AMERICAN UNIVERSITY OF BEIRUT

SCREENING COMMON LEBANESE GRAPEVINE VARIETIES FOR HIGH ACCUMULATION OF BENEFICIAL PHYTOCHEMICALS

by
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ABSTRACT
OF THE THESIS OF

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Title: Screening Common Lebanese Grapevine Varieties for High Accumulation of Beneficial Phytochemicals

Grapevine germplasm enhancement is critical to the viticulture sector. Conventional breeding efforts have been somewhat effective in producing new commercial cultivars with higher quality produce under current production regimes over the years. Nonetheless, due of the severe heterozygosity of the grape genome, which is exacerbated by inbreeding depression, the breeding method has limited utility for producing unique value-added cultivars. in this study, we characterized phytochemicals and antioxidant activity of 10 grape seed and pulp extracts from commercial varieties growing in Lebanon. The target is to identify potential advanced selections that can be promoted into new commercially accessible cultivars for fresh fruit consumption and seed extract. the goal of this study was to compare some of the phytochemical features extracted from 10 commercial grape varieties. data on the phytochemicals and antioxidant capacity of grapes consumed in Lebanon revealed that some of the phytochemical content was recorded to be higher in local varieties such as "Baytamouni" in flesh and seeds while this correlation was not shown in other local and imported varieties. This research study will generate substantial fundamental knowledge that will not only enrich the characteristics of the Lebanese grape varieties but will also assist grape breeders worldwide to exploit molecular mechanisms underlying valuable traits.

Keywords: Grapes, Phytochemicals, Polyphenol, FRAP, DPPH, Anthocyanin, Flavonoid, antioxidant activity

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ABBREVIATIONS

%	Percentage
ANOVA	Analysis of Variance
B.C.	Before Christ
DF	Dilution Factor
dm	Dry Matter
DMSO	Dimethyl Sulfoxide
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
Et al.	et alia
FRAP	Ferric Reducing Antioxidant Power
g	Grams
LDL	Low-density lipoprotein
MeOH	Methanol
mg	Milligram
mg GAE. L-1	Milligram gallic acid equivalents per liter
mg QE/g	Milligram quercetin per gram
mg/g	Milligram per gram
mg/g GAE	Milligram Gallic Acid Equivalents per gram
mg/l	Milligram per Liter
mg/ml	Milligram per milliliter
ml	Milliliter
mM	Millimolar
mol/l	Mol per liter

MW	Molecular Weight
nm	Nanometer
^{o}C	Degrees Celsius
ORAC	Oxygen Radical Absorbance Capacity
TBARS	Thiobarbituric acid reactive substances
TP	Total polyphenol
TPTZ	2,4,6-Tripyridyl-S-triazine
v/v	Volume per volume
ε	Molar Extinction Coefficient
µl	Microliter
µM	Micromolar

CHAPTER I

INTRODUCTION

Grapes have been a part of our societies and civilizations since the first recordings of history. Grapes were introduced to mankind as a symbol of luxury and wealth, where it was portrayed by many paintings and works of art to be the food of the extravagant. Their long-lasting presence within the everyday lives of humanity have pushed many individuals to experiment and see how grapes can have a long and differentiated positioning when it comes to the different industries of the world. Deeply present within the gastronomic sector, grapes are essential elements that find themselves in every cuisine in the world, a solid base for what can be considered a good meal. In addition to that, grapes are seen in many parts of the world as an original fruit which gives a sense of particularity for the region, as well as being an item to be compared between regions and determine the superiority of each variety. For example, rivalries between the best grape harvests were widely frequent in the areas of Lebanon and especially in the Bekaa region which is famous for the exceptional grape varieties that it produces. This rivalry was even adopted in the works of great authors, and especially Lebanese ones, who romanticize the image of grapes and associate it with home.

The fruit of grapes is one of the most ancient fruits to ever be cultivated in the ancient societies. The world which was known by regions such as Arabia, Egypt, Greece, and ancient Rome was highly associated with the cultivation of grapes and their exquisite art of wine making.

Grapes are categorized within the class of true berries, and that is so because of their fleshy fruit wall, their rich inside, and the fleshy pericarp which covers the entire

fruit. The grape plant is more often than not a simple wooden vine that has many branches or tendrils, which climbs up walls and sometimes forms natural canopies providing shade from the sun in a beautiful manner. The length of the grape vine could reach up to 17 meters if given the chance to grow without checking, however it is usually a lot shorter than that to ensure a better and more harvest, in addition to a more organized and aesthetic image, and it takes an erect shape, or an upward shrub when grown in relatively arid regions such as Bekaa. The grape fruit is not the only part of the plant that can be eaten, for the edible leaves were also integrated into many Arabian cultures, and especially the Lebanese and the Syrian, which used the leaves to create culturally particular foods.

Grape cultivation is considered a historic practice which is revered among the work of art, and whose products are known all around the world and throughout history, even in the life of Jesus Christ whose one miracle is to turn water into wine, a drink made out of grapes (Xia et al, 2010). Furthermore, the first proof of grape cultivation shows that it dates back to the year 3000 B.C. by the ancient Egyptians, which is evident in the ancient inscriptions in holographic upon the walls of their ancient ruins. In addition to that, the historical works of many scholars and historians confirm that the ancient Greeks and the ancient Romans developed grapes and used its products in many an event, and were even part of their culture. Roman emperors were great consumers of grapes, and even considered grapes, fruits and plants, to be part of their royal persona and became a symbol of their extravagance and specific foods. More interestingly, the Greek famous author of the Odyssey, Homer, mentions the cultivation of grapes and wine, which was a regular and revered drink for the Greeks. Furthermore, the first attempt to transport wine and the plants of grapes around the world was by the hands of

the Phoenicians, who delivered to the French territories in Europe in 600 B.C. These efforts were reinforced by the Romans who planted the grape plant in the Rhine valley around the second century (This et al, 2006). Grapes were then mentioned in many history books and the works of literature, and perhaps one of the most prominent figures to talk about grapes was Pliny the elder, who mentioned 91 different types of grapes which can produce more than 40 different types of wine. In the same timeline, the grape plant was transported to the Chinese territories in the far east through the lands of India, and was even taken by popularity in the new world of colonies in the Americas, Australia, and New Zealand, were they gained much favor with the people there. The world today knows more than 200 types of grape plants which are distributed across all regions in the world. However, there are two main species of grapes in the world, the Mediterranean, also known as European or the Vitis vinifera, which is known by a wine-like flavor and slip-skin shape, and the North American grape, also known as V. labrusca. The main difference in shape between these two species is that the skin does not come off easily from the pulp from the Mediterranean species, which makes it easier for the making of wine. While the other species are harder than the latter and is called the fox grape which is used for the making of grape juice and jellies, items that are widely famous in the United States. These two species are commonly coming from the cultivars of Vitis vinifera, or the common grapevine, the genus Vitis of the species in the family of the flowering plant, the Vitaceae (Christenhusz & Byng, 2016)

The cultivation of grapes requires a very specific set of conditions depending on the area in which they are cultivated. The region must host a dry and warm summer that lasts for at least three months and cool winters for the plant to grow as intended. Under no circumstances should grapes be grown in extreme winter conditions such as extreme

cold or heavy rain, because the frost would kill the roots and the rain would destroy the fragile wooden vines. Furthermore, the type of soil in which the plant must grow is not as specific as the weather conditions, as the grape plant adapts to a multitude of soil types such as the shallow and deep soils, or the blow sands and the clay loams, in addition to varying levels of nutrient rich soils that allow greater or lower levels of fertility.

Grape cultivation in many regions around the world, but most famously in regions such as California in the United States, and the Mediterranean region which extends from the Basin of Portugal till the shores of Lebanon and Syria. The grape plant is cultivated across the Mediterranean which are famous for their distinctive tastes and flavors, in addition to the lofty wines in many regions which have established a brand for themselves with a certain level of exclusivity.

Grape harvesting has many usages, one of which is winemaking, a process which begins with the grape picking step in the beginning of autumn in the northern parts of the world, meaning that it occurs between august and late October. However, in the southern parts of the world, the harvest happens between late February and April to produce the finest wines. The term late harvest or ice wine depends on grapes that are harvested months after the initial process.

The Mediterranean region is known for foods that contain a great deal of phytochemical materials such as olive oil. However, grapes contain many doses of phytochemical materials, phenolic acids, flavanols, flavon-3-ols, myricetin, peonidin, flavonoids, resveratrol, quercetin, tannins, anthocyanins, kaempferol, cyanidin, ellagic acid, and proanthocyanins.

But despite grapes being the foundations of a good wine or a filling meal, these interesting fruits seem to have a broad and compelling scientific explanation. The complicated lineage and the large variety of the different types of grapevines and their various placements around the world, sheds light on many important aspects about grapes. Despite their vast history, grapes have grown to have an important aspect to the human being in terms of the enhancing properties that it helps in achieving. But there is much more that meets the eyes to grapes than their adoption and presence and belonging within a specific family of plants and a genus.

Given the numerous varieties of grapes present around the world, an interesting and scrutinous eye will be placed on the different kinds of grapes in Lebanon and briefly discuss in the specific background of each one of them. Finally, grapes have always been famous and applauded for the multitude of health benefits that is offers. In this manuscript, we will be discussing the health benefits of grapes, seeing how chemically these fruits are able to better the health of the human body, specifically going into detail on their chemical compositions and the phytochemicals present in grapes, notably the flavonoids, antioxidants, anthocyanins and polyphenols. Throughout this manuscript, the use of various previous scientific journals will not only be mentioned and adapted, but also rigorously reviewed and related to other journals.

This study will explore the phytochemical content in flesh and seeds of ten chosen grape varieties in Lebanon such as antioxidants, anthocyanins, flavonoids, and phenols. The study will address the varying spectrum of colors in which the many grape varieties are specific. In addition to that, the study will explore the different uses of Lebanese grapes as well as their relative size.

CHAPTER II

LITERATURE REVIEW

Many scholars studied the aspects of grapes and its production, and they have gone in length on the countries that produce grapes, the uses of grapes in Lebanon, its benefits, and its phytochemical composition.

A. Grape Varieties in Lebanon

While numerous other countries in other continents such as the Americas have also started their own production of grapes, the country of Lebanon on the eastern coast of the Mediterranean Sea proves to be a compelling site for the production as well. According to Lebanese author and wine writer, Michael Karam, Lebanon is amongst one of the oldest and sites of wine production in the world (McGovern, 2003, as quoted in Karam, 2005). The country has a long history of wine trade, dating back to the age of Phoenicians and their trading ships that travelled throughout the Mediterranean Sea (Karam, 2005). Annually, and according to Karam's research, and backed by other statistics, the country produced nearly 110,600 tons of grapes in 2005 (Karam, 2005), (Tilasto, 2017). The production of grapes seems to have been decreasing throughout the years, going from its peaks of in 1992, with 362,315 tons of grapes to 74,140 tons in 2017 (Tilasto, 2017). Many of the well-known wineries in Lebanon, such as Chateau Kefraya and Ksara, have enjoyed international recognition, with Lebanese wine being consumed in many parts of the world. However, one element of viniculture in Lebanon that supplies with interesting and eye-opening data is the different grape varieties in Lebanon. Generally, the grape varieties in Lebanon seem to be from noble varieties and possess specific characteristics (LaMar, 2001). According to research conducted by the

faculty of Agriculture in the Lebanese University, there exists 10 kinds of grape varieties in Lebanon that adhere mainly to the red and white grape varieties (Mohasseb & Sassine, May 2019). The grape varieties in Lebanon can be noted as follows:

1- Cinsault: a traditional red variety in Lebanon and traditionally found in the region of Keferaya. It is typically used in blends and is high-yielding, early-ripening and a hot weather grape
2- Cabaret Sauvignon: Originally from France, it is the commonly recognized red wine grape variety in the world. It is small in size and has a purple black color, late seasoning ripening and self-fertile, this grape variety is known for its resistance to disease.
3- Syrah or Shiraz: Its origin can be traced back to the region of Shiraz in Persia, Syrah is a very vigorous red variety with a spreading growth habit. Growth can be excessive on deep, fertile soils and with high-vigor rootstocks.
4- Tempranillo: "Blue-black grape variety originated in Spain as a natural hybrid of Cabernet franc and Pinot noir. Vines are productive to very productive, capable of bearing medium to large crops of 20 to 30 tons per hectare. High yields may reduce acid level, while delaying harvest." (Mohasseb & Sassine, May 2019)
5- Pinot Noir: Regarded as one of the oldest cultivated varieties from the Vitis Genus, it has one of the earliest harvest dates and is a short season variety, it is susceptible to low temperatures and the early bud is at risk for spring frost.
6- Obeidi: Typical Lebanese traditional variety used for Aarak. It is fragile with a soft skin.

7- Clairette: The grapes produce a fruity wine, low in acidity levels and high in alcohol content, it is adapted to dry and infertile limestone soils. In order to get a good yield, the variety must be pruned hard.

8- Chardonnay: This variety begins to grown in spring, and the cool temperature that during the beginning of the spring period makes it variety susceptible to frost injury and a high percentage of seedless berries. However, it has low fruit yields compared to other higher-yielding varieties.

9- Sauvignon Blanc: Known to produce a very sweet wine, its time of harvest is imperative to its yield, considering that if the harvest is late a rot occurs. It is ill-advised to plant it in highly fertile and deep soils.

