CHARACTERIZATION AND CLASSIFICATION OF WINES FROM GRAPE VARIETIES GROWN IN TURKEY

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in Food Engineering

by
İlknur ŞEN

July 2014
İZMİR

We approve the thesis of **İlknur ŞEN**

Examining Committee Members:

Prof. Dr. Figen TOKATLI
Department of Food Engineering, İzmir Institute of Technology

Prof. Dr. Durmuş ÖZDEMİR
Department of Chemistry, İzmir Institute of Technology

Assoc. Prof. Dr. Banu ÖZEN
Department of Food Engineering, İzmir Institute of Technology

Prof. Dr. Yeşim ELMACI
Department of Food Engineering, Ege University

Prof. Dr. Ahmet YEMENİCİOĞLU
Department of Food Engineering, İzmir Institute of Technology

11 July 2014

Prof. Dr. Figen TOKATLI
Supervisor, Department of Food Engineering
İzmir Institute of Technology

Prof. Dr. Ahmet YEMENİCİOĞLU
Head of the Department of
Food Engineering

Prof. Dr. R. Tuğrul SENGER
Dean of the Graduate School of
Engineering and Sciences

ACKNOWLEDGEMENTS

I would like to thank to my supervisor Prof. Dr. Figen TOKATLI for her guidance and support throughout the thesis study. I also would like to express my thanks to the committee members, Prof. Dr. Durmuş ÖZDEMİR and Assoc. Prof. F. Banu ÖZEN for their valuable comments and advices.

I would like to thank to the research centers; Biotechnology and Bioengineering Research Center and Environmental Reference Research and Development Center for providing the HPLC and ICP-MS instruments. I also would like to thank to IYTE Scientific Research Projects Commission for funding my thesis with the projects 2008-IYTE-18 and 2010-IYTE-07.

Finally, I would like to thank to my family for their support, encouragement, love and patience.

ABSTRACT

CHARACTERIZATION AND CLASSIFICATION OF WINES FROM GRAPE VARIETIES GROWN IN TURKEY

The wines of Turkish grapes from four vintages (2006-2009) were classified according to variety, geographical origin and vintage based on their chemical composition (element, polyphenol, color, acid, sugar, alcohol, pH, total phenol and brix) by using multivariate statistical techniques.

In the varietal classification of red wines, the partial least square-discriminant analysis (PLS-DA) demonstrated the discrimination of Boğazkere-Öküzgözü, Kalecik Karası, Syrah and Cabernet Sauvignon varieties from each other as the significant element, polyphenol, organic acid, sugar and alcohol parameters were combined in the model. Boğazkere and Öküzgözü wines of East Anatolia were characterized with their high coumaroylated anthocyanin derivatives, while Syrah wines of West Anatolia were rich in anthocyanins and flavonols. Kalecik Karası wines were the poorest in terms of total phenol content. In the classification of white wines, Emir wines of Kapadokya region were characterized with their high Li, Sr and resveratrol contents. Sultaniye wines were the lowest in polyphenol content and Muscat wines were the richest in hydroxycinnamic acids.

The regional discrimination of red and white wines was achieved with the significant element and polyphenol compositions. The western region wines were characterized with their higher Pb content which may be due to the industrialization of West Anatolia. Moreover, red wines of Tekirdağ region were recognized with their low flavonol-glycoside contents. 2009 vintage red wines were characterized with their high anthocyanin and flavonol contents. In the same way, 2009 vintage white wines had higher flavonols, flavonol-glycosides, phenolic acids and flavan-3-ols.

ÖZET

TÜRKİYE'DE YETİŞTİRİLEN ÜZÜM ÇEŞİTLERİNDEN ÜRETİLEN ŞARAPLARIN KARAKTERİZASYONU VE SINIFLANDIRILMASI

Türk üzümlerinden elde edilmiş, dört hasat yılına ait (2006-2009) şaraplar kimyasal içeriklerine (element, polifenol, renk, asit, şeker, alkol, pH, toplam fenol ve brix) dayanarak çoklu değişkenli istatistiksel yöntemler kullanılarak çeşide, coğrafi bölgeye ve hasat yılına göre sınıflandırılmıştır.

