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Effect of altitude and berry maturity on chemical composition of Lebanese Grenache grapes and wines

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Your living is determined not so much by what life brings to you as by the attitude you bring to life; not so much by what happens to you as by the way your mind looks at what happens.

Khalil Gibran

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Abstract

The global warming that affects the chemical composition of wine grapes during ripening would lead to an upward shift of the winegrowing regions worldwide. This study aimed to elucidate the influence of altitude and maturity on the chemical composition of Lebanese Grenache grapes and wines. Grenache berries from three vineyards with different altitudes were sampled at three ripeness levels in 2018 and 2019. Laboratory microvinifications were performed and the quality parameters of the resulting wines were determined. The results showed that the grapes and wines at the high sites had higher acidity due to the lower temperature in 2018. But the lower night temperatures in 2019 resulted in increased acidity at the lowest vineyard. Overall, the altitude had a positive effect on the seed phenolics and a negative effect on the skin proanthocyanins. The most prominent factor was the soil in 2018 and the vintage in 2019. In fact, the highest levels of skin phenolics were found in the vineyard with the poorest soil in 2018 and in the vineyards with the highest increase in the diurnal shift, in 2019. The levels of seed phenolics did not vary much over the ripening period. In 2018, the vineyard with the least fertile soil had the highest content of total individual anthocyanin compounds. For the skin phenolics, the maturity effect was more often dependent on the altitude effect. The skin phenolics decreased mostly at the end of ripening at the lowest vineyard due to the hotter weather, and increased mostly at the end of ripening at the highest vineyards due to the cooler weather. The wine produced from the vineyard with the most infertile soil had the highest amount of total volatiles. Altitude had a positive effect on the wine color intensity as it was greater at the highest sites. The wines from the high altitude vineyards had their aroma compounds more diversified since they had a higher number of volatile compounds with greater levels than those in the lowest vineyard.

Keywords: altitude, maturity, grape, polyphenols, aromas

Abbreviations

1B2M: 1-Butanol-2-Methyl

1He: 1-Hexanol

3MH: 3-mercaptohexanol

4CL: CoA Ligase

4MMP: 4-mercapto-4-methylpentan-2-one

AAA: Aromatic Amino Acids

Ab: Ain Bourday

ACC: Acetyl-CoA Carboxylase

ADH: Arogenate Dehydrogenase

ADT: Arogenate Dehydratase

AMG: Anthocyanidin Monoglucosides

ANOVA: Analysis of Variance

ANR: Anthocyanidin Reductase

asl: above sea level

ATP: Adenosine Triphosphate

BC: Before Christ

Ben: Benzaldheyde

C4H: Cinnamic Acid 4-Hydrolase

Cat: catechin monomers

CDP-MEP: 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate

CHI: Chalcone Isomerase

CHS: Chalcone Synthase

CI: Color Intensity

Citol: Citronellol

CM: Chorismate Mutase

CNI: Cool Night Index

CoA: coenzyme A

ChSy: Chorismate Synthase

Cy: Cyanidin

Cy-glu: Cyanidin 3-O-glucoside

DAD: Photodiode Array Detector

DAHP: 3-Deoxy-D-Arabino-Heptulosonate7-Phosphate

DFR: Dihydroflavonol 4-Reductase

DHD: 3-Dehydroquinate Dehydratase

DHQS: 3-Dehydroquinate Synthase

DMADP: dimethylallyl diphosphate

Dp: Delphinidin

Dp-glu: Delphinidin 3-O-glucoside

DP: Polymerization Degree

DSTmin: monthly mean minimum temperature

DXP: 1-deoxy-d-xylulose-5-phosphate

DXS: DXP synthase

E4P: Erythrose-4-Phosphate

EAc: Ethyl Acetate

EAnsk: Extractable anthocyanins in grape skins

EBu: Ethyl Butanoate

EC: Electrical Conductivity

Ed: Eddeh

Ede: Ethyl Decanoate

Edo: Ethyl Dodecanoate

Ehe: Ethyl Hexanoate

EOc: Ethyl Octanoate

Epi: Epicatechin

EpiG, EGC: Epigallocatechin

Epig: Epicatechin gallate

EPose: Extractable polyphenols in grape seeds

EPosk: Extractable polyphenols in grape skins

EPrse: Extractable proanthocyanidins in grape seeds

EPrsk: Extractable proanthocyanidins in grape skins

F3′5′H: Flavonoid 3′5′-Hydroxylase

F3′H: Flavonoid 3′-Hydroxylase

F3H: Flavanone 3-Hydroxylase

FDP: Farnesyl Diphosphate

FDPS: Farnesyl Diphosphate Synthase

$FeSO4.7H_20$: Ferrous Sulphate Heptahydrate

FID: Flame Ionization Detector

FLS: Flavonol Synthase

GA: Gallic Acid

GAE: Gallic Acid Equivalent

GAP: D-glyceraldehyde-3-phosphate

GC/MS: Gas Chromatography/Mass Spectrometry

GD: Galloylation Degree

GDD: Growing Degree Days

GDP: Geranyl Diphosphate

GDPS: Geranyl diphosphate synthase

GGDPS: Geranylgeranyl diphosphate synthase

Glu: Glutamate

GSP: Average Growing Season Precipitation

GST: Growing Season Temperature

HMBDP: 1-Hydroxy-2-Methyl-2-(E)-Butenyl-4-Diphosphate

HMG-CoA: hydroxymethylglutaryl-CoA

HPLC: High-Performance Liquid Chromatography

HPP-AT: 4-Hydroxyphenylpyruvate Aminotransferase

HSD: Tukey's honestly significant difference

IAc: Isoamyl Acetate

IBHP: 3-Isobutyl-2-Hydroxypyrazine

IBMP: 3-Isobutyl-2-Methoxypyrazine

IDP: Isopentenyl Diphosphate

IPCC: Intergovernmental Panel on Climate Change

IPSL: Institute Pierre Simon Laplace

LAR: Leucoanthocyanidin Reductase

LARI: Lebanese Agricultural Research Institute

LDOX: Leucoanthocyanidin Dioxygenase

Lim: Limomene

LOX: Lipoxygenase

m: meters

MA: Malic acid

Md: Mdoukha

mDP: mean Degree of Polymerization

MEP: 2C-Methyl-D-Erythritol-4-Phosphate

MIBP: 2-Methoxy-3-Isobutylpyrazine

MOET: Ministry of Economy and Trade

Msa: Methyl salicilate

Mv: malvidin 3-O-glucoside

MVA: Mevalonic Acid

NRCS: Natural Resources Conservation Service

OAV: Odor Activity Value

OIV: International Organisation of Vine and Wine

OMT: O-Methyltransferase

PAL: Phenylalanine Ammonia-Lyase

PC: Procyanidin Oligomers

PC1: First Principal Component

PC2: Second Principal Component

PCA: Principal Component Analysis

PDH: Prephenate Dehydrogenase

PDMS/DVB/CAR: Polydimethylsiloxane/Divinylbenzene/Carboxen

PDT: Prephenate Dehydratase

PEA: Phenyl Ethyl Alcohol

PEP: Phosphoenolpyruvate

pH: Potential of Hydrogen

Phe: Phenylalanine

Pi: Inorganic Phosphate

Pn: Peonidin 3-O-glucoside

PPA-AT: Prephenate Aminotransferase

PPY-AT: Phenylpyruvate Aminotransferase

Pt: petunidin 3-O-glucoside

RS: Reducing sugars

SAc: Sum of Acetylated Anthocyanins

SAHcy: S-Adenosylhomocysteine

SAM: S-Adenosyl-L-Methionine

SCo: Sum of P-Coumaroylated Anthocyanins

SDH: Shikimate Dehydrogenase

SK: Shikimate Kinase

SM: Seed Maturity index

SPME: Solid-Phase Microextraction

TA: Total Anthocyanin

TaA: Tartaric acid

TAL: Tyrosine Ammonia Lyase

TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene

TF: Touriga Francesa

TLA: Total Leaf Area

Tm: mean temperature

TMA: Total Monomeric Anthocyanins

Tmax: maximum temperature

Tmin: minimum temperature

TN: Touriga Nacional

TP: Total Polyphenols

TPA: Total Proanthocyanidins

TPI: Total Polyphenol Index

TSS: Total Soluble Solids

TT: Total Tannins

TTA: Total Titratable Acidity

Tyr: Tyrosinec

UFGT: Flavonoid-3-O-Glucosyltransferase

UV: Ultraviolet

UVL: Union Vinicole du Liban

VOC: Volatile Organic Compounds

VvOMT: O-methyltransferase protein

WAV: Weeks After Véraison

WHO: World Health Organization

A-KG: A-Ketoglutarate

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Introduction

Wine has been frequently related to religious ceremonies and feasting activities throughout history. Our knowledge concerning wine, mankind's most commonly consumed liquor, is about ten thousand years old (Fehér et al., 2007). The Iron Age (1200-500 BC) stands out as a time in which the art of wine production and consumption spread to many regions of the Mediterranean. Recent excavations at the Tell el-Burak coastal site, 9 km south of Sidon in Lebanon, have produced extensive new data indicating the presence of local wine production during this period (Orsingher et al., 2020).

Most remarkably, winemaking in Lebanon, an ancient and beautiful country sitting in an outstanding position on the shores of the eastern Mediterranean Sea, has been undergoing a renaissance that has the potential to put this mountainous country on the international wine map. Among the first nations in the world, Lebanon has traditionally produced wine and table grapes (Chalak et al., 2016) and the foundation of its modern wine industry can be traced back to the 19th Century when in 1857, French Jesuit missionaries imported vines and viticultural techniques from the French colonies in Algeria. During the ruling period of the Ottoman Empire (1516 - 1916), wine production was forbidden except for religious purposes (Bou-Antoun, 2014). After that, Lebanon was governed by a French military administration in 1923 and thousands of French soldiers and civil servants came to the country leading to the promotion of a wine drinking culture and an increase in the demand for wine making. In contrast, the Lebanese civil war (1975-1990) held back the progress of the wine sector after which the modern winemaking resumed.

Modern Lebanese winemaking has moved away from the ancient port cities of the Phoenicians to the most important viniculture area, the Bekaa Valley. Between the two large mountain ranges, Lebanon and Anti-Lebanon Mountains that stretch across nearly the whole length of the country, the long, elevated and fertile basin, the Bekaa Valley is home to the most important vineyards and wineries in Lebanon. The famous Bekaa Valley is considered as an agricultural oasis where most vineyards are planted between 1000 and 1200 meters above sea level (asl), making it very similar to Mendoza in Argentina.

In the Northeastern Bekaa, some vineyards are planted on hillside slopes at an altitude reaching 1500 m asl. Pure Land & Super-high Altitude Vineyard (China) in Lhasa, Tibet, China, set the record for the highest vineyard at 3,563.31 m altitude on September 27, 2018 (Guiness World

Records, 2018) and it is reported that a vineyard in Argentina (Salta) is located at 3100 m, followed by vineyards such as in Lebanon at 1700 m (Santos, E. A. dos, Florisbal, L. M., Loss, A., Besser, M. L., & Dortzbach, 2019). The second most productive wine region after the Bekaa is Batroun in North Lebanon, where the vineyards are planted either sea facing or further inland between 300 and 1300 meters asl.

The production of secondary metabolites in fruits is affected by altitude, but not consistently. As a result, more research is needed because this factor is still understudied (Gouvinhas et al., 2020). Since it is directly correlated with different environmental factors like humidity, sunlight exposure, UV radiation and temperature that affect grape ripening, altitude may have a major effect on the mesoclimate (Alessandrini et al., 2017). The hilly Mediterranean geography of Lebanon has an influence on viticulture making altitude and the related climate act as essential factors. On the other hand, Lebanon has experienced, since around 1970, an average mean temperature rise of about 0.3 degrees per decade and this long-term warming pattern is clear, according to the Intergovernmental Panel on Climate Change (IPCC), leading to expose grapes, among other fruit crops, to sunburn and early ripening (Verner et al., 2018). There is a clear and alarming decrease in different water resources such as rivers and springs creating a major problem for the Lebanese agriculture sector (Shaban, 2011).

The expected increase of the air temperature in Lebanon because of the global warming incites the winegrower to use more heat-tolerant cultivars like “Grenache”. Grenache cultivar, which is widely used by Lebanese producers (UVL, n.d.), thrives in a hot, dry Mediterranean climate which is found in Lebanon but as far as we know, there are no studies conducted in Lebanon about this grape variety. Also, to the best of our knowledge, there are no studies that have been concerned with the effect of altitude on chemical composition of grapes and wine in Lebanon at different ripening stages. Hence, the aim of this study is to investigate how altitude affects the chemical composition of Lebanese Grenache grapes at different ripeness levels, and the quality parameters of the resulting wines.

This thesis is part of partnership between the Ph.D. School of the University of Sassari in Italy and the Lebanese University, Faculty of Agriculture. The Grenache grape samples were collected through the cooperation of three Lebanese wineries aiming to encourage scientific research:

Chateau Saint Thomas and Chateau Trois Collines in the Bekaa valley and Chateau of Coteaux de Botrys in Batroun region.

The experimental analyses related to this research work were performed in the laboratory of food science in the Department of Agriculture of the University of Sassari and in the laboratories of the Lebanese Agricultural Research Institute (LARI).

This research work is performed in order to:

– Evaluate the impact of altitude and the related climate conditions on the phenolic content of Grenache grapes grown in three Lebanese regions with different altitudes for two consecutive vintages (2018 and 2019).
– Improve the knowledge of the effect of maturity degree on the concentration of anthocyanins, proanthocyanins and total polyphenol content in skins and seeds of Grenache berries.
– Investigate the influence of altitude on the concentrations of phenolic and volatile organic compounds in Grenache wines.

The obtained results will help researchers better understand the accumulation of polyphenols and volatiles in Grenache grapes from regions with peculiar climate conditions. It is not easy for the winegrower to select the suitable grape cultivar for the appropriate site and if a wrong choice was made then it is very difficult to correct it by cultural practices or during winemaking. Therefore, the choosing of a vineyard's site and cultivars is a critical decision that will have a long-term impact on its financial performance. The right choice will help the winegrower to reach its market-oriented targets. The knowledge about the phenolic and volatile compounds of Grenache grapes grown at widely different altitudes in a Mediterranean climate allow the productive sector to direct the type of wine to be produced.

Chapter I. State of the art

I.1 Grape berries

Grape berries have a long and rich history, and they were valued for their use in winemaking during the ancient Greek and Roman civilizations. European grapes (*Vitis vinifera*), North American grapes (*Vitis labrusca* and *Vitis rotundifolia*) and French hybrids are currently the main species. The increased demand for grape culture grew over time, and grapes are now one of the world's most important fruit crops. According to the 2019 statistical report on world vitiviniculture (OIV, 2019), the world production of grapes reached in 2018, 77.8 millions of tons: 57% of wine grape, 36% of table grape, and 7% of dried grape. Grape products such as fruit, wine, juice, and raisins are common among consumers and the grape berries contain several nutrient elements like minerals, vitamins, edible fibers, carbohydrates, and phytochemicals.

I.2 Lebanese grape varieties

The diversity of the Lebanese terroirs together with the possibility of cultivating vines at different altitudes have created a mosaic of wine grape varieties that are grown all over the country (Lelay G. & Roger T., 2003). The Jesuit fathers who had introduced and applied their winemaking experience in Lebanon, imported Cinsault, Carignan and Grenache grape varieties from Algeria (Karam, 2020). Then Lebanon's period under French rule was and is still evident in the cultivars that are most heavily planted such as Cabernet Sauvignon, Syrah, Merlot, Cinsault, Grenache, Carignan and Mourvedre (MOET, 2010). The wine industry is currently oriented towards substituting old grape varieties with noble ones in order to obtain high quality wine (Ghantous & Sassine, 2016). The Lebanese wine vineyards had been nearly totally planted by noble hybrid grape varieties imported from Europe and the USA until in 2012-2013, a winery located in western Bekaa called Chateau Saint Thomas used successfully the local indigenous variety "Obeidy" (Chalak et al., 2016). The most grown cultivars are in a decreasing order: Cabernet Sauvignon, Syrah, Tempranillo, Merlot, Chardonnay, Viognier, Sauvignon Blanc, Cinsault and Grenache. Other cultivars such as Mourvedre, Carignan and two indigenous white wine cultivars, Obeidy and Merweh have a relatively low production rate (Mohasseb et al., 2020). Malbec has been recently introduced to Lebanon and it is present in North and West of the Bekaa (Ghantous & Sassine, 2016).

According to the official association of wine producers in Lebanon, Union Vinicole du Liban (UVL, n.d.), Lebanese wineries commonly use Grenache to produce wines with a high alcoholic content and Cabernet Sauvignon as a varietal wine or as a blend component. Cinsault has been used for decades in Lebanon as well as Carignan which produces wine with great acidity and a remarkable dark hue. Merlot is used in most of upper and mid-range wines and Mourvèdre produces well-structured wines. Tempranillo could be the most important Spanish grape variety for some Lebanese producers and Syrah is valued for its longevity and its aromas and flavors of berry fruits, spices and prunes. Because of its high tannin content and very dark color, Petit Verdot is used for its ability to contribute extensively to a wine's aging potential. Cabernet Franc is a new cultivar to Lebanon and is planted in the two main winegrowing regions, the Bekaa and Batroun.

Different studies were conducted on several grape varieties grown in Lebanon such as the comparative study of phenolic and technological maturity of Cabernet Sauvignon, Merlot, Syrah and Cabernet Franc (Rajha et al., 2017), the impact of different winemaking techniques on polyphenols in wines from Syrah and Cabernet Sauvignon (Ghanem, 2017), the morphological characterization of 35 indigenous varieties which have a potential interest in winemaking including Obeidy and Merweh (Chalak et al., 2016) and the effect of different cultural practices on the behavior and the performance of Malbec (Ghantous & Sassine, 2016).

I.3 Grenache cultivar

Grenache is one of the most widely planted red wine grape variety in the world (Figure 1) and is predominantly grown around the north-western Mediterranean coast where France, Spain and Italy are the top producers (Tardaguila et al., 2008) (Figure 2). It has been linked to Grenache noir in France, Garnacha tinta in Spain and Cannonau in Italy, where Sardinia is the primary region for its cultivation (Petretto et al., 2021; Vacca et al., 2009). It is commonly grown in hot and dry winemaking areas, such as California or Australia and used to make rosé or fortified wines because it has thin skin and ripens late (Rentzsch et al., 2007). It is highly vigorous, extremely drought tolerant, adapts easily to various types of soil and produces wine with a high alcoholic content (De Andrés-de Prado et al., 2007; OIV, 2017). The vine withstands strong winds and drought due to its strong wood canopy and upright growth. Its clusters are compact, winged, conical and large. The berries are blue-black, rounded, covered in bloom, medium in size with abundant juice (Radden, 2021). It thrives at low yield in the hot, dry Mediterranean climate and it is best suited to warm, poor, dry, gravely, slightly acidic and stony soils with low limestone content and high heat-reflection capacity allowing it to ripe slowly. This late-ripening variety has a high sugar accumulation capacity and produces wine lacking acid, tannin and color and it is often blended with Carignan, Mourvèdre, Syrah, Tempranillo and Cinsault. Grenache grapes generally yield a light on the palate wine characterized by its berry flavor, spicy notes and an exquisite aromatic intensity (ultraripe black fruits, prunes). The flavor of Grenache wines results from the interaction of around 40 odorous compounds of which the terpenols, some norisoprenoidic derivatives, the ethyl esters, lactones and the volatile phenols are the most important. The presence of very small levels of a rather extensive list of aroma compounds results in the subtle flavor of Grenache grapes (Ferreira & Lopez, 2019) and the produced wine can develop peach, chocolate, kirsch and even flowery notes (López et al., 2004) in addition to liquorice, dry fig, pepper and cherry jam notes (Segurel et al., 2009).

Figure 1: Grenache plantings worldwide. Adapted from: (CIVR, 2017)

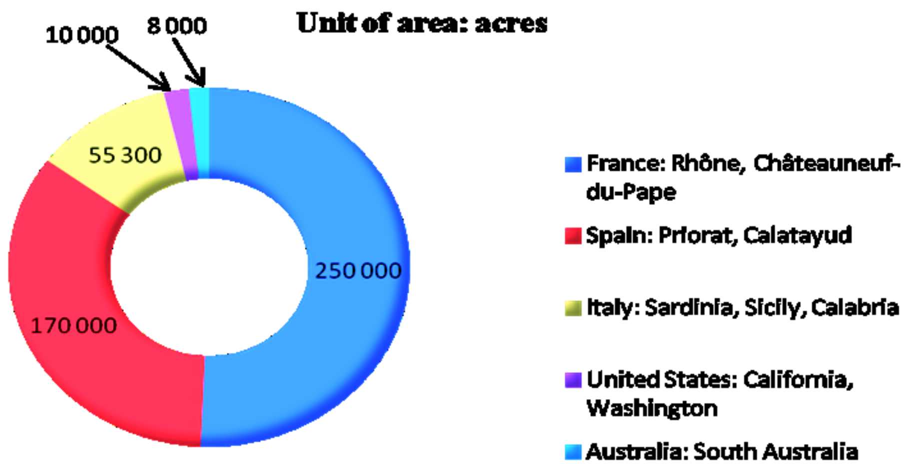


Figure 2: Acreage of Grenache in major growing countries. Adapted from: (Puckette, 2019)

High alcohol content and low acidity of the produced wine impart exquisite aromatic intensity, a wonderful structure and an appealing roundness and its sensitivity to oxidation leads to display notes of dried fruits (raisins, figs, nuts) and spice (caramel, coffee, cocoa) in fortified sweet wines (CIVR, 2017). Regarding the cultivation of Grenache in Lebanon, in the mid nineteenth century, French Jesuit monks imported to the country Grenache grapes which were regarded at that time as "les meilleurs cépages" or the best varietals from Algeria (Karam, 2020). Grenache is currently planted mainly in the Bekaa in addition to the regions of North and Mount Lebanon and is used in 15 out of 54 active wineries to make varietal and mostly blended wines (Mohasseb & Sassine, 2019).

I.4 Bibliographical review

Major parts of the paragraphs from **I.4.1** to **I.4.5.3** are included in a review article entitled: "**Altitude effect on chemical composition of grapes and wine: a review"** that is submitted for publishing.

I.4.1 Altitude effect

The term "terroir", derived from the Latin word "terre" or "territoire", is very popular in wine literature. It is related to the interactions between environmental factors and applied vitivinicultural practices that provide uniqueness to the produced wine. OIV, the International Organisation of Vine and Wine, defined the vitivinicultural terroir in the Resolution OIV/VITI 333/2010 (OIV, 2010a). One of the factors that contribute to the terroir of a region's wine, thus influencing the physicochemical behavior, the phenolic and volatile compounds of grape berries and consequently the sensory characteristics of the resulting wine, is the altitude (Xing et al., 2016). The altitude of a growing site which affects temperature, humidity, UV-B radiation, sunlight hours, water deficits and other environmental factors can strongly influence climatic conditions. The importance of the researches conducted on the impact of geographical parameters like altitude on the main chemical components in the grapes arises from the beneficial impacts of these components on product sensory attributes and human health, such as antioxidant activities (Cory et al., 2018). Their evolution is very important for winegrowers in the decision-making process about the optimal geographical conditions that lead to the production of a wine with distinctive characteristics. The influence of altitude in grape berries chemical composition such as sugars, acids, non-flavonoid compounds including copigments and antioxidants (stilbenes), flavonoid compounds including anthocyanins, oligomers and polymers of flavan-3-ols, and flavonols in addition to volatile organic compounds has been recognized and highlighted in very few recent papers (Alessandrini et al., 2017; de Oliveira et al., 2019; Rienth et al., 2020), showing that, up to now, the altitude impact on grape and wine chemical composition has been poorly investigated. It is important to underline here that global warming could lead to changes in wine geography and an upward shift of the growing regions has been forecasted to happen (Pomarici & Seccia, 2016). In fact, among the new viticultural strategies that can be adopted to mitigate the negative impacts of global warming on grape and wine quality, particularly to delay grape ripening, altitude is one of the most effective (Gutierrez-Gamboa et al., 2021). The possibility of cultivating wine grapes under predicted hotter temperatures in the future in high-altitude regions characterized by a cooler climate needs to be studied. This review aims to explore the recent studies related to the effects of altitude on the chemical components mostly the polyphenols and volatile compounds of grapes and wine, and to find new insights for future research also in the light of climate changes that will necessarily modify global viticulture.

I.4.1.1 Origin, structure and function of phenolic compounds in grapes and wine

Polyphenols are a group of organic molecules widely distributed in the plant kingdom. They are made up of a complex assembly of several thousand compounds ranging from small compounds containing only one single aromatic ring carrying one to three hydroxyls to several such structural units (De Pascual-Teresa & Clifford, 2017). Plant secondary metabolism produces phenolics during normal growth and as defense response to stress conditions like wounding, infection and UV radiation (Naczk & Shahidi, 2006).

I.4.1.1.1 The flavonoid biosynthesis

The phenylpropanoid pathway and the polyketide (or acetate) pathway combine for biosynthesis of flavonoids as shown in Figure 3. The phenylpropanoid pathway provides p-coumaroyl-CoA and the polyketide pathway provides malonyl-CoA for C2 chain elongation from acetyl-CoA by acetyl-CoA carboxylase (ACC). The first step in flavonoid biosynthesis is catalyzed by chalcone synthase (CHS) which yields naringenin chalcone from the substrates p-coumaroyl-CoA and malonyl-CoA. Chalcone isomerase (CHI) catalyzes the stereospecific cyclization of naringenin chalcone into naringenin, which is a general precursor for flavones, flavonols, anthocyanins, proanthocyanidins and isoflavones. Flavanone 3-hydroxylase (F3H) converts naringenin to dihydrokaempferol and the subsequent hydroxylation of the C3′ position of dihydrokaempferol is catalyzed by Flavonoid 3′-Hydroxylase (F3′H) to produce dihydroquercetin. Whereas the subsequent hydroxylation of the C3′/C5′ positions of dihydrokaempferol is catalyzed by flavonoid F3′5′-hydroxylase (F3′5′H) to produce dihydromyricetin. Dihydrokaempferol, dihydroquercetin and dihydromyricetin are also converted by flavonol synthase (FLS) to kaempferol, quercetin and myricetin, respectively. Dihydroflavonol 4-reductase (DFR) catalyzes the reduction of dihydroquercetin and dihydromyricetin into leucocyanidin and leucodelphinidin, respectively. Furthermore, leucocyanidin and leucodelphinidin are respectively converted to cyanidin and delphinidin by leucoanthocyanidin dioxygenase (LDOX). Leucocyanidin is converted to catechin by leucoanthocyanidin reductase (LAR) while cyanidin and delphinidin are respectively converted to epicatechin and epigallocatechin by anthocyanidin reductase (ANR) (Yonekura-Sakakibara et al., 2019). Uridine diphosphate-glucose: flavonoid-3-O-glucosyltransferase (UFGT) catalyzes the formation of cyanidin-based anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside) from cyanidin on one hand, and of delphinidin-based anthocyanins (delphinidin-3-glucoside, petunidin-3-glucoside and malvidin-3-glucoside) from delphinidin on the other hand (Jeong et al., 2006). The flavonoid biosynthesis and accumulation have been shown to be influenced by environmental parameters like temperature, fungal elicitors, UV radiation, light and soil moisture content (Sparvoli et al., 1994; G. Wang, 2015).

Polyphenols are located mainly in the seeds and skins of grapes. All classes of phenolic compounds have many structures differing in the number and position of hydroxyl and methoxyl groups on the basic backbone. They can be divided into non-flavonoid and flavonoid compounds.

I.4.1.1.1.1 Non-flavonoid compounds

Non-flavonoid compounds include phenolic acids, which are classified into benzoic and cinnamic acids but also other phenolic derivatives such as stilbenes, the most famous molecule of which is resveratrol. Phenolic acids are present in grapes pulp and skin and they are precursors of some volatile phenols. Compounds that are nonflavonoid serve as copigments (hydroxycinnamic and hydroxybenzoic acids) and antioxidants (stilbenes) (Niculescu et al., 2018). The phenolic acids are mostly present as hydroxybenzoic and hydroxycinnamic acids.