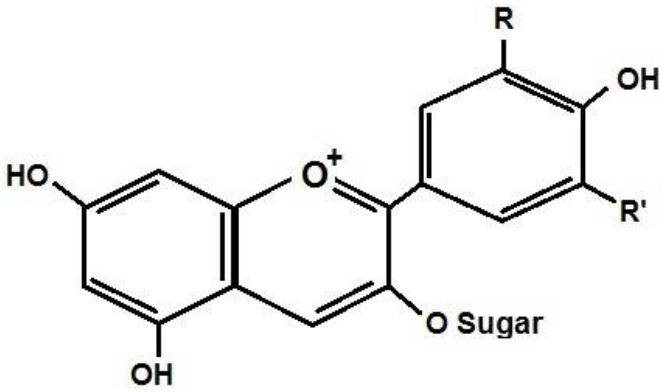
10- Muscat: sweet floral aroma grape varieties showing a high tolerance to heat and drought conditions. Tolerant to long distance transportation.

What is distinctive about the grape varieties in Lebanon is the striking diversity in the origins of the different varieties. The varieties present in in the country make for a multipurpose use for the grapes are being produced, and cutting differences in weather conditions and requirement for each type of grape makes for an important type of grape-related product. With the production of grapes in Lebanon being mainly concentrated to the creation of wine, and despite the absence of official data dictating the amount of wine output in Lebanon," FAO estimated the production of approximately 14,200 tons of wine in Lebanon ranking it as 45th largest wine producer in the world with 0.05% of Global supply" (Blominvest, 2013). Lebanon can be seen as an example of the vast empire and industry of grape production and how in a small country with a rich history, the persistent presence of a grape-harvesting industry reflects itself on the well-being of the different consumers of grape related products, notably wine.

B. Phytochemicals in grapes

1. Anthocyanin

Table grapes are known to have many phytochemicals within them, one of which is anthocyanin (Downey et al, 2006). Anthocyanin is the red pigment which can be found in red and black grapes, and it is the pigment that gives color to the grape. Naturally, it is usually present in the skin and the flesh of the grape (Sitters et al, 2000). It is a water-soluble vacuolar pigment which can be seen in the colors of red, blue, and purple. These colors are determined according to the pH of the grape (Downey et al, 2006). Furthermore, anthocyanin is odorless and have no flavor, at the least in the minimum degree, for it contributes to taste in the least way possible, and as moderately as astringent sensations. The anthocyanin is found in the tissues of higher plants, and can mostly be observed in the leaves, stems, roots, fruits, and flowers (Downey et al, 2006). While this chemical attracts the insects and animals who pollinate the flower, they also attract the animals who eat grapes and help spread the seeds so that the family of grapes continues.



Anthocyanin

Figure 1 Anthocyanin chemical structure

The synthesis of anthocyanin in grapes happens by a biosynthetic way similar to that of tannin and flavones such as quercetin, kaempferol and phenolic acids such as caffeic acid, resveratrol. During berry development the main phase of anthocyanin biosynthesis occurs between veraison and harvest. Some viticulture management practices are known to influence anthocyanin levels in grapes and wine, which also has an effect on the color of the grape.

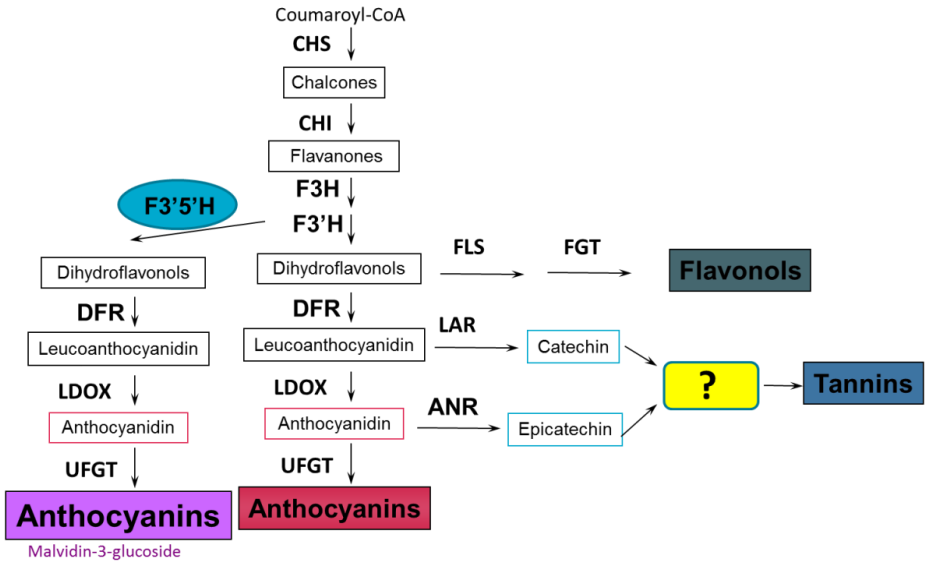


Figure 2 Anthocyanin biosynthetic path (Robinson, 2014)

Anecdotal evidence suggests that anthocyanin levels do vary substantially between different grape varieties, with some varieties considered high in anthocyanin, such as Shiraz, Petit Verdot, and Lagrein, and others comparatively low in anthocyanin, such as Pinot Noir, Sangiovese, and Nebbiolo (Kilmister, 2015), and certain table grape varieties lack the malvidin forms of anthocyanin. These differences in both concentration and types of anthocyanin can lead to differences in color and intensity overall.

C. Flavonoids

Flavonoids have almost 6000 structures which were identified and observed in many plants. They are a large family constituted of secondary metabolites, and their diversity of the chemical structure makes up for the wide range of physiological and biological activities. They have a very important role to play in plants such pollen fertilization, auxin transport regulation, pigmentation, defense against pests and pathogens, protection from ultraviolet radiations, and has many contributions to wine such as taste, color, and benefits. There are three main groups of flavonoids identified in grape berries, and the most common flavonoids found in grapes are anthocyanin (3-O-monoglucosides or 3,5-O-diglucosides of malvidin, cyanidin, peonidin, delphinidin, pelargonidin and petunidin as well as their acetyl-, p-coumaroyl- and/or caffeoyl-esters), flavonols (3-O-glycosides of quercetin , kaempferol , myricetin , laricitrin, isorhamnetin and syringetin), flavanols [(+)-catechin , (−)-epicatechin , (−)-epicatechin-3-O-gallate], dihydroflavonols (astilbin and engeletin) and proanthocyanidins. Flavan-3-ols include a range of polyphenolic compounds that include flavan-3-ol monomers, dimers, and various oligomers and polymers that are connected by interflavan linkages (C4–C8 or C4–C6) called condensed tannins or proanthocyanins (Deloire et al, 2019). Proanthocyanins are the most abundant class of grape phenols in the grape berry and are found in the seeds, skins, pulp, and stems. Flavonols are colorless compounds that accumulate after flowering and during ripening. They contribute to wine color by forming co-pigment complexes with anthocyanin. Furthermore, flavonoids act as a free radical scavenger and help in protecting the plant from the ultraviolet radiations which could be very dangerous to plants. In addition to that, studies show that flavonoids in grapes contribute in the interaction between plants

and pathogens. Quercetin-3-O-glucoside and quercetin-3-O-glucuronide have been identified as the main flavonols within the grape berries. Flavanols are present in grapes mainly in the form of (+)-catechin, (−)-epicatechin, and proanthocyanins (Deloire et al, 2019).

In white grape varieties such as Obiedi, flavanols represent 46% to 56% of total phenolics, whereas in red grapes such as Tfeifihi, they represent between 13% and 30% of total phenolic content. Flavonols are the second most abundant flavonoid found in table grapes. As such, they are represented by 3-O-glycosides in grape skins, but can be found also as aglycones (quercetin, kaempferol, myricetin, isorhamnetin) in wines and juices as a result of acid hydrolysis during processing and storage. Quercetin, kaempferol and isorhamnetin derivatives are found in both red and white grapes, whereas myricetin derivatives are found only in red varieties. The profile of flavonols strongly depends on grape cultivars, but in general quercetin-3-O-glucoside and quercetin-3-O-glucuronide are the predominant compounds present in most grapes. In muscadine grapes, quercetin-3-O-rhamnoside and quercetin aglycone have been identified as the major flavonols (Deloire et al, 2019).

Flavonoids are known to have many biological activities, yet they can vary according to many factors such as the degree of glycosylation, type of sugar residues, and subsequent acyl esterification. It is therefore possible to select different grape cultivars with unique flavonoid patterns having different health promoting effects on the human body.

D. Antioxidants

Flavonoids represent a large group of low molecular weight compounds with high antioxidant properties. Their specific chemical structure allows them to reduce oxidative stress through numerous mechanisms. For example, it was reported that, in vitro, flavonoids could act both as preventive antioxidants and chain breaking antioxidants [scavenging superoxide, peroxyl, alkoxyl and hydroxyl radicals as well as preventing low-density lipoprotein (LDL) oxidation. However, phytochemicals such as gallic acid, catechin, and epicatechin are present in the grape seeds and skins, and are also a source material for the production of antioxidants (Agric, 2004). Major flavonols and phenolic acids in seeds and skins of grapes of the Vitis vinifera and Vitis rotundifolia varieties of grapes were tested and then measured in one of the studies. The contribution of the major monomeric flavonols and phenolic acid to the total antioxidant capacity of grape seeds and skins was also determined. Gallic acid, monomeric catechin, and epicatechin concentrations were 99, 12, and 96 mg/100 g of dry matter (dm) in Muscadine seeds, 15, 358, and 421 mg/100 g of dm in Chardonnay seeds, and 10, 127, and 115 mg/100 g of dm in Merlot seeds, respectively (Agric, 2004). Concentrations of these three compounds were lower in winery byproduct grape skins than in seeds. These three major phenolic constituents of grape seeds contributed <26% to the antioxidant capacity measured as ORAC on the basis of the corrected concentrations of gallic acid, catechin, and epicatechin in grape byproducts. Peroxyl radical scavenging activities of phenolics present in grape seeds or skins in decreasing order were resveratrol > catechin > epicatechin = gallocatechin > gallic acid = ellagic acid. The results indicated that dimeric, trimeric, oligomeric, or polymeric procyanidins account for most of the superior antioxidant capacity of grape seeds (Aric, 2004).

E. Phenols

Studies explore the distribution of phenolic compounds in grapes. Grape is considered as one of the plants that are most rich in phenol, a chemical also known as phenolics that are spread across the plants. This chemical can be found in the skin of the grape, as well as the stem and the leaf of the grape, in addition to the seeds instead of their rich middle sections (Akoh et al, 2003). Studies also indicate that phenolic concentration reaches total concentration of phenolic 2178.8, 374.6, 23.8, and 351.6 mg/g GAE (gallic acid equivalent) in seed, skin, flesh, and leaf, respectively (Akoh, 2003). Furthermore, these studies show that the concentration of phenolic varies with many other variables. For example, cultivar and soil composition play an important role in determining the concentration of phenolic in the grape because of their major role in determining the nutrients that are absorbed by the plant, and hence it regulates the concentration of these chemicals in the fruit. Climate and geographical origin also have a substantial effect on the concentration of phenolic in the grape as well as the cultivation practices and the exposure to diseases that might cause abnormalities for the plant (Bruno, 2007).

The compounds mainly included proanthocyanins, anthocyanin, flavones, flavones, resveratrol and phenolic acids (LaMar, 2001). Proanthocyanin is considered to be the most prominent and certainly the most effective phenolic are the major phenolic compound found in the grape seed (Kennedy et al, 2009). Furthermore, studies show that another important phenol found in the grape is the pigment, which naturally determines the color of the group. Interestingly, the skin of the grape, which is the outer level of the grape, is colorless, and as such the pigments affect the inside of the grape instead of the skin. This means that the main part for the color of the grape is

determined by the flesh, and not the skin. This was determined and deduced by studying the composition of the skin and finding that it did not contain anthocyanin, which is the chemical responsible for the pigmentation and coloring (Bertelli, 2006).

One of the most studied forms of chemical compounds in wine and grapes is resveratrol, "a polyphenolic compound naturally found in peanuts, grapes, red wine, and some berries." (Oregon State University, 2015). This natural phenol and phytoalexin, also known by its IUPAC naming as 3,4', 5 trihydroxy stilbenes, and its chemical formula as C14H12O3, presents and offers several types of biological effect that are positive and beneficial to the human body. One of the many benefits that come with the consumption of grapes and resveratrol is its ability to aid and help with the process of metabolism through antioxidants and flavonoids. In a study on the biological effects of resveratrol on the human body, Lucie Frémont notes that the biological activities in regards to phytochemical compound, depends on the bioavailability of living organisms (2000). The proportion of resveratrol that seems to enter the circulation in the living beings finds an affinity in the cardiac area, as well as in the liver and kidneys, yielding effects that help to explain how "an average drinker of wine can particularly in the long term, absorb a sufficient quantity of resveratrol to explain the beneficial effect of red wine on human health." (Frémont, 2000). In terms of its antioxidant activities, it was observed that due to the phenolic compound of resveratrol, rich within the grapes, was attributed to its antioxidant prosperities. This can be explained and illustrated by the addition of trans-resveratrol to the porcine plasma, where the observations yielded that "(the distribution) between subsequently isolated lipoproteins and was associated with lipid as well as protein moieties. This may facilitate the protection of lipoprotein PUFA" (Frémont, 2000). Many of the benefits that come with the compound are

attributed to its stilbene structure, and how differentiated they are from that of flavonoids. This type of structure, in which Frémont extensively studied in her research, is considered and being studied to provide a form of cardiovascular protection, as well as the possessing an anti-inflammatory and anticancer protection (2000). While there is no direct influence and account that directly that says and complements this kind of information, and while there are many factors that come to play while observing the beneficial effects, much of this debate comes from the idea of what can be called as The French Paradox, which concludes that the high-fat and high-dairy diet of French people, which would typically cause high cardiovascular diseases, is countered by their consumption of red wine. An experiment was done on people who drank wine in large doses, namely the people Toulouse in France, and other who did not consume wine as often. The results showed that the French people from Toulouse who are known for their consumption of wine were less likely to have heart diseases that are fatal, even though they were noticed to have a much higher consumption of saturated fat, which is known to causing heart diseases. This experiment is the main reason behind the idea of the French Paradox, which as explained beforehand in this study, led to more studies and analysis in the region of France and the countries the surround it, in addition to other regions such as Spain and the Mediterranean in order to uncover possible explanations for the results.