Kırmızı şarapların varyeteye göre ayrımında, kısmi en küçük kareler-diskriminant analizi (PLS-DA), önemli element, polifenol, renk, organik asit, şeker ve alkol parametreleri modelde birleştirildiğinde, Boğazkere-Öküzgözü, Kalecik Karası, Şiraz ve Cabernet Sauvignon varyetelerinin birbirinden ayrımını göstermiştir. Batı Anadolu'nun Şiraz şarapları antosiyaninler ve flavonollerce zenginken, Doğu Anadolu'nun Boğazkere ve Öküzgözü şarapları içerdikleri yüksek kumaril antosiyanin türevleri ile karakterize edilmişlerdir. Kalecik Karası şarapları toplam fenol içeriği açısından en fakirdir. Beyaz şarapların sınıflandırılmasında, Kapadokya yöresinin Emir şarapları yüksek Li, Sr ve resveratrol içerikleri ile karakterize edilmişlerdir. Sultaniye şarapları polifenol içeriğinde en fakir ve Misket şarapları hidroksisinnamik asitlerce en zengindir.

Kırmızı ve beyaz şarapların bölgesel ayrımı önemli element ve polifenol içerikleri ile sağlanmıştır. Batı bölgelerinin şarapları yüksek Pb içerikleri ile karakterize edilmiştir ki, bu Batı Anadolu'nun sanayileşmesinden dolayı olabilir. Ayrıca, Tekirdağ bölgesinin kırmızı şarapları düşük flavonol-glikozit içerikleri ile dikkat çekmişlerdir. 2009 mahsulü kırmızı şaraplar yüksek antosiyanin ve flavonol içerikleri ile karakterize edilmişlerdir. Benzer şekilde, 2009 mahsulü beyaz şaraplar yüksek flavonol, flavonol-glikozit, fenolik asit ve flavan-3-ol içermektedirler.

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

LIST OF ABBREVIATIONS

(-)-epicat	(-)-epicatechin
$^1O_2$	Singlet oxygen
a	Ankara
a*	Red/green chromaticity
ABA	Abscisic acid
ACTC	Acetic acid
ANOVA	Analysis of variance
AOC	Appellation d'Origine Contrôlée
b	Bozcaada
b*	Yellow/blue chromaticity
B	Boğazkere
Bl	Blue%
c	Diyarbakır
C*	Chroma
C	Cabernet Sauvignon
C_{18}	Octyldecylsilyl
C_8	Octylsilyl
caffe	Caffeic acid
CD	Color density
CI	Color intensity
COO^-	Carboxyl free radical
CTRC	Citric acid
d	Denizli
DA	Discriminant analysis
Da%	Proportion of red coloration
DAD	Photodiode array detector
del3G	Delphinidin-3-glucoside
del3Ga	Delphinidin-3-glucoside acetate
del3Gc	Delphinidin-3-glucoside coumarate
Dlcatec	(+)-catechin
DO	Denomination of Origin

DOC	Controlled Denomination of Origin
e	Elazığ
E	Emir
ECD	Electrochemical Detector
ETH	Ethanol
FAAS	Flame atomic absorption spectroscopy
ferul	Ferulic acid
FLD	Fluorescence detector
FRU	Fructose
fru/gly	Fructose/glycerol ratio
gallic	Gallic acid
GFAAS	Graphite furnace atomic absorption spectroscopy
GLU	Glucose
glu/fru	Glucose/fructose ratio
glu/gly	Glucose/glycerol ratio
GLY	Glycerol
h	Denizli-Tekirdağ-İzmir
H	Chardonnay
H*	Hue
H_2O_2	Hydrogen peroxide
HCA	Hierarchical cluster analysis
$HO^{\bullet}$	Hydroxyl radical
HOCl	Hypochlorous acid
$HOO^{\bullet}$	Hydroxyperoxyl radical
HPLC	High pressure liquid chromatography
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
I	İzmir
k	Kapadokya
K	Kalecik Karası
kaemp	kaempferol
KK	Logarithmic color density
L	Çalkarası