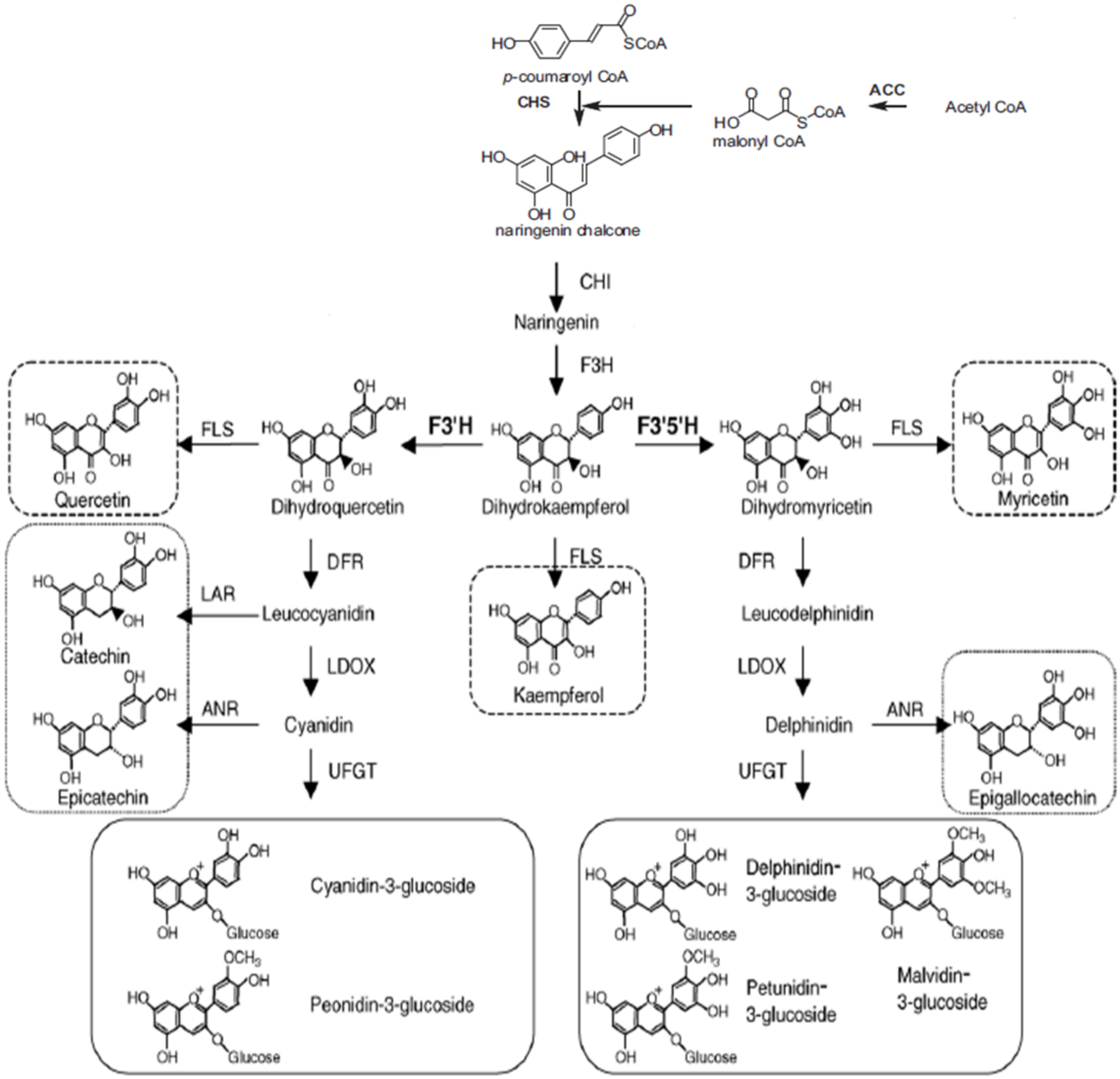


Figure 3: Biosynthesis of flavonoids. Adapted from: (Jeong et al., 2006). CHS: Chalcone Synthase; ACC: Acetyl-CoA Carboxylase; CHI: Chalcone Isomerase; F3H: Flavanone 3-Hydroxylase; F3′H: Flavonoid 3′-Hydroxylase; F3′5′H: F3′5′-Hydroxylase; FLS: Flavonol Synthase; DFR: Dihydroflavonol 4-reductase; LDOX: Leucoanthocyanidin Dioxygenase; LAR: Leucoanthocyanidin Reductase; ANR: Anthocyanidin Reductase; UFGT: Flavonoid-3-O-glucosyltransferase

I.4.1.1.1.1.1 Hydroxybenzoic acids

Hydroxybenzoic acids are differentiated by the substitution of their benzene nucleus. Being included in condensed tannins and the precursor of all hydrolyzable tannins, the principal hydroxybenzoic acid in grapes is gallic acid (Garrido & Borges, 2013) which has antiinflammatory, antioxidant, anti-cancer, antiviral and antifungal properties (Georgiev et al., 2014) (Figure 4).

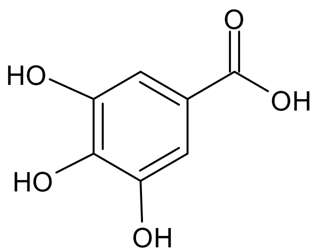
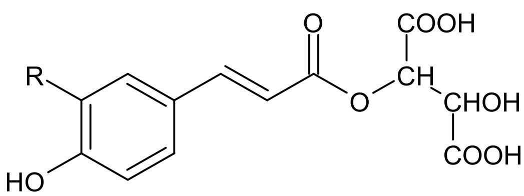


Figure 4: Chemical structure of gallic acid (García-Pérez et al., 2019)

I.4.1.1.1.1.2 Hydroxycinnamic acids

Hydroxycinnamic acids are predominantly present in grapes in the form of tartaric esters (Figure 5): caffeoyl tartaric (caftaric), p-coumaroyl tartaric (coutaric) and feruloyl tartaric (fertaric) acids. They display mostly in the trans isomeric forms but also exist in the cis forms (Garrido & Borges, 2013).



R = H: p-coumaroyl tartaric (coutaric acid)
R = OH: caffeyol tartaric (caftaric acid)
R = OCH_3: feruloyl tartaric (fertaric acid)

Figure 5: Chemical structures of some hydroxycinnamic ester derivatives in grapes (ITV, 1998)

I.4.1.1.1.1.3 Stilbenes

Stilbenes are natural phenolic compounds and they contain two aromatic nuclei joined by a molecule of ethanol or ethylene. Grapes are considered the most important food source of these substances. Among the stilbenes, the trans isomer of resveratrol (Figure 6) which is mainly found in the skin is involved in health benefits due to its antioxidant action (Jackson, 2000) and after being identified in wine and related to the " French paradox ", it has aroused scientists' interest (Katalinic´ et al., 2010).

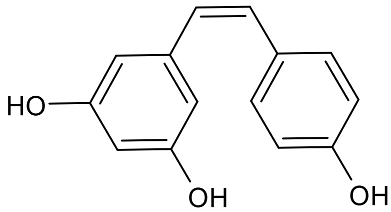


Figure 6: Chemical structure of Resveratrol (Ribéreau-Gayon et al., 2000)

I.4.1.1.1.2 Flavonoid compounds

The specific chemical composition of grape flavonoids makes them one of the most potent nutraceuticals in food. They are also used as phytopharmaceutical products, able to alleviate oxidative stress due to their effect in scavenging free radicals (Georgiev et al., 2014). Flavonoid compounds have a common basic structure of 15 carbon atoms comprising two aromatic nuclei, joined by a five-carbon, central, oxygen-containing ring (Figure 7), but they differ according to the substituents. Flavonoids are mainly synthesized in the skins and seeds of grapes, and up to 75 to 90% of them are found in the seeds (Jackson, 2000). They are mainly composed of anthocyanins and flavonols, predominantly existing in grape skins, and of flavan-3-ols present in grape seeds and skins (Xing et al., 2016).

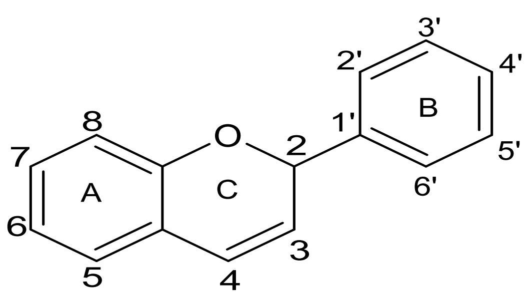


Figure 7: Basic skeleton of flavonoid compounds (Jackson, 2000)

I.4.1.1.1.2.1 Anthocyanins

Anthocyanins are water soluble flavonoid pigments and this favors their transfer into the must and wine during vinification (Moreno & Peinado, 2012). They represent an important family of polyphenol compounds in grape skins and are responsible for the color of the produced wine which is a notable sensory attribute (Mateus et al., 2002). They can also accumulate in the pulp of a few special grapes of *Vitis vinifera* cultivars like Garnacha Tintorera which is a hybrid of

Petit Bouschet and Grenache (He et al., 2010). The health benefits of anthocyanins have received a lot of attention, as epidemiological studies have shown that moderate consumption of anthocyanin products like red wine is linked to a lower risk of cardiovascular disease (Mori et al., 2007). The structure of anthocyans comprises two benzene rings linked by an oxygenated, unsaturated and cationic heterocycle, the flavylium cation (Ribéreau-Gayon et al., 2000). Anthocyanins are glycosides of anthocyanidin that constitutes the chromophore group of the pigment. The individual grape anthocyanins are the 3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin (Figure 8).

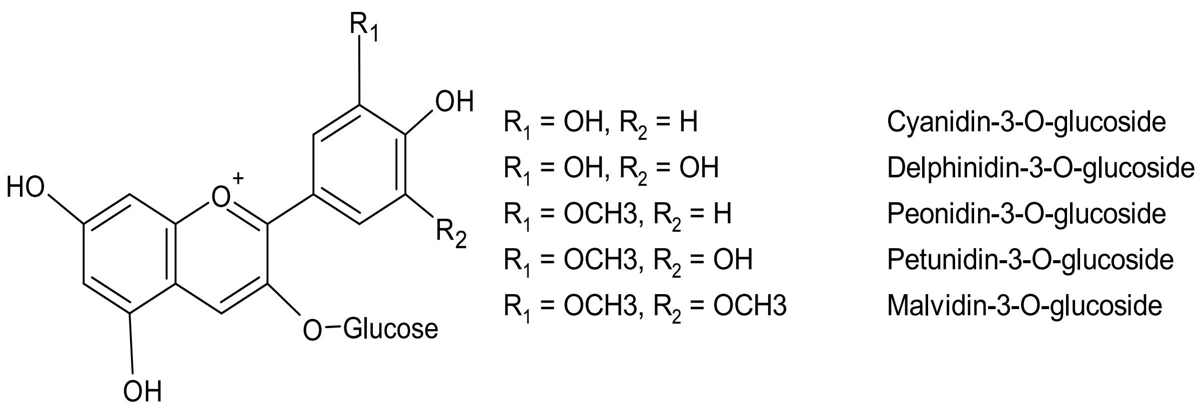
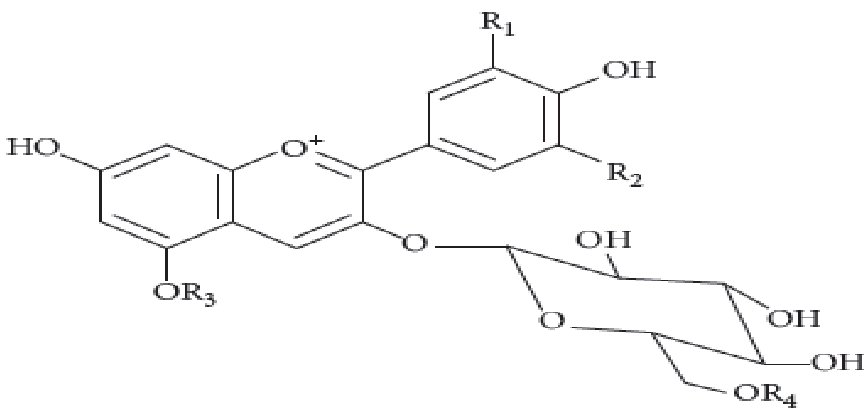


Figure 8: Chemical structure of grape anthocyanins (Benmeziane et al., 2016)

The latter is the most abundant molecule in all grape cultivars and may be considered to form the basis of the color of red grapes and their resulting wine (Ribéreau-Gayon et al., 2000). Glycosylated derivatives of these anthocyanins are esterified at the C6 position of glucose with caffeic, acetic or para coumaric acid (Moreno & Peinado, 2012) (Figure 9).



R_3 = H, glucose

R_4 = H, acetyl, p-hydroxycinnamyl (cis, trans), caffeoyl

Figure 9: Chemical structure of esterified monoglycosidic anthocyanins (Flamini, 2013)

I.4.1.1.1.2.2 Flavan-3-ols

Flavan-3-ols exist in grapes as oligomers and polymers, common names given to condensed tannins or proanthocyanidins, due to their capacity to undertake hydrolysis under hot acidic conditions that leads to the production of anthocyanidin pigments, procyanidins (polymers of catechin and epicatechin) when release cyanidin and prodelphinidins (polymers of gallocatechin and epigallocatechin) when release delphinidin. In tannins of grape skins, procyanidins and prodelphinidins are found but in tannins of grape seeds, only procyanidins are present (Jackson, 2014). Flavan-3-ols are transferred into the must through the steps of the winemaking process such as crushing, maceration and fermentation. They are extracted from grape, seeds and skins and they confer astringency to red wine (Conde et al., 2007) due to their affinity with the saliva proteins. The structure of tannins, specifically polymerization degree (DP) and galloylation degree (DG) of flavan-3-ols is related to astringency and bitterness sensations of wine (Flamini, 2013). Flavanols undergo polymerization reactions and can form, by reaction with themselves or with anthocyanins, numerous tannin-tannin or anthocyanin-tannin derivatives in wines. For the production of stable red wine color, polymeric pigments produced through ethyl linkages between grape tannins and anthocyanins are essential (Palade & Popa, 2018; Teng et al., 2019). Grape tannins have been shown to possess various therapeutic properties including antioxidant plasma activity and they are used for the treatment of skin inflammation and injuries and circulatory disorders (Flamini, 2013; Sieniawska, 2015). Tannins mainly comprise subunits of (-

)-epicatechin, but also important amounts of epigallocatechin, (+)-catechin, and epicatechin-3-O-gallate (Fournand et al., 2006) . Flavan-3-ols contain a pyran heterocycle with no double bond and hydroxylated in position 3 of the flavonoid skeleton. The hydroxyl group at position 3 can be esterified with gallic acid and the greater the concentration of gallic acid in tannins, the more bitterness and astringency in wines (Moreno & Peinado, 2012) (Figure 10).

In addition to the nature of the flavan-3-ols monomeric building units, the procyanidins structure depends on the number of these units, called the degree of polymerization, as well as by the type and the position of the intermonomeric bonds (Benmeziane, 2018). Two types of procyanidins are distinguished (Figure 11): type B is characterized by a C4-C8 (B1 to B4) or C4-C6 (B5 to B8) interflavan bond and type A contain an ether bond between carbons C2-C5 or C2-C7 in addition to the interflavan bond (Moreno & Peinado, 2012).

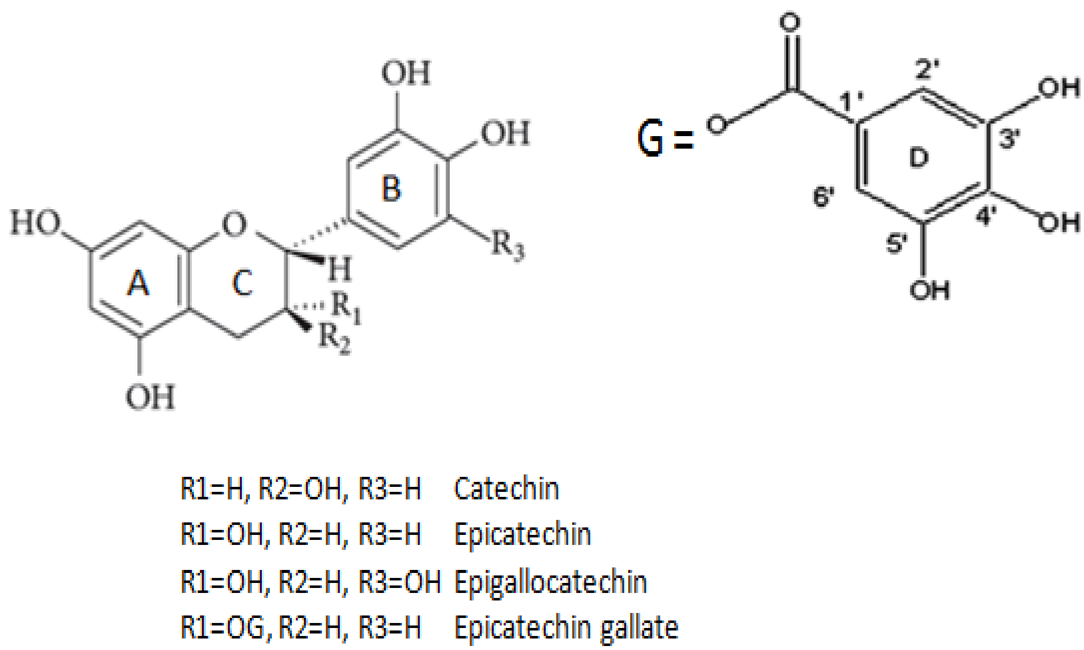


Figure 10: Flavanol monomers. Adapted from: (Ghosh et al., 2008)

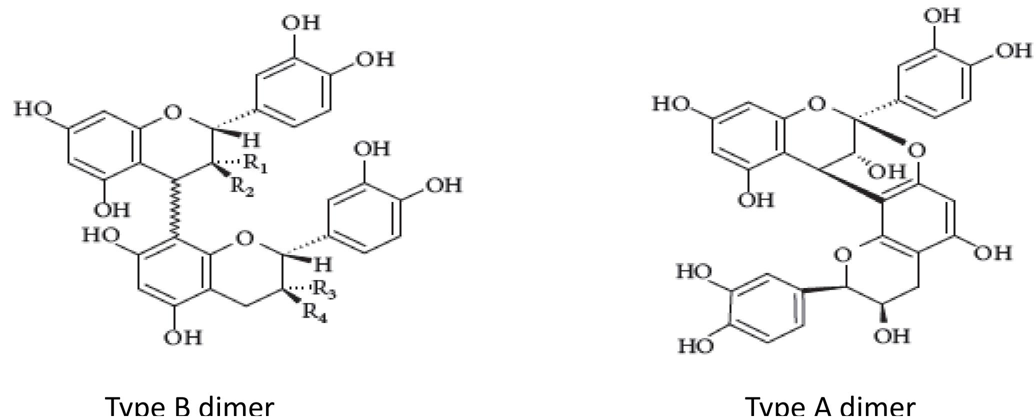


Figure 11: Types of procyanidins. Adapted from (Flamini, 2013)

A generalized proanthocyanidin polymer is represented in Figure 12. Seed proanthocyanidins have a relatively short mean degree of polymerization (mDP), with a subunit structure composed of a combination of flavan-3-ols in both terminal and extension subunits whereas skin proanthocyanidins have a long mDP and they consist primarily of catechin terminal subunits and epicatechin and epigallocatechin extension subunits (Downey et al., 2003).

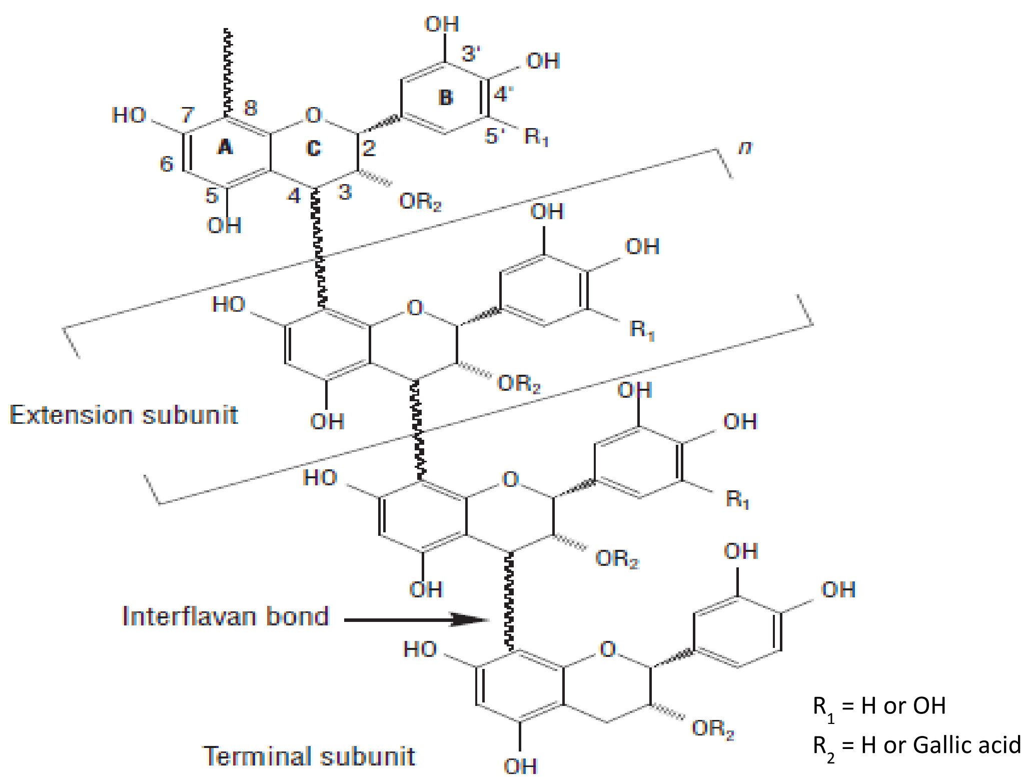


Figure 12: Generalized proanthocyanidin polymer. Adapted from (Downey et al., 2003)

I.4.1.1.1.2.3 Flavonols

Flavonols are pale yellow pigments containing a pyrone heterocycle and comparing with other grape flavonoids, they are found with the lowest concentration. They can serve as natural sunscreen for the grapes and are present in the skins in glycosylated forms in the C3 position of the flavonoid skeleton forming glucosides, galactosides, rhamnosides, rutinosides, and glucuronides. The principal flavonols are found in wine as aglycones (kaempferol, quercetin, myricetin and isorhamnethine) (Figure 13) where they act as copigments with anthocyanins (Jackson, 2000; Palade & Popa, 2018). In grapes and wine, flavonols are considered as cofactors for color development and they are synthesized as anthocyanins along the same pathway (Tarara et al., 2008). The intake of flavonols has been linked to a number of health benefits, including antioxidant capacity and a lower risk of vascular disease (Panche et al., 2016). Quercetin's primary biological function is to prevent human platelets from aggregating, and it appears that it also prevents carcinogens and cancer cell development in human tumors (Flamini, 2013).

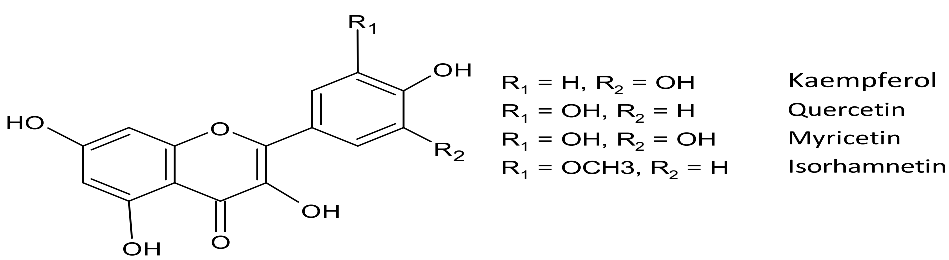


Figure 13: Aglycone structure of principal flavonols. Adapted from (Ribéreau-Gayon et al., 2000)

Phenolic compounds in edible plants are originated from tyrosine (Tyr) and phenylalanine (Phe) which are essential aromatic amino acids (AAAs) for protein production in all living cells. These AAAs also act as precursors in plants for a vast range of natural products that are important for plant reproduction, growth, defense, development and environmental responses (Maeda & Dudareva, 2012). Tyr and Phe are produced from the shikimate and aromatic amino acids (AAA) pathways and serve as starting points for the production for phenylpropanoids, relating primary to specialized metabolic pathways.

I.4.1.1.2 The shikimate pathway

As shown in Figure 14, the shikimate pathway begins with the condensation of phospho-enol-pyruvic acid (PEP) with erythrose-4-phosphate (E4P). This aldol condensation is catalyzed by 3-Deoxy-D-arabino-heptulosonate7-phosphate (DAHP) synthase to produce DAHP.

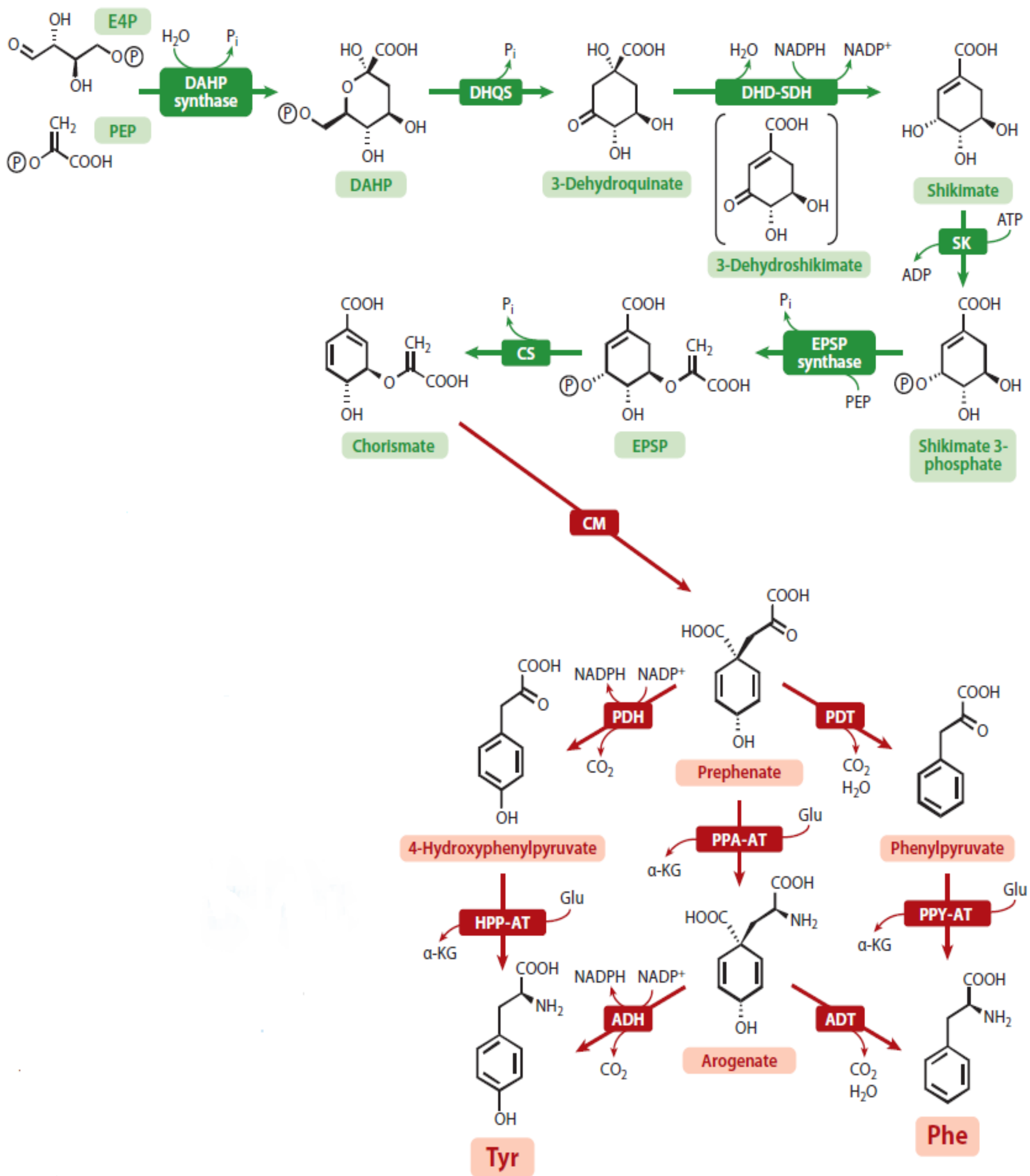


Figure 14: Shikimate and phenylpyruvate pathways. DHD and SDH form a bifunctional DHD-SDH enzyme in plants. The intermediates of the DHD-SDH enzyme-catalyzed reactions (3-dehydroshikimate) are shown in brackets. DAHP: 3-deoxy-D-arabino-heptulosonate 7-phosphate; E4P: D-erythrose 4-phosphate; EPSP: 5-enolpyruvylshikimate 3-phosphate; Glu: glutamate; α-KG: α-ketoglutarate; PEP: phosphoenolpyruvate; Phe: phenylalanine; Pi: inorganic phosphate; Tyr: tyrosine. Enzyme abbreviations: ADH: arogenate dehydrogenase; ADT: arogenate dehydratase; CM: chorismate mutase; CS: chorismate synthase; DHD: 3-dehydroquinate dehydratase; DHQS: 3-dehydroquinate synthase; HPP-AT: 4-hydroxyphenylpyruvate aminotransferase; PDH: prephenate dehydrogenase; PDT: prephenate dehydratase; PPA-AT: prephenate aminotransferase; PPY-AT: phenylpyruvate aminotransferase; SDH: shikimate dehydrogenase; SK: shikimate kinase. Adapted from (Maeda & Dudareva, 2012)

DAHP is converted to 3-dehydroquinate by 3-Dehydroquinate synthase (DHQS) in five consecutive chemical reactions: alcohol oxidation, β-elimination of inorganic phosphate, carbonyl reduction, ring opening, and intramolecular aldol condensation. Then, two enzymatic reactions in the shikimate pathway follow: the dehydration of 3-dehydroquinate to 3-dehydroshikimate to introduce the first double bond in the ring, and the reversible reduction of 3-dehydroshikimate into shikimate using nicotinamide adenine dinucleotide phosphate (NADPH). 3-Dehydroquinate dehydratase (DHD) and shikimate dehydrogenase (SDH) catalyze the respective reactions.

After that, the C3 hydroxyl group of shikimate is phosphorylated using ATP as a cosubstrate to produce shikimate 3-phosphate. This phosphorylation is catalyzed by Shikimate kinase (SK). The second to last step of the shikimate pathway that yields 5-enolpyruvylshikimate 3-phosphate (EPSP) is catalyzed by EPSP synthase, by transferring the enolpyruvyl moiety of PEP to the 5-hydroxyl position of shikimate 3-phosphate. Finally, the last step of the shikimate pathway, the 1,4-antielimination of the 3-phosphate and C6-pro-R hydrogen from EPSP that produces chorismate is catalyzed by chorismate synthase (CS).

I.4.1.1.3 The phenylpyruvate pathway

The Phe and Tyr pathways start when chorismate is converted to prephenate by chorismate mutase (CM) as shown also in Figure 14. The subsequent conversion of prephenate to Phe and Tyr may take place via the phenylpyruvate pathway which is primarily used by most microorganisms studied to date. In the phenylpyruvate pathway, prephenate is subjected to dehydration/decarboxylation by prephenate dehydratase (PDT) or to dehydrogenation/decarboxylation by prephenate dehydrogenase (PDH) to yield phenylpyruvate and 4-hydroxyphenylpyruvate, respectively. Then, phenylpyruvate is converted to Phe by phenylpyruvate aminotransferase (PPY-AT) and 4-hydroxyphenylpyruvate is converted to Tyr by 4-hydroxyphenylpyruvate aminotransferase (HPP-AT).