The reason for the French Paradox is explained by the chemical composition of grapes and their effects. Fruits and vegetables have phytochemicals that play a significant role in limiting chronic disease risk. Moreover, grapes are known to be one of the most consumed fruits on earth, and one of the fruits that are known for their abundant phytochemical composition. Furthermore, there is an epidemiological

evidence suggesting that the chemical composition of table grapes is the reason for its health benefits on fronts such cardiovascular diseases and cancer. Antioxidants, for example, play a role in preventing cancer cell proliferation and suppressing platelet aggregation. Furthermore, antioxidants found in grapes are also known to reduce body cholesterol. The anti-cancer factor is also present in the phenolic compounds that have properties contributing to inhibiting cancer in many of its forms such as colon, lung, liver, and skin. Furthermore, resveratrol that is one of the phytochemicals present in grapes is known to have a cancer-fighting feature which slows down the cell transformation from their normal state to cancerous. In addition to that, it is also known to improve DNA repair and stopping DNA damage. Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes; it mediated anti-inflammatory effects and inhibited cyclooxygenase and hydro peroxidase functions. However, the French paradox is explained by the presence of polyphenols and its effects on cardiovascular diseases (Lopez, 2014).

F. Effect of Polyphenol on Cardiovascular outcomes

However, other studies that were released more recently and better capture the nature of wine consumption and its benefits shed the light on the epidemiological aspects that support the theory of wine's benefits against diseases which was before referred to the presence of ethanol contents in high degrees as the only reason for the benefits of wine in the health spectrum (Baur, 2006). While that notion was true for the time in which it was conducted, new, more recent studies prove otherwise, and uncovered other reasons for why wine has many health benefits. The previous premise in the existing body of literature on wine benefits and wine consumption links the many

benefits that wine has to the alcoholic element, which is manifested in the fermentation process which makes up the bulk of that notion. However, if that were so, then other alcoholic drinks would have had the same benefits, when the case is not so. Recent studies show that the benefits of wine go well beyond the alcoholic element, but are rather more linked to grapes more than the fermentation process. Studies show that the productive role of wine is not exclusive to the alcoholic element which is evident because wine has many more health benefits that other alcoholic beverages (Rotondo et al, 2006). To validate that theory, Rotondo et al conducted an experiment to test how valid their theory was by comparing the cardio protective effect of both wine and beer. Beer is known as one of the most famous alcoholic drinks in the world, and it is highly demanded in countries such as Lebanon and the United States of America. However, the purpose of their experiment was to better explain the French paradox and uncover the true benefits of wine, and also determine if they were exclusive to wine. In other words, their experiment aimed to show that the wine health benefits were only exclusive to wine because of its chemical composition of grapes, and not only because it is an alcoholic drink. The authors analyzed the results of 209,418 participants and the results of 13 other studies of the same purpose by giving beer intakes to certain people and wine to others. The results show that beer intake did not have any cardio protective elements while wine did. At the end of the experiment, the explanation for the presence of a cardio protective element in wine was referred to grapes rather than fermentation. However, even with these results, the French paradox was still subject to other interpretations such the Mediterranean diet that is full of ethanol chemicals.

G. The origin of Grape Benefits

Grapes were given a special emphasis on being the origin of the health benefits of wine and table grapes because of their chemical composition. Studies show that grapes have many polyphenol compounds which include flavonoids, phenolic acids, and resveratrol. These specific compounds were given significant attention when studying the chemical composition of grapes because epidemiological studies have evidence that indicates that these compounds and their regular or dietary intake plays a substantial role in preventing cardiovascular diseases, or rather reduces the tendency for a person to die from a heart disease (Blacburn et al, 1995). Other researches delved into the matter of the cardio protective element of grapes present in wine, and expanded their research to include animals and humans for the validation and reliability of their methods. The experiments done in the existing body of literature concerning grapes ad their chemical composition aim to explain the effects that might benefit both humans and animals in the cardiovascular spectrum. These experiments tested the effect of grape polyphenols against the factors that usually come at play when discussing the issue of cardiovascular diseases and mortality rates in both animals and humans. Studies began their experiments by gathering animals and human subjects that were divided into two groups. The first group was composed of cohorts who reported and shown significant consumption of relevant flavonoids such as flavonols, flavones, and falavn (Hollman et al, 2001), while the other group did not report such high level of consumption of the same chemicals. The results of the study showed that the subjects with more reported consumption of these chemicals were less likely to experience cardiovascular diseases. In addition to that, another study containing more than thirty-four thousand postmenopausal women from the United States of America were subject

to a similar experiment. The experiment studied the association of dietary intake of foods that have Flavanones and anthocyanidins. After studying how much they indulged in these foods, these women were tested for the likeliness of having cardiovascular diseases and mortality. The results showed that the women who were more associated with these foods were less likely to have heart diseases, and had a decreased cardiovascular disease chance and all-mortality (Mink, 2007).

The reason behind the health benefits of table grapes in the cardiovascular domain and its effects on preventing heart diseases and mortality goes back to the chemical composition of table grapes. This revelation comes as a response to the views that claim these benefits go back to the alcohol factor in wine that is made from grapes. The grape is known for its high concentration of grape polyphenols, which in turn are most abundantly found in the skin of the grape as well its stem and its seeds. Furthermore, studies show that the table grapes that have the most abundant composition of these chemicals, the greater the health benefits. The assumption that wine is attributed with health benefits is falsified because the longer these chemicals are preserved from grapes into wine, the greater the benefit. A study shows that the content of phytochemicals in red grapes are ten times more prominent in white grapes, ultimately creating the assumption that red wine made from red grapes is much more beneficial than white wine (Sparwel et all, 2009). Furthermore, in order to validate the difference between red grapes and white grapes, cohorts from North America were gathered and given specimens of both red wines, made from red grapes, and white wine, made from white grapes. While all the cohorts did not report any differential effect between red wine and white wine, the results concerning the chemical composition were different. The results highlighted on a greater antioxidant effect of red wine over

white wine. In addition to that, the results also show that there was a more favorable effect of lipid metabolism of red wine over white wine (Velden, 2002).

Grapes have held an influential and decisive role and positioning in various different parts, and their importance can be seen in various different levels, including that of scientific origins. From their historical relevance in various different cultures and their concepting into a worldwide trading and business operations, the fruits of grapes can be found in nearly every country, with Lebanon being a specific example of how the globalized environment comes at play with the different varieties of grapes. When it comes to finding and pinpointing what exact benefits characterize the consumption of grapes, the benefits can be explained through a chemical and scientific lens, specifically with seeing how the phytochemical structure of resveratrol, the most commonly studied chemical compound found within grapes, can aid in diminishing cardiac, inflammatory and cancer related illness, through the various activities relating to their antioxidant, flavonoids and polyphenol activities. Grapes hold an interesting place among the various fruits, and given their special attributes, even enough to find a specific way for it to become incorporated into the other elements of everyday life in order to harvest and better utilize the health benefits present within them.

H. Opposing Views

Many studies were in favor of the health benefits of wine and their role of fighting and preventing the mortality of heart diseases through playing a protective role against cardiovascular diseases. However, many other studies are not as encouraging of that theory.

Even though there are many theories supporting by a significant body of evidence that emphasize on the relationship between the polyphenol composition of wine and grapes, and their relative consumption on the probability of mortality from cardiovascular diseases and reduced risk of mortality, many other studies do a different way. A variety of observational and experimental studies indicate that there is not association between polyphenol consumption and reduced mortality of cardiovascular diseases (Sesso et al, 2003). The results of these studies could be explained by a multitude of factors and elements. The previous results obtained from previous experiments that addressed the effect of these chemicals on the mortality reduction outcome and their health benefits were based only on dietary input harvested by questionnaire. These questions and their corresponding answers could be inaccurate, and even if they if they were accurate, they do not provide a sufficient base of information to create a theory supported by scientific evidence. These methods were described as imperfect and insufficient to measure and evaluate the polyphenol intake. In addition to that, previous studies only focused on few compounds while ignoring many others that might have a pivotal effect on these outcomes. Furthermore, one other aspect that might affect the reliability of these studies and therefore their credibility in being evidence for the proposed theory is that they do not take into account the difference in the socioeconomic class which tends to affect the ability of individuals to have the proper medical care and quality of food. In addition, other dietary and concomitant factors play a major role in disturbing the accurate and sufficient collection of data (Vita, 2003).

CHAPTER III

MATERIALS AND METHODS

After collecting the grape samples, digital refractometer was used to determine the total soluble solid (TSS)of each sample. The titratable acidity was quantified by measuring the initial pH Sodium Hydroxide (NaOH 0.1M) was added to increase the pH of each sample to 8.2The flesh and seeds of each sample were separated and put in liquid nitrogen and then stored at – 80 °C. Later, the samples were freeze dried at to crystalize and remove the water and suspension medium by sublimation. The samples were subjected to methanol extract for 24 hours and then totally dried. The samples were kept in the dark for analysis. The Folin assay, FRAP assay, Anthocyanin assay, Flavonoids assay and DDPH assay were performed on the different samples to determine the phytochemical content of each.

A. Total phenolic assay

1. Preparation of Sodium carbonate (7.5 % v/w)

Measure exactly 15.0 g Sodium carbonate and quantitatively transfer it into 200 mL volumetric flask. Mix well and adjust the volume to 200 mL with ddH_2O. The solution can be stored at 4 °C for one month. Temperate before use.

2. Preparation of Folin–Ciocalteu reagent

In plastic test tube (sterile 50 mL) mix exactly 5.0 mL Folin–Ciocalteu stock solution and add 45.0 mL ddH_2O (use analytic pipettes). Mix on vortex and immediately use for analysis.

3. Preparation of Gallic acid (or caffeic acid) standard with varied concentrations

Prepare a 1.0 mg/mL stock solution: In plastic test tube (sterile 15 mL) measure exactly 10.0 mg Gallic or Caffeic acid and add exactly 10.0 mL methanol (use HPLC grade methanol and analytic pipette). Mix on vortex until whole amount is dissolved. Use this stock solution to prepare range of concentrations according to the table below.

Table 1 Preparation of Gallic acid stock solution.

Stock solution (1.0 mg/mL), mL	MeOH, mL	Final concentrations, mg/L GA
0.05	9.95	5
0.1	9.9	10
0.2	9.8	20
0.3	9.7	30
0.5	9.5	50
1	9	100
2	8	200
3	7	300

In test tube (15 mL disposable test tubes) add on the following order 1000 µL of Folin–Ciocalteu reagent, 200 µL of standard solutions (GA or CA) or investigated sample, mix on vortex and add 800 µL Sodium carbonate. Incubate in darkness for exactly 30.00 minutes at room temperature (21 °C). A blank sample, containing 200 µL methanol instead of sample was developed as well. Measure the changes in absorption (at 765 nm) of investigated samples against the blank sample.

The results are expressed as mg Gallic (or Caffeic) acid equivalents per gram sample.

B. FRAP assay

1. Preparation of Sodium Acetate buffer (pH 3.6)

Measure exactly 1550.0 mg Sodium acetate trihydrate and quantitatively transfer it into 500 mL volumetric flask. Dissolve with about 300 mL ddH_2O and add exactly 8.0 mL glacial acetic acid. Adjust the volume to 500 mL with ddH_2O, mix well and

measure pH. If necessary, correct the pH to 3.6 by using NaOH or glacial acetic acid. The buffer can be stored on cold (4 °C) for no more than one month and in this case, it must be temperate before preparing the FRAP reagent for analysis.

2. ***Preparation of HCl solution (40 mM)***

Add 300 mL ddH_2O into 500 mL volumetric flask and then add exactly 2.03 mL concentrated HCl. Adjust the volume to 500 mL by gently adding ddH_2O and mix well. The solution can be stored at room temperature for up to three months.

3. ***Preparation of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution (10 mM)***

In plastic test tube (sterile 15 mL) measure exactly 31.0 mg of freshly prepared 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and add exactly 10.0 mL 40 mM HCl (use analytic pipette). Mix on vortex until whole amount is dissolved.

4. ***Preparation of Iron(III) chloride solution (20 mM)***

In plastic test tube (sterile 15 mL) measure exactly 54.0 mg of freshly prepared $FeCl_3x6H_2O$ and add exactly 10.0 mL ddH_2O (use analytic pipette). Mix on vortex until whole amount is dissolved.

5. ***Preparation of FRAP reagent***

Mix Acetate buffer, TPTZ solution and Iron(III) solution in ratio 10:1:1.

6. ***Preparation of Trolox standard with varied concentrations***

Prepare a 10 mM Trolox stock solution in plastic test tube (sterile 15 mL) measure exactly 25.0 mg (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and add exactly 10.0 mL methanol (use HPLC grade methanol and analytic pipette). Mix on vortex until whole amount is dissolved. Use this stock solution to prepare range of concentrations according to the table below.

Table 2 Preparation of Trolox stock solution.

Stock solution (10 mM Trolox), mL	MeOH, mL	Final concentrations, µM Trolox
0.5	9.5	50
0.3	9.7	100
0.25	9.75	200
0.2	9.8	250
0.1	9.9	300
0.05	9.95	500

Mix in test tube (15 mL disposable test tubes) 3000 µL FRAP reagent and 100 µL of Trolox standard solutions or investigated sample, dissolved in methanol; shake on vortex and left for exactly 15.00 minutes at room temperature (21 °C). A blank sample containing 3000 µL FRAP reagent and 100 µL methanol was developed as well. After 15 min measure the changes in absorption (at 593 nm) of investigated samples against the blank sample. The results are expressed as µM Trolox equivalents per gram sample.

