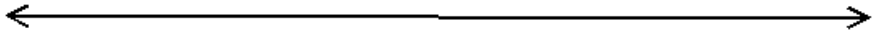
Sugar candy/Lollipop

light pronounced



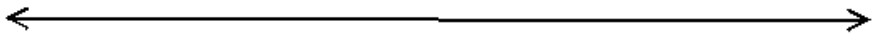
Woody/Vanilla

light pronounced



Chocolate/Mocha

light pronounced



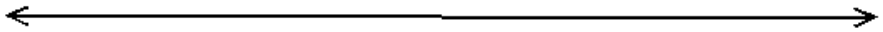
Appendix 3 (continued)

Retro-nasally (on the palate)

Please take a sip of wine in your mouth and shake it in order to solicit your palate and your cheeks . İt is not necessary to swallow the sample

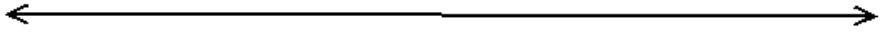
Red fruits

Light pronounced



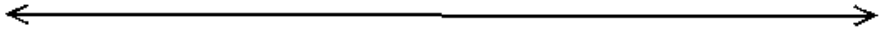
Dark fruits

Light pronounced



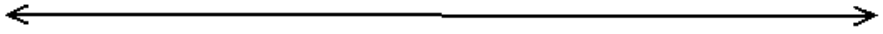
Dry fruits

Light pronounced



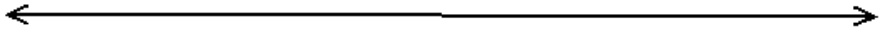
Ripe fruits

Light pronounced



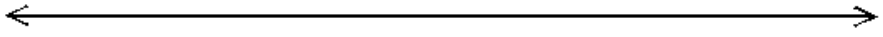