I.4.1.1.4 The phenylpropanoid pathways

The phenylpropanoid pathways serve as rich sources of metabolites in plants and include the flavonoid, stilbenoid, benzenoid, anthocyanin, and lignin pathways (Manela et al., 2015). As shown in Figure 15, the first and essential step of the general phenylpropanoid pathway is the transformation of phenylalanine into trans-cinnamic acid by the action of phenylalanine

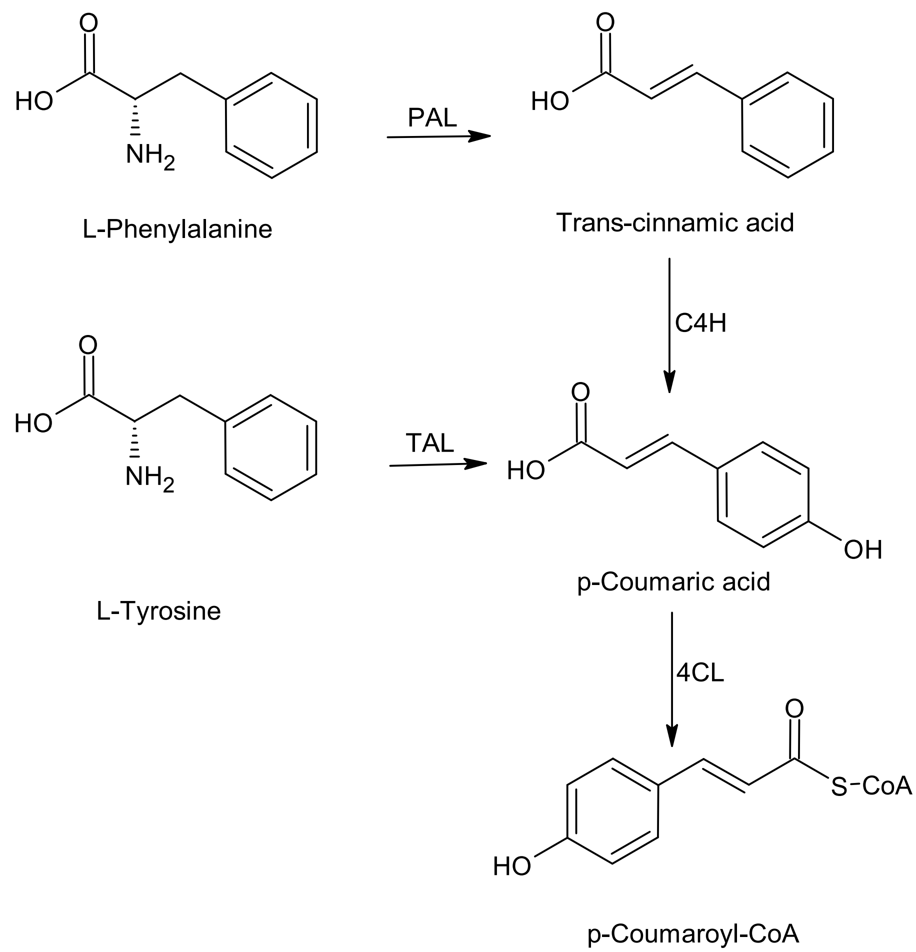


Figure 15: the general phenylpropanoid pathway. Adapted from (Emiliani et al., 2009). PAL: phenylalanine ammonia-lyase; C4H: Cinnamic acid 4-hydrolase; TAL: tyrosine ammonia lyase; 4CL: CoA ligase

ammonia-lyase (PAL). Cinnamic acid 4-hydrolase (CH4) catalyzes the transformation of trans-cinnamic acid into p-coumaric acid, which is then converted by p-coumaroyl: CoA ligase (4CL) to p-coumaroyl-CoA. In addition to the PAL activity, some plants have a tyrosine ammonia lyase (TAL) activity that is responsible for the production of p-coumaric acid directly from tyrosine, which leads to the subsequent formation of p-coumaroyl-CoA which is the precursor of the flavonoid biosynthesis that involves complex highly branched pathways (Emiliani et al., 2009).

I.4.1.2 Origin, structure and function of aroma compounds in grapes and wine

Volatile organic compounds are generally classified as varietal aroma compounds when come directly from the grapes, prefermentative aroma compounds formed during grape processing, fermentative aroma compounds formed by yeast and bacteria and postfermentative aroma compounds that are formed during conservation and ageing of wine (Zhu et al., 2016). It is recognized that the most important period for biosynthesis, accumulation and preservation of grape volatiles is between véraison and harvest (Alessandrini et al., 2017). Grape skins contain more than half of the volatile compounds found in the grape berries and their cell walls act as a barrier to the diffusion of aroma compounds (Martínez-Lapuente et al., 2019). The human olfactory system cannot perceive aroma compounds at levels below their olfactometry threshold (Manolache et al., 2019). Due to the fact that the olfactory impact of a volatile is determined by whether it is present at concentrations above its perception threshold, odor activity values (OAVs) were introduced to help choose impact odorants (Jiang et al., 2013). OAV is defined as the concentration of a single aroma compound divided by the odour threshold for that compound. (Yang et al., 2015). Aroma compounds with odor activity values (OAVs) greater than 1 contribute to the overall aroma character of a wine; nevertheless due to additive effects, some volatile compounds contribute to wine's aroma even if their OAV is lower than 1 (Jiang et al., 2013). The terpenic alcohols such as linalool, geraniol and citronellol from muscat-related grapes are responsible for varietal wine aroma nuances. The peppery flavor of Sauvignon varieties and the kerosene flavor in aged Riesling wines are caused by methoxypyrazines and some nor-isoprenic compounds, respectively (Ferreira et al., 1998; Hellín et al., 2010; Styger et al., 2011). Aroma-active volatiles can be classified as terpenes, methoxypyrazines, alcohols, aldehydes, esters, fatty acids, ketones and volatile thiols imparting different aromas to wine when they are present at higher than their odor perception threshold.

I.4.1.2.1 The terpenoid pathway

Terpenoids are biosynthesized through the condensation of the two precursors, isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP). These two universal precursors are synthesized by two different biochemical pathways: the mevalonic acid (MVA) pathway and the 2C-methyl-d-erythritol-4-phosphate (MEP) pathway which is also known as the 1-deoxy-D-xylulose-5-phosphate (DXP) pathway (Figure 16).

The MVA pathway begins with the condensation of two acetyl-CoA units to yield acetoacetyl-CoA by acetoacetyl-CoA thiolase, followed by condensation with a third acetyl-CoA unit by 3-hydroxy-3-methylglutaryl-CoA synthase to produce hydroxymethylglutaryl-CoA (HMG-CoA). Then HMG-CoA reductase converts HMG-CoA into mevalonate which is subsequently phosphorylated by mevalonate kinase to form mevalonate-5-phosphate. A second phosphorylation by phosphomevalonate kinase follows to yield mevalonate 5-diphosphate. The final step of the MVA pathway is catalyzed by mevalonate diphosphate decarboxylase to produce IDP which can be reversibly isomerized into DMADP by IDP isomerase.

The first step of the MEP pathway, the condensation of pyruvate and D-glyceraldehyde-3-phosphate (GAP) that produces 1-Deoxy-d-xylulose 5-phosphate (DXP), is catalysed by DXP synthase (DXS). Then, DXP reductoisomerase reduces DXP into 2C-methyl-D-erythritol 4-phosphate (MEP) that is subsequently converted to 4-diphosphocytidyl-2C-methyl-D-erythritol by 2C-methyl-D-erythritol 4-phosphate cytidyltransferase. The following step which yields 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate (CDP-MEP) is catalyzed by 4-(cytidine 5'-diphospho)-2C-methyl-D-erythritol kinase. After that, 2C-methyl-D-erythritol-2,4-cyclodiphosphate synthase converts CDP-MEP to 2C-methyl-D-erythritol-2,4-cyclodiphosphate which is then reduced to 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate (HMBDP) by 4-hydroxy-3-methylbut-2-enyl diphosphate synthase. HMBDP is finally reduced by HMBDP reductase to yield both IDP and DMADP (Bergman et al., 2019).

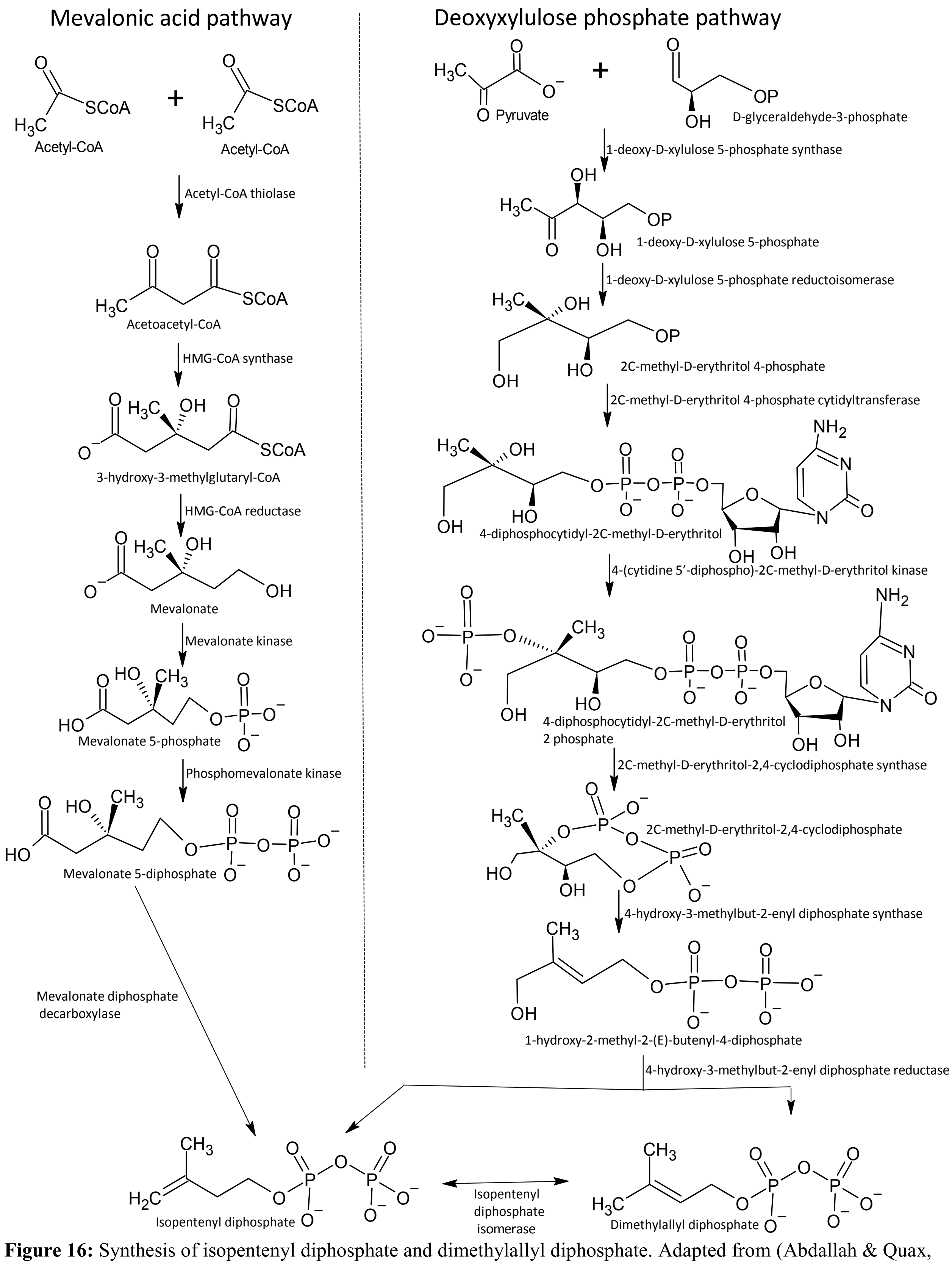


Figure 16: Synthesis of isopentenyl diphosphate and dimethylallyl diphosphate. Adapted from (Abdallah & Quax, 2017)

As shown in Figure 17, IDP and DMADP are condensed by the action of geranyl diphosphate synthase to produce geranyl diphosphate (GDP) from which longer prenyl chains such as farnesyl diphosphate (FDP) and geranylgeranyl diphosphate as well as terpenes are formed. Geranyl diphosphate synthase (GDPS), farnesyl diphosphate synthase (FDPS) and Geranylgeranyl diphosphate synthase (GGDPS) catalyze the condensation of IDP and DMADP to form GDP, FDP and GGDP respectively. Hemiterpene synthase is responsible for the conversion of DMADP into hemiterpenes (5 carbon atoms: C5), the most famous of which is isoprene. Monoterpene synthases catalyze the conversion of GDP to different monoterpenes (C10), such as linalool in lavender and thymol in thyme. Sesquiterpenoids (C15) like caryophyllene, are synthesized from the substrate FDP by sesquiterpene synthases and diterpenoids (C20) like taxol, are derived from the substrate GGDP by the action of diterpene synthase enzyme family.

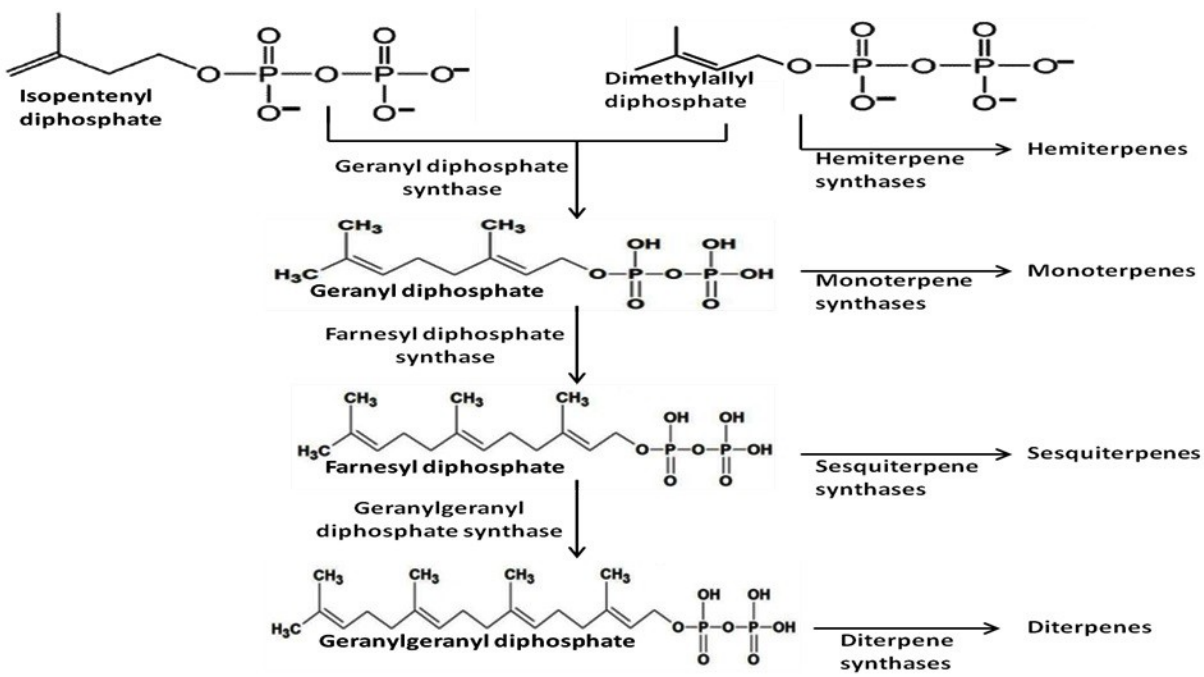


Figure 17: Synthesis of terpenes. Adapted from (Abdallah & Quax, 2017)

Terpenes are involved in a variety of fundamental plant activities, including photosynthesis, respiration, growth, defense, and environmental adaptability. Pigments, fragrances and agrochemicals are only a few of the industrial applications (Carrau et al., 2008). Terpenes occur in grapes both in free and more frequently as odorless glycosylated precursors linked to sugar moieties and are substantially responsible for fruity and floral aromas, while some have resin-like odors such as alpha-terpinene, p-cymene, myrcene, and farnesol (González-Barreiro et al., 2015). Among them, monoterpene alcohols with 10 carbon atoms notably linalool, nerol and geraniol evoke rose-like notes, citronellol gives off an odor of lemongrass and alpha-terpineol emits the odors of lily of the valley. Some sesquiterpenes with 15 carbon atoms such as rotundone confer the black pepper character to Shiraz grapes and wine (Wood et al., 2008) (Figure 18).

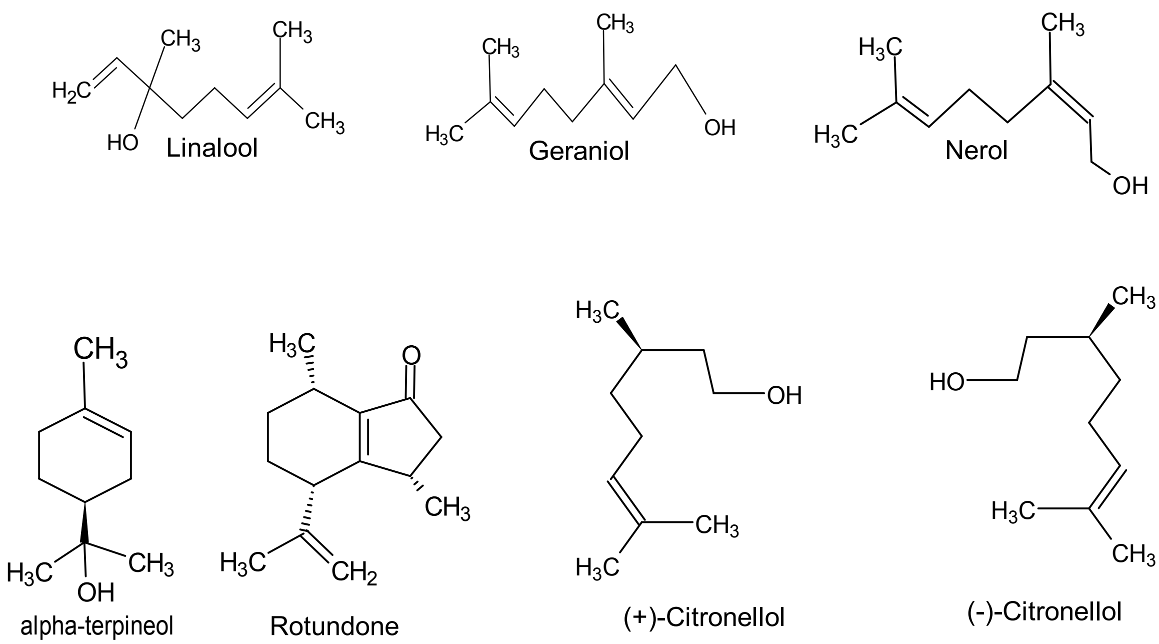


Figure 18: Chemical structures of some terpenes

Another family of terpenes includes carotenoids with 40 carbon atoms such as β-caroten, neoxanthin and lutein. Their oxidative degradation during grape ripening leads to the formation of norisoprenoid compounds with 13 carbon atoms, called C13-norisoprenoids. Even though they are present at trace levels, these compounds contribute significantly to the aroma potential of many wine varieties due to their low sensory thresholds. They are mostly present in glycosylated form and the enzymatic cleavage or acid hydrolysis during fermentation and conservation allows the liberation of free odor-contributing compounds (González-Barreiro et al., 2015). Among the most important C13-norisoprenoids are the β-damascenone, β-ionone and TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) (Figure 19) which impart to wine a pleasant stewed apple, a characteristic violet-like and distinctive kerosene-like odor, respectively.

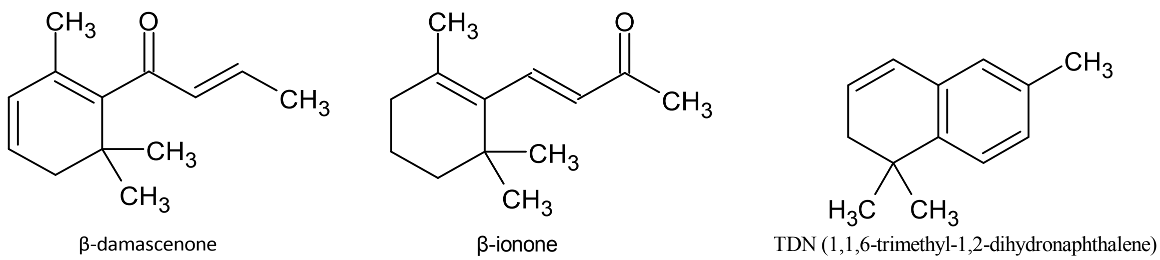


Figure 19: Chemical structures of some C13-norisoprenoids

I.4.1.2.2 Biosynthesis of methoxypyrazines

Methoxypyrazines are nitrogen heterocyclic compounds found in both grapes and wine and they result from the metabolism of amino acids. An important compound which contributes to the bell pepper odor characteristic of Sauvignon blanc cultivar is 3-isobutyl-2-methoxypyrazine (IBMP) (Styger et al., 2011). Some studies suggest that the pathway for IBMP biosynthesis begins with the amino acid leucine but the remainder of the biosynthetic pathway is not yet described. The final step in the biosynthesis of IBMP which has been elucidated in grapevine (Lin et al., 2019), involves the methylation of 3-isobutyl-2-hydroxypyrazine (IBHP) which is catalyzed by an O-methyltransferase (OMT) in the presence of S-adenosyl-L-methionine (SAM) as the methyl group donor (Vallarino et al., 2011) (Figure 20).

I.4.1.2.3 The lipoxygenase pathway

Straight-chain alcohols, aldehydes, esters, acids, and ketones are produced from the lipoxygenase (LOX) pathway and the majority of aliphatic volatiles in grapes are short-chain aldehydes and alcohols, which are considered as "green" aromas.

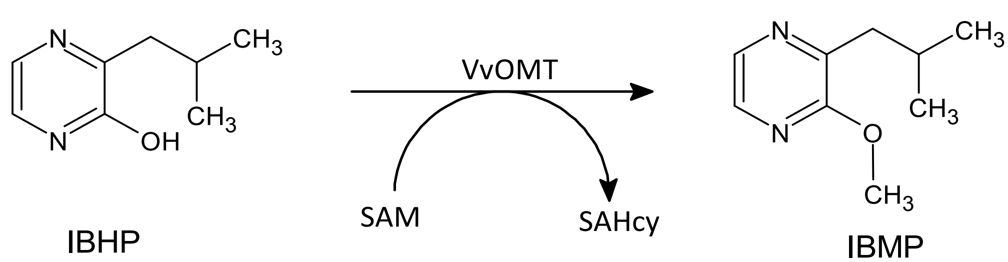


Figure 20: Proposed biosynthesis pathway for 3-isobutyl-2-methoxypyrazine (IBMP). Adapted from (Vallarino et al., 2011). IBHP: 3-isobutyl-2-hydroxypyrazine; VvOMT: O-methyltransferase protein; SAM: S-adenosyl-L-methionine; SAHcy: S-adenosylhomocysteine

Lipoxygenases are a class of fatty acid dioxygenases that catalyze the oxidation of polyunsaturated fatty acids like linoleic and linolenic acids to form straight-chain aliphatic volatiles (Liu et al., 2015). C6 and C9 aldehydes and alcohols are involved in the defense response against pests, diseases and plant wounds (Lin et al., 2019). During the winemaking and aging processes, alcohols can be converted into the respective acetate esters, which have an impact on the aroma quality of the resulting wine. C6 aldehydes and alcohols are present in grapes at a quantity related to the degree of maturation. During crushing of the harvested grapes, large quantities of C6 aldehydes are formed due to the oxidation of the existing unsaturated fatty acids in the must such as linoleic acid and linolenic acid. Then, during alcoholic fermentation, these aldehydes such as hexanal and trans-2-hexenal lead respectively to the formation of hexanol and trans-2-hexenol which are responsible for the herbaceous and vegetable aromas in wine (Figure 21) (Sabon et al., 2002).

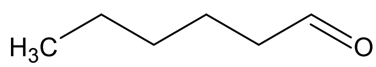
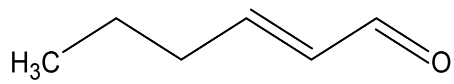
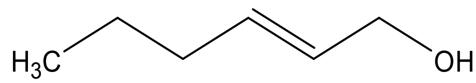
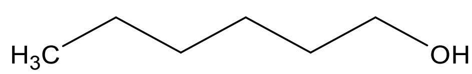
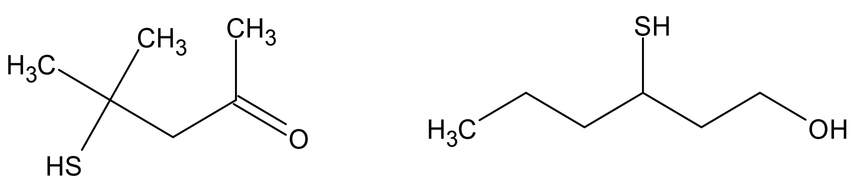
Compound	Structure	Odor descriptor
Hexanal		Herbaceous
(E)-2-Hexenal		Grass
(E)-2-Hexenol		Green
1-Hexanol		Green

Figure 21: Structure and odor properties of C6 aldehydes and alcohols. Adapted from (Ferreira & Lopez, 2019)

I.4.1.2.4 Biosynthesis of volatile thiols.

Although many thiols (mercaptans) generate off-putting odors, not all sulfur-containing compounds, nor even all thiols, are detrimental for wine quality (González-Barreiro et al., 2015). In fact, powerful odoriferous thiols including 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexanol (3MH) compounds are known as polyfunctional thiols, and are distinguished from simple thiols or mercaptans because of their pleasant aroma. 4MMP and 3MH belong to the class of varietal aromas because they result from the cleavage mechanism of odorless precursors present in grapes or musts. These precursors are cleaved during alcoholic fermentation due to the yeast through its beta-lyase activity (Roland et al., 2011). Cysteinylated (cys) and glutathionylated (glut) conjugates have been proposed as thiol precursors. 3MH could also be produced from E-2-hexenal, a "green leaf volatile", according to another proposed pathway in which E-2-hexenal may combine directly with H_2S to produce 3MH, or it may react with cysteine and/or glutathione to produce precursors that yeast biochemically transforms to thiols (Santiago & Gardner, 2015). 4MMP and 3MH (R isomer) are responsible for the black currant and grapefruit odor characteristics of Sauvignon blanc wine, respectively (Figure 22) (Styger et al., 2011).



a b

Figure 22: Structures of: (a) 4-Methyl-4-mercaptopentan-2-one, (b) 3-Mercapto-1-hexanol

I.4.1.2.5 Biosynthesis of higher alcohols

Higher alcohols, having more than two carbon atoms include aliphatic alcohols like isobutyl (methyl-2-propanol-1), methyl-2-butanol-1 and methyl-3-butanol-1, described as smelling of fusel oil, malt, or solvent and aromatic alcohols like 2-phenylethan-1-ol with a rose scent (Figure 23) (Cameleyre et al., 2015). A number of them are produced during fermentation from grape amino acids or directly from sugars and the increase of the fermentation rate due to several factors such as oxygenation and high temperature leads to the increase of their formation (Ribéreau-Gayon et al., 2000b). In the yeast cell, branched-chain amino acids leucine, isoleucine and valine produce higher alcohols which are isoamyl alcohol, optically active amyl alcohol and isobutanol respectively via the Ehrlich reaction (Swiegers et al., 2005). The Ehrlich pathway begins with a transamination reaction, in which the amino group of an amino acid is transferred to α-ketoglutarate, resulting in an α-keto acid and glutamate. Then, the α -keto acid is decarboxylated into an aldehyde that can either be reduced by a NADH-dependent reaction to its correspondent higher alcohol or it can be oxidized by a NAD^+-dependent reaction into a volatile carboxylic acid (Styger et al., 2011).

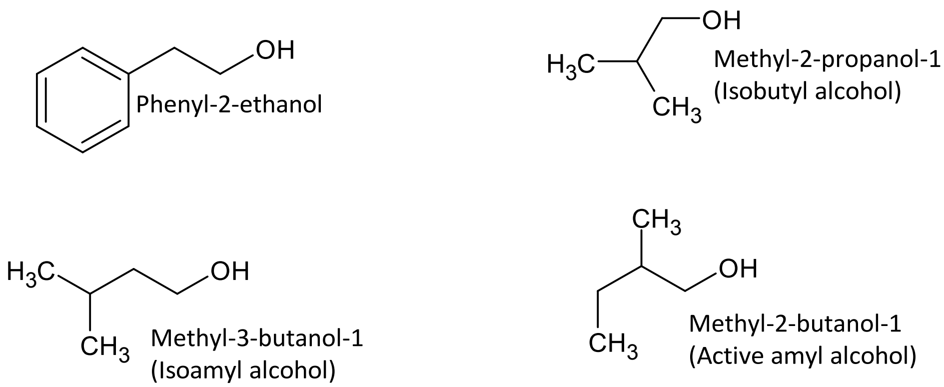


Figure 23: Structure of some higher alcohols in grapes

I.4.1.2.6 Biosynthesis of esters

The esters whose functional group is R_1-CO-O-R_2, can be classified as follows (Moreno & Peinado, 2012): alcohol acetates, the most volatile types; ethyl esters such as tartrate, lactate and malate, whose volatility depends on the volatility of the acid from which they are derived; and other esters like caffeoyl tartrate, lactates, pyruvates and succinates of alcohols other than ethanol. Esters are considered to be among the most important classes of volatile aromas compounds and their production during fermentation affects largely the fruity flavors associated with wine. Among the most significant ones are isobutyl acetate, isoamyl acetate and ethyl acetate (Figure 24) which impart banana, pear-drops and solvent-like aromas to wine, respectively (Swiegers et al., 2005). Regarding the enzymatic formation of esters, an acid is first activated by combining it with coenzyme A (CoA), before reacting with an alcohol to yield an ester. Acetyl-CoA (from pyruvate) or any of the acyl-CoA compounds produced by the enzyme acyl-CoA synthetase, can be used as a coenzyme donor. Therefore, the ethanolysis of acyl-CoA, an intermediate metabolite of fatty acid metabolism, leads to the production of ethyl esters like ethyl octanoate, ethyl hexanoate, and ethyl butanoate. On the other hand, the reaction between acetyl-CoA and alcohols that are produced from the degradation of amino acids, carbohydrates, and lipids, leads to the formation of acetate esters like phenethyl acetate, hexyl acetate, propyl acetate and isoamyl acetate (Styger et al., 2011).