L*	Lightness
LCTC	Lactic acid
LDL	Low density lipoproteins
LOD	Limit of detection
LOOH	Lipid peroxide
m	Manisa
M	Merlot
mal3G	Malvidin-3-glucoside
mal3Ga	Malvidin-3-glucoside acetate
mal3Gc	Malvidin-3-glucoside coumarate
MLC	Malic acid
myric	Myricetin
myric3G	Myricetin-3-glucoside
N	Narince
NIPALS	Nonlinear iterative partial least squares
NIR	Near Infrared
O	Öküzgözü
$O_2^{\bullet -}$	Superoxide anion
O_3	Ozone
o-coum	o-coumaric acid
OIV	International organization of vine and wine
OMLC	Original malic acid
ORS	Octopole reaction cell
P	Papazkarası
PB1	Procyanidin B_1
PC	Principal component
PCA	Principal component analysis
p-coum	p-coumaric acid
PDO	Protected designation of origin
peo3G	Peonidin-3-glucoside
peo3Ga	Peonidin-3-glucoside acetate
pet3G	Petunidin-3-glucoside
pet3Ga	Petunidin-3-glucoside acetate
PGI	Protected Geographical Origin Indication

pinA	Pinotin-A
PLS	Partial least squares
PLS-DA	Partial least squares-discriminant analysis
POD	Peroxidase
PPO	Polyphenoloxidase
PRESS	Predictive residual sum of squares
Q3galact	Quercetin-3-galactoside
Q3glucosi	Quercetin-3-glucoside
Q3glucuron	Quercetin-3-glucuronide
quer	Quercetin
r	Tokat
R	Red%
R_2	Goodness of fit
R^2_{pred}	Goodness of prediction
RF	Response factor
RI	Refractive index
RID	Refractive index detector
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of validation
$RO^{\bullet}$	Alkoxyl radical
$ROO^{\bullet}$	Peroxyl radical
RPD	Residual predictive deviation
RSS	Residual sum of squares
rutn	Rutin
S	Syrah
SIMCA	Soft independent modeling of class analogy technique
SS_{TOT}	Total sum of squares
SUCCN	Succinic acid
SVD	Singular value decomposition
t	Tekirdağ
T	Muscat
TA	Total Acidity
Tace	Total of acetylated malvidins
Tcoum/Tcoum	Total of acetylated malvidins/ Total of coumaroylated malvidins

TART	Tartaric acid
Tcoum	Total of coumaroylated malvidins
TP	Total Phenol Content
tresv	Resveratrol
U	Sultaniye
UVD	UV-Vis Detector
vanill	Vanillic acid
VDQS	Vin Delimité de Qualité Supérieure
VIP	Variable importance in the projection
vitA	Vitisin-A
vitA/pinA	Vitisin-A/pinotin-A
w	Denizli-Diyarbakır
WCS	Wavelet compression spectra
x	Denizli-Ankara
Y	Yellow%

CHAPTER 1

INTRODUCTION

Wine consumption is primarily based on consumers' preference for taste. The wide variety of grapes, different soil and climate conditions, and various winemaking and viticultural practices affect the quality, taste and appearance of wine. Hence, the consumers naturally demand for information regarding the properties of wine such as from which grape variety it was produced, where the vineyard was, or in which vintage it was produced. Besides the appearance and taste, the label of wine is the primary source of information including the geographical origin, ingredients, shelf life, vintage and wine type. Today, the wine makers are more responsible to assess the authenticity of their products to compete in the global market. The declarations on the wine label can be confirmed by characterizing the vineyards and grape varieties and by the optimization of process conditions which may prevent fraudulent production as well. This requires the determination of chemical composition of wine and comparison of the data collected over years with the use of multivariate statistical methods. The chemical composition of wine is very complex due to the presence of a wide variety of organic and inorganic compounds. Therefore, the detection of chemical compounds is more beneficial and practical with the aid of instrumental methods. The instruments such as high pressure liquid chromatography, gas chromatography or inductively coupled plasma mass detectors enable the multiple detection of analytes at one analysis time. The resulting data sets are then handled with multivariate statistical methods like principal component analysis, discriminant analysis or cluster analysis to combine all the information coming from different analysis for the characterization of wines. This enables the classification of products with respect to grape variety, geographical region or vintage.

Turkey has large surface area for vineyards due to its valuable soil and climate conditions for grape production. Additionally, it has many native grape varieties for wine production besides the non-native, widely cultivated types. The majority of these varieties still have not been registered under the geographical indication label which is considered as one of the problems of wine sector in Turkey.