Sugar candy/Lollipop

Light pronounced



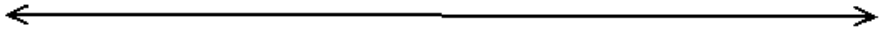
Woody /vanilla

Light pronounced



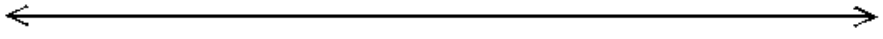
Chocolate/Mocha

Light pronounced

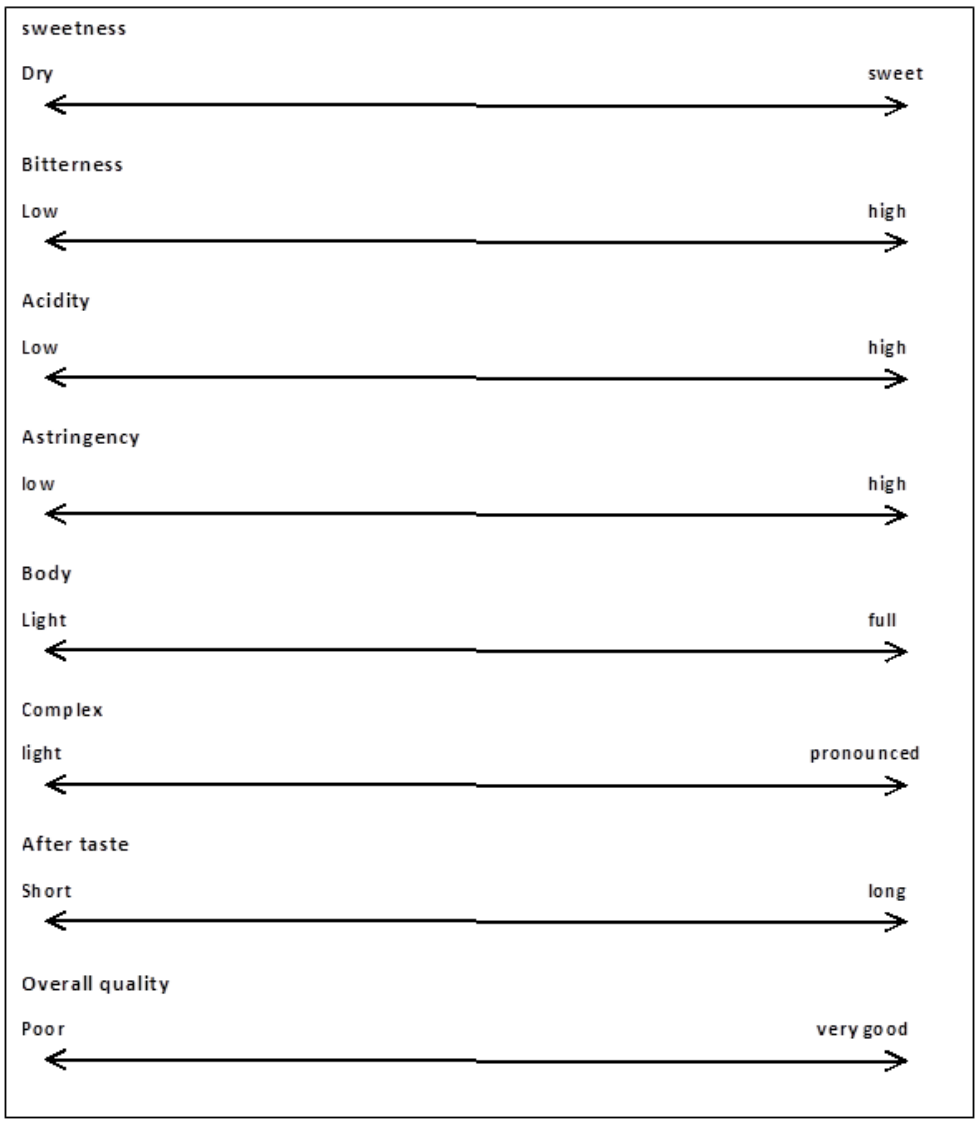


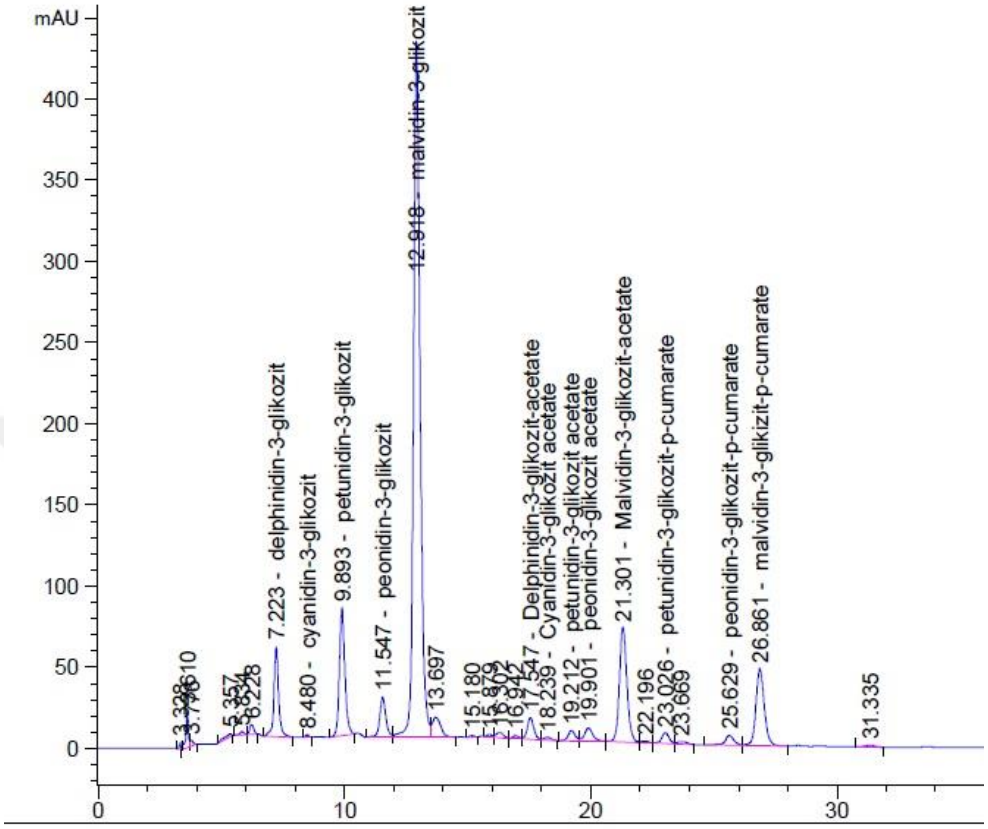
Spicy

Light pronounced

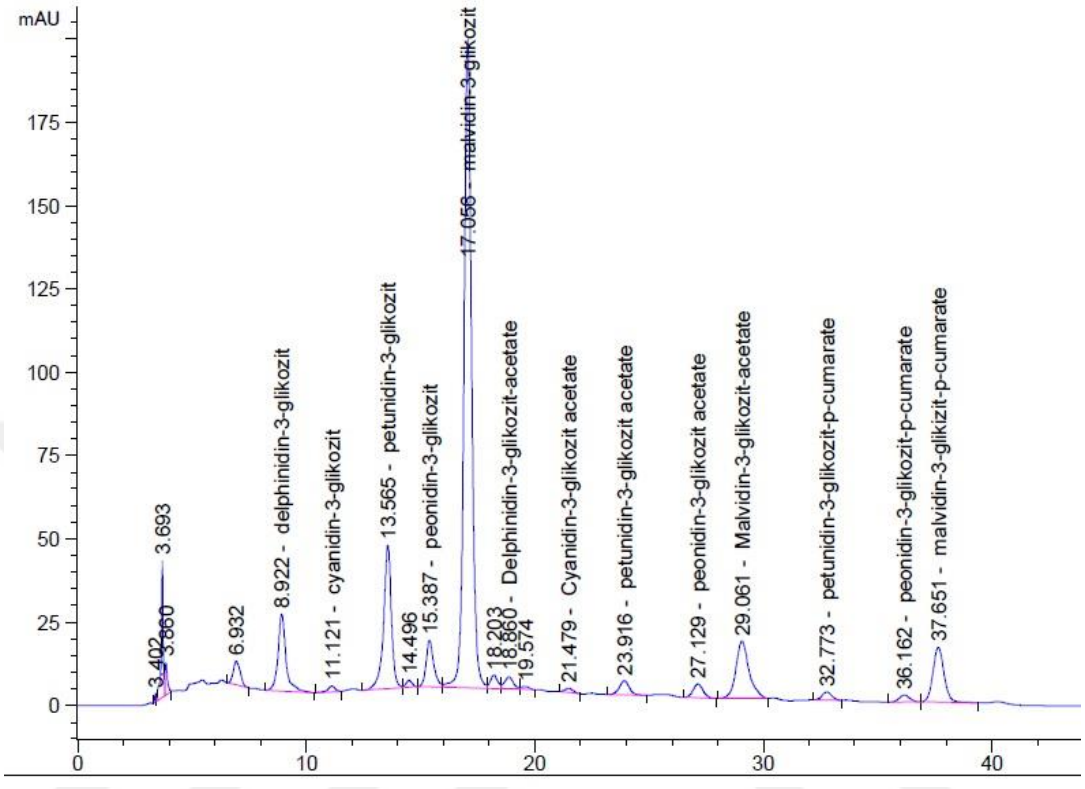


Appendix 3 (continued)