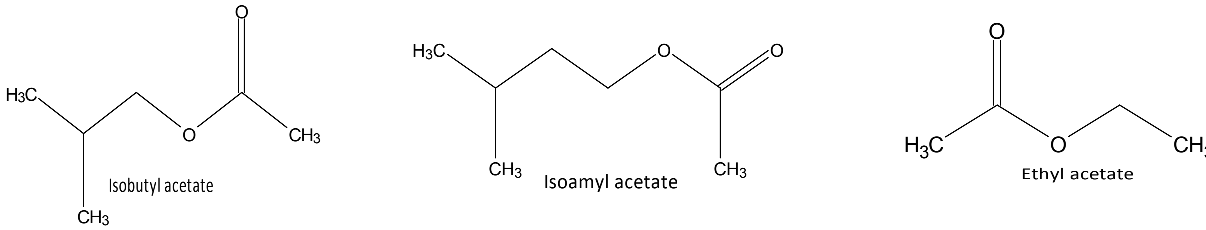


Figure 24: Structure of some esters in grapes

I.4.1.3 Effect of the altitude on grapes and wine

Altitude with its associated environmental conditions exerts important influences on grapes and wine. The key findings of the studies performed on the impact of altitude on physicochemical behavior, phenolic and aroma compounds of grapes and wine are listed in Table 1. In the following paragraphs, the altitude effect is presented in two parts. The first part discusses the altitude effect on the physicochemical behavior of grapes and wine and the second part discusses the altitude effect on the phenolic and aroma compounds of grapes and wine.

I.4.1.3.1 Altitude effect on physicochemical behavior of grapes and wine

Chasselas grapes cultivated on steep terraced slopes at altitudes between 375 and 575 meters were monitored by Rienth et al. (2020) in the AOC-Lavaux region in Switzerland. For all the three consecutive years of the study, altitude was the main driver of precocity and was consistently associated with the date of budburst and flowering. The temperature decreases with altitude, usually in the range of 0.65 to 1.0 °C for 100 m gain in elevation, let the plots at higher elevations show a delay in budburst and flowering. Because these plots ripen later during cooler periods of the year, they have the highest potential for producing high-quality grapes (with higher acidity and lower alcohol levels) under future warmer temperatures leading to a maximum terroir expression. The production of high-quality grapes is linked to the daily thermal amplitude, because of the nighttime temperatures usually lower in plots with high elevation (Gutiérrez-Gamboa et al., 2021). The grapevines that are cultivated in vineyards with low night temperatures present higher potential for color and volatile compounds (Gutiérrez-Gamboa et al., 2018). In cool-climate viticulture, the best expression of terroir is achieved when the grapevine variety's precociousness permits it to ripen its fruit at the end of the growing season (Van Leeuwen & Seguin, 2006) when the grapes contain balanced levels of soluble solid, acidity, phenolic, nitrogenous, and aroma compounds. The wines produced from high-altitude sites are generally fresh, with high acidity, high aromatic quality and a lower alcohol degree (Gutiérrez-Gamboa et al., 2021).

Cabré & Nuñez (2020) used climatic projections from the IPSL-CM5A-MR climate model of Institute Pierre Simon Laplace (France) for the near (2015–2039) and the far (2075–2099) future with the aim to evaluate the impacts of future climate on Argentinean winegrowing regions.

Table 1: Effect of increasing altitude on physicochemical behavior and on phenolics and aromas of grapes and wine

Cultivar	Affecting features	Effect on Grapes	References
Chasselas	Weather: lower temperature	Acidity ↑ and alcohol levels↓	Rienth et al., 2020
Niágara Rosada and Isabel	Weather: lower temperature	No effect on TSS. TTA ↑, pH and TSS/TTA ↓	Meneghelli et al., 2018
Syrah	Weather: lower temperature	Malic and succinic acid ↑. Citric acid, glucose and fructose ↓	Oliveira et al., 2019
Ancellotta, Garganega and Fiano	Weather: lower temperature	At harvest, TSS reached satisfactory values for producing quality wines	Malinovski et al., 2016
Lambrusco, Negroamaro and Nero d'Avola		At harvest, TSS did not reach satisfactory values for producing quality wines	
Chardonnay	Weather: lower temperature. Higher precipitations	Acidity ↑. TSS ↓ in the first year of the study. No effect on TSS in the second year of the study. At harvest, TSS reached satisfactory values for balancing acidity values	Regina et al., 2010
Pinot Noir		Acidity ↑, TSS ↓. At harvest, TSS did not reach satisfactory values for balancing acidity values	

Not reported	Weather: lower temperature	In the Argentinian region where the biggest grapevine production is concentrated, the annual mean temperature is expected to increase from 4 to 7 °C by 2099 under RCP8.5 emission of greenhouse gases scenario. High altitude sites and cool climate varieties seem to be benefited	Cabré & Nuñez, 2020
Muscat of Bornova	Higher percentage of lime in the soil	Trans-caftaric and trans-coutaric acids ↓	Karaoğlan et al., 2015
	Higher solar radiation	Quercetin-3-O-glycoside ↑	
Carignan	Weather: higher evapotranspiration, higher annual accumulated growing degree days and higher average temperature. Limited water access Higher solar radiation	Anthocyanins and total polyphenol index (TPI) ↑. Seed maturity index (SM) ↓	Edo-Roca et al., 2013
Grenache	Weather: lower temperature	No significant effect on anthocyanins and SM. TPI ↓	
Ekşikara	Weather: lower temperature Higher solar radiation	<u>The following parameters ↑:</u> anthocyanins in skins and whole berries; tannins and trans-resveratrol in skins and seeds; TP and antioxidant activity in skins, seeds and whole berries; petunidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside, acylated anthocyanins, gentisic acid, catechin, rutin and isorhamnetin-3-glucoside in whole berries; caffeic acid in seeds; chlorogenic acid in seeds and whole berries; epicatechin in skins. <u>No effect on the following parameters:</u> gallic acid in seeds and whole berries; rutin and isorhamhetin-3- glucoside in skins and seeds; procyanidin B1 and procyanidin B2 in seeds and whole berries. Malvidin-3-O-glucoside in skins and whole berries ↓	Coklar, 2017

Cabernet Sauvignon	Weather: lower temperature and large diurnal temperature range. Brown sandstone soil	Quercetin derivatives and cyanidin-derived anthocyanins ↑. Myricetin derivatives and delphinidin-derived anthocyanins ↓	Li et al., 2011
Cabernet Sauvignon, Carmenere, Syrah and Merlot	Weather: lower temperature, humidity and rainfall. Higher diurnal temperatures	Anthocyanins and trihydroxylated flavonols ↑. Dihydroxylated flavonols and acylated anthocyanins ↓	Liang et al., 2014
Cabernet Sauvignon	Weather: lower temperature and humidity, and higher diurnal temperatures. Stronger sunlight and abundant UV-B radiation	Cyaniding-type anthocyanins and quercetin-type flavonols ↑. No effect on flavan-3-ols	Xing et al., 2015
Syrah	Weather: lower temperature and bigger temperature difference between daytime and night time	Total phenols, non-flavonoids, flavonoids, total anthocyanins and skin tannins ↑. Trans-resveratrol and seed tannins ↓	Barreto de Oliveira et al., 2019
Touriga Nacional and Touriga Francesa	Weather: lower temperature, higher humidity	Carotenoids ↑	Oliveira et al., 2004
Merlot	Weather: lower temperature and bigger temperature difference between daytime and night time	Isoamyl acetate and ethyl hexanoate ↑. Total aroma compounds ↑ from 214 m to 450–600 m then ↓ up to 1100 m	Jiang et al., 2013
Cabernet Sauvignon		Isoamyl acetate, ethyl hexanoate and ethyl octanoate ↑. Total aroma compounds ↓	
		Grape stems	
Moscatel	Weather: lower temperature and absence of significant thermal stress. Absence of water stress	Total phenols, ortho-diphenols, flavonoids, most of individual phenolics, and antioxidant activity ↓	Gouvinhas et al., 2020

		Grapes and wine	
Touriga Nacional	Weather: lower temperature	Anhocyanins in skins and in wine ↑. Cat and PC in skins ↓. TPA in skins and seeds ↓. PC, color and astringency of wine ↓	Mateus et al., 2001
Touriga Francesa		Anhocyanins in skins and in wine ↑. Cat, PC and TPA in skins ↓. Cat and TPA in seeds ↑. Color and astringency of wine ↓	
		Wine	
Cabernet Sauvignon	Weather: lower night-time temperature. Stronger sunshine	No significant effect on aromatic profile. Isoamyl acetate, ethyl octanoate and fruity aromas ↓	Bao Jiang, 2012
Merlot	Weather: lower temperature. Higher rainfall and diurnal temperatures	Total phenolics, total flavonoids, total anthocyanins, sum of individual phenolics, salicylic acid and antioxidant capacity ↑. No effect on quercetin, trans-resveratrol and tannins	Jin et al., 2017
Cabernet Sauvignon	Higher sunshine hours and increased UV radiation	Total phenolics, total flavonoids, total anthocyanins, sum of individual phenolics, salicylic acid, antioxidant capacity, quercetin, trans-resveratrol and tannins ↑	
Glera	Weather: higher heat accumulation degrees	Total aroma compounds, elegance and floral aroma ↑	Alessandrini et al., 2017
Cabernet Sauvignon	Weather: lower temperature	MIBP ↑. No effect on α- and β-ionone and β -damascenone	Falcão et al., 2017

↑: increased; ↓: decreased; TSS: total soluble solids; TTA: total titratable acidity; TPI: total polyphenol index; SM: seed maturity index; TP: total polyphenols; Cat: catechin monomers; PC: procyanidin oligomers; TPA: total proanthocyanidins; MIBP: 2-methoxy-3-isobutylpyrazine

The study was performed under a moderate emission scenario of greenhouse gases (RCP4.5) and a higher emission scenario (RCP8.5), which is considered the most pessimistic one. Aiming to explore the suitability changes and the possible geographical shifts of Argentinean winegrowing regions, the average growing season temperature (GST), the Cool Night Index (CNI), the average growing season precipitation (GSP) and the monthly mean minimum temperature (DSTmin) during the dormant season were the assessed bioclimatic indices. The CNI index is considered as the mean minimum night temperatures during the later maturity stages of the ripening period and it gives a measure of ripening potential indicating the suitability of a winegrowing region notably with relation to secondary metabolites (polyphenols, aromas) in grapes and wines. The GST index is used to determine a cultivar's suitability in a given site and its projected changes could be useful for identifying the best appropriate grapevine varieties for current and future locations, as well as for moving, enlarging, or reducing the surface of the existing viticultural areas to keep current quality. The results showed that CNI and GST are projected to increase mainly by far future for RCP8.5 scenario (more than 6 °C for CNI and between 4 and 7 °C for GST). The predicted higher latitudes and higher altitudes displacement of the current GST and CNI spatial trend could result in modifications in the choice of cultivars and alteration of grapevine quality. Hence, in the near future and under both scenarios, the traditional Argentinean winegrowing areas such as Mendoza and San Juan provinces appear to be favored because they contain a great variety of climate maturity groups for selecting grapevine varieties of diverse thermal requirements. Nevertheless, in the far future, except for high altitudes in the southwest of Mendoza, this area seems to be disadvantaged, mainly under the RCP8.5 scenario. The results showed also that the growing sites at higher elevations such as Catamarca and Salta seem to be benefited in the near and far future and that cool climate varieties may have more and new suitable areas for being cultivated, while warm and hot climate varieties may have less suitable areas to maintain current quality.

A study on the evaluation of the influence of altitude and climate on wine grapes quality was conducted on ‘Niágara Rosada’ and ‘Isabel’ cultivars in vineyards located at three different altitudes (250, 500 and 650 m asl) in the state of Espírito Santo, Brazil (Meneghelli et al., 2018). At harvest, the total soluble solids (TSS) values of both cultivars in the three locations were not significantly different. The vineyard with 650 m altitude and lower mean air temperature favored higher values of total titratable acidity (TTA) and lower values of pH and of the ratio (TSS/TTA) for the two grape varieties as compared with the values of the vineyard at 500 m altitude. The lower values of acidity in the site at 500 m altitude are due to the higher temperature that led to the degradation of malic acid. Relatively high values of TTA were obtained at 250 m altitude and this was attributed to the excess of leaf nitrogen that induces the vines to be very vigorous, the period of vegetative growth to extend and the fruit maturity to be delayed. Upon cultivar comparison at the same altitude, 'Isabel' cultivar presented higher values of TTA and lower values of pH and TSS/TTA ratio than 'Niágara Rosada' cultivar. This could be related to the characteristics of 'Isabel' cultivar itself and its longer ripening period leading to greater levels of acidity when harvested at the same time with 'Niágara Rosada'.

The chemical composition of Syrah grapes grown in two locations with different altitudes (350 and 1.100 m asl) in northeast Brazil was analyzed by Barreto de Oliveira et al. (2019) for two consecutive years. Overall, the altitude exerted a significant effect on berry composition. In fact, the high-altitude grapes were characterized by higher content of malic acid, ranging from 2.75 to 2.87 g/Kg of fresh fruit, while the concentrations in the low-altitude region ranged from 2.41 to 2.43 g/Kg of fresh fruit. This could be a result of the high temperatures at lower altitude that contributed markedly to the degradation of malic acid in the grapes. In contrast, significantly higher citric acid concentrations were found at lower altitude, ranging from 35.03 to 76.7 mg/Kg of fresh fruit, whereas these values at higher altitude ranged from 2.30 to 2.55 mg/Kg of fresh fruit. The low content of citric acid in the grapes at higher altitude may be linked to its use in the biosynthesis of malic acid. The higher levels of glucose, ranging from 192.96 to 271.7 mg/Kg of fresh fruit, and of fructose, ranging from 158.67 to 192.44 mg/Kg of fresh fruit at lower altitude may be due to the higher temperature in this region. The highest levels of succinic acid were obtained in the second year of the study, during which its concentration in the high altitude site (158.10 mg/Kg of fresh fruit) was slightly higher than in the low altitude site (154.67 mg/Kg of fresh fruit). However, the highest levels of tartaric acid were obtained in the first year of the study, during which its concentration in the must at lower altitude (5.54 g/kg of fresh fruit) was greater than in the must at higher altitude (4.93 g/kg of fresh fruit).

The physico-chemical quality of 6 Italian native cultivars, 'Ancellotta', 'Lambrusco', 'Negroamaro', 'Nero d'Avola', 'Fiano' and 'Garganega' cultivated at 1.300 m asl in Água Doce, state of Santa Catarina, Brazil, was examined during two consecutive growing seasons (Malinovski et al., 2016). It was concluded that the effects of high altitude regions on viticultural performance of grapevines differ from one cultivar to another. 'Ancellotta' and 'Garganega' varieties exhibited statistically higher total soluble solids (TSS) values than the other cultivars at harvest. And these two cultivars in addition to 'Fiano' reached satisfactory levels of TSS, i.e., above 18 °Brix, for the elaboration of quality wines. In contrast, the TSS values of ‘Lambrusco’, ‘Nero d’Avola’ and ‘Negroamaro’ at harvest were below 18 °Brix and the quality of the wines to be produced from these three cultivars may be compromised. ‘Nero d’Avola’ cultivar originating from Sicily and ‘Negroamaro’ cultivar originating from Puglia in Southern Italy, had difficulties in the development of their berry components like sugars and acidity during ripening. This could be due to the difference in annual air temperature, being warmer in their region of origin. Whereas ‘Ancellotta’ cultivar, originating from Central Italy with similar thermal conditions as Água Doce stood out by good qualitative indices and showed to be well adapted to the locality. The second year of the study, which had lower sunshine hours, higher number of rain days and shorter phenological cycle during grape maturity than the first year, showed higher levels of titratable acidity and lower pH.

The maturity potential of Chardonnay and Pinot Noir for the production of sparkling wines in two vineyards at different altitudes (873 and 1150 m asl) in Minas Gerais, Brazil was evaluated by Regina et al. (2010) for two consecutive years. At harvest and for both cultivars, lower acidity (ranging from 98.67 to 99.33 meq/L for Chardonnay and from 113 to 122 meq/L for Pinot Noir) was obtained at the lower altitude region for both years of the study. Due to the higher ambient temperature at the low-site vineyard that contributed to a greater accumulation of total soluble solids (TSS) in the berries, higher content of TSS was obtained at the lower altitude region in Pinot Noir grapes for both years of the study (ranging from 16.07 to 19.3 ˚Brix) and in Chardonnay grapes for the first year of the study (16.87 ˚Brix). In the second year of the study, which was characterized by lower rainfall and humidity, Chardonnay grapes had their total soluble solids values at the low altitude (19.67 ˚Brix) similar to the values at the high altitude (19.73 ˚Brix). The high-site vineyard, characterized by lower air temperature and higher precipitations, presented berries with bigger size and mass and with more appropriate concentrations of malic acid (except for Chardonnay grapes at the first year of the study) to produce sparkling wines of organoleptic quality (ranging from 5.21 to 8.04 g/L). On the other hand, Chardonnay grapes reached at harvest satisfactory values of TSS for balancing acidity values, unlike Pinot noir grapes that did not reach satisfactory values of TSS for balancing acidity values mainly in the higher altitude site.

I.4.1.3.2 Altitude effect on phenolic and aroma compounds of grapes and wine

I.4.1.3.2.1 Effect on phenolic compounds

The levels and the composition of phenolics in grapes vary significantly across different parts of the fruit like the skins and the seeds as a result of biotic and abiotic stresses (Coklar, 2017). Altitude has an influence on the ripening and polyphenolic composition of grapes and consequently, on the sensory characteristics of the wine produced (Xing et al., 2016). UV radiation levels increase approximately by 10% with every 1000 meters increase in altitude (WHO, 2002) and increased UV radiation noted at higher altitudes promotes the synthesis of skin anthocyanins. This behavior can explain why wines produced from high-altitude regions have more color intensity (Jin et al., 2017). At higher altitudes, the increase in UV-B radiation can reach 8% per decade leading to the enhancement of color, flavonol, and tannin synthesis in red grapes on one hand (van Leeuwen & Darriet, 2016) and to higher concentrations of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) that can give the wine an intense, and sometimes unpleasant, smell of hydrocarbons on the other hand.

The increase in the altitude of vineyards does not necessarily lead to an increase in total phenolic content in the produced wines. In fact, a study performed on wines of Muscat of Bornova grapes grown in Turkey showed that their phenolic content was the highest for Halilbeyli sub-region (115 m altitude), followed by the Menderes (90 m altitude) and Kemaliye (245 m altitude) sub-regions (Karaoğlan et al., 2015). In the same study, the difference of the concentrations of hydroxycinnamic acids, which are associated to the wine browning process and that act as powerful antioxidants was significant among the three sub-regions. As a matter of fact, the concentrations of trans-caftaric and trans-coutaric acids in the produced wines from Kemaliye sub-region (39 and 5.8 mg/L respectively) were lower than those of Menderes sub-region (55 and 23.6 mg/L respectively) and of Halilbeyli sub-region (80 and 25.3 mg/L respectively). The lowest values obtained at Kemaliye could be related to the soil composition of this sub-region which is characterized by its highest percentage of lime. The flavonol compounds can serve as natural sunscreen for the grapes and their synthesis is induced by light. Thus, the highest amount of quercetin-3-O-glycoside (5.16 mg/L) was obtained for the wine from Kemaliye which had the highest solar radiation value (587,465 watts per square meters). Furthermore, the obtained amounts of quercetin-3-O-glycoside for Halilbeyli and Menderes wines (2.22 and 3.88 mg/L, respectively) were correlated with the corresponding solar radiation values (542,875 and 553,782 watts per square meters, respectively).

The phenolic content of Carignan grapes grown in two different locations, called early and late ripeness parcels corresponding to higher (370 m asl) and lower (305 m asl) altitude respectively, was studied by Edo-Roca et al. (2013) over three years. The average temperature, the evapotranspiration, and the annual accumulated growing degree days (GDD) in the early parcel were higher than those in the late parcel. Consequently, for the three years of the study, the kinetics of anthocyanin accumulation in the early (warm) parcel were much faster than in the late (temperate) parcel, resulting in a higher concentration of total and extractable anthocyanins at harvest. Also the shallow stony soil of the early vineyard parcel provides higher drainage capacity than the deeper soil of the late vineyard parcel. This leads the vines in warm parcel that have limited access to water to produce smaller berries with increased anthocyanin content. At the same time, the total leaf area (TLA) in the early parcel (3.4 m^2/vine) was smaller than the TLA in the late parcel (4.8 m^2/vine). As a result, the grapes in the warm parcel were more exposed to solar radiation and synthesized bigger amounts of anthocyanins. At harvest, the total polyphenol index (TPI) was higher in the early treatment unlike the seed maturity index (SM) which was greater in the late treatment. The obtained percentage of SM values in the late terroir (60-70 %) causes high levels of astringency and it is not adequate for completing the phenolic ripening of the seeds. The lack of seed maturity and anthocyanin content in the late parcel leads to the production of a young wine with a lighter and fresher style. However, the anthocyanin content (700 mg/L) and the percentage of seed maturity (42-55 %) obtained in the early terroir represent the desirable levels required for the production of a reserve wine.

In the same study, the phenolic content of Grenache grapes grown in two different locations, called early and late ripeness parcels corresponding to lower (236 m asl) and higher (422 m asl) altitude respectively, was also determined. The recorded climatic data revealed differences between the early (warm) and late (temperate) vineyard parcels and the shallow stony soil of the early terroir is characterized by a higher drainage capacity. Still, and unlike the Carignan results, total and extractable anthocyanins contents and SM values at harvest were not significantly different between the two parcels. However, the total polyphenol index (TPI) was significantly higher in the early treatment. On the other hand, grape anthocyanins presented a decline in the last ripening control in the warmest year of the study during which dryness and high temperatures have occurred with a 3-day heat wave (temperatures reaching an unusual 40 °C) one week before harvest. For both early and late parcels, Grenache did not reach the optimal seed maturity due to the high percentage of SM values (70-80 %) at harvest indicating some risk of astringency in the wines produced. This could be related to the genetic characteristics of this variety itself rather than the climate conditions of the growing parcel.

The stilbene berry concentration was tested at harvest in three *V. vinifera* L. varieties: 'Barbera', 'Croatina' and 'Malvasia di Candia aromatica' cultivated in four commercial vineyards in Tidone valley, Italy, located at 150, 240, 320, 420 m asl (Bavaresco et al., 2007). The concentration of trans-resveratrol increased up to elevation 3 (320 m) and then declined a bit at elevation 4 (420 m). Trans- and cis-piceid contents were enhanced up to elevation 3 and then significantly decreased at elevation 4. When comparing cultivars, 'Barbera' and 'Croatina' showed similar trans-resveratrol concentrations (71 and 76 µg/kg berry FW, respectively) that were greater than that of 'Malvasia di Candia aromatica' (24 µg/kg berry FW). 'Barbera' had the highest trans- and cis-piceid levels (235 and 136 µg/kg berry FW, respectively) whereas 'Malvasia di Candia aromatica' had the lowest concentrations (13 and 1 µg/kg berry FW, respectively). Fungal pressure becomes more severe as humidity rises, stimulating the production of stilbenes. The response to such stimulation was mostly evident for the concentration of cis-piceid in 'Barbera' cultivar, which was the highest in the third year of the study that was characterized by the highest relative humidity and the lowest degree days at the end of ripening. However, the vintage effect was not evident for trans-resveratrol and trans- and cis-piceid in 'Croatina' and 'Malvasia di Candia aromatica' varieties, showing that different cultivars have a different response despite being affected by the same stressful biotic and abiotic factors.

Different findings were obtained regarding the altitude effect on a Turkish grape cultivar Ekşikara, native to Mediterranean region (Coklar, 2017) when comparing two vineyards located at 1000 and 1500 m asl. Under the effect of sunlight exposure and low temperatures in high elevation vineyards, the total anthocyanin content of grape berries increases. Consequently, total monomeric anthocyanin content in whole berries and skins at high altitude were significantly higher than those at the lower altitude. Also, the tannin levels in grape seed and skin at the high site were significantly greater than those at the low site. Moreover, higher values of total phenolic content in whole berries, in skins and in seeds were obtained at the high site. Regarding the vintage effect in the high altitude area, the total phenolic contents of seeds and whole grapes in the first and the second year of the study were similar unlike the total phenolic content of skins and the tannin content of seeds which showed a decrease in the following year. Phenolic compounds in berries such as hydroxycinnamates, anthocyanidins, flavan-3-ols and flavonols are well-known antioxidants. Hence, it was seen that there was a strong correlation between total phenolic content and antioxidant activity of the analyzed grape fractions. With the increasing altitude, significant increases in the antioxidant activities in whole berries, in skins and in seeds were obtained courtesy of the increase in total phenolic content in berry, skin, and seed extracts. Discrepancies in temperature, sunshine exposure, and water deficits are all potential effects of altitude on the synthesis of individual anthocyanins in grape berries. With decreasing altitude, the relative amount of malvidin-3-O-glucoside in both skin and whole berry increased, whereas that of petunidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside and acylated anthocyanins in whole berry decreased. Concerning the identified non-flavanoid compounds, a substantial decline in the amounts of gentisic acid in whole berries and of caffeic acid in seeds was noted as altitude decreased. No significant changes in gallic acid concentration were obtained in whole berries and in seeds from the two vineyards, whereas the amount of chlorogenic acid in seeds and in whole grapes dropped with decreasing altitude. The trans-resveratrol content in skins and seeds increased with increasing altitude. High altitude generally corresponds to more intense sunlight and lower temperature, both of which are important factors stimulating the synthesis of flavonoids. An increase in the concentrations of catechin in whole berries and of epicatechin in skins was shown with an increase in altitude. Among the identified flavonols, rutin (quercetin-rutinoside) and isorhamnetin-3-glucoside showed a considerable decrease of their amounts in the whole berry, as the altitude increased. Nevertheless, as a result of the greater altitude, no significant variations in rutin and isorhamhetin-3-glucoside contents of skin and seeds were obtained. Likewise, the contents of procyanidin B1 and procyanidin B2 in all fractions were unaffected by altitude.

Another altitude effect was observed for grape skins of Touriga Nacional (TN) and Touriga Francesa (TF) red *Vitis vinifera* varieties which had higher levels of anthocyanins and total anthocyanidin monoglucosides (AMGs) at higher altitudes between 250 and 350 meters in comparison to the lower altitudes, between 100 and 150 meters (Mateus et al., 2001; Mateus et al., 2002). In addition, the produced wines made from high altitude grapes had higher anthocyanins content, especially for the TN cultivar. At the lower cultivation locations, the average maximal temperature values during ripening were between 33 and 38ºC and the average minimal temperatures were reported near 15ºC with humidity levels close to 95-100 % during the night. These low altitude climatic conditions seem to be unfavorable for the biosynthesis of AMG in grape skin and wine. On the other hand, altitude had a positive effect on the levels of catechin monomers (Cat) and total extractable proanthocyanidins (TPA) in the seeds of TF grapes. However, higher concentrations of Cat and low molecular procyanidin oligomers (PC) in grape skins of both varieties, of TPA in skins of both cultivars and in seeds of TN, and of PC in the TN wine, were obtained at the lower altitudes. The general outcome of the sensorial analysis was that the TN and TF wines made from low altitude grapes between 100 and 150 meters were more appreciated. The wine produced from TN grapes at the lower altitudes had higher color intensity despite having lower anthocyanins content. This lower content of anthocyanins could be explained by the considerably greater amounts of low molecular procyanidin oligomers (PC) existing in the TN wine at the lower altitude, where it's possible that some interactions between these low molecular PC and AMGs occurred as a result of copigmentation. Furthermore, covalent associations between anthocyanins and PC can take place by direct condensation or through ethyl linkages leading to higher complex structures responsible for the production of a stable and intensified wine color. In addition, the low altitude TN wine showed a higher astringency due to its containment of higher levels of PC and TPA that contribute importantly to a greater ability to precipitate bovine serum albumin (BSA), in comparison with the high altitude wine.

A study was performed in China on the phenolic compounds of Cabernet Sauvignon grapes from five wine growing regions with different terroir characters (Li et al., 2011). The results showed that similar phenol profiles were obtained between wines from regions geographically close to each other at altitudes between 1036 and 1214 meters with cool-warm climate, and between wines from regions at altitudes between 40 and 214 meters with warm climate. The phenol profiles in the wines from the regions at the highest altitude (1900-3500 m asl) with warm-arid climate were significantly different from those from all the other regions with lower altitude. The terroir components including soil type, climate and topography are involved in the regulation of the phenolic compound biosynthesis in grape berries, resulting in the regional characteristics of the produced wines. The branch points of the biosynthetic pathway of flavonoids in grape berries are regulated by a number of enzymes such as flavonoid 3'-hydroxylase (F3'H) and flavonoid 3', 5'-hydroxylase (F3'5'H) which convert dihydrokamepferol into dihydroquercetin and dihydromyricetin, in addition to their derivatives, respectively. Cyanidin-derived anthocyanins and delphinidin-derived anthocyanins are synthesized from dihydroquercetin in the F3'H branch pathway and dihydromyricetin in the F3'5'H branch pathway, respectively, in the downstream pathways of these two branches. The regional wines with (1900-3500 m) altitude had the greatest levels of both quercetin derivatives and cyanidin-derived anthocyanins, while the regional wines with 214 m altitude had the second highest level of myricetin derivatives and the highest levels of delphinidin-derived anthocyanins. This could be related to different factors like climate, soil and vineyard aspect that might promote the directional flow of carbon into the myricetin synthetic branch or to the quercetin synthetic branch. The region with the highest altitude is characterized by a brown sandstone soil, warm-arid climate and a large diurnal temperature range in addition to specific soil water availability and specific water retention curve (the relationship between soil water content and soil water pressure head). This could incite the flow of more carbon to the F3'H branch pathway leading to higher concentrations of quercetin derivatives and cyanidin-derived anthocyanins in the grapes and in the resulting wines. Conversely, the region with altitude 214 m asl with warm, semi-humid climate and clay and sandy soils, might stimulate the directional flow of carbon into the F3'5'H branch pathway and result higher levels of myricetin derivatives and delphinidin-derived anthocyanins in the grape berries and in the produced wines.

The environmental factors had a significant impact on the amounts of anthocyanin and flavonol compounds in skins of four grape varieties (*Vitis vinifera* L.), Cabernet Sauvignon, Carmenere, Syrah and Merlot, cultivated in five major wine regions in China (Liang et al., 2014). These regions are located at widely different altitudes ranging from 39 m to 1214 m asl. Higher anthocyanin content was observed in grapes from the high altitude western regions which were characterized by lower humidity, rainfall as well as big temperature difference between daytime and night time. Moreover, the grapes with a higher amount of trihydroxylated flavonols were generated in the high altitude vineyards that were near the mountains, with an arid continental monsoon environment and low rainfall throughout the growing season. However, the grapes from the low altitude eastern regions near the sea which were characterized by higher daily minimum temperature, humidity and rainfall as well as low temperature difference between daytime and night time, had a higher proportion of dihydroxylated flavonols. On the other hand, the ratio of the acylated anthocyanins to total anthocyanins, from the western to the eastern regions displayed an increasing trend. The high altitude regions with their related environmental factors such as limited yearly rainfall seem to offer favorable conditions for the production of a high proportion of anthocyanins monoglucosides. Conversely, the low altitude regions with their higher temperature and humidity favored the production of grapes with greater content and higher proportion of acylated anthocyanins.