To the best of our knowledge, no comprehensive study about the characterization of Turkish wines from different grape varieties has been reported in literature so far. The objective of this thesis study was to characterize monovarietal wines produced from major grape varieties based on their chemical composition and to classify them using chemometric tools. For this purpose, 136 monovarietal commercial wine samples from four vintages between 2006 and 2009 were collected from local markets and analyzed for their mineral contents with inductively coupled plasma mass spectroscopy and optic emission spectroscopy; polyphenol, organic acid, sugar and alcohol contents with high pressure liquid chromatography; color and total phenol contents with spectrophotometric methods and several quality parameters like pH, brix and total acidity. Principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), soft independent modeling of class analogy (SIMCA) and hierarchical cluster analysis (HCA) were the multivariate statistical techniques employed for the classification of wine samples according to their grape variety, vintage and geographical region.

CHAPTER 2

WINE

2.1. History of Winemaking

Most researchers believe that the domestication of wine grape (*Vitis vinifera*) and the discovery of winemaking originated from the southern Caucasia which accounts to today's north-eastern Turkey, northern Iraq, Azerbaijan and Georgia. From Caucasia, the winemaking and grape cultivation spread towards Mesapotamia, Palestine, Syria and Egypt (Jackson, 2000). Based on the consumption of wine, winemaking spread around Mediterranean.

Jackson (2000) reported that the ancestral strains of wine yeast *Saccharomyces cerevisiae* was not naturally present on the grape flora. The natural habitat might be the oak trees and the overlap of yeast with the grape might be due to the natural habit of grapevine climbing up the trees, such as oak or the co-harvest of grapes and acorns. Louis Pasteur was the first scientist proposing the presence of wine yeasts on the surface of grapes. The following studies either failed or confirmed this proposal due to the difficulty of finding *Saccharomyces cerevisiae* on the grapes. It was also shown that the damaged grapes contained high number of microorganisms including *S.cerevisiae*. These microorganisms were believed to be carried to the grape by insects such as bees and wasps (Mortimer & Polsinelli, 1999). Today, with the establishment of wine yeast population finding enough time and suitable environment for survival and growth, they may originate from surface of grapes, the surfaces of winery equipment and also from inoculum cultures.

The ancient wines resembled to dry or semidry table wines which turn vinegary by spring based on the lack of SO_2 usage and poor protection from O_2. Concentration was a common application in Greece or Rome yielding syrupy wines. Better quality wine with aging potential was able to be produced by seventeenth century with the use of sulfur in barrels and stable sweet wines started to be produced by mid-1600's such as the Tokaji wines of Hungary. This was followed with the production of sparkling wines with the invention of strong glass bottles that could resist high pressures generated with

the CO_2 production. The development of cylindrical glass bottles with industrial revolution made the storage of laid position of bottle possible. Hence, the cork remained wet isolating the wine from O_2. And, wines with complex aroma and fragrance were able to be produced. The development of alcohol distillation started by the Arabians in the eleventh century A.D. and the technique was spread to Europe. Therefore, the production of fortified wines like Sherries is of recent origin with the addition of distilled spirits to fermenting juice to stop fermentation prematurely (Jackson, 2000).

2.2. Quality of Wine

The French oenologist, Emile Peynaud, has defined wine quality as the totality of its properties which render it desirable. It is the subjective pleasure provided by drinking which conditions judgment. Quality can be present only if the individual has the ability to perceive and approve it (Charters, 2003). Another study by Moran & Saliba (2012) reported that the major motivation of man to drink wine and other alcoholic beverages was taste which was followed by the enhancement of well-being. Therefore, the preference of wine consumption may rely on the personal experience, taste and the perception of quality. However, there are still quantitative components to define wine quality. For instance, the methodology of winemaking, geographical origin and the chemical composition are described to define wine with its other aspects.

According to the Turkish Food Codex (2008a) wine is defined as the fully or partially fermented product of crushed or uncrushed grapes or grape must with or without the protected designation of origin or protected geographical origin indication labels. It also covers natural and artificial sparkling and semi-sparkling wines and liqueur wines. The alcohol content should not be less than 9%. The total acidity should be at least 3.5 g/L in terms of tartaric acid. The sugar contents are defined for different wine types such as dry, semi-sweet or sweet wines.