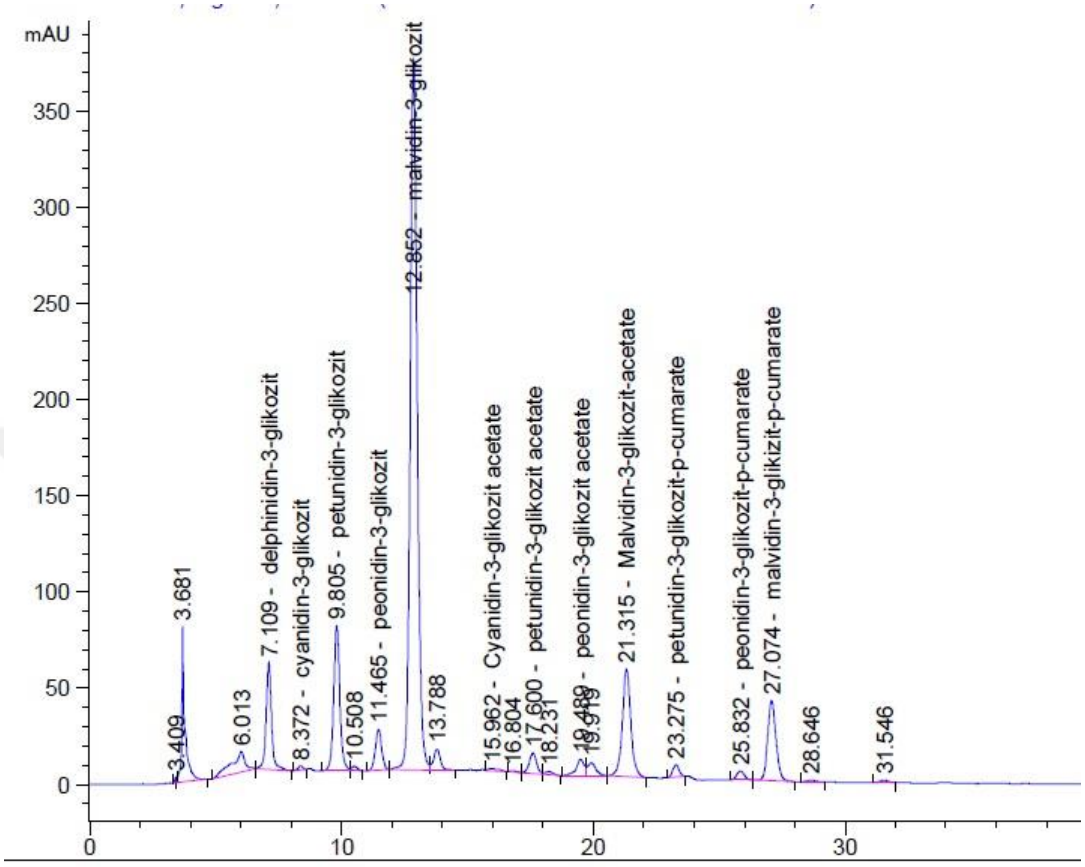




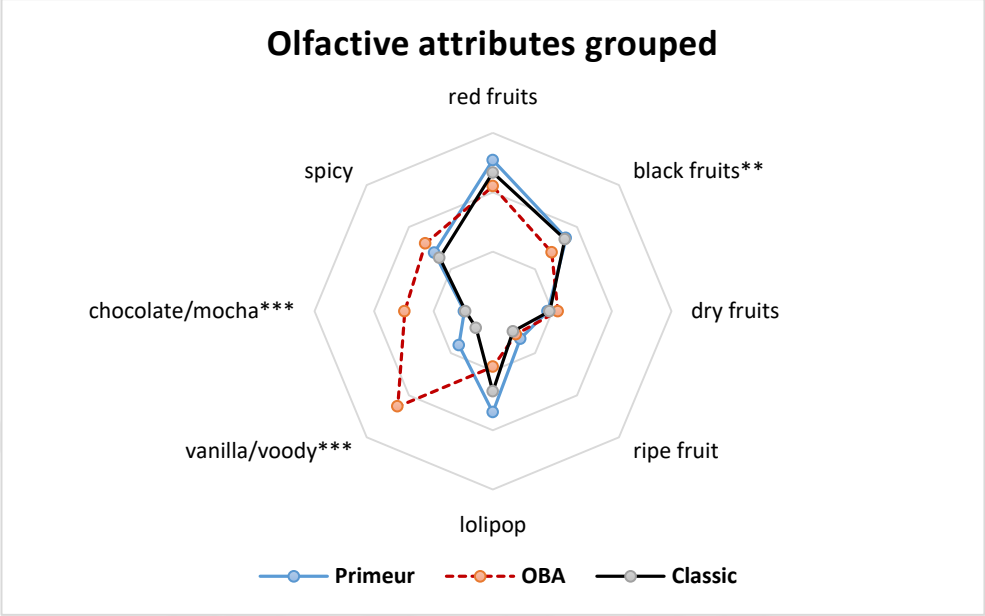
Appendix 4. Chromatogram of anthocyanin monomers of CM Öküzgözü red wine from 2019 vintage



Appendix 5. Chromatogram of anthocyanin monomers of Classic Öküzgözü red wine from 2019 vintage



Appendix 6. Chromatogram of anthocyanin monomers of OBA Öküzgözü wine from 2019 vintage



Appendix 7 olfactive attributes of the three styles of Öküzgözü wine evaluated nasally and retronasally grouped under a unique plot