C. Total monomeric anthocyanin and polymeric color assay

1. Preparation of Potassium chloride buffer (0.025 M, pH 1.0)

Measure exactly 932.0 mg Potassium chloride and quantitatively transfer it into 500 mL cup. Put 490 mL ddH2O, mix well and adjust pH to 1.0 with concentrated HCl. Quantitatively transfer into 500 mL volumetric flask and adjust the volume to 500 mL with ddH_2O. The solution can be stored at 4 °C for one month. Temperate before use.

2. Preparation of Sodium acetate buffer (0.4 M, pH 4.5)

Measure exactly 6562 mg Sodium acetate and quantitatively transfer it into 500 mL cup. Put 490 mL ddH_2O, mix well and adjust pH to 4.5 with glacial acetic acid. Quantitatively transfer into 500 mL volumetric flask and adjust the volume to 500 mL with ddH_2O. The solution can be stored at 4 °C for one month. Temperate before use.

3. Preparation of Bisulfite solution

In plastic test tube (disposable 15 mL) measure exactly 500.0 mg Potassium bisulfite and add exactly 2.5 mL ddH_2O (use analytic pipette). Mix on vortex until whole amount is dissolved. The solution must be freshly prepared before analysis.

In test tube (disposable 15 mL) add known amount of investigated sample and dilute with potassium chloride buffer (pH 1.0) so the absorption of obtained solution (at 520 nm) to be lower than 1.5. Calculate the dilution factor by dividing the final volume by the initial volume of sample. Use the obtained dilution factor to prepare two dilutions of sample, one with potassium chloride buffer (pH 1.0) and one with sodium acetate buffer (pH 4.5). Mix well and left the tubes to equilibrate for 15 min. Measure and record the absorptions of both samples against ddH_2O at maximum of absorbance (520 nm) and absorbance of haze (700 nm).

The monomeric anthocyanin concentration in the original sample was calculated using:

Monomeric anthocyanin=(A*MW*DF*1000)/(ε*1), mg/L

MW is the molecular weight (for Petunidin-3,5-diglu MW=641.4); DF is the used dilution factor; ε is the molar extinction coefficient (for Petunidin-3,5-diglu ε =33040).

The results are expressed as mg Petunidin-3,5-diglu equivalents per gram sample.

The degradation index was calculated using the following formula:

Degradation index = $(A_{520}-A_{700})_{pH1.0}/[(A_{520}-A_{700})_{pH1.0}-(A_{520}-A_{700})_{pH4.5}]$

To prepare bleached sample for determination of polymeric color: Dilute the sample in test tube (disposable 15 mL) with ddH_2O by using the same dilution factor,

used for monomeric anthocyanins assay. Transfer 2.8 mL of diluted sample into one test tube, containing 200 µL ddH_2O and another 2.8 mL to other test tube, containing 200 µL Potassium bisulfite. Mix well and left them to equilibrate for 15 min. Measure and record the absorptions of both bleached and non-bleached samples against ddH_2O at 420 nm, 520 nm and 700 nm.

The color density of the control sample (treated with ddH_2O) was calculated using:

Color density = $[A_{420}-A_{700})+(A_{520}-A_{700})]*DF$

The polymeric color of the bleached sample (treated with Potassium bisulfite) was calculated using:

Polymeric color = $[A_{420}-A_{700})+(A_{520}-A_{700})]*DF$

The percent polymeric color was calculated using:

Percent polymeric color = (Polymeric color/ Color density)*100, %

The browning index of the bleached sample (treated with Potassium bisulfite) was calculated using:

Browning index = $A_{420}-A_{700}$

D. Total flavonoids assay

1. Preparation of 10% Aluminum chloride

Measure exactly 1.0 g Aluminum chloride in sterile test tube (50 mL). Add exactly 10.0 mL 96% Ethanol (HPLC grade).

Cool down the solution before analysis. The solution can be stored at 4 °C for one month. Temperate before use.

2. Preparation of 1M potassium acetate

Measure exactly 19.63 g potassium acetate and transfer it into 200 mL volumetric flask. Add approximately 100 mL dd H2O and mix to obtain clear solution. Adjust the volume to 200 mL with ddH_2O. The solution can be stored at 4 °C for few months. Temperate before analysis.

3. Preparation of Quercetin standard with varied concentrations

Prepare a 0.1 mg/mL stock solution: measure exactly 20.0 mg Quercetin and transfer it into 200 mL volumetric flask. Add approximately 100 mL 80% ethanol (use HPLC grade ethanol). Mix on vortex until whole amount is dissolved and adjust the volume to 200 mL with 80% ethanol. Use this stock solution to prepare range of concentrations according to the table below.

Stock solution (0.1 mg/mL), mL	80% EtOH, mL	Final concentrations, mg/L Q
2.0	0.0	100
1.0	1.0	50
0.5	1.5	25
0.25	1.75	12.5

Table 3 Preparation of Quercetin standard

In test tube (15 mL disposable test tubes) add on the following order 1.5 mL 96% ethanol, 0.5 mL sample, 0.1 mL 10% aluminum chloride, 0.1 mL 1M potassium acetate and 2.8 mL dd H2O. Mix well and incubate at room temperature for exactly 30.00 minutes. A blank sample is developed for the each sample on the same way but the 0.1 mL 10% aluminum chloride was replaced with 0.1 mL ddH2O. Measure the changes in absorption (at 415 nm) of investigated sample against the corresponding blank sample.

The results are expressed as mg Quercetin equivalents per gram sample.

E. DPPH assay

Measure exactly 0.0080 g DPPH (2,2-DIPHENYL-1-PICRYLHYDRAZYL) and quantitatively transfer into 200 mL volumetric flask. Dissolve with MeOH and mix well on vortex. Adjust the volume in volumetric flask with MeOH. Cover the flask with aluminum foil and keep in a dark and cold (4 °C) place 1 hour before analysis.

Stock solution (10 mM Trolox), mL	MeOH, mL	Final concentrations, µM Trolox
0.5	9.5	50
0.3	9.7	100
0.25	9.75	200
0.2	9.8	250
0.1	9.9	300
0.05	9.95	500

Table 4 Preparation of Trolox stock solution.

Mix in test tube (15 mL disposable test tubes) 2350 µL DPPH solution and 150 µL of Trolox solution or investigated sample; shake on vortex and leave in darkness for exactly 15.00 minutes at room temperature (21 °C). A blank sample, containing 2350 µL DPPH solution and 150 µL MeOH was developed as well. Measure the absorption (at 517 nm) of both blank and investigated samples against MeOH. The decrease of DPPH was calculated by the equation:

% decrease of DPPH = (A517 blank – A517 sample)/A517 blank * 100

The results are expressed as µM Trolox equivalents per gram dry sample.

To determine EC_{50} value, dissolve the investigated sample in MeOH to obtain solutions with varying concentrations. Run the assay as described previously and build calibration curve (sample concentration vs. % decrease of DPPH). Find the EC_{50} value graphically as a concentration in which 50% decrease of DPPH occurred. The EC_{50} value is presented as milligram of dry sample, necessary to provide 50% decrease of

absorption of 1 mL 0.1 mM solution of DPPH for 15 min in darkness at room temperature (21 °C).

1. Preparation of 0.1 mM DPPH in 2-propanol for seed oil analysis

Measure exactly 0.0080 g DPPH (2,2-DIPHENYL-1-PICRYLHYDRAZYL) and quantitatively transfer into 200 mL volumetric flask. Dissolve with 2-propanol and mix well on vortex. Adjust the volume in volumetric flask with 2-propanol. Cover the flask with aluminum foil and keep in a dark and cold (4 °C) place for 1 hour before analysis.

Table 5 Preparation of 10 mM Trolox (0.025 g Trolox in 10 ml 2-propanol).

10 mM Trolox	2-Propanol	µM Trolox
0.5	9.5	50
0.3	9.7	100
0.25	9.75	200
0.2	9.8	250
0.1	9.9	300
0.05	9.95	500

Mix in test tube (15 mL disposable test tubes) 2350 µL DPPH solution and 150 µL of Trolox solution or investigated sample; shake on vortex and leave in darkness for exactly 15.00 minutes at room temperature (21 °C). A blank sample, containing 2350 µL DPPH solution and 150 µL 2-propanol was developed as well. Measure the absorption (at 517 nm) of both blank and investigated samples against 2-propanol. The decrease of DPPH was calculated by the equation:

% decrease of DPPH = (A517 blank – A517 sample)/A517 blank * 100

The results are expressed as µM Trolox equivalents per gram oil sample.

To determine EC_{50} value, dissolve the investigated sample in 2-propanol so to obtain solutions with varying concentrations. Run the assay as described previously and

build calibration curve (sample concentration vs. % decrease of DPPH). Find the EC_{50} value graphically as a concentration in which 50% decrease of DPPH occurred. The EC_{50} value is presented as milligram of oil sample, necessary to provide 50% decrease of absorption of 1 mL 0.1 mM solution of DPPH for 15 min in darkness at room temperature (21 °C).

F. Statistical Analysis

One way ANOVA was used to compare the levels of phytochemicals in the flesh and seeds of the 10 different grape varieties. The post hoc analysis was used to compare the significance between all varieties using SPSS software version 22. Significance was preestablished at $\alpha<0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

In this Chapter, the results of the research work will be illustrated and discussed.

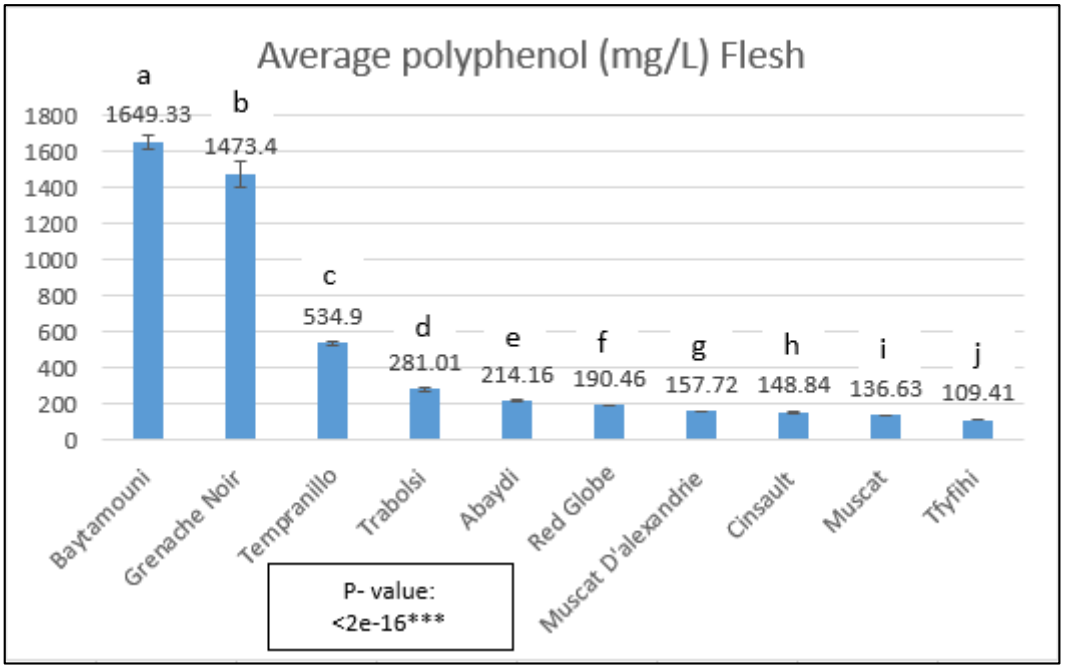


Figure 3 Average polyphenol (mg/L) in flesh of 10 grapevine varieties

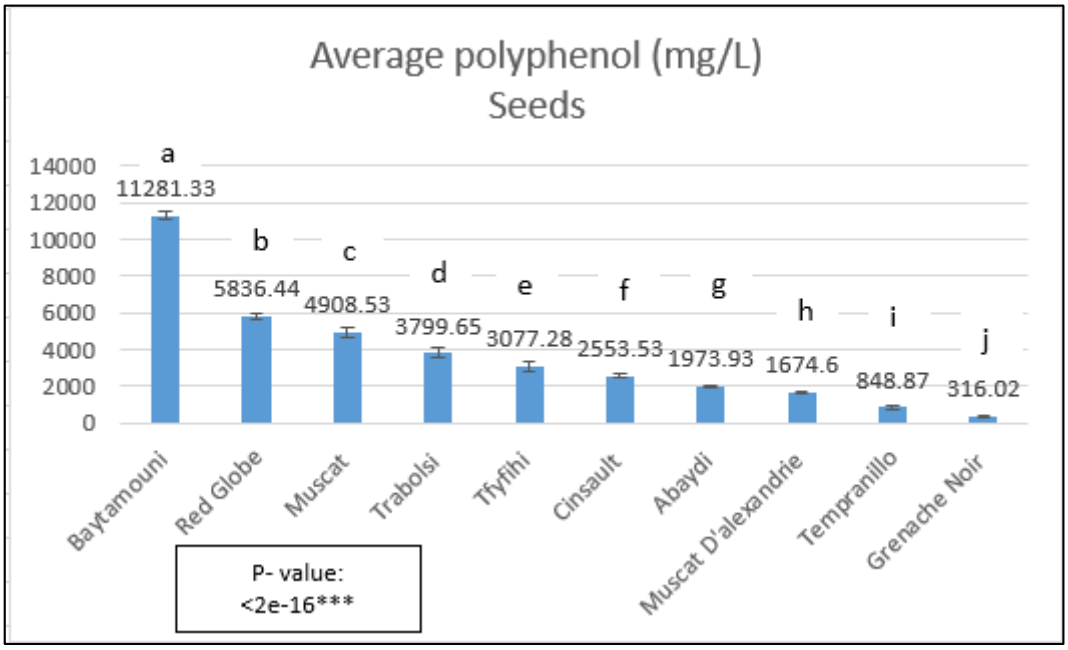


Figure 4 Average polyphenol (mg/L) in seeds of 10 grapevine varieties

In the present study and as shown in figures 3 and 4, the amount of total Polyphenol was analyzed in 10 commercial grape varieties. The quantification assay of Polyphenol in Cinsault revealed an average result of 148.84 mg/L as flesh less than that of the seed variant, which has an average of 2553.53 mg/L. Meanwhile, the quantification assay of Polyphenol in Tfyfihi revealed an average equal to 109.41 mg/L as flesh less than the 3077.28 mg/L of a seed. Moreover, Muscat d'alexandrie got an average result equal to 157.72 mg/L as flesh and 1674.40 mg/L as a seed. The study continues by testing Muscat as flesh and then seed, and the average result was 136.63 mg/L in the former less than that in the latter, which is equal to 4908.53 mg/L. The next test subject is Red globe, as flesh the average result was equal to 190.46 mg/L less than the 5836.44 mg/L of the seed variant. When the test is performed on Abaydi it scored an average result of 214.16 mg/L as flesh less than that its seed variant that has an average result of 1973.93 mg/L. Furthermore, Trabolsi revealed an average of 281.01 mg/L as flesh less than 3799.65 mg/L of the seed variant. The quantification assay of polyphenol in Tempranillo produced the following results: 534.9 mg/L as flesh and 848.87 mg/L as seed. Baytamouni had an average result as flesh in the experiment 1649.33 mg/L and as a seed; the average was 11281.33 mg/L, which is significantly greater than the flesh variant. Lastly, the average result for Grenache noir as flesh was 1473.4 mg/L greater than that of the seed variant that has an average of 316.02 mg/L. The observed total Polyphenol values were in the range of 109.41 (Tfyfihi) to 1649.33 mg/L (Baytamouni) as flesh, while in seed they were in the range of 316.02 (Grenache noir) to 11281.33 mg/L (Baytamouni). The richest amount of Polyphenol was documented in Baytamouni seeds and the lowest amount was documented in Tfyfihi flesh. The results of the