Stronger sunlight, lower temperature and humidity, a larger temperature difference between day and night, and more severe climatic conditions are all associated with higher altitude. These climate conditions existing in the high cultivation sites (approximately at 2900 m asl) in southwest China, induced increased production of anthocyanins and flavonols in the Cabernet Sauvignon grapes, particularly cyaniding-type anthocyanins and quercetin-type flavonols from the F3'H branch of the flavonoid biosynthetic pathway, when compared with the sites at lower altitudes (2300 m and 2150 m asl). Meanwhile, flavan-3-ols formed from both branches of the flavonoid biosynthetic pathway (F3'H and F3'5'H) were comparatively less affected by altitude and more by the harvest year (Xing et al., 2016). A higher effect of vintage than altitude was also observed for Chardonnay and Pinot Noir cultivated in Minas Gerais, Brazil (Regina et al., 2010). The growing season with a greater number of sunny days contributed to a greater accumulation of anthocyanins and phenolic compounds in the grapes. However, in another study performed by Barreto de Oliveira et al. (2019), the difference in altitude had a greater impact on Syrah grapes than the harvest year. The concentrations of total phenols (1,440 mg/ kg fresh fruit), non-flavonoids (200 mg/ kg), flavonoids (1,240 mg/ kg) and total anthocyanins (890 mg/ kg) at the site with 1,100 m altitude were significantly greater than those at the site with 350 m altitude (450, 130, 320 and 350 mg/ kg fresh fruit, respectively). This result could be explained by the fact that the high altitude region is characterized by a big temperature difference between daytime and night time and by the maximum temperatures during the productive cycle that stayed below 30 °C, favoring the accumulation and preservation of the phenolic compounds during the ripening period of the grapes. The grape skins at the high site contained higher levels of total condensed tannins, including monomeric, oligomeric and polymeric 3-flavanol whereas the grape seeds contained higher levels of these compounds at the low site. On the other hand, the concentrations of trans-resveratrol in the first and the second year of the study were 5.71 and 8.17 mg/Kg fresh fruit respectively at the low site and 4.11 and 4.72 mg/Kg fresh fruit respectively at the high site. The synthesis of stilbenes is induced by environmental stresses. Thus, the maximum daily temperatures that exceeded 30 °C during berry growth and ripening in the low altitude area could have stressed the vines, resulting in higher resveratrol levels. Vintage is one of the main factors that influence the concentrations of proanthocyanidins in grapes. In fact, among the monomeric and the small oligomeric compounds in the seeds, gallocatechin, epigallocatechin and B1, B2, B3 and B4 dimers showed higher levels at the higher altitude in the first year of the study while catechin and B2 dimers esterified with gallic acid revealed high concentrations at the lower altitude in the second year of the study.

The impact of altitude was studied on Moscatel grape stems sampled form three different regions in northern Portugal at 120, 670 and 730 m asl over two consecutive vintages (Gouvinhas et al., 2020). Total phenols, ortho-diphenols and flavonoids in the samples from the lowest altitude region presented the highest content for both years of the study. These compounds in all samples, except for the ortho-diphenols content of the lowest site, increased significantly in the second year of the study which was characterized by an atypical summer with a 3-day heat wave (temperatures above 40 °C) near the beginning of the harvest. Under such stress conditions that scalded the grapes, the plants produced secondary metabolites as a defense mechanism. The obtained lower levels of total phenols, ortho-diphenols and flavonoids in the high-altitude regions may be attributed to the absence of significant water or thermal stresses in these regions. Yet, higher biological capacities were induced by the high precipitations and the climate which has an Atlantic influence at the low-altitude regions. Also, for both years of the study, most of the individual phenolics found in the grape stems, such as caftaric acid, quercetin-3-O-glucoside and an unidentified hydroxycinnamic acid had their highest concentrations at the lowest altitude region. The samples from the lowest altitude region presented the highest antioxidant activity with no differences regarding seasons, and this activity increased significantly at the higher altitude regions from the first to the second year of study. The multivariate analysis showed that the high altitude regions generally induced a lower antimicrobial activity in the samples. This antimicrobial activity seemed to be more affected by the genetic characteristics of the grape stem varieties rather than by the climate conditions and the altitude of the growing sites.

The influence of high-altitude regions on the contents of total polyphenols (TP) and total monomeric anthocyanins (TMA) varies from one cultivar to another, as shown by a study performed by Malinovski et al. (2016) on six Italian cultivars: 'Ancellotta', 'Lambrusco', 'Negroamaro', 'Nero d'Avola', 'Fiano' and 'Garganega' grown in Santa Catarina, Brazil at 1300 m altitude. In fact, the results showed that 'Ancellotta' and 'Lambrusco' which are characterized by their small berries and high genetic similarity reached higher concentrations, though with different extents, of TP (2217 and 1538 mg/L, respectively) and TMA (3514 and 1585 mg/L, respectively) than the other tested cultivars, among which 'Nero d'Avola' contained the highest values (1135 mg/L of TP and 1415 mg/L of AMG). The year of the study which had a greater number of rainy days resulted in a greater amount of polyphenols in the grapes. This might be a result of the fungal diseases that are common in areas with high rainfall, which induces the plants to produce phenolic compounds as a stress response. The intensity of this response changed among grape varieties showing that different cultivars have a different behavior even if they are subjected to the same stressful climatic conditions.

In high-altitude areas in China (2282 m, 2435 m and 2608 m), a decrease in temperature and an increase in diurnal temperature, rainfall and sunshine hours were associated with an increase in altitude. As a result, total phenolic, total flavonoid, total anthocyanin content, sum of individual phenolics and antioxidant capacity of Merlot and Cabernet Sauvignon wines increased with altitude (Jin et al., 2017). While no significant effect of altitude on gallic acid was observed for Merlot and Cabernet Sauvignon wines, the amount of salicylic acid increased dramatically with altitude in the wines produced from both varieties. Salicylic acid might be synthesized by the grapes as a defense response against stress conditions such as UV radiation that increases with increasing altitude. Upon cultivar comparison, the contents of quercetin, trans-resveratrol and tannins in Cabernet Sauvignon wines increased with increasing altitude, but no significant effect of altitude was obtained on the same parameters in Merlot wines. Overall, the altitude followed by sunlight hours mostly affected the phenolic characteristics and antioxidant activity of tested red wines.

I.4.1.3.2.2 Effect on aroma compounds

Grape volatiles are affected by the vineyard location with its associated climatic conditions, which in turn affect the wine sensory properties. A study was performed by Alessandrini et al.

(2017) on the influence of altitude on the volatile compounds of Glera grapes cultivated in two vineyards (200 m and 380 m asl), in Col San Martino, Italy. The high vineyard was warmer than the low one and it was characterized by higher heat accumulation degrees that favored the accumulation and preservation of the aroma compounds and enhanced the elegance and the floral aroma of the produced wine. The minimum air temperatures values at the low site that were approximately 2 ˚C lower than those at the high site, were considered as the most responsible factor that limited the biosynthesis of the aroma compounds at the lower altitude.

Cabernet Sauvignon wines made from grapes grown at different altitudes (774, 960, 1350 and 1160-1415 m asl) were studied by Falcão et al. (2007) in Santa Catarina State, Brazil. There was no effect of altitude on the concentrations of α- and β-ionone and β –damascenone in the produced wines. However, in the highest altitude (1415 m asl) where the temperature was lower in comparison to the other sites studied, both in winter and summer, the MIBP levels were greater in comparison to wines from the other regions. Consequently, the wines from the highest altitude (1415 m asl) were correlated with a "bell pepper" aroma, whereas wines from the lowest altitude (774 m asl) were correlated with the "red fruits" aroma. The content of MIBP in the grape is closely linked to viticulture parameters like growing temperature (Allen et al., 1994) and according to previous study, lower temperatures during the time preceding véraison had a greater impact on the MIBP content in grapes than after they have matured (Lacey et al., 1991). Also, sunlight influences the formation of 3-Alkyl-2-Methoxypyrazines (MPs), and exposing immature berries to light enhanced the concentration of MPs (Pickering et al., 2021). The variability in microclimatic conditions of the grapevines, like light intensity and temperature, may be caused by the heterogeneity of the vine vigor (Asproudi et al., 2016). When compared to less vigorous vines, high vigor vines have usually lesser fruit exposure and a greater MIBP content in the fruit (Mendez-Costabel et al., 2014). Other factors that impacts the vine vigor and the biosynthesis/degradation of aroma compounds are the vine water status and water content and availability in soil (Willwerth & Reynolds, 2020). As a result of increasing water inputs from rainfall and irrigation, MIBP synthesis can increase (Mendez-Costabel et al., 2014).

Rotundone is described by Ferreira (2012) to act as an important aroma impact compound in wine and the anecdotal evidence that rotundone was more common in Shiraz wines from cool climate locations was corroborated in an Australian red wine survey (Black et al., 2015). Geffroy et al. (2016) evaluated the variability of its concentrations in Gamay N wines among four French wine-growing areas. The results showed that Auvergne which is the coolest vineyard over the whole wine-growing season and the ripening period, and the wettest during the véraison-harvest period, had the highest rotundone concentrations and the most intense peppery notes. Peppery aroma scores and rotundone concentration in wine were shown to have a significant correlation. In cooler vintages and vineyards, higher levels of rotundone in Vespolina grapes are expected to accumulate (Caputi et al., 2011). The highest levels of rotundone were obtained in Shiraz berries from vines exposed to less light and/or cooler temperatures, showing a within-vineyard variation and that the topography of the vineyard, particularly the aspect, was the most important factor in the formation of rotundone (Scarlett et al., 2014). In another study, rotundone was typically present at the top and in shaded areas of bunches of Shiraz grapes, which correlates to lower grape surface temperatures and its concentration was negatively affected by fruit temperatures above 25 °C (Zhang et al., 2015a). Furthermore, a study of 15 vintages of Shiraz wine produced from the same vineyard block at the same winery showed that wines from cooler and/or wetter seasons tend to have greater levels of rotundone (Zhang et al., 2015b).

A study conducted on Muscat of Bornova grapes grown in three Turkish terroirs, Menderes (90 m asl), Halilbeyli (115m asl) and Kemaliye (245 m asl) showed that for the first year of the study, the concentration of total terpene compounds in the grapes was the highest in Menderes, followed by the Kemaliye and Halilbeyli. However in the following year, this concentration was the highest in Kemaliye followed by Menderes and Halilbeyli (Celik et al., 2015). In this case, for the two years of study, the Muscat cultivated in the Halilbeyli terroir showed the lowest content of volatile compounds than the other terroirs, even though it can be noted a seasonal effect more than an altitude effect or terroir effect. In addition, another study conducted on the sensorial characteristics of three Muscat wines produced from the same Turkish terroirs with 90, 115 and 245 m altitude (Karaoğlan et al., 2015), showed that the wine at 115m asl was the least preferred because of its darkest color and its highest bitterness and astringency revealing a terroir effect rather than an altitude effect on wine sensory profile.

The latitude effect was more pronounced than the altitude effect for Malbec wines produced by different regions in Argentina. The vineyards of 31-33° latitudes produced the most desired sensory attributes in wine such as floral, sweetness, cooked fruit and raisin as opposed to regions outside these latitudes which exhibited sourness, bitterness and strong herbal aroma (Goldner & Zamora, 2007). A study on the effect of altitude on carotenoids of Touriga Franca and Touriga Nacional grape varieties was conducted by Oliveira et al., 2004 in the Douro Valley. The altitudes ranged from 85 to 145 to 180 m for Touriga Franca and from 90 to 155 to 210 m for Touriga Nacional. High-elevation terraces, presenting higher humidity and lower temperature, promoted the accumulation of carotenoids which degrade during the grape ripening period to form odor-active C13-norisoprenoids (C. Oliveira et al., 2004).

The produced young Cabernet Sauvignon wines from the flat land in Loess Plateau region (China) with 909.3 m altitude and from the slope land with 1280.5 m altitude were examined by Bao Jiang (2012). Loess Plateau region is characterized by its dryer climate, stronger sunshine and lower night-time temperature. The aromatic profiles for the two wines were similar and showed only quantitative but not qualitative differences. In a decreasing order, the alcohols, the esters and the fatty acids were the most represented compound classes in terms of the number and concentration of volatiles in the flat and slope lands wines. The ratios of alcohols, composed mainly of isoamyl alcohol, isobutyl alcohol, 2-phenylethanol and 2-octanol were 55.6 and 64.7% of the total volatile compounds detected in the flat and slope lands wines, respectively. On the other hand, the ratios of esters, composed mainly of ethyl acetate, isoamyl acetate, ethyl lactate and heptyl acetate, were higher in the flat land wine (36.2%) than in the slope land (26.3%) and the ratios of fatty acids, composed mainly of acetic acid were higher in the slope land wine (8%) than in the flat land wine (5.2%). Furthermore, the flat land wine was characterized by more intense fruity aromas (pineapple, pear and banana) with floral notes due to the fact that it contained higher odor active values of isoamyl acetate and ethyl octanoate.

Isoamyl acetate, ethyl octanoate and ethyl hexanoate which are byproducts of yeast metabolism, acted as the most powerful aroma compounds in Cabernet Sauvignon and Merlot wines produced from four wine grape-growing regions in China (214 m, 450–600 m, 1036 m and 1100 m asl). For both wines, the contents of isoamyl acetate and ethyl hexanoate were the greatest in the region with the highest altitude. The same result was obtained for ethyl octanoate only for Cabernet Sauvignon wine; while for Merlot wine, the content of ethyl octanoate was the greatest in the second highest region with 1036 m altitude. Upon region and cultivar comparison, the highest concentration of total aroma compounds was found in the Cabernet Sauvignon wines at the lowest altitude (214 m asl) and the lowest concentration at the second lowest altitude (450–600 m asl); whereas for Merlot wines, the highest concentration of total aroma compounds was found at the second lowest altitude and the lowest concentration at the second highest altitude (1036 m asl) (Jiang et al., 2013). The differences in aroma compound concentrations may be attributed to the grape variety and to the different ecological conditions in vineyard since for all treatments in this study, the same management practices, the yeast and the fermentation conditions were used. Precipitation, diurnal temperature range, soil conditions, and light intensity are thought to have a role in the regulation of grape volatiles, resulting in the regional characteristics of wines. Furthermore, it was concluded that the magnitude of the effect by environmental factors on the volatile compounds of young wine may be related to cultivar.

I.4.1.4 Conclusion

Altitude affects the chemical composition of grapes and wine to a large extent. Some studies showed that an increase in altitude resulted in a delay in budburst, flowering and grape ripening rendering the elevated winegrowing regions more adapted to climate change. Studies showed that high altitude cultivation favored higher content of acidity and of aroma compounds in grapes, anthocyanins, flavonols, total anthocyanidin monoglucosides and condensed tannins in grape skins. It induced also the accumulation of total phenolic, total flavonoid, total anthocyanin content, and optimal wine sensory characteristics such as the elegance, bell pepper and floral aromas. However, other studies showed that the highest phenolic content in grapes, the highest concentration of total aroma compounds and the highest bitterness and astringency in wine were obtained from a region in the middle, between the highest and the lowest regions in altitude. Yet, in other studies, higher content of condensed tannins and procyanidin compounds in grape seeds, of flavonols, trans-resveratrol and 3-O-acetylglucoside anthocyanins in grape skins and red fruits aroma and higher color intensity of the produced wine were obtained at lower altitude. Other factors that influence berry and wine composition such as cultivar, vintage and the presence or absence of significant water or thermal stresses emerged from the studies presented above. As explored in this review, vineyard location (altitude, latitude, slope, orientation), plant material (variety, clones, rootstock), training system, soil type and solar radiation are all factors that influence grape and wine quality and it is very difficult to separate the action of each individual variable on it. This is the reason why in the literature some articles contain contradictory findings.

To expand our knowledge concerning the altitude impact on grape and wine quality, with regards to the related climate and the evolution of phenolic and aromatic compounds during berry ripeness, more studies on different cultivars in different regions are needed. Moreover, the recent interest in altitude as strategy to delay grape ripening and to ensure that ripeness can occur at lower temperatures is due to the role of this important viticultural parameter in reducing the negative effects of the global warming.

I.4.2 Maturity effect

Grapes are fleshy berries with a shape that varies greatly between varieties but remains consistent within a single variety. They are characterized by different shapes and sizes, including flattened, lobular, elongated, ovoid and ellipsoid. They have a hard consistency and prior to véraison, they are green, contain chlorophyll, and can conduct photosynthesis (Moreno & Peinado, 2012). Post véraison, their consistency changes depending upon the variety and the berries of red varieties turn reddish-violet color whereas the berries of white grape varieties turn yellow. The size of the berries is determined by several factors, including seed growth, the soil, and the cultivation process in addition to the number of berries in the cluster. In varieties that contain seeds such as wine grapes, the fruit develops after fertilization of the ovary and the berries reach their maximum size when production conditions are favorable.

The fruit development goes through successive periods (Moreno & Peinado, 2012): herbaceous growth, during which the stalks reach their final size, the berries increase in volume, the sugar content is low and acids begin to accumulate; an approximate two weeks period called véraison when the color of the berries changes till reaching the typical color of the variety; ripening period characterized by a significant alteration of berry’s composition due to the transformation of the present substances and the accumulation of those that are derived from other organs; over-ripening period during which the yield of fruit juice and the acidity decrease while the sugar content increases by a partial water evaporation from the pulp.

The grape skin can be divided into the outermost layer, the cuticle, the intermediate epidermis and the inner layer, the hypodermis, which is composed of several cell layers containing most of the grape skin phenolics (Pinelo et al., 2006). Grape seeds which contain procyanidins, total gallic and caffeic acid at their outer layers, have strong antioxidant properties, scavenging free radicals and neutralizing them, are able to improve health status, in particular for the prevention and management of some chronic diseases (Cory et al., 2018) and play a significant role in anthocyanin stabilization and protein precipitation (Jackson, 2000).

I.4.2.1 Berry maturity effect on grapes and wine

The maturity of the grapes can be expressed as technological maturity when the pulp has reached an optimal content of sugar and acidity, and as phenolic maturity when the content and the ability for the extraction of anthocyanins, polyphenols and tannins have reached an optimum (Jediyi et al., 2019). Time of harvest and grape ripeness levels have a considerable impact on the phenolic and aroma compound concentrations of grapes and winemaking decisions are based on the fate and the extractability of the anthocyanins, flavanol monomers and proanthocyanidins of red grapes during ripening.

In fact, a study conducted by Allegro et al. (2016) on Sangiovese grapes showed that the advanced ripening levels increased the extractability of anthocyanins and skin tannins regardless of the accumulation of their total concentration. This could be due to the alteration of the structure of the skin cell wall during berry maturation. Kennedy et al. (2001) also found a positive effect of berry ripening on proanthocyanidin mDP in Shiraz grape skins, the proportion of (-)-epigallocatechin extension subunits, and the level of anthocyanins associated with the proanthocyanidin fraction. In contrast, the ethanol-extractable skin tannin content of Cabernet Sauvignon did not increase with advancing ripeness (Bindon et al., 2014). This reduction in extractability could be explained by the increase of the cell wall porosity during ripening leading to a rise in the amount of skin tannin bound by noncovalent interactions within cell wall pores.

Another study conducted by Fournand et al. (2006) on Shiraz grape skins showed that on the third week after véraison, free anthocyanins and total red pigments accumulated. Afterwards, free anthocyanins showed a small decrease although the content of total red pigments remained almost constant, suggesting that free anthocyanins were converted to derived pigments. For the same grape variety, the accumulation of proanthocyanidin in seeds and skins started early in berry development and maximum levels were reached around véraison (Downey et al., 2003). This pattern was not the same in hotter growing regions (Hanlin & Downey, 2009), where skin tannin content was highest at fruit set before declining toward véraison, indicating impact of environmental conditions.

A similar influence was observed for Pinot noir grapes by Del Rio & Kennedy (2006) who found that an increase in the heat amount between fruit set and véraison was correlated with an increase in the proanthocyanidin content in grapes and wine over three consecutive vintages. The anthocyanin content and the values of total polyphenol index (TPI) of Cabernet Sauvignon, Merlot, Syrah and Cabernet Franc grapes grown in Lebanon were highest at the hottest and driest years and, during ripening, they increased to reach a maximum then decreased (Rajha et al., 2017).

In Thessaloniki, northern Greece, the accumulation of total anthocyanins during ripening was studied for four red grape cultivars, the Greek indigenous cvs. Agiorgitiko and Xinomavro, alongside Grenache noir and Syrah. In overall terms, the grape skin anthocyanins showed an increasing pattern during maturation, and then exhibited a decreasing trend 10-30 days before harvest; this decrease could be related to the degradation and inhibition of the biosynthetic pathway of anthocyanins due to the higher temperatures of the cluster zone at the last stages of the ripening period. At harvest, malvidin-3-*O*-glucoside was predominant in the skins of the four cultivars and its highest content was found in Grenache noir skins (Theodorou et al., 2019).

The maturity effect was evident in vineyards located in AOC Montsant, Spain, when higher TPI, anthocyanin and proanthocyanidin concentrations and mDP in Grenache wine were obtained from riper grapes harvested at 7 weeks after véraison (Pascual et al., 2016). The same trend was observed in northern Aragon, Spain, when Grenache grapes harvested at around 8 weeks after véraison produced wine with higher TPI, higher concentrations of varietal aroma compounds such as rotundone and linalool and lower acetaldehyde concentration (Arias et al., 2019). Also in Valladolid, Spain, harvesting Tinto Fino and Cabernet Sauvignon grapes at around one week after the conventional time, led to an increase in skin phenols, free anthocyanin levels and quality characteristics of the resulting wines. When aged, these wines showed stability in their color intensity because of the increase of their anthocyanin derivatives content. Still, extending the harvest date for two weeks after the usual time rather than one week was not beneficial for the produced wine (Pérez-Magariño & González-San José, 2004).

Nebbiolo grapes grown in Piedmont, Italy, showed a strong increase of their total and individual anthocyanins between the starting and the end of véraison (Locatelli et al., 2016). Their flavonols (quercetin-3-O-glucoside and quercetin-3-O-galactoside) mostly increased during ripening whereas their tannin content remained substantially unchanged. In another study, the levels of oligomeric procyanidins and monomers from seeds and skins in Castelão Francês and Touriga Francesa cultivars from south of Lisbon, Portugal were examined during ripening (Jordão et al., 2001). Regarding seeds, the concentration of oligomeric procyanidins showed generally a rapid decreasing trend in the first ripening stages that slowed in the last stages. Nevertheless for Castelão Francês, B4 and B2 (dimeric procyanidins), T2 (trimeric procyanidin) and B2-3-0-gallate (galloylated dimeric) exhibited a small increase during the last ripening stages. For Castelão Francis also, a consistent decrease in the monomers: (+)-catechin and (-)-epicatechin during maturation occured, except for epicatechin, which exhibited a slight increase in the first stages of maturation. For Touriga Francesa, these monomers showed a rapid increase followed by a decrease in the last ripening stages. Regarding skins, the concentration of oligomeric procyanidins in both varieties generally showed a rapid decrease in the first stages of maturation. Then at the last ripening stages, it decreased gradually except with the dimeric Bl and the trimeric T2 that showed a small increase.

The behavior of skin anthocyanins in Syrah grapes was investigated from véraison to full maturity in experimental plots close to Chateauneuf-du-Pape, France (Vian et al., 2006). The content of total anthocyanins exhibited an increasing trend and achieved a maximum 28 days after véraison followed by a decrease until harvest. The hot climatic conditions induced skin anthocyanin accumulation and their highest content was reached at the highest temperature registered during maturation. Also, the water deficits registered over 47 days after véraison might have favored anthocyanin biosynthesis during ripening. The concentration of the individual anthocyanins, 3-O-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin showed an increasing trend for 28 days after véraison then decreased for 7 days before persisting fairly constant until the last stages of ripening. The contribution of Malvidin 3-O-glucoside was the highest to the total anthocyanin concentration followed by the contributions of delphinidin 3-O-glucoside, petunidin 3-O-glucoside, and peonidin 3-O-glucoside, which were nearly the same and then the lowest contribution was for cyanidin 3-O-glucoside. Regarding the acylated anthocyanins, the contribution of acetyl and p-coumaryl derivatives of malvidin 3-O-glucoside to the total anthocyanin concentration increased during ripening and was greater than that of acetyl and p-coumaryl derivatives of peonidin 3-O-glucoside which persisted almost constant.

Harbertson et al. (2002) monitored the evolution of tannins in seeds and skins of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. Regarding Cabernet Sauvignon cultivar and in the first year of the study, tannin levels in seeds on a per berry basis exhibited a rapid decrease three weeks after véraison, followed by a rapid increase with a subsequent decrease that remained till harvest. This behavior of decreasing and increasing was due to a qualitative modification in seed phenolic composition rather than a quantitative oscillation in total seed phenolics. For the two consecutive years of the study, the levels of seed tannins at véraison were more than twice the levels of skin tannins then these levels reached a similar value by harvest. Regarding Syrah cultivar, tannin levels in seeds reached a peak around véraison followed by a rapid decrease but they persisted higher than tannin levels in skins throughout the season. With respect to Pinot noir cultivar, the levels of tannin in seeds reached a peak after véraison followed by a decrease and in skins they persisted almost constant; these levels had similar value by harvest.

The accumulation patterns of benzenoids, terpenes and norisoprenoids volatile compounds in Glera grapes from two different sites in Col San Martino, Italy, were studied during ripening for two consecutive years (Alessandrini et al., 2017). At both sites and in both years, the benzenoid concentration was particularly high at véraison then its trend was not consistent showing oscillation during the ripening period whereas terpene levels gradually increased from véraison to maturity. However, regarding the synthesis of norisoprenoids which is temperature-dependent, it showed an increasing trend during all the ripening period in the first year of the study but in the following cooler year, it remained constant in one site and slightly decreased in the other. This behavior might be explained by the low temperatures that occurred in the middle and final stages of ripening.

The effect of ripening stage on grape carotenoid content was investigated in the Douro Valley for eight cultivars: Touriga Brasileira, Touriga Nacional, Tinta Barroca, Tinta Amarela, Tinta Roriz, Souzão, Touriga Franca and Tinto Cão during the last month of maturation (C. Oliveira et al., 2004). Overall, results showed that carotenoid content decreased during ripening, except for

Touriga Franca in which it increased from véraison till reaching its maximum after 10 days then it decreased. Among carotenoids, lutein and chlorophyll a presented the largest percentage decreases. Vilanova et al. (2012) investigated the volatile composition of four cultivars from Betanzos, NW Spain: Blanco lexitimo, Agudelo, Godello and Serradelo in two different ripening stages. The results showed that the free and bound fractions of the aroma compounds in these cultivars was strongly influenced by the cultivar and the ripening stage and that for most of the quantified volatiles, there was a significant interaction "cultivar x ripening date". A positive effect of berry ripening was obtained for free monoterpenes in Blanco lexitimo due to the higher content of linalool at the last ripening date. The concentrations of bound C6-compounds and monoterpenes increased during ripening in Agudelo and Godello cultivars but decreased in Serradelo. In all cultivars, free hexanoic acid increased during ripening in addition to the glycosidically bound compounds except in Blanco lexitimo cultivar. The high levels of C6-compounds, (E)-2-hexen-1-ol, 1-hexanol and (E)-2-hexenal allowed a significant increase of free total volatile concentration between the two ripening dates studied for Godello and Serradelo grape varieties.

The concentrations of α-ionone, β-ionone and β-damascenone in Pinot noir and Barbera wines produced from grapes harvested at three different maturity levels (-15d, -7d and 0d, indicating the days before full-ripeness) were investigated (Asproudi et al., 2018). The year of the study was characterized by mild winter and water availability in June, resulting in a significant advance of the vine phenological phases and β-damascenone and β-ionone levels in wines particularly produced from Barbera grapes decreased in concordance with grape maturity while, α-ionone was less influenced by the grape maturity stage. Harvesting early notably for Barbera cultivar at 7 days before full-ripeness, resulted in good technological parameters, lower wine alcohol levels and a higher content of β-damascenone.

Asproudi et al. (2016) studied the evolution of the hydrolytically released C13-norisoprenoids in Nebbiolo berries during ripening as influenced by the vineyard microclimatic characteristics including the vineyard aspect (South and West) and vine vigor. The results showed that the accumulation of the majority of norisoprenoid glycosides identified in the grapes and expressed as μg/100 berries, occurred early starting from pre-véraison and continuing until 3-4 weeks post-véraison for two consecutive vintages. Then during the pre-harvest period, a slowdown accumulation of total norisoprenoids was observed until harvest in the blocks with the most vigorous vines whereas an important decline of their content was noticed in the south less vigorous blocks. This decline may be due to the higher heat experienced by the south blocks during the last phase of ripening and it appeared earlier in the second year of the study when a greater number of hours of fruit exposure to temperatures exceeding 35°C were registered in September, as compared to the first year. On the other hand, increased light exposure as a consequence of the lower vigor promoted norisoprenoids accumulation until the post-véraison stage in the less vigorous blocks while low light exposure in the most vigorous vines penalized the maximum content of norisoprenoids.

A complementary study was conducted by Asproudi et al. (2020) on the link between bunch microclimate, defined by different vineyard aspect and vine vigor, and carotenoid evolution in Nebbiolo grapes during ripening. For the two consecutive years of the study, the lutein content per berry showed an increase after véraison till reaching a peak at about 4 weeks after véraison, in proportion to the vine vigor, thus more evident in the most vigorous vines. This increase could be related to high vigor which often indicates a higher synthesis of chlorophyll and, hence, of lutein and/or to the cooler conditions in the most vigorous plants that lower the degradation of lutein. Conversely, the peak of lutein after véraison was less prominent in the south less vigorous blocks where high temperatures and increased sunshine exposure might have induced carotenoid degradation. Furthermore and at harvest, the lowest lutein levels were found in the less vigorous and warmest vineyard facing south whereas the west-exposed and the more vigorous vineyard maintained the highest levels. The neoxanthin content showed a similar behavior to the lutein content during ripening when a concentration peak at 4 weeks after véraison was observed especially in the cooler parcels. Regarding β-carotene, a consistent content was obtained during ripening in all experimental plots in the first year of the study however a significantly lower content was obtained in the south less vigorous blocks in both years at harvest. In the second year of the study, the high temperatures of south plots led to a notable degradation of β-carotene during véraison that was greater than the degradation observed at the end of ripening. Nevertheless, the west-facing vineyards which have lower temperatures and are less sunlit, a slower degradation of β-carotene was observed allowing the maintenance of constant levels during ripening in both years.