The taste and mouth-feel sensations, color, odor and flavor of wine describe its quality and are dependent on chemical composition, winemaking techniques, grape varieties and geographical origin. The quantitative assessment of wine quality can be either an evaluation with trained/untrained panels or an analysis with panels trained specifically. The sensory analysis provides detailed characterization of wine's sensory attributes and can give a monetary value to wine or can be a research and development

tool (Fanzone et al., 2012; Jackson, 2002). The tasting of wine includes several sensory perceptions such as sweetness, sourness, saltiness and bitterness sensed by tongue, and astringency, metallic, burning, body or prickling sensations as the mouth-feel sensations sensed by one or more of the trigeminal receptors. These receptors are heat and cold sensors, touch sensors, pain sensors and movement and position sensors. The combination of all these perceptions determines the quality term balance (Jackson, 2000; 2002).

A balanced wine has a harmony in all dimensions, such as; acidity, sweetness, tannins and alcohol. Body is a term related to the alcohol strength of wine. A good body exhibits a feeling of weight (substance) in the mouth. On the other hand, a poor body can be described as watery. All wines possess a sweet taste based on the presence of sugars (mainly glucose and fructose), as well as ethyl alcohol and glycerol. Other grape sugar components can be arabinose, xylose, glycol, inositol and sorbitol that occur during fermentation. Mannitol and mannose can be detected in wine only as a result of bacterial spoilage. Sourness is a yield of presence of complex function of acids and wine pH. The most significant acids are the organic acids. Among them, the grape organic acids produce similar taste perceptions such as tartaric acid defined with a hard taste (hard taste: overly tannic wine), malic acid considered as green (sourness of green apples) and citric acid yielding a fresh taste. The acids of microbial origin produce more complex tastes. Lactic acid possesses a light, fresh, sour taste (milky flavor), while acetic acid has a sharp, sour taste and distinctive odor. This acid is the only volatile acid to affect wine fragrance negatively (vinegary taste). The succinic acid possesses salty, bitter tastes (Jackson, 2000; 2002).

Saltiness is a result of presence of metal cations such as Na^+ ions. On the other hand, metallic sensation which is commonly found in dry wines can be related to the presence of Fe or Cu ions. The bitter taste and astringent (dry, puckery, and dust in the mouth sensation) perceptions arise from the phenolic compounds and their derivatives. For instance, the complex tannins exhibit an astringent taste, while monomer tannins tend to show bitterness. They are also responsible for the formation of an important quality characteristic, color. Besides the phenolic compounds, many alcohols occur in wine. Among them, ethanol is the most important having effect on the wine sensory characteristics. It has a distinctive odor and it enhances the perception of sweetness. Moreover, it stimulates burning and weight (substance) sensations in the mouth. Another common alcohol in wine is glycerol which creates no odor but contributes to

the perception of body sensation and has a mild effect on the sweetness (Jackson, 2000; 2002). Aroma (bouquet) plays an important role in the quality of wine. The aroma of wine is caused by several hundred volatile compounds such as esters, alcohols, volatile acids, lactones, sulfur and nitrogen containing volatiles, acetals, phenols, terpens aldehydes and ketones. The sources of undesired aroma components can be the cultivar (strawberry, foxy, black-currant), fermentation and microbial spoilage (sauerkraut-like, mousiness), or maturation (cork-off flavor, kerosene) (Rapp, 1998).

2.3. The Necessity of Wine Characterization

Wine is a valuable and widely consumed beverage which is highly affected from various factors such as geographical origin, vintage, grape variety, growing conditions, climate and technological processes. It covers a wide variety of products from different grape varieties, vintage, geographic origin or winemaking techniques including sweet or dry wines, red, rose or white wines, all with different sensory characteristics. Each of these characteristics fulfills specific consumer demands. The consumer's food consumption habits depend on socio-cultural and economic factors. They have a greater awareness and demand about knowing the information of wine they consume. For instance, 16000 people in 16 western European countries were interested in the geographical origin of food and preferred traditional over mass production methods (Ilbery & Kneafsey, 2000). This trend forces the producers to indicate the information of their products on wine label. The label may include such information as brand, wine type, vintage, grape variety, origin of product and quality criteria. These identify the quality of wine which is appreciated by consumers, and strongly influence the price level of the product. Overall, the indication of geographic origin and vintage of a specific grape variety is mainly triggered by economic reasons which determine the quality and price of wines from different regions as well as it is an issue of trademark and consumer protection (Jaitz et al., 2010).