average polyphenol in the flesh and seeds were all significantly different with a p-value of (<2e-16).

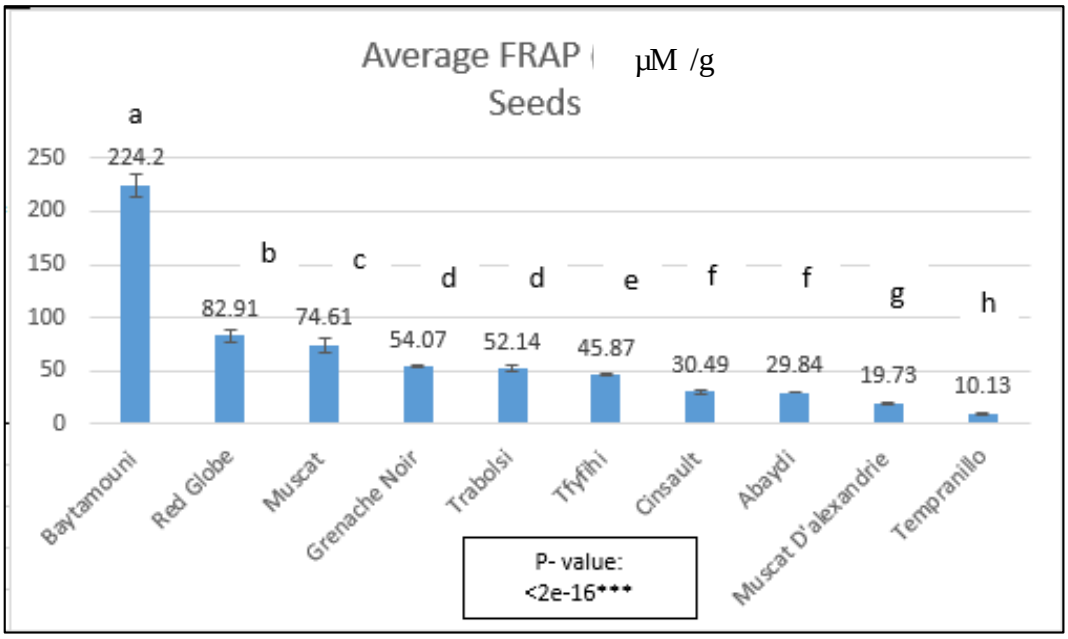


Figure 5 Average FRAP (µM/g) in seeds of 10 grapevine varieties

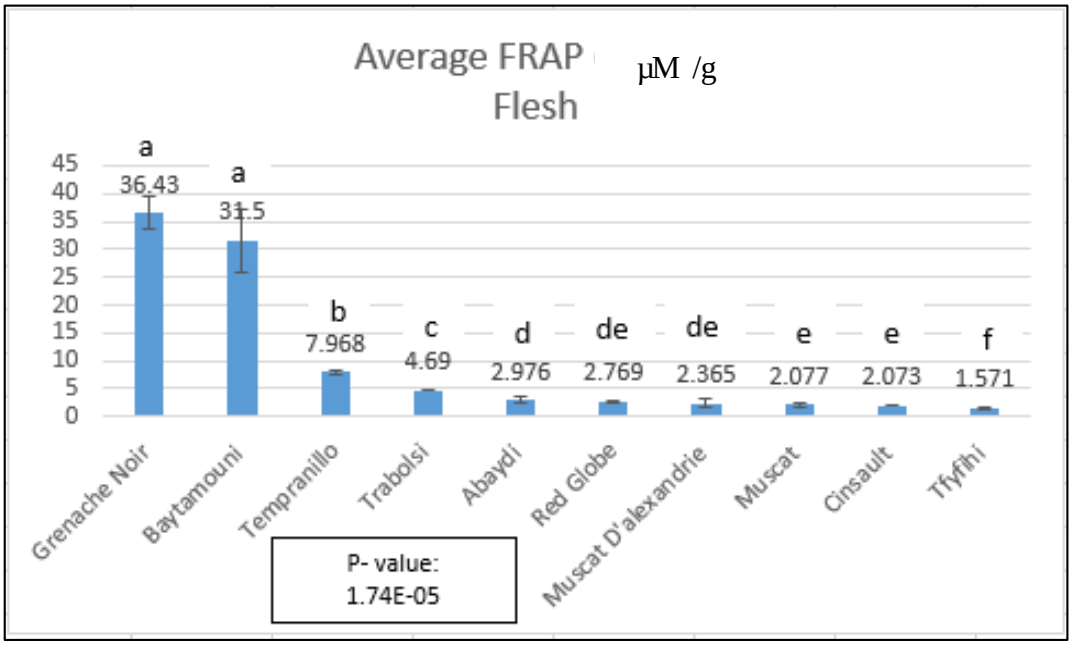


Figure 6 Average FRAP (µM/g) in flesh of 10 grapevine varieties

Figures 5 and 6 represent the results of the quantification assay of FRAP. Cinsault revealed an average result of 2.073 µM/g multiplied by the dilution factor as flesh which less than that of the seed variant which has an average of 30.49 µM/g. Meanwhile, the quantification assay of FRAP in Tfyfihi revealed an average equal to 1.571 µM/g as flesh compared to 45.87 µM/g as a seed. Moreover, Muscat d'alexandrie got an average result equal to 2.365 µM/g as flesh less than 19.73 µM/g, which is that of the seed. The study continues by testing Muscat as flesh and then seed, and the average result was 2.077 µM/g in the former less than that in the latter, which is equal to 74.61 µM/g. The next test subject is Red globe, as flesh the average result was equal to 2.769 µM/g less than the 82.91 µM /g of the seed variant. When the test is performed on Abaydi it scored an average result of 2.976 µM /g as flesh less than that of its seed variant that has an average result of 29.84 µM /g. The quantification assay of FRAP in Tempranillo produced the following results: 7.968 µM /g as flesh and 10.13 µM /g as seed. Baytamouni had an average result as flesh in the experiment 31.5 µM /g and as a seed; the average was 224.2 µM /g, which is significantly greater than the flesh variant. Lastly, the average result for Grenache noir as flesh was 36.43 µM /g greater than that of the seed variant that has an average of 54.07 µM /g. The observed total FRAP values were in the range of 1.571 to 36.43 µM /g as flesh, while in seed they were in the range of 10.13 to 224.2 µM /g. The richest amount of FRAP was documented in Baytamouni seeds and the lowest amount was documented in Tfyfihi flesh. The results of the average FRAP were divided into 6 clusters. Grenache Noir and Baytamouni were the most significant while Tfyfihi was the least significant. The remaining varieties were skewed in the middle. The p-value of the average FRAP in flesh was 1.74E-05. Similarly, in the seeds, Baytamouni was the most significant. However, Tempranillo

was the most insignificant in comparison to the other varieties that were divided into 6 clusters and skewed in between the varieties of highest and lowest significance. The p-value of the average FRAP in seeds was <2e-16***.

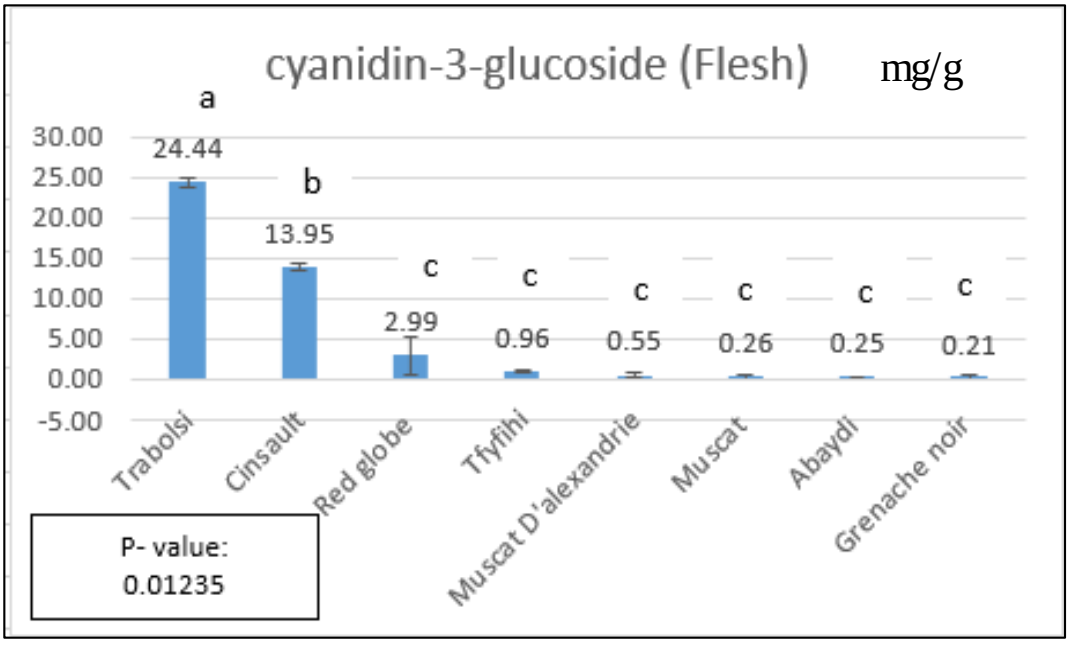


Figure 7 Cyanidin-3-glucoside (mg/g) in flesh of 8 grapevine varieties.

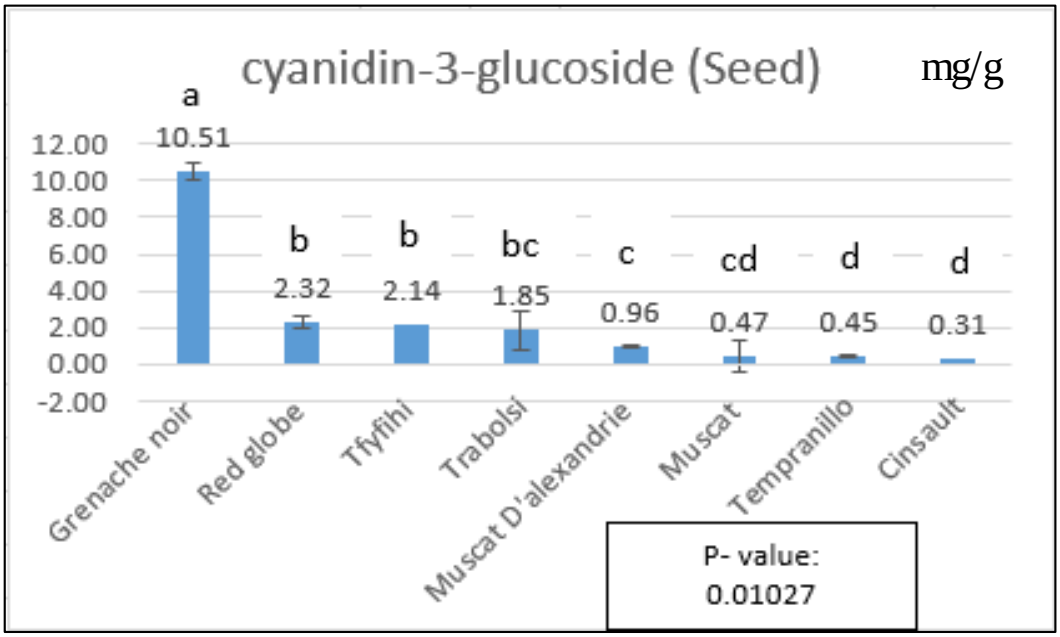


Figure 8 Cyanidin-3-glucoside (mg/g) in seed of 10 grapevine varieties.