I.4.2.2 Conclusion

From the studies presented above, we can conclude that several factors affect the evolution of the phenolic compounds during ripening, including the grape variety and the environmental conditions. Advancing ripeness led to an increase in total and individual anthocyanins and flavonols in grapes, phenols and extractable anthocyanins and tannins in grape skins, proanthocyanidins in grape seeds and TPI, mDP, free anthocyanins, rotundone and linalool levels in the produced wine of some cultivars. For other cultivars, lutein and neoxanthin contents in grapes peaked at about 4 weeks after véraison, skin free anthocyanins reached the maximum at the third week after véraison while tannin content remained constant in grapes and was highest at fruit set or at around véraison in grape skins depending on the heat amount during ripening. More research on diverse cultivars in varied terroirs is needed to increase our understanding of the evolution of phenolic and aromatic compounds during berry maturity.

Chapter II. Altitude and berry maturity effects on Lebanese Grenache grapes and wines

II.1 Introduction

The Lebanese wine is currently very known and praised worldwide and it showed a growing increase in exports to more than 35 countries of which UK, USA and France are the top destinations. Wine is one of the top Lebanese agricultural exports and in 2018, demand for Lebanese wine grew by an annual 14.3%, the volume of wine exports reached 2,322 tons and the value of wine exports reached 20.3 millions of dollars (BANK, 2019).

Lebanon is characterized by its highest percentage of agricultural land in the Middle East (64.3% of total land area) and its mild climate, rich soil, and abundant water resources which are the key elements of agricultural production (IDAL, 2017). Nearly half of its total surface area, though with different productivity levels, could be cultivated (MoE/UNDP, 2011). It has a natural environment favorable to the production of grapes with high oenological potential (Lelay G. & Roger T., 2003). Due to the topographic features and the complex landform consisting of sloping and steep lands, Lebanese terroirs present differentiated microclimates in a relatively limited area (Bejjani et al., 2014). The variations in scenery are more related to altitudes than to geographical distances.

In 20 years from now, it is predicted that the maximum air temperature (Tmax) in Lebanon would increase 1 to 2 degree and the rainfall amount to decrease 10 to 20% from the coastal to the inland regions (MoE, 2011). Climatic projections show that Tmax and precipitation quantities for rainfed wine grapes will both result in a possible decline in the grape yield and quality, mainly in the low altitude regions with low precipitation amounts (MoE/UNDP, 2011).

Global warming would apparently lead to changes in wine geography and an upward shift of the growing regions has been forecasted to happen (Pomarici & Seccia, 2016). Moreover, it is important to study the behavior of grape berries especially the evolution of their chemical components during their ripening period, which would be altered because of the increase in global temperature. On the other hand, because of the present and expected future changes in the Lebanese climate, winegrowers could shift to late-ripening, more drought and heat tolerant cultivars such as “Grenache”. The scientific reliable information regarding the influence of environmental conditions and fruit maturity on grape and wine characteristics is provided by terroir studies which are scarce regarding Lebanese grapes and wines. For that reason, the purpose of this work is to determine the effects of altitude and berry maturity on the phenolic composition of Lebanese Grenache grapes on one hand and the effect of altitude on the phenolic and aroma compounds of Lebanese Grenache wines on the other hand. Due to their importance in the quality of grapes and wine and their numerous beneficial effects on human health, the phenolic and volatile compounds in Grenache grapes are investigated in this research work.

II.2 Materials and Methods

II.2.1 Chemicals and standards

All the following chemicals were purchased from Sigma Aldrich, unless noted otherwise and were at the maximum purity grade available: tartaric acid, sodium bisulfate, acetonitrile (Panreac, Spain), methanol, ethanol, sodium carbonate, ferrous sulphate heptahydrate, sulphuric acid (VWR chemicals, France), formic acid (Carlo Erba Reagents, Italy), Folin-Ciocalteu's phenol reagent, NaOH, HCl, ethyl acetate, ethyl butanoate, isoamyl acetate, limonene, 1-butanol-2-methyl, ethyl hexanoate, 1-hexanol, ethyl octanoate, benzaldheyde (Alfa Aesar, USA), ethyl decanoate, citronellol, methyl salicilate (Alfa Aesar, Germany), ethyl dodecanoate, phenyl ethyl alcohol, ethyl tetradecanoate, 3-octanol, malvidin-3-glucoside chloride, hydrocarbon mixture from C8–C23.

II.2.2 Grape samples

Representative samples of Grenache grapes were collected from three Lebanese regions with different altitudes called "Eddeh" in Batroun, "Mdoukha" in South of the Bekaa valley and "Ain Bourday" in North of the Bekaa valley (Table 2).

Table 2: Altitude and climate of the selected vineyards

Vineyard Region	Altitude Climate in general
Eddeh	300 m above sea level Hot and rain-free summer, pleasant fall and spring, and cool, rainy winter
Mdoukha	1000 m above sea level Dry, warm summers and wet, often snowy winters
Ain Bourday	1500 m above sea level Hot, dry summers and relatively cold, sometimes snowy winters

Their locations on the Lebanese map are represented in Figure 25. Mdoukha and Eddeh vineyards are only rainfed while for Ain Bourday vineyard, the water is delivered to the plants through rainfall and drip irrigation. The meteorological data (temperature and precipitation) of the three vineyards were provided by LARI weather stations. The nearest stations were in Kfarhata region (GPS coordinates X= 35.741313 and Y= 34.28905) for Eddeh, in Rachaya el

Fakhar region (GPS coordinates X= 35.662762 and Y= 33.358787) for Mdoukha and in Doures region (GPS coordinates X= 36.153133 and Y= 34.00312) for Ain Bourday.

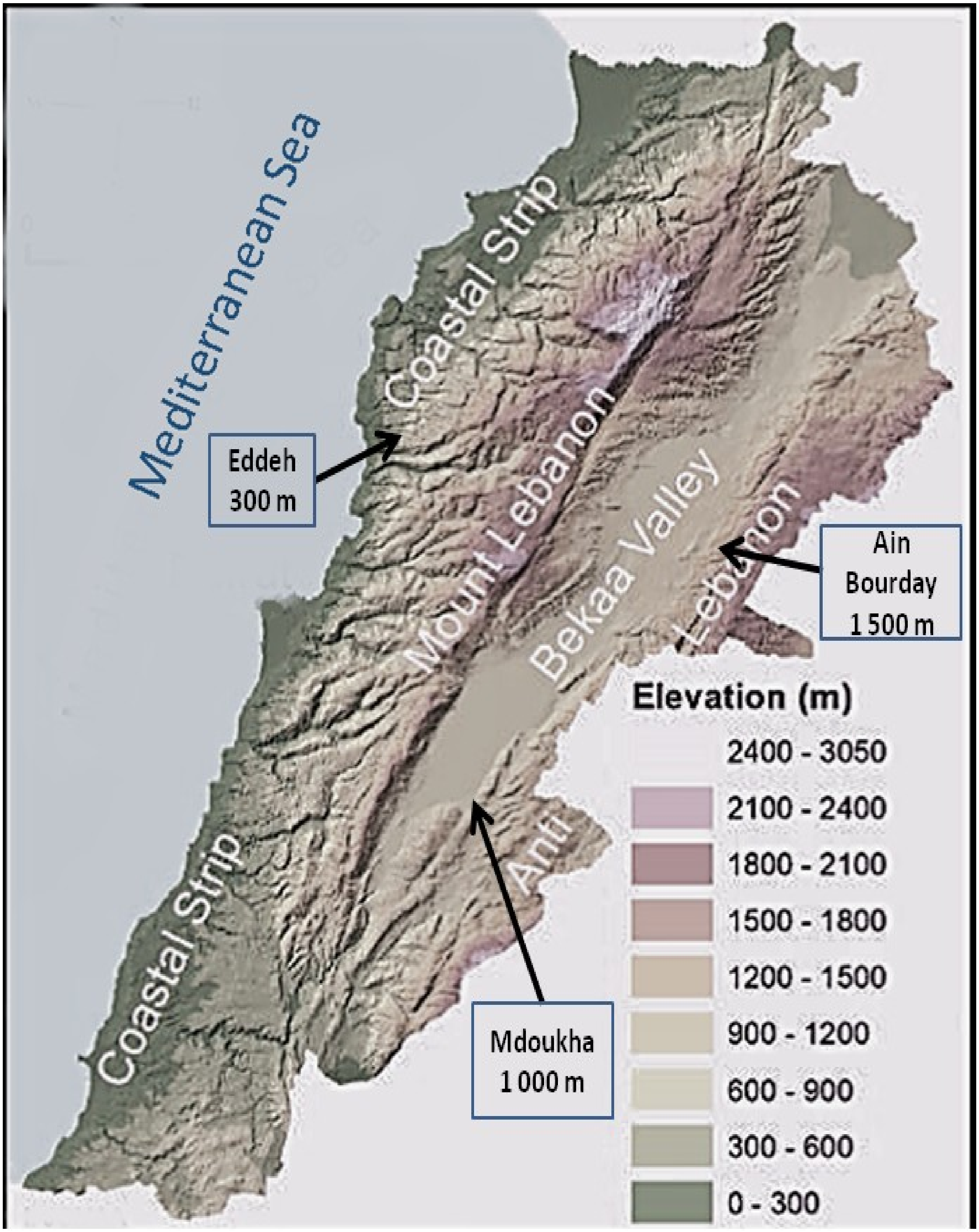


Figure 25: Geographical origin of grape samples. Adapted from: (Verner et al., 2018).

The photos of the three vineyards are represented in Figure 26.

Figure 26: Photos of the 3 vineyards

For the analysis of fruit chemistry and grape skin and seed phenolics, berry samples were collected at 3 maturity levels, before, during and after the harvest time for commercial winemaking that is related to different factors including commercial maturity of grapes (TSS at 22-26 °Brix). Other factors that affect the harvest time include the availability of workers for grape picking and of space in wineries. In 2018, the first sampling corresponded to the first maturity level in Eddeh and was conducted on August 9. The second sampling corresponded to the harvest time decided by winegrowers but the day of harvest was procrastinated several times due to lack of labor and lack of space in the wineries. The second sampling in Eddeh was conducted 22 days later, on August 31. The grapes may be damaged by insects or break down due to rot if they are left on the vine for too long, reducing quality of the sampling. Still in 2018, the quality of the sampling in the three vineyards was not negatively affected and the grape samples were collected at four, seven and eight weeks after véraison (when around 50% of the grapes change color) for Eddeh, at five, seven and eight weeks after véraison for Mdoukha and at four, six and seven weeks after véraison for Ain Bourday. But for the second year (2019), the berry samples from the three vineyards were collected irrespective of the harvest day decided by the winegrowers, at three, five and seven weeks after véraison.

In each vineyard and for each sampling, around 5 kg of berries were randomly picked from the vines at both sides as walking through the row, from top, middle and bottom of selected clusters. From these collected berries, 2 replicates of 100 berries were sampled for the analysis of total soluble solids, pH and total acidity (g/L of tartaric acid) using a digital refractometer model PAL-α (ATAGO, Japan) and expressed as °Brix, a WTW inolab 7110 pHmeter and by titration with indicator (bromothymol blue) with NaOH 0.1 mol/L until the color changes to blue-green, respectively. And 2 replicates of 100 berries were sampled for the analysis of grape skin and seed phenolics. Also, around 15 kg of berries were collected randomly from the three vineyards as mentioned before for laboratory microvinification and their harvest time is mentioned in the following paragraph.

II.2.3 Maceration and fermentation procedures

In 2018, the grapes for laboratory microvinification were collected at the same time they were harvested for commercial winemaking. From Eddeh and Mdoukha, the grapes were collected in 2018 at seven weeks after véraison (24.9 and 26.4 ˚Brix, respectively) and from Ain Bourday at six weeks after véraison (24.5 ˚Brix). In 2019, the grapes for laboratory microvinification were collected when their Brix reached 24 to 26 degrees; from Eddeh, the grapes were collected in 2019 at seven weeks after véraison (24.1 ˚Brix) while from Mdoukha and Ain Bourday at five weeks after véraison (26.1 and 26.2 ˚Brix, respectively). The collected grape samples from the three selected vineyards were vinified in the laboratory in order to analyze the main chemical components of the resulting wines. The laboratory microvinification was carried out in triplicate. Around 2 Kg of grapes per replica were crushed manually then they were transferred into 2 L glass Erlenmeyer flasks after adding sodium metabisulphite (50 mg of $NaHSO_3$/kg). The flasks were continuously shaken at a controlled temperature (25°C) and the yeast (Levospark, saccharomyces cerevisiae, SCK 1, Tecnofood Italia) was added according to the instruction written on the packaging box: 10-40 g/hL. From time to time, the floating skins and seeds were punched down to be in contact with the must at the bottom, to increase color, aroma, and tannin extraction. The alcoholic fermentation was performed until total or cessation of sugar consumption and the malolactic fermentation was not performed because the concentration of malic acid in the three produced wines was equal or below 2 g/L. Fourier-transform infrared spectroscopy (FTIR) was used according to OIV/OENO Resolution 390/2010 (OIV, 2010b) using FOSS WineScan (Hilleroed, Denmark) to determine total acidity, tartaric acid, malic acid, pH, reducing sugars and color intensity of the produced wines. Spectrophotometric and volatile organic compounds (VOCs) of the resulting wines were also analyzed as described below.

II.2.4 Soil analysis

Soil samples were taken from each vineyard at depths of 30–40 cm and they were analyzed in the soil laboratory of the Lebanese Agricultural Research Institute (LARI) using the methods according to AFNOR (1994) (Table 3).

Table 3: Soil parameters analyzed with the corresponding references

Parameters	Reference
Texture (%)	AFNOR X31-107 Bouyoucos Hydrometer Method USDA Texture Triangle
pH water (1: 5)	ISO 10390:2005
Electrical Conductivity ($mS.cm^{-1}$)	ISO 11265: 1994
Organic Matter %	Walkley-Black 1947
Total $CaCO_3$ (%)	NF X 31-105
Active $CaCO_3$ (%)	NF X31-106:1998
Organic Nitrogen (%)	By Calculus
Available P_2O_5 (ppm)	ISO 11263:1994 (Olsen Method)
Exchangeable K_2O (ppm)	NF X31-108:1998
Exchangeable CaO (ppm)	NF X31-108:1998
Exchangeable MgO (ppm)	NF X31-108:1998
Exchangeable Na (ppm)	NF X31-108:1998

II.2.5 Spectrophotometric determinations

II.2.5.1 Grape skin and seed phenolics

II.2.5.1.1 Preparation of skin and seed extracts

The extraction of phenolics in grape skins and seeds was performed according to the method of Fanzone et al. (2011). From the grape samples of each maturity level, the skins and seeds of 100 berries were manually separated, then weighed and grounded with 30 mL of ultrapure water. To the resulting grounded material (skins and seeds), 40 mL of a prepared hydroalcoholic solution (12:88 v/v ethanol/ ultrapure water) containing 5 g/L of tartaric acid was added. Ultrapure water was added in order to adjust the weight of the obtained suspension to 200g then NaOH or HCl were added to adjust the pH of extracts to 3.6. Maceration for 2 hours was performed for the extracts at 25°C and at 200 rpm by using an orbital shaker, and a centrifugation for 15 min at 2038g followed. The absorbance measurements for the obtained extracts were made with a UV-VIS spectrophotometer, HP 8453, Palo Alto, CA.

II.2.5.1.2 Extractable polyphenols in grape skins

The extractable polyphenols in grape skins and seeds were determined according to the method of Di Stefano et al. (1989) which allows their isolation on a Sep-Pak C18 cartridge to remove salts, sugars and proteins interferences. The extract was diluted with 1 N sulphuric acid (1:5 for the seeds and 1:10 for the skins) then 1 mL of it was loaded to a previously activated Sep-Pak C18 cartridge (Waters, USA) with 2 mL of methanol and 5 mL of 0.01 N sulfuric acid. The phenolic compounds were eluted with 2 mL of methanol and 5 mL of ultrapure water in 25 mL flasks after washing the cartridge with 2 mL of 0.01 N sulfuric acid. A volume of 1 mL of Folin-Ciocalteu reagent was added to the eluted sample. Then after 3-5 minutes, 4 mL of 10% sodium carbonate were added followed by ultrapure water to volume. The absorbance at 700 nm against a blank was measured after 90 minutes on a 1 cm cell. The blank was prepared in the same way but with ultrapure water instead of the extract. The results (mg/kg of grape) were expressed as (+) catechin = 186.5* E700 * d; d = dilutions.

II.2.5.1.3 Extractable anthocyanins in grape skins

The extractable anthocyanins in grape skins were determined according to the method of Di Stefano et al. (1989). The skin extract was diluted with 1 N sulphuric acid (1:10) then 10 mL of it were loaded to a previously activated Sep-Pak cartridge with 4 mL of methanol and 10 mL of 0.01 N sulfuric acid. The anthocyanins were eluted with 3 mL of methanol in 25 mL flasks. Then, 3 mL of concentrated hydrochloric acid were added to the eluted sample followed by a solution of hydrochloric ethanol (ethanol: water: HCl conc 70: 30: 1) to volume. The absorbance was read from 380 to 700 nm in a 1 cm cell and hydrochloric ethanol was used as blank. The total anthocyanins were expressed as mg of malvidin per kg of grapes. Total anthocyanins = E (1 cm, X max vis) * 26.5 * d; d = dilutions; X max = 536-540 nm.

II.2.5.1.4 Extractable proanthocyanidins in grape seeds

The extractable proanthocyanidins in grape seeds were determined according to the method of Di Stefano et al. (1989). The seed extract was diluted with 0.1 N sulphuric acid (1:2) then 4 mL of it were loaded to a previously activated Sep-Pak cartridge with 4 mL of methanol and 10 mL of

0.01 N sulfuric acid. The proanthocyanidins were eluted with 6 mL of methanol in a 100 mL distillation flask after washing the cartridge with 8 mL of sulfuric acid 0.1 N. Then, 9.5 mL of absolute ethanol and 12.5 mL of concentrated hydrochloric acid containing 300 mg/L of ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) were added to the eluted sample followed by heating at 100°C for 50 min. Afterwards, the flask was placed in the dark in a cold water bath for 10 minutes and the absorbance was read from 380 to 700 nm in a 1 cm cell. The blank was prepared in the same way but without heating and the flask was immediately immersed in ice. Absolute ethanol was used as the instrument blank. Once the absorption spectrum between 380 and 700 nm was obtained, the tangent to the absorption curve was traced starting from the minimum point and ending at the maximum point. The height of the segment parallel to the ordinate axis that joins the maximum point of the absorbance curve with the tangent was measured and expressed in absorbance units. A similar measurement was made on the blank. The content of proanthocyanidins was expressed in mg of cyanidine chloride per kg of grapes and was calculated as follows: Cyanidin mg / Kg = ∆E'1 cm * 1162.5 * d; ∆E': difference in absorbances between the sample and the blank; d: dilutions.

II.2.5.1.5 Extractable proanthocyanidins in grape skins

The extractable proanthocyanidins in grape skins were determined according to the Bate-Smith method as described by (Ribéreau-Gayon & Stonestreet, 1966). The skin extract was diluted 50 times with ultrapure water then 4 mL of the diluted sample, 2 mL of ultrapure water and 6 mL of hydrochloric acid were added in a test tube. The latter was water bathed at 100 °C for 30 minutes and a blank was prepared in the same way in another test tube but without heating and it was stored in dark. After heating, 1 mL of ethanol was added to each tube and the tubes were left in dark until the heated tube was cooled. The absorbance of each test tube was read at 550 nm using ultrapure water as the blank. The absorbance difference was multiplied by the factor 19.33 to calculate the final concentration of proanthocyanidins which was expressed as mg/Kg of grapes, considering the dilution and the weight of the used berries.

The calculated values of the different parameters were obtained by taking into account the used amount of grapes and the volume of the extract, the molecular weights of the different compounds and the respective molar extinction coefficients (determined with calibration curves).

II.2.5.2 Wine phenolics

II.2.5.2.1 Total Polyphenol Index (TPI) in wine

TPI was measured according to Ribéreau Gayon et al. (1998a). After dilution (1:100, wine: water), the absorbance was measured at 280 nm under 1 cm optical path and the result was obtained by multiplying the absorbance by the factor of 100.

II.2.5.2.2 Total anthocyanins in wine

Total anthocyanins were quantified according to Ribéreau-Gayon & Stonestreet (1965). A wine solution containing 1 mL of wine, 1 mL of ethanol and 20 mL of 2 % hydrochloric acid was prepared. 10 mL of the wine solution was put in two different test tubes then 4 mL of water was added in a tube while 4 mL of sodium bisulphite solution (15% bisulphite v/v in water) was added to the other. After 20 min, the difference in absorbance at 520 nm (1 cm path length) between the two tubes was recorded then multiplied by the factor 875 to calculate the final concentration of total anthocyanins which was expressed as mg/L.

II.2.5.2.2 Total tannins in wine

The same method of Bate-Smith described above for the determination of the extractable proanthocyanidins in grape skins was used. Total tannins concentration was determined by measurement of the absorbance at 550 nm after acid hydrolysis of the wine samples and a blank, and was expressed in mg/L.

II.2.6 HPLC determination of anthocyanins in skin extracts

The skin extracts prepared from the grapes collected at the harvest time for commercial winemaking for the first year of the study and at three, five and seven weeks after véraison for the second year were submitted to HPLC determination. The anthocyanins were analyzed using an HPLC system consisting of Agilent 1260 (Santa Clara, CA 95051, United States) equipped with a quaternary pump, an autosampler and a photodiode array detector (DAD) and a Phenomenex Gemini C18 110 A column (4.6 mm x100 mm, 3 µm). 100 µL of skin extract, previously filtered through a 0.45 µm pore size nylon membrane, was injected onto the column. The mobile phase consisted of solvent A (water/formic acid, 90:10, v/v) and solvent B (acetonitrile) with a gradient program as follows: from 0 to 12 min, 96-85% A and 4-15% B; from 12 to 22 min, 85-85% A and 15-15% B and from 22 to 35 min, 85-70% A and 15-30% B and followed by washing (100% methanol) and re-equilibration of the column. The flow rate was set at 1.1 mL/min from 0 to 22 min and at 1.5mL/min from 22 to 35 min and the separation was performed at 25°C. The photodiode array detection was performed from 210 to 600 nm and the anthocyanins were quantified by peak area measurements at 520 nm, according to the method of Fanzone et al. (2010) and expressed by using malvidin-3-glucoside chloride as standard for a calibration curve.

II.2.7 GC-MS analysis of aroma compounds in wine

II.2.7.1 SPME conditions

A solid phase microextraction (SPME) coupled with gas chromatography was performed to determine the chemical composition of the VOCs in the produced wines according to Petretto et al. (2021). Following the instruction of the manufacturer, a 100 µm PDMS/DVB/CAR (Polydimethylsiloxane/Divinylbenzene/Carboxen) coated fiber (Supelco, Sigma Aldrich, St. Louis, MO, USA) was preconditioned. 10 mL of wine and 100 µL of internal standard (3-octanol, 225 mg/L) were added in a 20 mL SPME vial (75.5 x 22.5 mm) which was firmly closed with a screw cap with Viton 1A septum. After 5 min of equilibration at 60 °C, the conditioned fiber was inserted through the septum and suspended in the headspace to isolate the volatile compounds. After being exposed to extract the VOCs for 30 min at 60 °C, the fiber was retracted, removed from the vial and desorbed directly into the injection port of the gas chromatograph at 250 °C for 5 min in a splitless mode. A bake-out step was performed for the fiber at 250 °C for 5 min before and after each use.

II.2.7.2 GC-MS analysis

The GC/MS used for the analysis of the VOCs extracted in the fiber was Agilent 7890 GC equipped with a Gerstel MPS autosampler and coupled with an Agilent 7000C MSD detector. The chromatographic separation was performed using an Agilent VF-Wax column of 60 m x 0.25 mm i.d. and 0.5 µm film thickness. The oven temperature was programmed as follows: 40°C hold for 4 min, to 150°C at 5.0°C/min, hold for 3 min, to 240°C at 10°C/min, hold for 12 min; carrier gas, helium; constant flow, 1.8 mL/min. Using a Gerstel µFlowManager µsplit 2-way, the flow was split at the end of the column into the MSD detector and a Flame Ionization Detector (FID) at a ratio of 2:3. Mass Hunter Workstation B.06.00 SP1 was used for data analysis and the compound identification was carried out by comparing with co-injected pure compounds and by matching the MS fragmentation patterns and retention indices using the built-in libraries, the collected literature data, or commercial mass spectral libraries (NIST/EPA/NIH 2008; HP1607 purchased from Agilent Technologies). For obtaining the linear retention indices, a hydrocarbon mixture from C8-C23 was injected in the same HS-SPME/GC-MS conditions. The main compounds detected in the headspace of the produced wines and the calibration equations used for quantification of compounds are represented in Table 4. The concentrations of the individual compounds in the headspace were quantified using calibration curves obtained by the internal standard method. 3-Octanol was used as internal standard. Standards for each quantified compound were accurately weighed and dissolved in 10 mL of ethanol. The resulting stock solutions were added of internal standard and diluted with ethanol:water (12:78 v/v) to obtain five levels according to the linearity range. Tartaric acid was added to adjust the pH of each sample to 3.5. The VOCs concentrations were expressed as mg/L of wine.

Table 4: Main compounds detected in the headspace of the produced wines and calibration equations used for quantification of compounds. RI, experimental linear retention indexes calculated on an WF-Wax column

Compound	**RI**	**Calibration equation**	**R^2**	**Linearity range (μg mL^{-1})**
Ethyl Acetate	882	y=0.0321 - 0.0027	0.9993	1.8 - 37
Ethyl butanoate	1076	y=0.2721 - 0.0149	0.9973	0.014 - 2.11
Isoamyl acetate	1105	y=0.4346x - 0.0388	0.9562	0.009 - 1.305
Limonene	1182	y=15.918x - 0.0157	0.9792	0.001 - 0.07
2-methyl-1-butanol ovlp 3-methyl-	1200	y=0.0221x + 0.0561	0.996	0.4 - 180
Ethyl hexanoate	1215	y=0.09258x + 0.281	0.996	0.052 - 7.83
1-Hexanol	1335	y=0.088 + 0.0443	0.9954	0.018 - 2.715
Ethyl octanoate	1419	y=9.0921x + 0.1693	0.9999	0.002 - 2.955
Benzaldehyde	1547	y=1.297x + 0.0185	0.9982	0.008 - 0.395
Ethyl decanoate	1628	y=22.818x + 0.2955	0.9995	0.002 - 3.24
Citronellol	1795	y=0.7479x + 0.00091	0.9948	0.001 - 0.23
Methyl salicylate	1810	y=2.7173x + 0.0099	0.9995	0.0001 - 0.08
Ethyl dodecanoate	1814	y=80.923x + 0.123	0.9958	2E-05 - 0.022
2-phenylethanol	1846	y=0.0257x + 0.0239	0.9995	120.4 - 10.04

II.2.8 Statistical analysis

Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test for mean separation, with a significant level of 95% ($p < 0.05$) and principal component analysis (PCA) were performed using Xlstat 2021.2.

II.3 Results and discussion

II.3.1 Soil composition

Soil has an important effect on vineyard performance and on grapes and wines composition. The results of the analyzed soil samples are presented in Table 5. The three vineyards have similar soil texture, defined as sandy clay loam. The soils from Eddeh and Mdoukha vineyards are richer in clay (34.25 and 32.12%, respectively) than the soil from Ain Bourday vineyard (22.04%). Clay-rich soils have good water retention ability but poor drainage. The soils from Ain Bourday and Eddeh are richer in loam (14.66 and 13.58%, respectively) than the soil of Mdoukha (10.7%). Loam-rich soils are fertile, composed of roughly equal amounts of silt, sand and clay. The soil pH at the three sites ranged from 8.18 to 8.52, which classified them as alkaline soils. The values of the soil electrical conductivity (EC), representing the soil salinity, at the three vineyards belonged to the non-saline ($0<EC<2$) class, according to the classes of salinity and EC in the Natural Resources Conservation Service (NRCS) Soil Survey Handbook (Cheng et al., 2014). The most fertile soil belongs to Eddeh due to its containment of the highest levels of nitrogen (0.339%), phosphor (100.64 ppm), potassium (705.83 ppm) and organic matter (5.65%), followed by Ain Bourday (0.237%, 91.76 ppm, 684.67 ppm and 3.94%, respectively) and then by Mdoukha (0.116%, 32.17 ppm, 189.82 ppm and 1.93%, respectively). Infertile soil, rather than fertile soil, has a higher composite and inorganic ion concentration which could up-regulate the gene expression related to flavonoid metabolism and activate flavonoid synthesis (Li et al., 2011; Ma et al., 2014) and wines from poorer soils exhibit higher total phenolic contents and color intensity (Wang et al., 2015).

II.3.2 Weather conditions

The weather data represented in Table 6 and Figure 27 corresponds to the period between véraison and grape harvest (from July till September) for the three vineyards, in 2018 and 2019. For both years, Mdoukha had the lowest mean air temperature (ranging from 23.5 to 24.16 ˚C), followed by Ain Bourday (ranging from 24.29 to 24.32 ˚C) then by Eddeh (ranging from 25.04 to 25.52 ˚C); Ain Bourday had the highest maximum temperatures (ranging from 34.25 to 34.95 ˚C) followed by Eddeh (31.55 ˚C) then by Mdoukha (31.22 to 31.35 ˚C); and Ain Bourday had the lowest minimum temperature (ranging from 13.2 to 14.03 ˚C).