Today, the wine makers which would like to make fair trade with fair prices in the competitive global wine market are responsible to assure the authenticity of their products. Authentication of wine confirms the declarations on the label and ensures that the product has no fraudulent conditions. The fraudulent production of wine can be dilution of wine with water, addition of alcohol, color and flavoring compounds,

blending high quality wine with a lower quality one and mislabeling. For instance, a recent incidence of mislabeling occurred at late 90's in Chile by the confusion of Carmenere grapes with a special clone of Merlot (Versari et al., 2014).

For the authentication of wine products vineyards and varieties have to be characterized, and wine production has to be optimized which requires the knowledge of specific characteristics of wine. The classical way is the determination of wine chemical composition and comparing these data with previously established databases from authentic or commercial wines using multivariate data analysis methods. Since wine has a very complex matrix of water, sugar, alcohol, and a great variety of organic and inorganic components (phenolic compounds, mineral compounds, organic acids, aroma compounds, color compounds etc.), the indication of origin or variety can be possible with the use of a high number of parameters (González-Fernández et al., 2012; Schlesier et al., 2009).

The quality label terms, Protected Designation of Origin (PDO) and Protected Geographical Origin Indication (PGI) were introduced in 1992 by EU regulations 2081/92 and 2082/92 to label the geographical origin of agricultural foods. For PDO, the food products with this label must be produced, processed and prepared using unique methods within a particular geographical area where the quality and characteristics of the product are based onto that geographical area. The geographical link must occur at all stages of production, process and preparation (for instance the Eskişehir meerschaum, Aegean cotton, Malatya apricots, Ezine cheese). On the other hand, PGI is used to indicate food produced, processed or prepared within a specific geographical area where the food has a reputation or certain qualities attributable to that area. The geographical link occurs at least in one of the stages of production, process and preparation (for instance Isparta carpets, Siirt blankets, Mersin cezeriye) (Castro et al., 2011; Ilbery & Kneafsey, 2000; Saavedra et al., 2011). As of June 2008, 779 products have been labeled under PDO (446) and PGI (333) in Europe. France and Italy together account for more than 40% of the total registrations. In addition to these two countries, 90% of total registrations were shared between Germany, Greece, Portugal and Spain. It should be recognized that the protected products are generally from Mediterranean countries and a very limited number of products were protected from the northern Europe. This indicates the cultural importance of territorial products. Most of the labeled products were meat and meat products and cheese which were followed by some fruits, cereals and olive oil (http://ec.europa.eu/; Tekelioğlu & Demirer, 2008). In

Turkey, the geographical indication labeling has started since 1996 and 178 products have been labeled so far including various food products such as meat and meat products, dairy products, fruits, olives, olive oil and deserts (http://www.tpe.gov.tr/).

There are several quality label terms for wine as well, such as Controlled Denomination of Origin (DOC) used in Italy and Portugal, Appellation d'Origine Contrôlée (AOC) used in France, Denomination of Origin (DO) used in Spain. They indicate the idea of labeling geographical origin of wine. The geographical indication of wine products has been employed to guarantee the provenance of wine and to prevent fraudulent production. The identification of wine with its original territory indicates the standard quality and characteristic taste of the product desired by the consumers. The denomination of vitivinicultural origins and the vitivinicultural geographical units which are recognized and protected by law includes most of the European countries (France, Spain, Italy, Portugal, the Czech Republic, Romania, Austria, Belgium, Bulgaria, Luxembourg, Slovenia and Switzerland) as well as Argentina, Brazil, Chile, Australia, New Zealand, Georgia, Canada and USA (Castro et al., 2011; Fabani et al., 2010; Gonzálvez, et al., 2009; http://www.oiv.int/oiv/info/enlisteindication; Marengo & Aceto, 2003; Martin, Watling, & Lee, 2012; Saavedra et al., 2011).