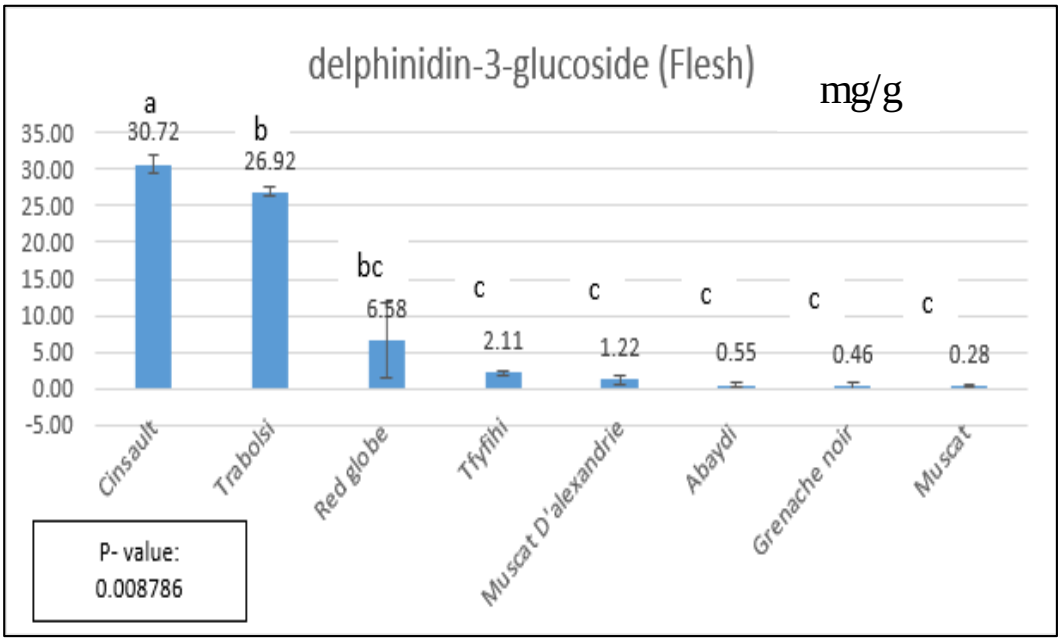


Figure 9 Delphinidin-3-glucoside (mg/g) in flesh of 8 grapevine varieties.

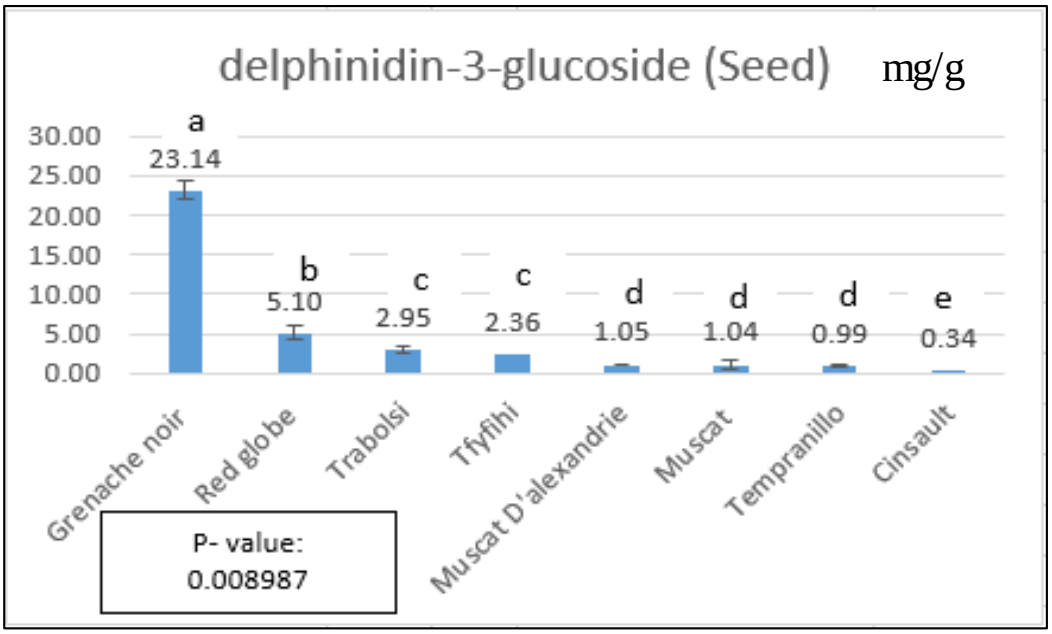


Figure 10 Delphinidin-3-glucoside (mg/g) in seed of 8 grapevine varieties.

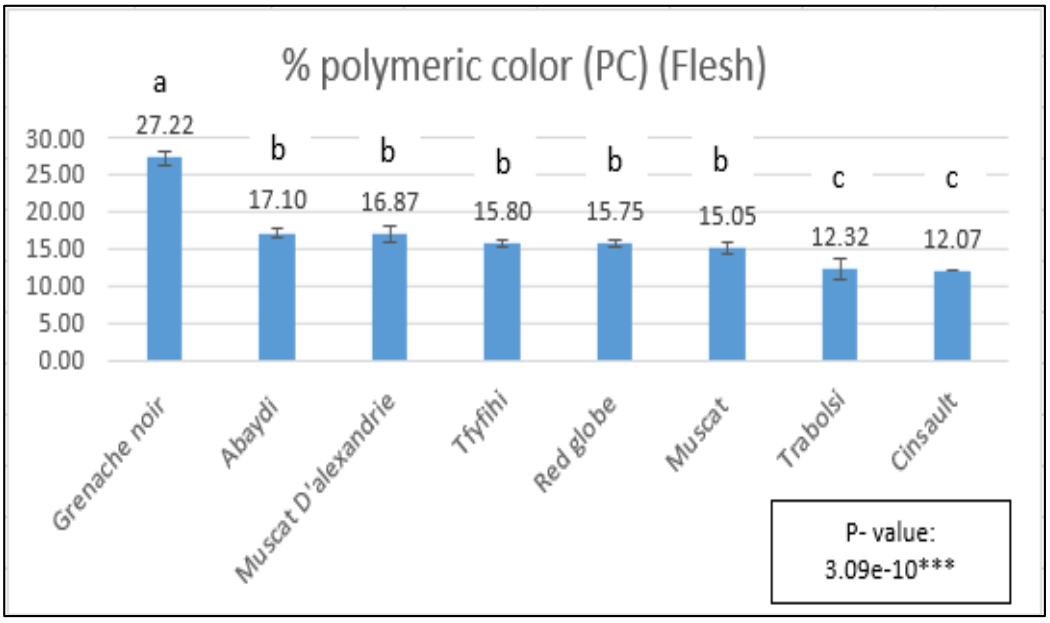


Figure 11 Percent polymeric color in flesh of 8 grapevine varieties.

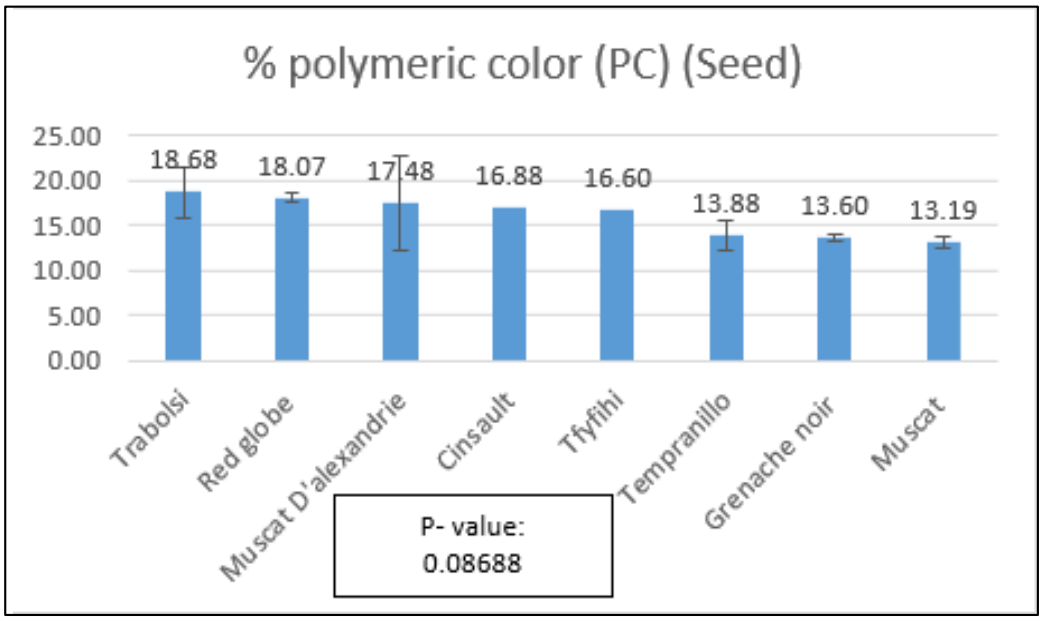


Figure 12 Percent polymeric color in flesh of 8 grapevine varieties.

The study goes on to determine the average total anthocyanin in Cyanidin – 3-glucoside (mg/g) and in delphinidin – 3- glucoside (mg/g) for flesh seeds (figures 7, 8, 9 and 10). Moreover, the average polymeric color (figures 11 and 12), average color density, average monomeric anthocyanin and average browning index were determined

in both flesh and seeds. The average total anthocyanin of the flesh of Muscat d'alexandrie was 0.55 mg/g. However, due to the lack of enough sample quantity, the average total anthocyanin in the seed of the same variety could not be determined. The average percentage polymeric color for this variety was higher in the seeds (17.5%) than in the flesh (16.9%). Similarly, the average color density, average monomeric anthocyanin and average browning index were higher in the seeds of Muscat d'alexandrie than in the flesh. In the flesh, the average color density was 0.54 while it was 2.51 in the seeds. The average monomeric anthocyanin was 2.95 in the flesh while it was 5.09 in the seeds. The average browning index in the flesh was 0.09 whereas it was 0.14 in the seeds. The average polymeric color was the lowest in this variety.

The average total anthocyanin in the flesh of Cinsault variety (13.95) was higher than that of the seeds (0.31). The average percentage of polymeric anthocyanin in Cinsault variety was also higher in the seeds (16.9) than flesh (12.1). The average color density, average monomeric color and average browning index of the flesh of Cinsault were 1.21, 74.13 and 0.13 while those of the seeds were 2.86, 1.63, and 0.18 respectively. The highest average monomeric anthocyanin was recorded in the flesh of this variety. The average browning index was the lowest in this variety.

Similarly, the average total anthocyanin in flesh of Muscat (0.26) was higher than the average total in seeds (0.01). The average percentage of polymeric anthocyanin in Muscat variety was also higher in the flesh (13.2) than seeds (15). The average color density, average monomeric color and average browning index of the flesh of Muscat were 0.85, 1.37 and 0.05 while those of the seeds were 5.71, 0.04, and 0.65 respectively. The average color density was the highest in the seeds of this variety while

the average monomeric anthocyanin was the lowest. Moreover, the average browning index was the highest in both Muscat and Red globe varieties.

The average total anthocyanin of the flesh of Tfyfihi was 0.96 mg/g. However, the average total anthocyanin in the seed (2.14) was greater than that of the flesh. The average percentage polymeric color for this variety was higher in the seeds (16.6%) than in the flesh (15.8%). In addition to that, the average color density, average monomeric anthocyanin and average browning index were higher in the seeds of Tfyfihi than in the flesh. In the flesh, the average color density was 1.14 while it was 5.29 in the seeds. The average monomeric anthocyanin was 5.10 in the flesh while it was 11.39 in the seeds. The average browning index in the flesh was 0.21 whereas it was 0.32 in the seeds.

Furthermore, the average total anthocyanin in flesh of Red Globe (2.99) was higher than the average total in seeds (2.32). The average percentage of polymeric anthocyanin in Red Globe variety was also lower in the flesh (15.7) than seeds (18.1). The average color density, average monomeric color and average browning index of the flesh of this variety were 1.3, 15.88, and 0.2 while those of the seeds were 4.49, 12.31, and 0.65 respectively.

The average total anthocyanin of the flesh of Abaydi was 0.25 mg/g. However, due to the lack of enough sample quantity, the average total anthocyanin in the seed of the same variety could not be determined. The average percentage polymeric color for this variety was only determined in the flesh and the average color density, average monomeric color and average browning index of the flesh of this variety were 0.63, 1.33, and 0.09 respectively.

Additionally, the average total anthocyanin in the flesh of Trablosi variety (24.44) was higher than that of the seeds (1.85). The average percentage of polymeric anthocyanin in Trabolsi variety was also higher in the seeds (18.7) than flesh (12.3). The average color density, average monomeric color and average browning index of the flesh of Trabolsi were 2.39, 129.91 and 0.16 while those of the seeds were 5.38, 9.82, and 0.60 respectively. The highest average total anthocyanin and percentage polymeric color were recorded in the flesh of this variety.

The total anthocyanin in the flesh of Tempranillo wasn't determined due to the lack of sufficient sample quantity. However, the total anthocyanin of the seeds of that same variety was 0.45. Similarly, no values for the average color density, average monomeric anthocyanin and average browning index of the flesh were determined. However, those of the seed were 13.9, 1.05, 2.38, and 0.11 respectively.

The average total anthocyanin in the flesh of Grenache Noir variety (0.21) was lower than that of the seeds (10.51). The average percentage of polymeric anthocyanin in Grenache Noir variety was lower in the seeds (13.6) than flesh (27.2). The average color density, average monomeric color and average browning index of the flesh of Grenache Noir were 0.15, 1.10, and 0.03 while those of the seeds were 1.95, 55.85, and 0.29 respectively.

Finally, no data regarding the average total anthocyanin in the flesh of Baytamouni was recorded and the average in the seeds was recorded to be negative (-0.4). Similarly, no average polymeric color for this variety was determined. No values for the average color density, average monomeric anthocyanin and average browning index of the flesh were determined. Nevertheless, the average color density and average

browning index of the seed were 4.62 and 0.52 respectively. The average monomeric anthocyanin for the seed of this variety was negative with a value of 2.1.