Table 5: General composition of the soils from the three selected vineyards

Parameters	**Analysis Result**		
	Eddeh (300m asl)	**Mdoukha (1000 m asl)**	**Ain Bourday (1500 m asl)**
Texture (%)	Clay: 34.25 Loam: 13.58 Sand: 52	Clay: 32.12 Loam: 10.7 Sand: 57.18	Clay: 22.04 Loam: 14.66 Sand: 63.3
	Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam
pH water (1: 5)	8.18	8.52	8.35
Electrical Conductivity (mS.cm-1)	0.237	0.092	0.365
Organic Matter %	5.65	1.93	3.94
Total $CaCO_3$ (%)	5.6	9.39	42.4
Active $CaCO_3$ (%)	-	-	6.82
Organic Nitrogen (%)	0.339	0.116	0.237
Available P_2O_5 (ppm)	100.64	32.17	91.76
Exchangeable K_2O (ppm)	705.83	189.82	684.67
Exchangeable CaO (ppm)	691.55	296.03	968.54
Exchangeable MgO (ppm)	583.49	249.77	817.19
Exchangeable Na (ppm)	53.52	15.15	79.66

Table 6: Air temperature and rainfall data in Eddeh (300 m asl), Mdoukha (1000 m asl) and Ain Bourday (1500 m asl) in 2018 and 2019

	Air temperature (˚C)				Σ Rainfall (mm)
	Maximum	**Average**	**Minimum**	**Range**	
2018					
Eddeh	31.55	25.52	19.66	11.89	45.6
Mdoukha	31.22	24.16	17.73	13.49	4.4
Ain Bourday	34.25	24.32	14.03	20.22	4.6
2019					
Eddeh	31.55	25.04	18.47	13.08	2.6
Mdoukha	31.35	23.50	17.5	13.85	2.2
Ain Bourday	34.95	24.29	13.20	21.75	14

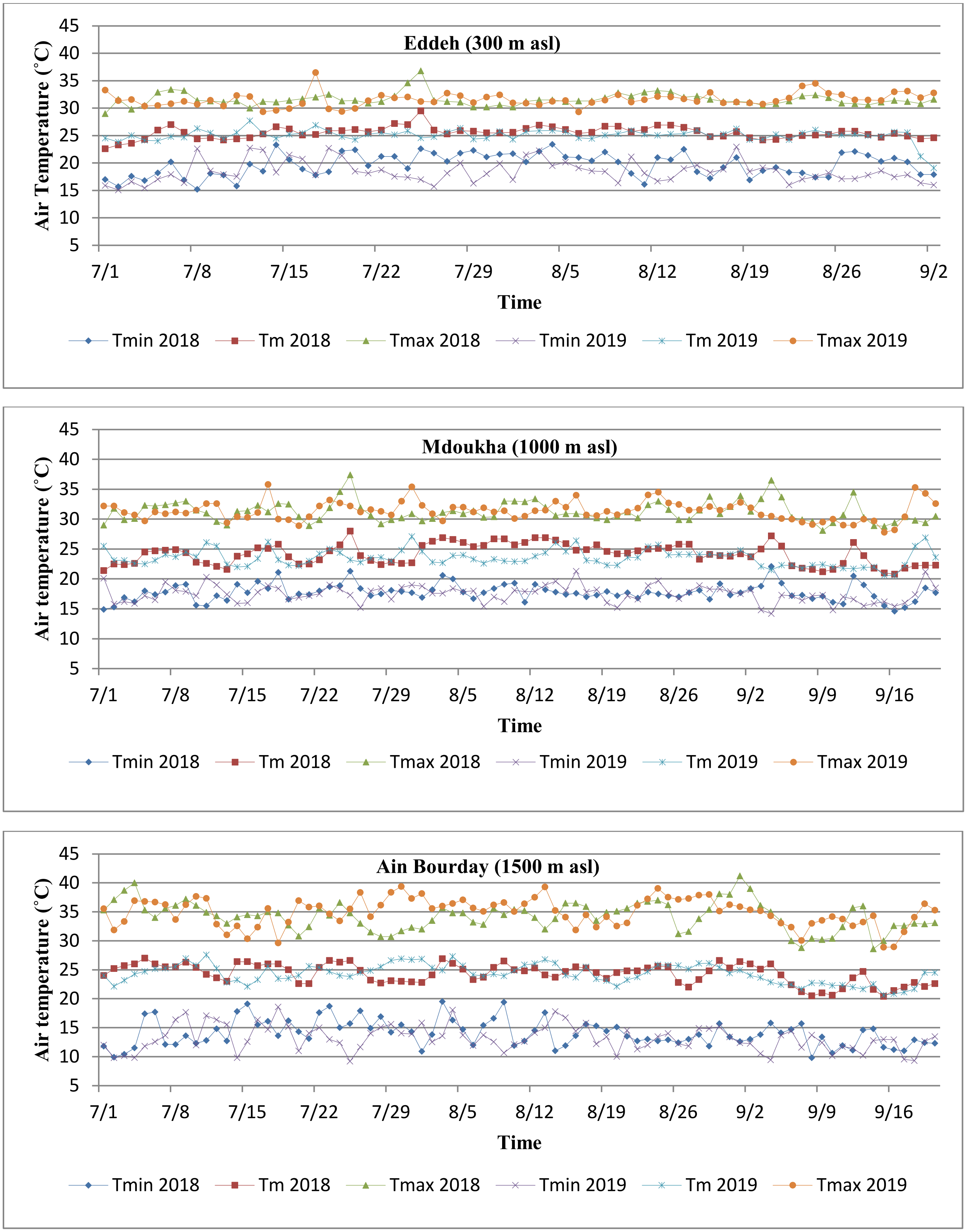


Figure 27: Evolution of maximum, mean and minimum daily temperature (˚C). Tmax: maximum temperature; Tm: mean temperature; Tmin: minimum temperature

Ain Bourday had also the largest diurnal temperature range (ranging from 20.22 to 21.75 ˚C), followed by Mdoukha (ranging from 13.49 to 13.85 ˚C) then by Eddeh (ranging from 11.89 to 13.08 ˚C). The daily thermal amplitude of the three vineyards increased from 2018 to 2019 due mainly to the decrease in nighttime temperatures. For the three vineyards and for both years, the daily maximum temperature exceeded 30 °C for most of the days in July, August and September. For these same months in the three vineyards, a higher number of days with maximum temperature exceeding 32 °C were observed in 2019 than in 2018. Relatively low precipitations were obtained in the three vineyards ranging from 2.2 mm for Mdoukha in 2019 to 45.6 mm for Eddeh in 2018.

II.3.3 Fruit chemistry analysis

The values in Table 7 are the overall means of sugar content, total acidity (TTA) and pH obtained from the grape samples of the three selected vineyards in 2018 and 2019. As mentioned before, the grapes were sampled at 3 ripeness levels, before, during and after the usual harvest time in 2018 and at three, five and seven weeks after véraison in 2019. For both years, between the first, the second and the third maturity level, the total soluble solids (TSS) increased significantly; the pH tended to increase whereas the TTA tended to decrease, although the differences were not always significant. The statistical analysis related to the altitude and the vintage effects were performed for the samples having the same maturity degrees. In the second year of the study characterized by higher values of maximum temperature (Tmax) than those of the first year at the end of ripening (Figure 27), TTA levels of Mdoukha and Ain Bourday vineyards at the third maturity level (2.85 and 3.15 g/L tartaric acid, respectively) were greater than those at the second maturity level (2.48 and 2.78 g/L tartaric acid, respectively). This could be explained by the concentrating effect of fruit dehydration during the later stages of berry ripeness, when the phloem's transfer of water and solutes between the vine and the berries slows (Sherman et al., 2017). In 2018, Mdoukha reached higher values of TSS than those of Eddeh and Ain Bourday for the third ripening level and in 2019, Mdoukha and Ain Bourday had higher values of TSS than those of Eddeh for the three ripening levels. The wines obtained from high-altitude vineyards are generally fresh, with high acidity (Gutiérrez-Gamboa et al., 2021) and, in 2018, Mdoukha and Ain Bourday vineyards had higher levels of total acidity and lower levels of pH than those of Eddeh courtesy of the cold climate of the high altitude regions (Brighenti et al., 2013). Also, the soils from Ain Bourday and Mdoukha are more alkaline and richer in calcium carbonate (42.4 and 9.39 %, respectively) than the soil of Eddeh (5.6 %). Calcareous soils don't retain heat and their cooler temperatures normally delay ripening (MacNeil, 2001) tending to yield more acidic grapes.

However, the average minimal temperature decreased the most in Eddeh from 2018 to 2019 (from 19.66 to 18.47 ˚C) and its average maximal temperature remained constant whereas the average maximal temperatures in Mdoukha and Ain Bourday increased from 2018 to 2019. This could have limited the degradation of acids in Eddeh in 2019 and resulted in higher levels of acidity for the second and third maturity levels and lower levels of pH for the three maturity levels than those in Mdoukha and Ain Bourday in 2019. Regarding the vintage effect for the same vineyard at the same maturity level, Eddeh showed higher values of TSS and pH and lower values of acidity in the first year than those of the following year.

Sugar accumulation is promoted by high temperatures (Costa et al., 2020) and for Mdoukha, the higher number of days with daily maximum temperature exceeding 32 °C and reaching 35.8 °C in 2019, could have contributed to higher values of TSS and pH and lower values of acidity in 2019 (except for the sample at 7 weeks after véraison for acidity) than those in 2018. No vintage effect was observed in Ain Bourday except for the pH which was higher at 7 weeks after véraison in 2019 than that in 2018.

Table 7: Fruit chemistry results (mean values and standard deviations) from the three vineyards in 2018 and 2019

V	Y	WAV	TSS	pH	TTA
Ed	2018	4	23.25* ± 0.11 cα	3.63 ± 0.01 cα	2.26 ± 0.01 aβ
		7	24.9± 0.07 bαA	3.86 ± 0.01 bαA	1.65 ± 014 bβB
		8	26.7± 0.07 aβ	3.95 ± 0.01 aα	1.5 ± 0.02 bβ
	2019	3	19.35 ± 0.21 cβ	3.19 ± 0.08 bγ	5.93 ± 0.74 aα
		5	22.45 ± 0.07 bβ	3.29 ± 0.09 bβ	4.88 ± 0.11 aα
		7	24.1 ± 0.14 aβB	3.8 ± 0.01 aγB	4.58 ± 0.11 aαA
Md	2018	5	24.55 ± 0.04 cB	3.29 ± 0.01 bB	3.9 ± 0.00 aA
		7	26.4 ± 0.14 bαB	3.4 ± 0.03 aγB	2.85 ± 0.00 bαA
		8	28.1 ± 0.14 aα	3.47 ± 0.01 aβ	2.75 ± 0.07 bα
	2019	3	23.65 ± 0.21 cα	3.86 ± 0.03 bα	5.33 ± 0.53 aα
		5	26.05 ± 0.07 bαA	3.92 ± 0.01 bαA	2.48 ± 0.32 bβB
		7	29.65 ± 0.64 aαA	4.16 ± 0.00 aαA	2.85 ± 0.21 bβA
Ab	2018	4	22.75 ± 0.04 cα	3.14 ± 0.01 bβ	5.54 ± 0.01 aα
		6	24.5 ± 0.14 b	3.49 ± 0.01 a	3.35 ± 0.03 b
		7	26.4 ± 0.14 aαA	3.52 ± 0.01 aβB	3.15 ± 0.02 cαA
	2019	3	24.25 ± 0.21 cα	3.64 ± 0.02 cβ	5.93 ± 0.11 aα
		5	26.15 ± 0.07 bα	3.75 ± 0.02 bα	2.78 ± 0.11 cβ
		7	28.4 ± 0.00 aαA	4.01 ± 0.01 aβA	3.15 ± 0.00 bβA

V: vineyard; **Y**: year; **Ed**: Eddeh; **Md**: Mdoukha; **Ab**: Ain Bourday; **WAV**: weeks after véraison; **TSS**: total soluble solids (˚Brix %); **TTA**: total acidity (g/L tartaric acid). * Values are the mean of two replicates for each extraction. Different lowercase Latin letters within the same column indicate significant differences among several maturity levels in the same vineyard and the same year. Different Greek letters within the same column indicate significant differences among vineyards at the same maturity level and the same year. Different uppercase Latin letters within the same column indicate significant differences between years for the same vineyard at the same maturity level.

II.3.4 Phenolic evolution of grapes

The evolution of the phenolic composition during grape ripening in the three vineyards in 2018 and 2019 is shown in Table 8. In overall terms, the concentrations of skin and seed phenolics were similar among the grape berries sampled at the three maturity levels from each vineyard and year. However, the skin phenolics of Eddeh for both years (except for skin proanthocyanidins in 2018) and the skin anthocyanins and total polyphenols of Mdoukha in 2018 increased between the first and the second ripeness level before declining at the third level although not always significantly. In a study on phenolic maturity of Cabernet Sauvignon, Merlot, Syrah and Cabernet Franc grapes grown in Lebanon conducted by Rajha et al. (2017), the anthocyanin content and the values of total polyphenol index (TPI) increased to reach a maximum then decreased. The authors reported that the decrease of the anthocyanin content could be due to the combination of anthocyanins and tannins.

In another study by Fournand et al. (2006), free anthocyanins and total red pigments accumulated on Shiraz grape skins up to the third week after véraison. Following that, free anthocyanins decreased somewhat, while overall red pigment concentration remained almost unchanged, suggesting that free anthocyanins were transformed to derived pigments. In the same trend, the skin proanthocyanidins of Eddeh declined in 2019 at the third ripeness degree after increasing between the first and the second ripening controls. Del Rio & Kennedy (2006) found that the decline of skin proanthocyanidins of Pinot noir grapes amount at harvest, to 65% of the maximum observed near véraison, could be linked to the formation of stable associations with cellular components such as polysaccharides or proteins which might make proanthocyanidins less extractable.

While following an erratic pattern between the first two maturity levels, the skin phenolics of Ain Bourday in both years and of Mdoukha in 2019 and the skin proanthocyanidins of Mdoukha in 2018 increased between the second and the third ripening stages although not always significantly. This increase can be explained by the fact that the grapes in Mdoukha and Ain Bourday are exposed to higher levels of UV radiation due to their higher altitudes and synthesized bigger amounts of anthocyanins on one hand and by the cooler night temperatures of these two vineyards that could have prolonged anthocyanin and tannin synthesis on the other hand. Cool night temperatures favored two enzymes in the anthocyanin biosynthesis pathway in the Darkridge (*Vitis vinifera* L. x *Vitis labrusca* L.) berry skin (Costa et al., 2020) and Sherman et al. (2017) found that an increase in the total per berry anthocyanin and skin tannin concentrations of Merlot grapes was concomitant with an increase in maturity, indicating that biosynthesis could occur still after traditional harvest maturity had been reached.

Except for seed proanthocyanidins in 2018 and seed polyphenols in 2019 in Ain Bourday that both reached their maximum levels in the first maturity degree, then fell to a lower value in the second maturity degree, only to increase again in the third maturity degree, the concentrations of seed phenolics in the three vineyards were not significantly different between the three ripeness stages for both years indicating that seed maturation was complete prior to the first ripeness level. Upon vineyard comparison, in 2018 and excluding skin proanthocyanidins at the last two stages of ripening, the skin and seed phenolics in Mdoukha had the highest values among the three vineyards even if not always significantly. This could be explained by the fact that Mdoukha has the poorest soil with the least levels of nitrogen, phosphor, potassium and organic matter and the least rainfall amount that induced the vines to produce smaller berries with increased polyphenol and anthocyanin content. The synthesis of polyphenols is stimulated by low nitrogen supply (Van Leeuwen et al., 2018) and according to a study by Cheng et al. (2014), higher anthocyanin concentrations in Cabernet Sauvignon grape skins were obtained from soils with less water and organic matter.

Nevertheless, in the following year, it is possible that the vintage factor overruled the soil factor. At the last ripening level in 2019, the seed and skin phenolics of Eddeh and Ain Bourday reached similar values to those of Mdoukha, and some values of the skin phenolics of Eddeh particularly at the first two ripening controls, were significantly higher than those of Mdoukha and Ain Bourday. In fact, the diurnal shift increased the most in Eddeh and Ain Bourday from 2018 to 2019 and led to an increase in polyphenol and anthocyanin content in these vineyards from the first to the second year of the study. The temperature variation between day and night exerts a significant impact on anthocyanin synthesis. Within a specific temperature range, the accumulation of anthocyanins is induced by a higher daytime temperature resulting in a bigger photosynthesis, and a lower nighttime temperature which leads to a reduced plant respiration (Liang et al., 2014). Furthermore, under the influence of low temperatures and sunlight exposure that are associated with high altitude sites, the seed phenolics of Mdoukha and Ain Bourday were higher than those of Eddeh for both years excluding seed extractable polyphenols in Mdoukha in 2019. A similar result was obtained in a previous study by Coklar (2017) on Ekşikara grapes native to Mediterranean region, whose total polyphenol and tannin levels in the seeds at the high elevation vineyard were significantly greater than those at the low elevation vineyard.

Table 8: Phenolic evolution of grapes (mean values and standard deviations) from the three vineyards in 2018 and 2019

V	Y	WAV	EPosk	EAnsk	EPrsk	EPose	EPrse
Ed	2018	4	267.11 ± 54.99 aα*	253.63 ± 36.85 aα	1062.67 ± 116.13 aα	355.45 ± 49.14 aβ	360.34 ± 15.87 aβ
		7	426.18 ± 83.88 aαA	277.50 ± 20.95 aβA	1108.32 ± 28.12 aαA	356.59 ± 75.66 aαB	222.74 ± 70.15 aαB
		8	256.57 ± 63.97 aα	230.90 ± 50.92 aα	1240.21 ± 754.12 aα	378.39 ± 198.57 aα	234.06 ± 30.88 aα
	2019	3	558.35 ± 12.96 abα	316.96 ± 0.07 aα	1683.72 ± 68.03 aα	987.12 ± 62.98 aα	897.45 ± 99.78 aα
		5	619.15 ± 23.06 aα	348.05 ± 3.63 aα	1818.03 ± 8.27 aα	707.31 ± 35.15 aβ	660.00 ± 152.47 aα
		7	509.44 ± 33.5 bαA	346.52 ± 22.57 aαA	1279.64 ± 139.74 bαA	972.26 ± 107.27 aαA	757.93 ± 96.46 aαA
Md	2018	5	509.36 ± 105.74 aA	365.87 ± 85.1 aA	1705.35 ± 232.48 aA	913.25 ± 187.18 aA	440.42 ± 67.22 aB
		7	580.97 ± 82.55 aαA	469.85 ± 50.63 aαA	723.19 ± 246.35 bαA	813.58 ± 233.27 aαA	474.43 ± 177.07 aαB
		8	437.15 ± 56.85 aα	411.03 ± 55.88 aα	1190.15 ± 73.19 abα	695.00 ± 243.26 aα	392.02 ± 71.38 aα
	2019	3	371.08 ± 72.13 aα	166.44 ± 7.48 cβ	927.18 ± 69.99 bβ	977.78 ± 10.58 aα	949.74 ± 121.37 aα
		5	404.49 ± 4.52 aβA	245.16 ± 2.94 bβA	938.42 ± 32.92 bβB	935.00 ± 21.06 aαA	1030.85 ± 53.67 aαA
		7	532.94 ± 45.89 aαA	343.53 ± 1.48 aαA	1299.96 ± 46.87 aαA	875.79 ± 42.95 aαA	1192.35 ± 6.92 aαA
Ab	2018	4	294.04 ± 38.88 aα	163.24 ± 42.3 aα	620.35 ± 45.63 aα	974.72 ± 185.3 aα	590.48 ± 21.76 aα
		6	291.54 ± 36.85 a	205.90 ± 10.73 a	428.83 ± 76.27 a	700.64 ± 96.88 a	320.65 ± 62.88 b
		7	387.88 ± 13.68 aαB	235.43 ± 45.15 aβA	1067.03 ± 166.39 aαA	611.99 ± 28.53 aαB	424.09 ± 31.23 aαB
	2019	3	574.68 ± 76.01 aα	307.74 ± 33.25 aα	957.68 ± 71.9 aβ	1013.27 ± 30.27 aα	1185.24 ± 134.27 aα
		5	510.61 ± 66.34 aαβ	375.63 ± 12.84 aα	1069.53 ± 78.48 aβ	914.21 ± 10.17 bα	1067.79 ± 139.16 aα
		7	532.08 ± 41.15 aαA	407.86 ± 50.14 aαA	1093.23 ± 170.72 aαA	989.36 ± 4.48 abαA	1136.63 ± 154.72 aαA

* Values are the mean of two replicates for each extraction. Different lowercase Latin letters within the same column indicate significant differences among several maturity levels in the same vineyard and the same year. Different Greek letters within the same column indicate significant differences among vineyards at the same maturity level and the same year. Different uppercase Latin letters within the same column indicate significant differences between years for the same vineyard at the same maturity level. **EPosk**: Extractable polyphenols in grape skins (mg of catechin /kg of grape); **EAnsk**: Extractable anthocyanins in grape skins (mg of malvidin /kg of grape); **EPrsk**: Extractable proanthocyanidins in grape skins (mg of cyanidin chloride /kg of grape); **EPose:** Extractable polyphenols in grape seeds (mg of catechin /kg of grape); **EPrse:** Extractable proanthocyanidins in grape seeds (mg of cyanidin chloride /kg of of grape).

However in another study by Mateus et al. (2001), while altitude exerted a positive effect on the total extractable proanthocyanidins (TPA) in the seeds of Touriga Francesa grapes, it exerted a negative effect on TPA in the seeds of Touriga Nacional grapes showing that the altitude factor seemed to be cultivar-dependent.

Upon vintage comparison, the seed phenolics in the three vineyards in 2019 were greater than those in 2018 even if not always significantly. The year 2019 was characterized by a larger temperature difference between day and night than that in 2018 and a higher number of days with daily maximum temperature exceeding 32 °C and reaching 36.5 °C in Eddeh, 35.8 °C in Mdoukha and 39.4 °C in Ain Bourday. These weather conditions in the second growing season might have favored more the biosynthesis of phenolics in the grape seeds. In the present study, altitude had a positive effect on the seed proanthocyanidins and a negative effect on the skin proanthocyanidins. In fact, Mdoukha and Ain Bourday had lower values of skin proanthocyanidins although not always significantly, except for the sample from Mdoukha at 7 weeks after véraison in 2019. In contrast, Barreto de Oliveira et al. (2019) found that the skins of Syrah grapes at the high site with 1.100 m altitude contained higher levels of total condensed tannins than those at the low site with 350 m altitude and that the total condensed tannins of Syrah seeds at the low site were greater than those at the high site. The altitude factor appears to impact differently in different parts of the same cultivar in addition to its different influence between cultivars.

II.3.5 Evolution of the individual anthocyanin compounds

HPLC analysis was performed on skin extracts produced from grapes collected at the harvest time for commercial winemaking for the first year of the study and at three, five, and seven weeks following véraison for the second year. Delphinidin 3-O-glucoside (Dp), cyanidin 3-O-glucoside (Cy), petunidin 3-O-glucoside (Pt), peonidin 3-O-glucoside (Pn), malvidin 3-O-glucoside (Mv), sum of acetylated anthocyanins (SAc) and sum of p-coumaroylated anthocyanins (SCo) were determined (Table 9). In 2018, Mdoukha which contains the poorest soil with the least amounts of organic matter and water, had the highest levels of acylated and non-acylated anthocyanins even if not always significantly, except for Pn which was the greatest in Eddeh. In a study conducted by Cheng et al. (2014), higher quantities of 3′5′-substituted, O-methylated and acylated anthocyanins were found in Cabernet Sauvignon grapes from soils with less water and organic matter, which was a favorable trait that would give more persistent coloration to the corresponding wine in the future. However, a decrease was observed for the total amount of the individual anthocyanins of Mdoukha in 2019 reaching significantly lower levels than those of Eddeh and Ain Bourday at the last ripening control. This decrease could be linked to the degradation and inhibition of the biosynthetic pathway of anthocyanins courtesy of the higher temperatures that characterized the year 2019. In a study on Grenache noir, Xinomavro, Agiorgitiko and Syrah cultivars, Theodorou et al. (2019) related the decrease in anthocyanin accumulation with the greater temperatures of the cluster zone due to the smaller vine leaf area at the end of the ripening period. Regarding Eddeh and Ain Bourday, the increase in diurnal temperature might have favored the accumulation of the individual anthocyanins in the grape skins.

Table 9: Individual anthocyanin compounds (mean values and standard deviations (mg/Kg of grape skin)) from the three vineyards in 2018 and 2019

V	Y	WAV	Dp*	Cy	Pt	Pn	Mv	SAc	SCo	Total
Ed	2018	7	11.47 ± 1.25 βB	11.05 ± 1.53 αA	18.74 ± 1.86 βB	86.12 ± 23.89 αA	380.95 ± 17.68 βB	9.25 ± 0.18 αA	16.66 ± 1.51 α βB	534.22 ± 47.9α βB
	2019	3	13.92 ± 0.42 cβ	4.99 ± 0.22 bβ	23.52 ± 0.66 cβ	45.29 ± 2.86 bβ	293.04 ± 14.58 bβ	9.66 ± 0.16 aβ	11.57 ± 0.12 bγ	402.00 ± 17.95 bβ
		5	20.73 ± 2.45 bβ	5.89 ± 0.52 bβ	34.67 ± 3.6 bβ	54.98 ± 4.44 bβ	503.64 ± 44.45 aβ	21.99 ± 3.32 aα	30.49 ± 4.55 bα	672.39 ± 63.32 aβ
		7	29.93 ± 0.42 aAβ	9.57 ± 0.59 aAβ	46.92 ± 0.01 aAβ	70.46 ± 1.83 aAα	571.89 ± 12.17 aAαβ	20.42 ± 5.32 aAα	55.86 ± 8.83 aAα	805.05 ± 23.49 aAαβ
Md	2018	7	37.95 ± 6.16 αA	14.43 ± 2.15 αA	56.58 ± 8.7 αA	64.02 ± 6.81 αA	647.21 ± 74.09 αA	11.49 ± 0.91 αA	20.18 ± 0.8 αA	851.86 ± 98.02 αA
	2019	3	20.50 ± 0.61 bβ	6.96 0.00 aβ	29.67 ± 1.42 bβ	39.83 ± 0.17 bβ	276.96 ± 17.02 bβ	10.64 0.47 cαβ	13.86 ± 0.23 bβ	398.43 ± 18.52 bβ
		5	30.70 ± 0.23 aβ	8.97 ± 0.44 aβ	44.37 ± 0.84 aβ	68.42 ± 2.48 aαβ	472.12 ± 10.48 aβ	14.42 ± 0.46 bα	18.04 a± 0.2 bα	657.05 ± 14.13 aβ
		7	32.01 ± 3.2 aAβ	8.72 ± 0.93 aAβ	45.10 ± 4.34 aAβ	59.55 ± 6.5 aAα	487.95 ± 38.95 aAβ	17.45 ± 0.87 aAα	22.00 ± 1.99 aAβ	672.77 ± 56.78 aAβ
Ab	2018	6	12.69 ± 2.69 βB	9.77 ± 1.47 αB	18.62 ± 4.63 βB	29.54 ± 6.37 αB	186.45 ± 68.01 βB	nd	4.37 ± 1.93 βB	261.44 ± 76.99 βB
	2019	3	53.21 ± 4.62 aα	21.79 ± 1.42 aα	60.61 ± 2.59 aα	85.33 ± 3.05 aα	465.52 ± 5.24 bα	12.88 ± 0.88 bα	17.71 ± 0.35 aα	717.06 ± 5.91 bα
		5	64.16 ± 6.54 aAα	17.83 ± 1.93 aAα	80.90 ± 8.61 aAα	90.30 ± 9.8 aAα	756.47 ± 70.58 aAα	21.24 ± 1.78 aα	29.34 ± 3.37 aAα	1060.23 ± 102.62 aAα
		7	62.16 ± 4.63 aα	16.10 ± 2.26 aα	76.31 ± 5.42 aα	87.08 ± 9.26 aα	697.78 ± 50.78 aα	22.55 ± 1.6 aα	28.36 ± 4.16 aβ	990.32 ± 78.11 abα

***Dp** (delphinidin 3-O-glucoside), **Cy** (cyanidin 3-O-glucoside), **Pt** (petunidin 3-O-glucoside), **Pn** (peonidin 3-O-glucoside), **Mv** (malvidin 3-O-glucoside), **SAc** (sum of acetylated anthocyanins), **SCo** (sum of p-coumaroylated anthocyanins). Different lowercase Latin letters within the same column indicate significant differences among several maturity levels in the same vineyard and the same year. Different Greek letters within the same column indicate significant differences among vineyards at the same maturity level and the same year. Different uppercase Latin letters within the same column indicate significant differences between years for the same vineyard at the same maturity level.

For the three vineyards and for both years, the non-acylated anthocyanins were more prevalent than the acylated anthocyanins and the contribution of Mv was the highest to the total anthocyanin concentration followed by the contributions of Pn, Pt, Dp and Cy in descending order. Besides, the levels of SCo were always greater than those of SAc throughout ripening. Non-acylated anthocyanins predominate in the red wine grape cultivars traditionally grown in the Spanish area of La Mancha (Cencibel/Tempranillo, Garnacha/Grenache, and Garnacha Tintorera), with malvidin 3-monoglucoside being the major anthocyanin, especially in Grenache and p-coumaroyl derivatives being the main acylated anthocyanins (Hermosín Gutiérrez & García-Romero, 2004).

In 2019, the amount of the individual anthocyanins showed an increasing trend during the ripening period in Eddeh and Mdoukha with few exceptions, whereas in Ain Bourday it increased between the first and the second maturity stages then remained almost constant in the third maturity stage. Regarding the altitude effect between vineyards at the same year and maturity level, in 2018 and excluding Pn, the levels of the individual anthocyanins in Mdoukha were higher than those in Eddeh, though not always significantly. Also in 2019, an altitude effect was observed during ripening; all of the non-acylated anthocyanins in Ain Bourday, the highest site, had their levels significantly greater than those of Eddeh and Mdoukha (except for Pn in Eddeh at 7 weeks after véraison). A similar result was observed for the acylated anthocyanins only at 3 weeks after véraison. Altitude which is associated with lower temperatures and higher sunlight exposure, can affect differently the synthesis of individual anthocyanins in grape berries. When comparing two cultivation sites of Ekşikara grapes located at 1000 and 1500 m asl in Turkey, the relative amount of Mv, Pn and Cy in grape skins increased with decreasing altitude whereas Pt, Dp and acylated anthocyanins ratios decreased (Coklar, 2017).