The PDO label becomes more valuable for the Old World producers (France, Italy, Spain or Germany) with the introduction of New World wines (Chile, California, Australia, South Africa or New Zealand) into the global market. Moreover, the new grape varieties from the New World will become highly important for the wine industry (Martinez-carrasco, Brugarolas, & Martinez-poveda, 2005). For instance, Brazilian wines from either *V.vinifera* or their primary hybrids have gained position in the international market. Most Brazilian wines are produced from non-vinifera grape cultivars (*V. Labrusca* and *V. bourquina*), and new hybrid grape cultivars such as BRS Violeta are being developed to adapt it to the tropical, sub-tropical and temperate wine regions of Brazil (Lago-Vanzela et al., 2013). To cope with the production of misleading products and to control the authenticity of products, European Union has started a project in 2002 with the title "Establishing of a WINE Data Bank for analytical parameters for wines from Third countries". Within the project, the authenticity of wines from Eastern Europe (Bulgaria, Romania, Hungary, Moldavia and Macedonia) and from overseas countries (Argentina, Australia, Chile, California, South Africa) was assessed according to their geographical origin using multivariate data analysis methods based on the analytical parameters of wine (stable isotopes, organic matter, classical

wine analysis parameters, organic acids, volatile components and anthocyanin composition) (Schlesier et al., 2009).

The Turkish Food Codex (2008a) has also identified geographic origin indication for Turkish wines. According to it, wines with geographical origin indication should have the characteristic quality, reputation or other properties essentially attributed to the defined geographical region and should be produced or prepared within that region from at least 85% of the grapes originating from the defined region. From the native grape varieties, Elazığ Öküzgözü and Kalecik Karası grapes were labeled in 2007 and 2005, respectively. The other labeled varieties were Aegean Sultani (2003), Arapgir Köhnü (2006), Çimin (2000), İsabey seedless (2004) and Tarsus white (2003) (http://www.tpe.gov.tr/). Yet, there aren't any wine products in the list labeled with geographic origin and there are still valuable grape varieties for wine production which could be registered under the geographical indication label. Gumus & Gumus (2008) reported in their survey that lack of implementation of origin control was one of the problems of wine sector in Turkey, and 14 out of 42 enterprises strongly supported the practice of grape origin control.

2.4. Wine Types

Zhao (2005) reported that classification is an intellectual activity and without classification there will be chaos. Although wine has a wide variety of product range, there is not a generally accepted classification system for it. They can be grouped according to sweetness, color, alcohol content, CO_2 content, fermentation process, geographical region, grape variety or vintage.

Initially, according to the alcohol content they can be divided into table wines (9-14% v/v) and fortified wines (17-22% v/v). The table wines can be subdivided as still and sparkling wines based on their CO_2 contents. Most of the table wines belong to the category of still wines. Still wines can further be classified according to their color as red, rose and white wines which also classify the flavor, use and production methods. White wines are commonly consumed with meals, therefore possess an acidic character. The sweet white wines generally accompany a dessert or are consumed alone. Most of them are given no or very little maturation at oak barrels. On the other hand, most red wines are dry with bitter and astringent taste that balances with food proteins. The well-

aged ones with diminished tannins can be enjoyed after meal and does not require food. Most red wines are aged at oak barrels. The oak barrel aging prior to in-bottle yields subtle flavors (i.e. Pinot Noir, Cabernet Sauvignon, Syrah wines). Moreover, the wines suitable for aging are initially excessively tannic and those suitable for early consumption contain light flavors. The second class in the table wine category is the sparkling wines which employ yeasts to generate CO_2 to produce effervescence in a secondary fermentation either in bottles or in closed tanks, or CO_2 can be incorporated under pressure (at least 3 bar pressure should be in the bottle at 20 ^{o}C). Finally, fortified wines into which distilled spirits are added to elevate their alcohol level include wines such as Sherry-like, Port-like or Madeira-like wines (Jackson, 2000). In addition to these classifications, each wine type can be further subdivided according to the sugar content. According to the Turkish Food Codex (2008a), wines can be grouped into four classes according to their sugar content: Dry wines (≥4 g/L or <9 g/L for wines with total acidity in terms of tartaric acid <2 g from the residual sugar amount), demi-sec wines (<12 g/L or ≥4 g/L), semi-sweet wines (<45 g/L or ≥12 g/L) and sweet wines (minimum 45 g/L).