The results of the average Cyanidin – 3- glucoside in the flesh were the most significant for Trabolsi and least significant for Grenache Noir. Red globe, Tfyfihi, Muscat D'alexandrie, Muscat, Abaydi and Grenache Noir were all group into one cluster. The p-value of the average Cyanidin – 3- glucoside in flesh was 0.01235. However, for the average Cyanidin – 3- glucoside in seeds, the results showed that Grenache Noir was the most significant and Cinsault was the least significant. The p-value of the average Cyanidin – 3- glucoside in seed was 0.01027.

Moreover, the results of the average delphinidin – 3- glucoside in the flesh were the most significant for Cinsault and least insignificant for Muscat. The varieties were divided into 3 clusters with a p-value of 0.008786. However, for the average delphinidin – 3- glucoside in seeds, the results showed that Grenache Noir was the most significant and Cinsault was the most insignificant. The p-value of the delphinidin – 3- glucoside in seed was 0.008987.

In the results of the % polymeric color of the flesh, Grenache Noir was significant and Cinsault was least significant. The varitites were divided into 3 clusters with Abaydi, Muscat D'alexandrie, Tfyfihi, Red globe and Muscat skewed in the middle. The p-value of the % polymeric color in flesh was 3.09e-10***. All the values for the % polymeric color of seeds were insignificant with a p-value of 0.08688.

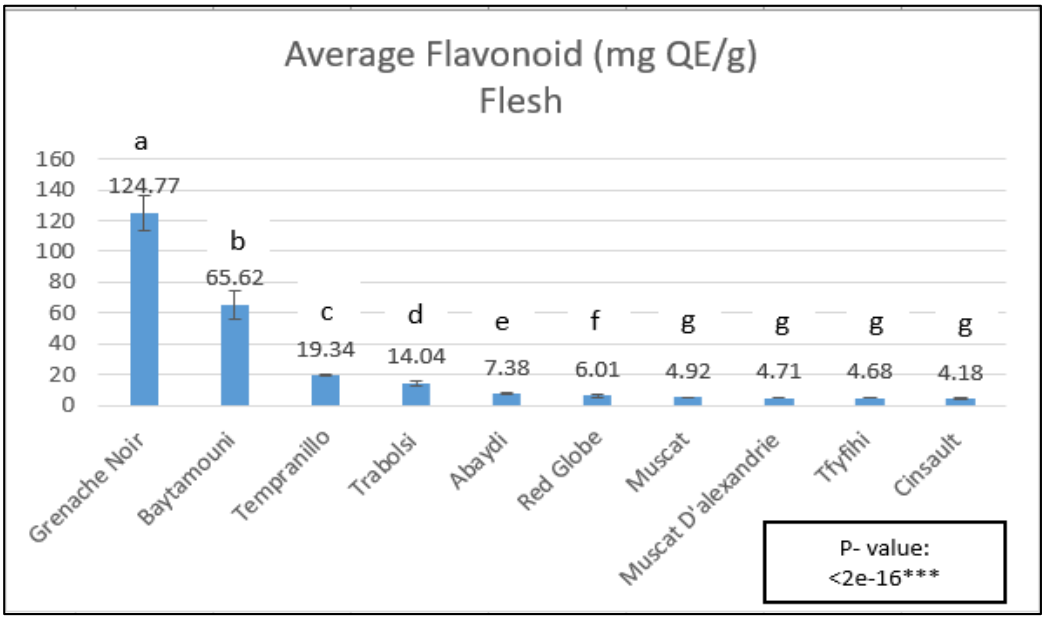


Figure 13 Average flavonoid (mg QE/g) in flesh of 10 grapevine varieties.

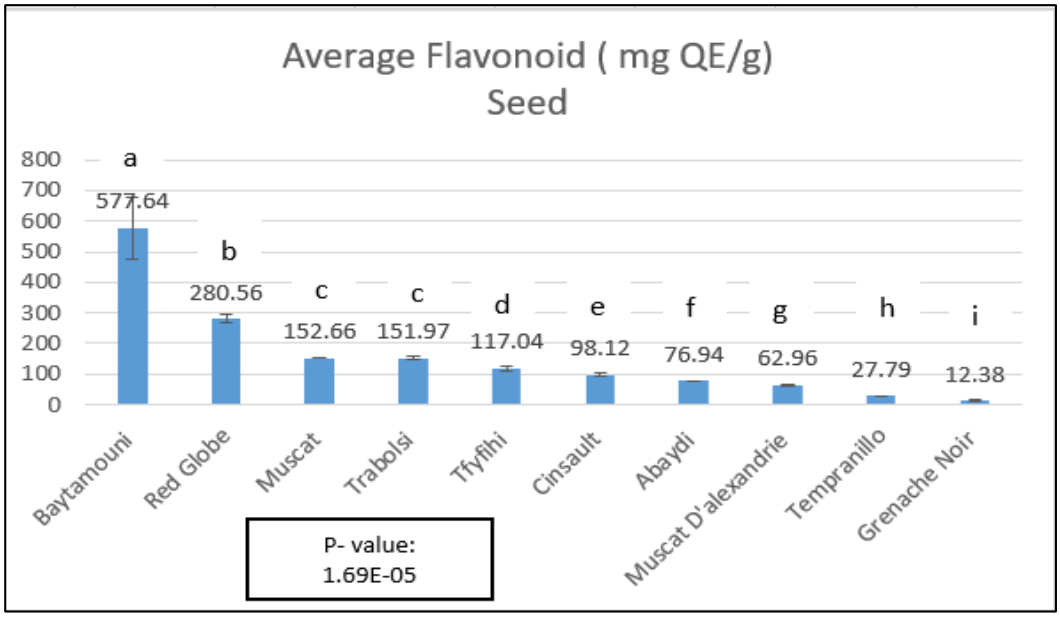


Figure 14 Average flavonoid (mg QE/g) in seed of 10 grapevine varieties.

The quantification assay of folavonoids is shown in figures 13 and 14. Cinsault revealed an average result of 4.18 mg QE/g as flesh which is significantly less than that of the seed variant which has an average of 98.12 mg QE/g. Meanwhile, the quantification assay of flavonoids in Tfyfihi revealed an average equal to 4.68 mg QE/g

as flesh compared to 117.04 mg QE/g as seeds. Moreover, Muscat d'alexandrie got an average result equal to 4.71 mg QE/g as flesh and 62.96 mg QE/g as seeds. The study continues by testing Muscat as flesh and then seeds, and the average result was 2.077 mg QE/g in the former less than that in the latter, which is equal to 152.66 mg QE/g. The next test subject is Red globe, as flesh the average result was equal to 6.01 mg QE/g less than the 280.56 mg QE/g of the seed variant. When the test is performed on Abaydi it scored an average result of 7.38 mg QE/g as flesh less than that its seed variant that has an average result of 76.94 mg QE/g. Furthermore, Trabolsi revealed an average of 14.04 mg QE/g as flesh less than 151.94 mg QE/g of the seed. The quantification assay of flavonoids in Tempranillo produced the following results: 19.34 mg QE/g as flesh and 27.79 mg QE/g as seed. Baytamouni had an average result as flesh in the experiment 65.62 mg QE/g and as seed, the average was 488.65 mg QE/g that is significantly greater than the flesh variant. Lastly, the average result for Grenache noir flesh was 124.77 mg QE/g greater than that of the seed variant that has an average of 12.38 mg QE/g. The observed total flavonoid values were in the range of 2.077 to 124.77 mg QE/g as flesh, while in seed they were in the range of 12.38 to 488.65 mg QE/g. The richest amount of flavonoid was documented in Baytamouni seeds and the lowest amount was documented in Muscat flesh. The results of the average Flavonoid in the flesh were divided into 7 clusters with Grenache Noir being the most significant anf Cinsault being the least. The p-value of the average FRAP in flesh was <2e-16***. However, for the average Flavonoid in seeds were divided into 9 clusters with Baytmouni ranking most significant and Grenache Noir ranking most insignificant. The p-value of the average Flavonoid in seed was 1.69E-05.

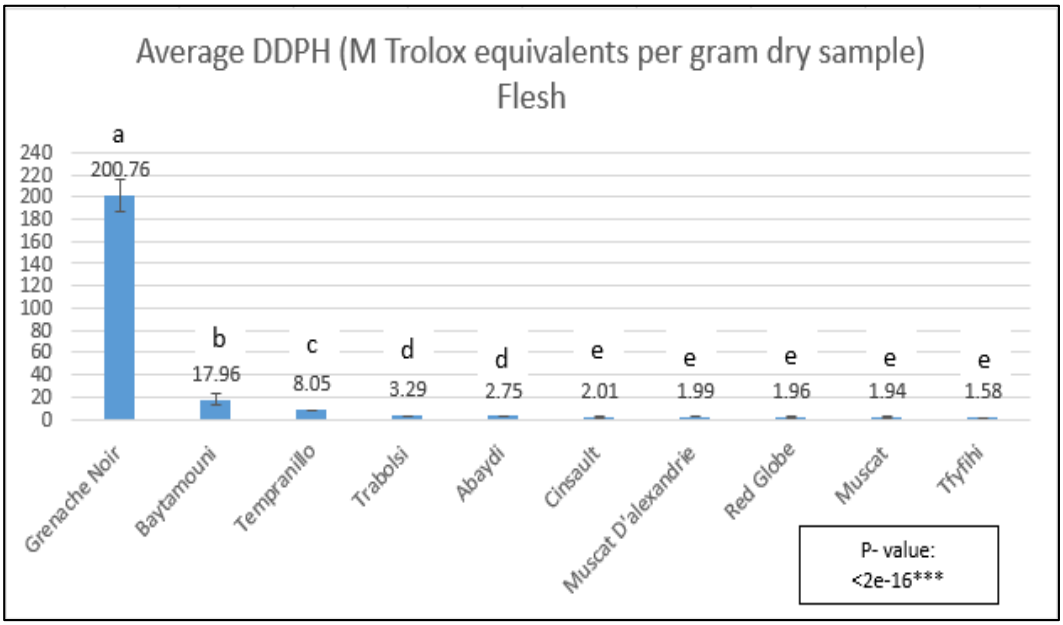


Figure 15 Average DDPH in flesh of 10 grapevine varieties.

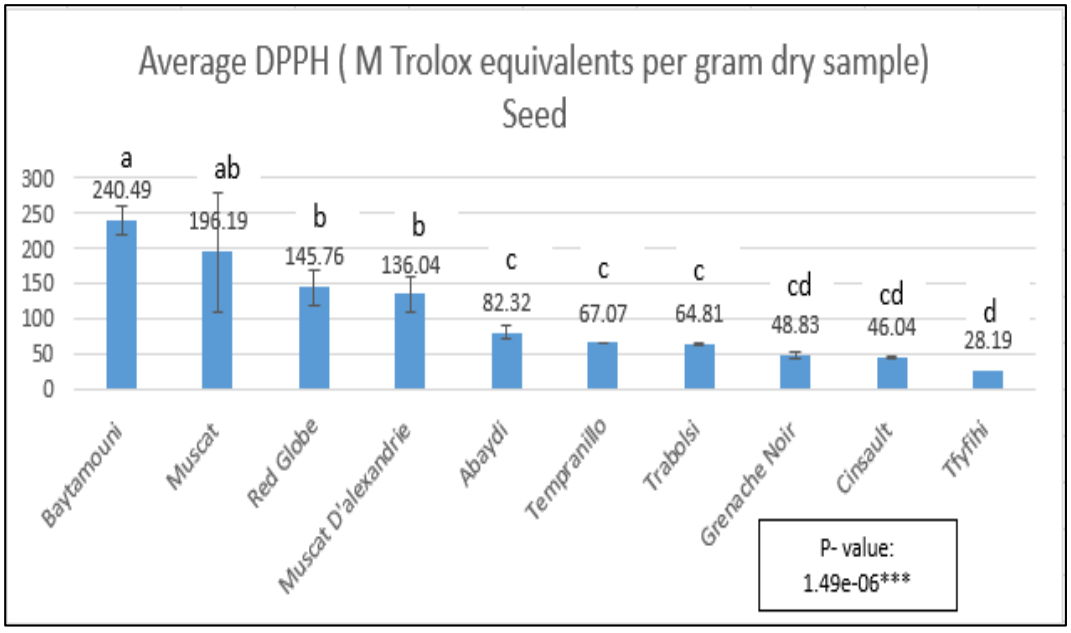


Figure 16 Average DDPH in flesh of 10 grapevine varieties.

The amount of total DPPH was analyzed in same grape varieties (figures 15 and 16). The quantification assay of DPPH in Cinsault revealed an average result of 2.01 g of M Trolox equivalents per gram dry sample as flesh, which is signifacntly less than that of the seed variant, which has an average of 46.04 g. Meanwhile, the quantification

assay of DPPH in Tfyfihi revealed an average equal to 4.68g as flesh less than the 31.65 g as a seed. Muscat d'alexandrie got an average result equal to 1.99g as flesh and 136.04 g as a seed. The study continues by testing Muscat as flesh and then seed, and the average result was 1.94 g in the former less than that in the latter, which is equal to 196.19 g. The next test subject is Red globe, as flesh the average result was equal to 1.96 g less than the 172.39 g of the seed variant. When the test is performed on Abaydi it scored an average result of 2.75g as flesh less than that its seed variant that has an average result of 82.32 g. Furthermore, Trabolsi revealed an average of 3.29g as flesh less than 64.81 g of the seed. The quantification assay of DPPH in Tempranillo produced the following results: 8.05g as flesh and 67.07g as seed. Baytamouni had an average result as flesh in the experiment 17.96 g and as a seed, the average was 240.49 g, which is significantly greater than the flesh variant. Lastly, the average result for Grenache noir as flesh was 200.76 g greater than that of the seed variant that has an average of 48.83 g. The observed total DPPH values were in the range of 1.94 (Muscat) to 200.76 g (Grenache noir) as flesh, while in seed they were in the range of 31.6 (Tfyfihi) to 240.4 g (Baytamouni). The richest amount of total DPPH was documented in Baytamouni seeds and the lowest amount was documented in Muscat flesh. The results of the average DPPH in the flesh were most significant for Grenache Noir, and least significant for Tfyfihi. The p-value of the average DPPH in flesh was <2e-16***. However, for the average DPPH in seeds, the results showed that Baytamouni and Muscat were most significant and Tfyfihi least significant with the remaining varieties skewed in the middle. The p-value of the average DPPH in seed was 1.49e-06***.