II.3.6 Wine chemistry analysis

Table 10 shows the data of the analysis performed on the produced wines as a function of harvested year and altitude. The collection time and the degree Brix of the grape samples for winemaking are mentioned in paragraph II.2.3 “Maceration and fermentation procedures”. Most of the parameter values lay within the range observed in previous studies performed on Grenache wines. The levels of pH (3.12 – 3.46), TPI (49 – 69), total anthocyanins (TA) (174 – 378 mg/L) and total tannins (TT) (316 – 2039 mg/L) were close to the levels found by Pascual et al. (2016)

(pH: 4.2 – 4.4; TPI: 42 – 55; TA: 280 – 365 mg/L; TT: 509 – 655 mg/L) and by Maza et al. (2020) (pH: 3.2 ; TPI: 39 – 46; TA: 308 – 478 mg/L; TT: 901 – 1041 mg/L). Also, the levels of total acidity (4.2 – 5.13 g/L tartaric acid) were close to those found by Garde-cerdán et al. (2013) (4.3 g/L tartaric acid) and Andrés et al. (2020) (4.2 – 4.4 g/L tartaric acid).
Upon altitude effect, in 2018, the total acidity and the tartaric acid levels in the wines from Mdoukha and Ain Bourday were higher than those in the wine from Eddeh due to the cold climate of these high altitude sites that limits the degradation of acids. However, in the following year, the decrease in the night temperature in Eddeh (1.19 ˚C drop) might have slowed the degradation of acids and resulted in higher amounts of total acidity and tartaric acid than those of Mdoukha. Still in 2019, these amounts remained lower than those of the highest site, Ain Bourday. The pH levels followed the opposite pattern of the total acidity levels, but the results of malic acid did not show a consistent pattern. The greatest content of reducing sugars (4.4 g/L) was found in Mdoukha at the end of the laboratory microvinification in 2018, which may be due to the highest degree Brix (26.4) of the grapes collected for laboratory microvinification from that vineyard in that year.

In 2018, there was a correlation between the changes in the phenolic content in the grapes between vineyards (Table 8) and the changes in the phenolic content of the corresponding wines. In fact, among the three vineyards, the vines of Mdoukha, containing the poorest soil, produced the smallest grapes in 2018 and consequently, more berries were required to produce the same volume of wine, enriching its final polyphenolic composition. As a result, the wine from Mdoukha in 2018 had the highest anthocyanin content (378 mg/L) and the greatest color intensity (3.31). Touriga Nacional grapes which are characterized by their lower average berry weight, contained higher content of total anthocyanidin monoglucosides and low molecular procyanidin oligomers in the resulting wine as compared with Touriga Francesa grapes (N. Mateus et al., 2001). Furthermore, the wine from Mdoukha contained the highest levels of TPI (69 mg/L) and total tannins (670 mg/L) in 2018.

Grapevines grown in high altitude vineyards with cooler nighttime temperatures have a greater potential for color (Gutiérrez-Gamboa et al., 2018) and an altitude effect was observed also in this study for the wines produced from the highest sites. For both years, the wines from Mdoukha and Ain Bourday had greater color intensity (ranging from 1.79 to 3.31) than that from Eddeh (ranging from 1.2 to 1.63) despite having lower amount of anthocyanins more often. This lower content of anthocyanins might be caused by copigmentations between low molecular procyanidin oligomers and total anthocyanidin monoglucosides existing in the produced wine as reported in a study conducted by Mateus et al. (2001) on Touriga Nacional (TN) grapes. The wine produced from TN grapes had higher color intensity even though having lower anthocyanins content. Moreover, anthocyanins and low molecular procyanidin oligomers can form covalent connections by direct condensation or ethyl linkages, resulting in highly complex structures that produce a persistent and intensified wine color.

A similar growing season effect was observed between the phenolic content of the grapes used for laboratory microvinification and the phenolic content of the corresponding wines. In fact, in Mdoukha, the decrease in polyphenol and anthocyanin content in the grape skins from 2018 to 2019 (Table 8) resulted in lower content of TPI and total anthocyanins in the corresponding wine in 2019. For Mdoukha also, the increase in grape skin and seed proanthocyanins between years resulted in higher content of total tannins in the corresponding wine in 2019. Regarding Eddeh and Ain Bourday, the increase in polyphenol and anthocyanin content in the grape skins and seeds of these vineyards from the first to the second year of the study resulted in higher content of TPI, total anthocyanins and total tannins in the corresponding wines in 2019.

In 2018, the levels of the total tannins in the wines produced from the grapes of the three vineyards harvested at the time decided by the winegrowers were significantly lower than those obtained in 2019, although the degree Brix was more or less the same between years. Hence, evaluating the phenolic maturity in addition to the technological maturity is critical for determining the optimal harvest time of the grapes.

Table 10: Chemical analysis (mean values and standard deviations) of the produced wines from the three vineyards in 2018 and 2019

V	**Ed**		**Md**		**Ab**	
Y	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
TTA	4.20 ± 0.09 γB	4.60 ± 0.15 βA	5.13 ± 0.05 αA	4.07 ± 0.05 γB	5.00 ± 0.09 βA	4.97 ± 0.1 αA
TaA	1.93 ± 0.05 γB	3.73 ± 0.1 βA	2.9 ± 0.15 βB	3.08 ± 0.08 γA	3.35 ± 0.16 αB	4.03 ± 0.08 αA
MA	2.02 ± 0.08 αA	1.45 ± 0.08 γB	1.83 ± 0.05 βA	1.70 ± 0.00 βB	1.72 ± 0.04 γB	1.87 ± 0.05 αA
pH	3.30 ± 0.01 αA	3.24 ± 0.02 βB	3.12 ± 0.00 γB	3.46 ± 0.02 αA	3.18 ± 0.02 βA	3.21 ± 0.04 βA
RS	1.87 ± 0.21 βA	1.97 ± 0.32 αA	4.40 ± 2.02 αA	1.05 ± 0.1 βB	0.95 ± 0.08 βB	1.27 ± 0.08 βA
CI	1.20 ± 0.06 γB	1.63 ± 0.4 βA	3.31 ± 0.25 αA	1.82 ± 0.08 βB	1.79 ± 0.26 βB	3.13 ± 0.23 αA
TPI	49 ± 3 βA	56 ±14 αA	69 ± 6 αA	41 ± 3 βB	56 ± 8 βA	56 ± 7 αA
TA	203 ± 10 βB	336 ± 59 αA	378 ± 58 αA	174 ± 16 βB	189 ± 44 βB	294 ± 26 αA
TT	338 ± 173 βB	2039± 300 αA	670 ± 140 αB	1211 ± 144 γA	316 ± 171 βB	1698 ± 88 βA

V: vineyard, **Y**: year, **Ed**: Eddeh, **Md**: Mdoukha, **Ab**: Ain Bourday, **TTA**: Total acidity (g/L), **TaA**: Tartaric acid (g/L), **MA**: Malic acid (g/L), **RS**: Reducing sugars (g/L), **CI**: Color intensity, **TPI**: Total polyphenol index, **TA**: total anthocyanins (mg/L), **TT**: Total tannins (mg/L). Different Greek letters within the same column indicate significant differences among vineyards at the same maturity level and the same year. Different uppercase Latin letters within the same column indicate significant differences between years for the same vineyard at the same maturity level.

II.3.7 VOCs in wine

In order to find the amount of the main volatiles in the three Grenache vineyards, we made the sum of the concentrations of each volatile from the three vineyards then the mean was calculated. The qualitative chemical characterization revealed the presence of 37 main compounds that included varietal compounds and compounds derived from yeast metabolism. From the results on the quantitative data, obtained by calibration curves of pure standards, presented in Table 11, we can conclude that the main volatiles of the three Grenache vineyards were in a decreasing order: 1-butanol-2-methyl (mean values 328.11 mg/L in 2018 and 455.44 mg/L in 2019), phenyl ethyl alcohol (mean values 78.79 mg/L in 2018 and 60.20 mg/L in 2019, as above) and ethyl acetate (mean values 61.29 mg/L in 2018 and 59.17 mg/L in 2019, as above). These findings are concordant with a recent study conducted on VOCs of Grenache wines produced from different vineyards located across Sardinia (Petretto et al., 2021). 1-butanol-2-methyl, produced from branched-chain amino acids leucine and isoleucine in the classical Erlich pathway, imparts to wine malty/solvent-like aroma while phenyl ethyl alcohol is reminiscent of flowery/honey-like notes (Czerny et al., 2008). The concentrations of ethyl acetate obtained in 2018 and 2019 ranged between 49.91 mg/L and 73.69 mg/L which are much higher than its estimated odor perception threshold (7.5 mg/L) contributing largely to the fruity/solvent-like flavors of the produced wines. Another ester of fermentative origin, isoamyl acetate was detected at a higher level (mean values 0.25 mg/L in 2018 and 1.42 mg/L in 2019) than its sensory threshold imparting banana, pear flavor (Swiegers et al., 2005). 1-hexanol, formed mainly during prefermentative production steps, was detected (mean values 1.26 mg/L in 2018 and 3.23 mg/L in 2019) and it is usually associated with grassy, herbaceous aromas (González-Barreiro et al., 2015).

The total amount of VOCs in Ain Bourday in 2018 was significantly lower than that in 2019. It was also lower than the total amount of VOCs in Mdoukha and Eddeh for both years. The variation in the diurnal shift at high-altitude regions during the growing season incites the grapes to develop flavor (Xing et al., 2016) and the high altitude vineyards Mdoukha and Ain Bourday had a higher number of volatile compounds with greater levels than those in Eddeh for both years and particularly in 2019, showing an altitude effect. Another altitude effect was shown since the wines from the high sites Mdoukha and Ain Bourday that are characterized by their dryer climate, stronger sunshine and lower night-time temperature, had higher amounts of ethyl acetate, ethyl butanoate and ethyl decanoate for both years as compared to those in Eddeh.

Therefore, these ethyl esters, ethyl acetate, ethyl butanoate and ethyl decanoate that evoke respectively solvent-like, floral fruity and floral soap aromas can distinguish the high altitude wines from the low altitude wines. Nevertheless, in a study conducted by Bao Jiang (2012) on young Cabernet Sauvignon wines produced from two cultivation sites at 909.3 m and 1280.5 m altitude, the low altitude wine had more pronounced fruity aromas (pineapple, pear and banana) with floral notes because of the greater odor active values of isoamyl acetate and ethyl octanoate. On the other hand, Mdoukha and Ain Bourday are characterized by higher levels of sand in their soil and the wines from sandy soils have greater contributions from the solvent aroma series (González-Barreiro et al., 2015). For both years, the total amount of VOCs was found the highest in Mdoukha. This could be explained by the fact that infertile soil, as in the case of Mdoukha, is characterized by higher fruit maturation versus vegetative growth, what favors flavor development (González-Barreiro et al., 2015).

Among the terpenoids that are a key component of varietal aroma and are unaffected by yeast metabolism during fermentation (Bao Jiang, 2012), citronellol and limonene were found in trace amounts. As compared with the other vineyards, Mdoukha had the highest values of these two terpenoids for both years. Thus, these two compounds could serve as potential indicators to distinguish the wine produced from Mdoukha. The esters and alcohols were the most represented compound classes in terms of the number and concentration of volatiles in the produced wines from the three vineyards. The neogenesis of most of the identified and quantified aroma compounds including 8 esters, 3 alcohols, and one aldehyde is attributable to yeast metabolism and their production is dependent on the yeast strain, the composition of the must and the fermentation conditions. At the same time, the amount of amino acids in the must has a direct impact on the quality of the wine in view of the fact that during alcoholic fermentation, amino acids undergo a number of changes that result in flavor-active metabolites like volatile fatty acids, higher alcohols and esters (Petropoulos et al., 2018). Furthermore, the content of yeast assimilable nitrogen (YAN), which affects yeast growth and fermentation activity in grape must, is reported to be positively correlated with altitude. In fact, in a study conducted on Chardonnay and Shiraz grape samples cultivated at different altitudes ranging from 700m to over 1200m in the Granite Belt, Australia, it was found that as altitude increased, so did average YAN values (Laboratories, 2012). This could explain the fact that the wines from the high sites Mdoukha and

Ain Bourday had higher amounts of ethyl acetate, ethyl butanoate and ethyl decanoate and a higher number of volatile compounds with greater levels than those in Eddeh for both years.

Table 11: GC-MS results (mean values and standard deviations) of the main VOCs in the produced wines from the three vineyards in 2018 and 2019

Compound	Eddeh mg/L		Mdoukha mg/L		Ain Bourday mg/L	
	2018	2019	2018	2019	2018	2019
Ethyl acetate	52.981 ± 8.2 βA	49.913 ± 6.3 βA	69.939 ± 6.67 αA	73.695 ± 3.1 αA	60.953 ± 4.9 αβA	53.911 ± 4.1 βA
Ethyl butanoate	0.473 ± 0.05 βA	0.274 ± 0.05 βB	0.633 ± 0.028 αB	0.93 ± 0.1 αA	0.665 ± 0.1 αA	0.57 ± 0.09 αβA
Isoamyl acetate	0.255 ± 0.03 βB	0.92 ± 0.13 γA	0.142 ± 0.033 γB	1.785 ± 0.18 αA	0.34 ± 0.05 αB	1.561 ± 0.094 βA
Limonene	0.002 ± 0.00 αA	tr	0.002 ± 0.00 αA	tr	0.002 ± 0.00 αA	tr
1-butanol-2-methyl	357.13 ± 50.75 αA	417.1 ± 53.48 αA	367.48 ± 51.6 αB	470.1 ± 9.75 αA	259.73 ± 26.06 βB	479.129 ± 14.47 αA
Ethyl hexanoate	0.654 ± 0.05 βA	0.167 ± 0.01 γB	0.757 ± 0.17 αβA	0.425 ± 0.01 αB	0.91 ± 0.06 αA	0.364 ± 0.01 βB
1-hexanol	0.65 ± 0.09 βB	1.526 ± 0.51 γA	1.731 ± 0.46 αB	4.525 ± 0.56 αA	1.412 ± 0.28 αB	3.64 ± 0.2 βA
Ethyl octanoate	0.062 ± 0.00 βB	0.214 ± 0.03 γA	0.04 ± 0.00 γB	0.638 ± 0.06 αA	0.11 ± 0.015 αB	0.521 ± 0.026 βA
Benzaldheyde	tr	tr	0.007 ± 0.00 α	tr	0.016 ± 0.00 α	tr
Ethyl decanoate	tr	tr	0.02 ± 0.00 αB	0.554 ± 0.14 αA	0.009 ± 0.00 βB	0.21 ± 0.12 βA
Citronellol	0.02 ± 0.00 αA	0.01 ± 0.00 βB	0.032 ± 0.016 αA	0.013 ± 0.00 αA	0.013 ± 0.00 αA	0.011 ± 0.00 βA
Methyl salicilate	0.006 ± 0.00 α	tr	0.003 ± 0.00 β	tr	0.005 ± 0.00 αβ	tr
Ethyl dodecanoate	tr	0.021 ± 0.00 γ	0.005 ± 0.00 B	0.149 ± 0.03 αA	tr	0.092 ± 0.023 β
Phenyl ethyl alcohol	89.75 ± 14.12 αA	66.07 ± 5.49 αB	94.16 ± 18.29 αA	54.14 ± 4.65 βB	52.46 ± 9.35 βA	60.38 ± 2.00 αβA
Total VOCs	501.982 ± 65.66 αA	536.206 ± 8.33 αA	534.951 ± 53.72 αB	606.947 ± 35.63 αA	376.675 ± 34.31 βB	600.388 ± 26.46 αA

Different Greek letters within the same column indicate significant differences among vineyards at the same maturity level and the same year. Different uppercase Latin letters within the same column indicate significant differences between years for the same vineyard at the same maturity level.

II.3.8 Principal component analysis (PCA)

Figures 28 (for the year 2018) and 29 (for the year 2019) show the PCA analysis of the data represented in Tables 9, 10 and 11 corresponding to the skin individual anthocyanins, the wine chemical compounds and the wine volatile compounds respectively.

In figure 28, the first two principal components explained 99.8% of total variability (PC1 vs. PC2), where PC1 was responsible by 63.18% and PC2 explained 36.62%. The scatter plot in Figure 28 shows that the samples from Mdoukha are the richest in all the wine phenolics and mostly all the skin individual anthocyanins, and in color due to the highest contents of skin and wine anthocyanins. As mentioned before, the soil factor seems to play a major role in the accumulation of phenolic compounds in the grapes and subsequently in the wine. The Grenache cultivar is known to be best suited to poor and dry soil which is the case in Mdoukha. Moreover, the wine from Mdoukha was mainly characterized by the volatiles ethyl acetate, ethyl decanoate, ethyl dodecanoate and limonene. The wine from Ain Bourday was characterized by the volatiles benzaldheyde, ethyl octanoate, isoamyl acetate, ethyl hexanoate, ethyl butanoate and 1-hexanol. The wine from Eddeh was characterized by the compounds methyl salicilate, peonidin and malic acid and by the highest pH.

In figure 29, the first two principal components explained 100% of total variability (PC1 vs. PC2), where PC1 was responsible by 61.51% and PC2 explained 38.49%. In 2019, the samples from Mdoukha were the richest in mostly all the aroma compounds. However, the samples from Ain Bourday, the highest site where the diurnal temperature increased the most (1.53 ˚C) from the first to the second year of the study, were the richest in mostly all the skin individual anthocyanins.

Furthermore, the wine from Ain Bourday was mainly characterized by the greatest color intensity and levels of TPI, total acidity, tatrtaric acid, and the volatile 1-butanol-2-methyl, while the wine from Eddeh was mainly characterized by the greatest levels of total tannins, p-coumaroylated anthocyanins and the volatile phenyl ethyl alcohol.

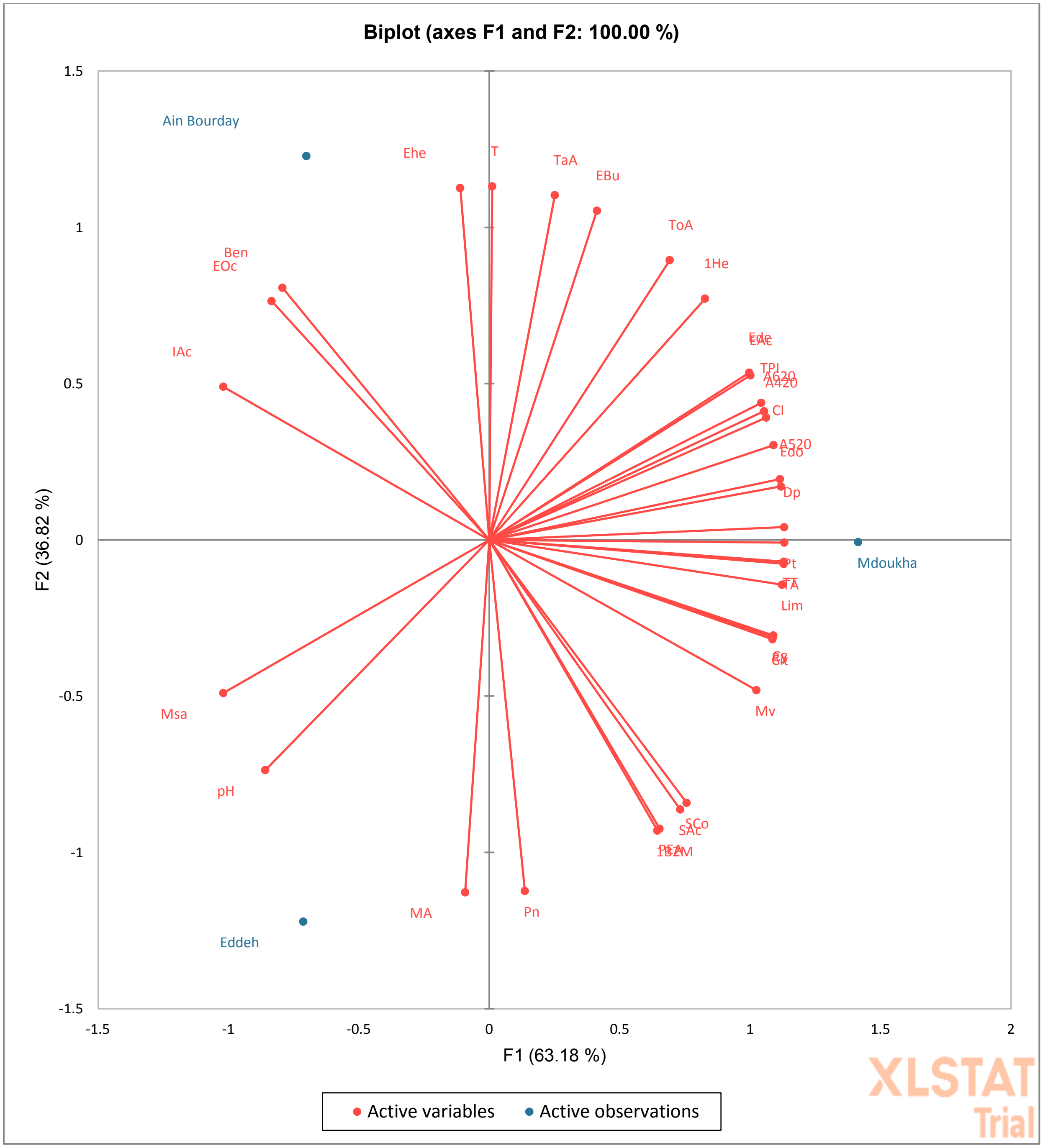


Figure 28: Principal component analysis of skin individual anthocyanins and wine chemical and volatile compounds in Eddeh, Mdoukha and Ain Bourday in 2018. **TTA**: total acidity; **TaA**: tartaric acid; **MA**: malic acid; **RS**: reducing sugars; **CI**: color intensity; **T**: tint; **TPI**: total polyphenol index; **TA**: total anthocyanins; **TT**: total tannins; **EAc**: ethyl acetate; **EBu**: ethyl butanoate; **IAc**: isoamyl acetate; **Lim**: Limonene; **1B2M**: 1-butanol-2-methyl; **Ehe**: ethyl hexanoate; **1He**: 1-hexanol; **EOc**: ethyl octanoate; **Ben**: benzaldheyde; **Ede**: ethyl decanoate; **Cit**: citronellol; **Msa**: methyl salicilate; **Edo**: ethyl dodecanoate; **PEA**: phenyl ethyl alcohol; **Dp**: delphinidin; **Cy**: cyanidin; **Pt**: petunidin; **Pn**; peonidin; **Mv**: malvidin; **SAc**: (sum of acetylated anthocyanins); **SCo** (sum of p-coumaroylated anthocyanins).

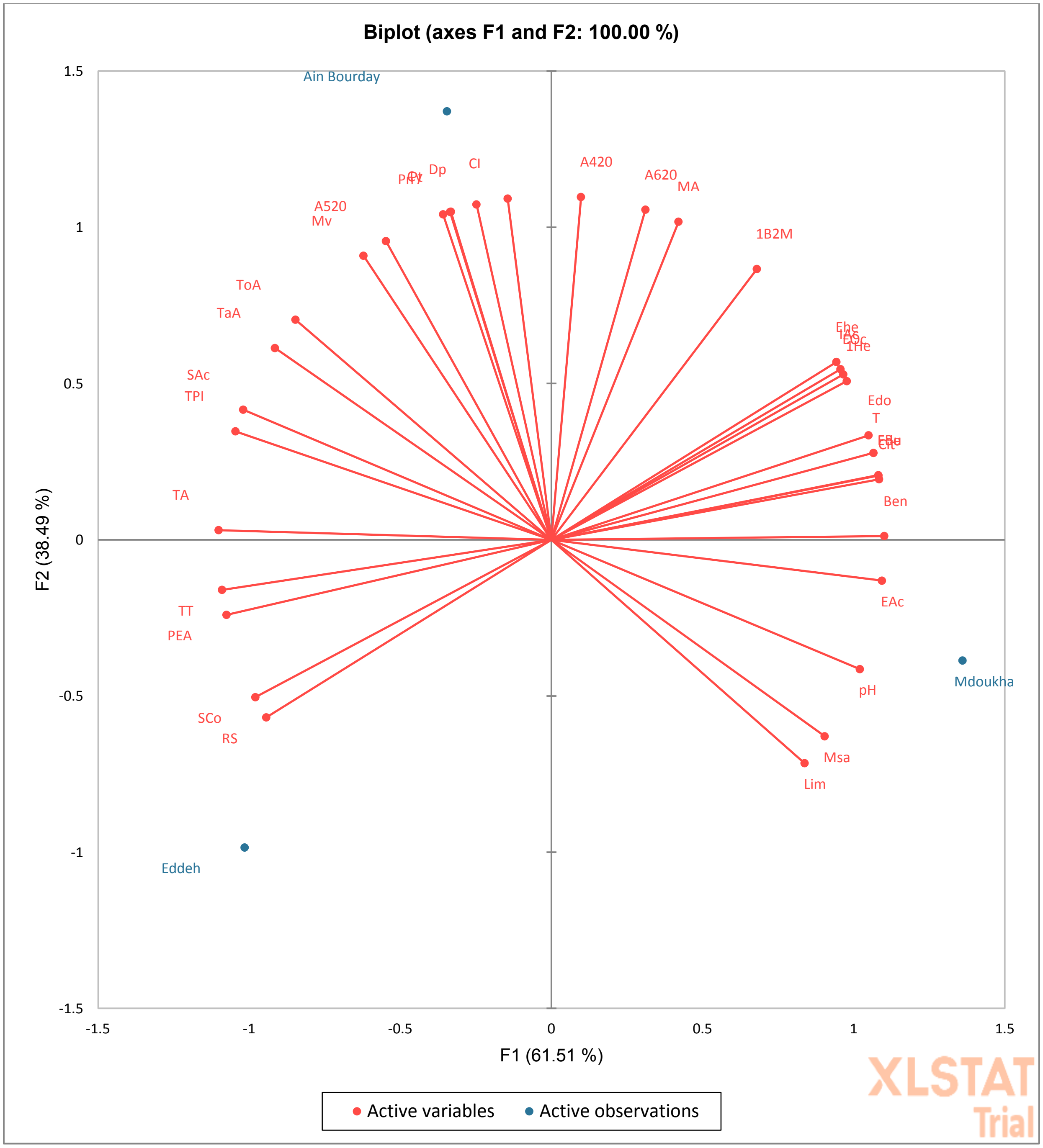


Figure 29: Principal component analysis of skin individual anthocyanins and wine chemical and volatile compounds in Eddeh, Mdoukha and Ain Bourday in 2019. **TTA**: total acidity; **TaA**: tartaric acid; **MA**: malic acid; **RS**: reducing sugars; **CI**: color intensity; **T**: tint; **TPI**: total polyphenol index; **TA**: total anthocyanins; **TT**: total tannins; **EAc**: ethyl acetate; **EBu**: ethyl butanoate; **IAc**: isoamyl acetate; **Lim**: Limomene; **1B2M**: 1-butanol-2-methyl; **Ehe**: ethyl hexanoate; **1He**: 1-hexanol; **EOc**: ethyl octanoate; **Ben**: benzaldheyde; **Ede**: ethyl decanoate; **Cit**: citronellol; **Msa**: methyl salicilate; **Edo**: ethyl dodecanoate; **PEA**: phenyl ethyl alcohol; **Dp**: delphinidin; **Cy**: cyanidin; **Pt**: petunidin; **Pn**; peonidin; **Mv**: malvidin; **SAc**: (sum of acetylated anthocyanins); **SCo** (sum of p-coumaroylated anthocyanins).

II.4 Conclusions

The data obtained in this work highlighted the major influence of the altitude and ripening-related changes in physicochemical behavior, phenolic and aroma compounds of Lebanese Grenache grapes and wines. The results demonstrated that the acidity of the grapes increased with altitude in the first year of the study due to the cooler environment. However, a vintage effect was observed when the cooler night temperatures at the low site in the second year of the study resulted in higher acidity in the grapes and in the corresponding wine. In general terms, the altitude tended to exert a positive effect on the seed phenolics and a negative effect on the skin proanthocyanins for the two consecutive vintages. However, in the first growing season, the soil factor was most prominent. In fact, the poorest soil in Mdoukha favored the increase of skin phenolics in 2018. And in the second growing season, the vintage factor was most prominent since the increase in the diurnal shift in Eddeh and Ain Bourday favored the increase of skin phenolics in 2019. The maturity effect was mostly dependent on the altitude effect for the skin phenolics. In fact, while the skin phenolics tended to decrease more often at the end of ripening at the lowest site Eddeh due to the hotter weather, they tended to increase more often at the end of ripening at the highest sites Mdoukha and Ain Bourday courtesy of the cooler weather. Overall, no significant changes were shown in the amounts of the seed phenolics during the ripening period.

On the other hand, the skin individual anthocyanins reached their maximum amount at the second maturity level in Ain Bourday whereas they reached it at the third ripening control in Eddeh and Mdoukha. Also, the poorest soil in Mdoukha favored the increase of the skin individual anthocyanins in the first year of the study except for Pn which was the highest in Eddeh, while the largest diurnal temperature range in Ain Bourday favored their increase in the second year of the study. Moreover, the soil factor resulted in the production of the wine from Mdoukha with the highest wine phenolics in 2018 and the vintage factor led to the increase of wine phenolics in Eddeh and Ain Bourday in 2019.

In 2018, the total amounts of VOCs in Mdoukha and Eddeh were significantly higher than that in Ain Bourday. For both years, and especially in 2019, the high altitude sites Mdoukha and Ain Bourday exhibited a larger number of volatile compounds with greater levels than those in Eddeh, indicating an altitude effect. This result shows that the wines produced at the high sites have their aroma compounds more diversified than those produced at the low sites. Citronellol and limonene might be used as possible indicators to differentiate Mdoukha wines. A vintage effect was observed in Ain Bourday where the total amount of VOCs in 2019 was greater than that in 2018 and in Mdoukha where a higher number of volatiles with the greatest levels were observed in 2019 than in 2018.

It is prominent from the results of this research work that the altitude and the berry maturity are not solely responsible for the evolution of the grape and wine phenolic and aroma compounds. Other factors that influence grape and wine composition like vintage and soil emerged from the results presented above and the influence of other factors such as humidity, UV radiation, sunlight exposure and viticultural practices should be investigated in future research. In addition, a wine sensory analysis could be carried out to examine the relationship between sensory attributes and the obtained VOCs in the produced wines.

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