Our results indicate that the grape seed extracts have higher polyphenol contents than the flesh extracts.

The quantification assay revealed that flavonoids present in the different 10 varieties of grapes: Cinsault, Tfyfihii, Muscat, Red globe Abaydi, Trabolsi, Tempranillo, and Grenache noir, is present in a larger quantity in the seeds rather than in the flesh of the grapes.

The same goes for the amount of total DPPH and the total amount of Polyphenol as well as the quantification assay of FRAP analyzed in the same varieties and being compared between the flesh and the seeds; the amount present is found in greater amounts in the seed of all 10 grapes.

As for the average monomeric anthocyanin found in the grapes, the amount found in the flesh was larger than the amount found in the seeds for four out of the 10 varieties while the results for the Abaydi, Muscat, Tempranillo, and the Baytamoni were not determined.

The total phenolic activity associated with the two different harvest times was not statistically significant. However, the differences between the hybrids were important.

In 2008, KXP10 with dark skin with seeds had the highest total phenol and antioxidant activity scores. The seeded black-skinned BX2149 scored the highest in both ingredients in 2009. BX2149 and KXP10 are available in the high phenolic, antioxidant grape juice industry. Upon examination, the seedless hybrids showed the lowest phenol content and antioxidant activity of both years, regardless of skin color.

In 2009, hybrids containing seeds were also analyzed and seeds were removed for total phenol and antioxidant activity. Decreased antioxidant activity has always been observed in seed-free hybrids. With white skin with seeds, FX110 gave the highest score for antioxidant activity. Similar to the antioxidant activity results, with one

exception, reduced phenol content was observed in seed-removed hybrids. The exception is the 53/1 hybrid, which has very small seeds on white skin and it is more valuable to remove the seeds. BX2149, which has dark skin with seeds, had the highest phenol content. These results may be related to the fact that the phenolic and antioxidant activity of the compound occurs predominantly in seeds. The best evidence of this is that seedless varieties have very low scores in both years.

Pastrana-Bonilla et al. produced comparable results (2003). The maximum antioxidant content was discovered in grape seeds, followed by skin, while the lowest antioxidant capacity was found in grape flesh. Total phenolic in Muscatine grape components was found to be 5 times more in the seed than in the skin and 80 times higher in the pulp, according to the researchers. This result could be explained by the high catechin content of the seed and the low presence of key phenolic in the pulp. The comparatively high value for total phenolic in skins compared to the sum of individual phenolic detected in them suggests that there may be other phenolics present in the skins that were not identified in this study.

Total phenolic and antioxidant activity analyses were chosen due to the rising importance of human health in recent years. Many researchers have utilized various methodologies to study these chemicals and have stated that the results may vary (Vrhovsek et al., 2001).

In this study, total phenolic and antioxidant activity were found to be closely associated. The total phenolic and antioxidant activity were higher in hybrids with seed and hybrids with pigmented skin. Furthermore, certain hybrids with a Muscat flavor yielded superior results. Even if they have pigmented skin, seedless hybrids were in the lowest group.

Color is the most important attribute used with other variables as an indicator of grape quality. This property is directly related to the phenolic composition of the juice and the anthocyanin present in the grape skin. Anthocyanin is involved in many reactions that promote color change in grape products, primarily through the formation of pigments and macromolecular pigments. The amount and composition of phenolic compounds and anthocyanin depend on the type, variety, maturity, weather, viticulture practices, and region of the grape. The various methods and treatments used to make grape juice also have a significant impact on the final phenolic composition compared to Natural fruits. These include the type of extraction and contact time, as well as heat and enzymatic treatment. The high temperatures used during extraction, storage, and pasteurization decomposed anthocyanin, resulting in a reduction in color and total phenol content.

Vicente et al. (2011) got similar findings with sugar cane spirit aged in oak casks. They looked at the antioxidant capacity, phenolic chemicals, and furfuraldehyde. With increasing age time, antioxidant capability and phenolic component content both rise, and there is a considerable association between the two. Total phenolic content and antioxidant activity in grapes might vary depending on the variety of compounds, the color of the skin, and the presence of seed, according to Orak (2007). They stated that most types are black in color and that the seeds have excellent antioxidant activity and total phenolic. The findings of this study were similar to those of the previous one.

Akond et al. (2011) analyzed Twenty-nine common bean anthocyanin, total polyphenols and antioxidant activity from different origins, and seed coat color. They also reported that in general, genotypes of beans high in anthocyanin and polyphenols

have high antioxidant activity. Similar results were obtained from this study, where the hybrid has higher antioxidant activity and at the same time has higher phenol content.

With 18 cultivars belonging to five Oriental Vitis species, *Vitis vinifera*, three Euro-Asian Hybrids, one Euro-American Hybrid, and muscadine grape (*Vitis rotundifolia*) planted in diverse sites throughout China, Xu et al. (2010) came to a similar conclusion. They looked at the grapes' phenolic components and antioxidant capabilities. The distribution of phenolic chemicals in seeds and skins differed significantly between them. Furthermore, substantial relationships were found between several antioxidant assays in both seeds and skins. The primary phenolic components were also shown to be highly linked with antioxidant capabilities.

The overall polyphenol contents have additionally been compared in research performed with exceptional juice kinds created from white and purple grapes acquired through traditional or natural agricultural methods. It became located that the purple grape juices had better phenolic contents than the ones of white grape juices, and natural juices had better phenolic contents than the ones of juices crafted from conventionally farmed grapes (DANI et al.,2007). In any other study, the implied total polyphenol (TP) values inside the natural juice (2639.18 mg GAE.L–1) have been additionally better than the ones observed within side the business juice (2282. 64 mg GAE. L–1). Incomparable research on TP content material in grape juices the use of the Folin-Ciocalteu technique performed via way of means of Frankel et al. (1998), the consequences for Concord grape juice ranged from 1654 - 1971 mg GAE.L–1; and for juices produced with a greater diversity of grapes, the TP variety became 1407-1541 mg GAE.L–1. The equal authors point out that the phenolic contents of grape juice can be stimulated via way of means of the processes hired inside the juice manufacturing and

reactions going on in the course of storage. Cabernet Sauvignon wines (*Vitis vinífera*) of the 2004 and 2005 vintages from four exceptional locations in Santa Catarina State have been analyzed for the full phenolic contents, displaying TP values starting from 1.008 to 1.597 mg GAE.L–1 (FALCÃO et al., 2007).

These values are decreased than the grape juice samples analyzed in any other study, additionally originating from Santa Catarina State. This indicates that grape juice may be taken into consideration as a supply of phenolic compounds without being alcoholic. In the technological context, the profile of the phenolic composition of grapes represents a determinant component in wine and grape juice high satisfactory. This profile also can be used to display the manufacturing method and the high-satisfactory manipulation of these beverages.

Most commercial juices and all homemade juice samples show a significant difference ($p < 0.05$) between monomeric anthocyanin and high molecular weight anthocyanin, with higher proportions of monomeric anthocyanin. Their contribution to the coloring of commercial juices ranged from 35.71 to 69.48%. However, the highest percentage was observed with homemade grapes (76.39%). The high molecular weight of anthocyanin in all juices averaged 31.73 to 65.71%. Malacrida and Motta (2005) reported that an average grape juice value of 81.6% in high molecular weight anthocyanin was associated with juice color. High levels of total anthocyanin and polyphenols were observed in the grape juice sample. This is similar to the level reported for red wine. Total phenol content, total monomer anthocyanin, and antioxidant activity varied significantly between commercial juice samples. A strong correlation has been observed between the antioxidant activity and phenol content of commercially available grape juice samples, and that grape juice is the source of these

compounds and provides an alternative option for those who need to avoid alcohol. This result suggests that the total polyphenols in grape juice can be used to characterize this product and can be used as a parameter to monitor the manufacturing process or for quality control studies.

The correlation between grape seed concentration and the total phenol content and DPPH radical capture capacity of the varieties of juice indicates that the correlation coefficient showed a high positive correlation between the seed concentration and total phenol content of all grapes increase. A high positive correlation was also found between grape seed concentration and the antioxidant capacity of the grapes.

Anthocyanin is extracted from plants have been used as food additives. Food additive E163 is one of the commercially available additives derived from fruit anthocyanin such as grape skin, a purple food additive used in the manufacture of purple jams, confectionery, and beverages. Recently, synthetic food colorants have received public attention for their adverse effects on safety and human health, especially their effects on neurological function and behavior. Anthocyanin is one of the bioactive ingredients considered in dietary supplements and traditional medicine. It is traditionally used as herbal medicine, an appetite stimulant, and to treat many other illnesses. These color pigments are powerful dietary supplements or pharmaceutical ingredients. As a dietary supplement, the bioavailability of anthocyanin is an important factor in maintaining good health. The health and therapeutic effects of anthocyanin are primarily due to their antioxidant properties. As reported in the literature, anthocyanin chalcones and quinoid bases with double bonds attached to keto groups are effective antioxidants for removing free radicals. In addition, the glycosylated bring-in structure

of anthocyanins contributes to high antioxidant activity, and ortho hydroxylation and ethoxylation significantly increase antioxidant activity to prevent disease.

CHAPTER V

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Grape seeds have a lot of antioxidant power. Antioxidant protection, anti-diabetic, anti-cholesterol, and anti-platelet capabilities are among their possible health advantages. Grape seed eating is regarded to have health benefits due to the bioactivities of its polyphenols. Several meta-analyses have demonstrated the potential health effects of dietary polyphenols on major chronic non-communicable diseases. As a result, further research into the screening of individual polyphenol elements in grape seeds that have health-promoting qualities is required. This is because a cause-effect relationship between grape seed consumption and health impacts can only be established if the composition of grape seeds has been fully described and standardized. The impact of incorporating these beneficial polyphenol elements from grape seeds into food systems utilizing sophisticated technologies would require extensive research.

The anti-oxidative impact of grape seed extracts on meat products has been studied. When grape seed extracts were added to cooked ground beef, it increased oxidative stability and lowered hexanal content by 97 percent after three days of refrigerated storage when compared to the control without the antioxidant. Another study found that turkey patties improved their oxidative stability. Furthermore, the authors discovered that when grape seed extracts were added to turkey patties at 1.0 percent and 2.0 percent, the thiobarbituric acid reactive substances (TBARS) readings were roughly 10-fold lower than the control. The wine odor and slightly bitter flavor of grape seed extracts added to turkey patties were also noticed.

Based on the entire research work that has been conducted, we have been able to classify the ten different grape varieties based on their phytochemical content. Some of the phytochemical content, was higher in the seeds compared to the flesh. This conclusion can offer an explanation for why the pharmaceutical industry is using the seed extract for resveratrol supplements and other polyphenol components. Moreover, it was concluded that there is a direct correlation between the color of the flesh and anthocyanin content. This data should open the door for further work on stilbene and the use of grape extract (seed and flesh) on toxicity assay.

APPENDIX

	Weather data 2020 North			
Month	**Air temperature [°C]**	**Relative humidity [%]**	**Precipitation [mm]**	**Solar radiation [W/m2]**
January	9.11 - 15.02	56.81 - 87.88	0 - 43.8	19 - 131
February	4.63 - 16.47	58.04 - 93.31	0 - 31.6	22 - 159
March	10.57 - 19.33	62.91 - 84.57	0 - 73.2	28 - 226
April	14.82 - 20.48	60.77 - 86.3	0 - 21.6	100 - 252
May	18.56 - 28.25	44.66 - 77.07	0 - 10.6	63 - 264
June	20.9 - 26.16	54.01 - 83.64	0	145 - 293
July	25.39 - 29.45	64.33 - 78.44	0	170 - 285
August	26.81 - 28.89	51.87 - 76.99	0	212 - 264
September	26.2 - 29.54	58.87 - 80.08	0	165 - 207
October	22.05 - 27.92	34.65 - 74.41	0	92 - 189
November	14.06 - 23.87	65.98 - 87.92	0 - 120.8	11 - 131
December	10.07 - 17.22	61.45 - 90.58	0 - 54.2	39 - 106
	Weather data 2021 North			
Month	**Air temperature [°C]**	**Relative humidity [%]**	**Precipitation [mm]**	**Solar radiation [W/m2]**
January	7.2 - 17.76	61.4 - 80.65	0 - 16	14 - 127
February	8.3 - 16.81	49.15 - 84.07	0 - 54.2	30 - 175
March	11.29 - 21.36	59.19 - 82.66	0 - 29.2	32 - 215
April	11.92 - 23.94	62.65 - 82.93	0 - 30.6	28 - 256
May	19.92 - 23.89	65.2 - 78.82	0	241 - 279
June	22.88 - 27.34	62.26 - 78.33	0	148 - 279
July	26.62 - 28.99	55.48 - 79.2	0	187 - 264
August	27.19 - 30.27	57.81 - 73.94	0	196 - 242
September	22.54 - 28.3	56.29 - 69.39	0 - 4.4	51 - 210
October	20.27 - 26.3	46.67 - 74.08	0 - 10.2	58 - 195
November	15.61 - 21.42	39.05 - 78.63	0 - 10.8	43 - 139
December	8.94 - 18.2	54.05 - 83.86	0 - 22.4	18 - 114

S1- Weather conditions in the nearby region where the grape samples were collected during years 2020-2021

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