The classification according to geographical origin indication has been employed in many countries and regulated with laws. Each country has introduced different classification systems with different names; however the idea is the same. For instance, in United States, according to the regulation 27 CFR 4.25 of Alcohol and Tobacco Tax and Trade Bureau, appellation of origin can be a country, a U.S. state or up to 3 states together (multi-state appellation), a U.S. county or up to 3 counties (multi-count appellation), a U.S. or foreign government recognized delimited grape-growing area (viticultural area) (http://www.ttb.gov/). The E.U. countries such as Italy label the products as PDO and PGI. In France, the system has the name *Apellation d'Origine Contrôlée* (AOC); in Spain it is called as *Denominacion de Origen* and in Germany it has the name *Qualitätswein bestimmte Anbaugebiete*. The German system calls the wine growing regions as *Anbaugebiet* and divides a wine growing region into districts called as *Bereich*. Each *Bereich* has collective sites which are *Grosslage* and the collective sites are composed of vineyards called as *Einzellage* (Jackson, 2000). The French classification system is primarily based on the geographic region and has four groups as *Vin de Table, Vin de Pays,* AOC and *Vin Delimité de Qualité Supérieure* (VDQS). *Vin de Table* is simply the table wine made anywhere in France. *Vin de Pays* means country wine giving an indication of where it comes from. VDQS indicates

superior quality wines than *Vin de Table* and *Vin de Pays*. At the top of classification system is the AOC wines with guaranteed origin and quality. It is further classified into regional (i.e. Bordeaux, Burgundy), village (Pauillac in Bordeaux) and *premier cru* and *grand cru* for vineyards in Burgundy. French classification system is based on geographic origin rather than grape variety. Grape varieties are restricted to vine regions and their distribution is regionalized. For instance, at least 70% of Cabernet Sauvignon grapes are cultivated in Bordeaux, and each vine region is authorized to cultivate less than 5 grape varieties that are believed to be the most suitable for the geographic environment and climate (Zhao, 2005). In Australia, the geographical indication system has been employed which follows a hierarchical system and the first level of classification is the state: Australia has six states at which each of them is a geographic origin indication on its own (Martin et al., 2012).

The grape variety is another motive to classify wines. According to the OIV's (International Organization of Vine and Wine) International Standard for the Labelling of Wines, the monovarietal wines should contain at least 75% of the grape variety giving the specific character to the wine (OIV, 2012a). In the U.S.A. according to the regulation 27 CFR 4.23, the single variety wine is defined as not less than 75% of the wine is derived from grapes of that variety and the entire 75% of which was grown in the labeled appellation of origin area (www.ecfr.gov). The classification of wine according to the American system is primarily based on grape variety which can be listed as generic wines, proprietary wines, and varietal wines. The generic wines are heavily blended with different grape varieties, thus, have only generic name. The proprietary wines have the name invented by its winery, and the varietal wines have the name of the grape variety unless 75% of its volume belongs to the defined grape variety (Zhao, 2005). On the other hand, E.U. identifies single variety wines to contain at least 85% of the named grape variety (http://www.food.gov.uk/). By the Australian laws, the single varietal wines should contain the represented variety by at least 85% (Martin et al., 2012).

In terms of vintage, OIV mentioned that the wines should be produced from 100% of the grapes from the defined vintage. However, according to U.S.A. at least 95% of the wine volume should be produced from the grapes harvested in the defined year (OIV, 2012a; Zhao, 2005).

2.5. Grape Varieties and Viticulture Regions in Turkey and in the World

Vitis, one of the most widely cultivated fruit in the world, is thought to have more than 15.000 varieties. Those which originate from Anatolia are over 1200 (Kızılgöz, Sakin, & Gürsöz, 2011). However, not all of the varieties are suitable for winemaking. The grapes should be cultivated in the suitable geographical region having appropriate soil characteristics and climate conditions to obtain high yield and produce the best quality wines. Generally, the viticulture regions in the world remain within the 10 and 20 ^{o}C annual isotherms (Figure 2.1).

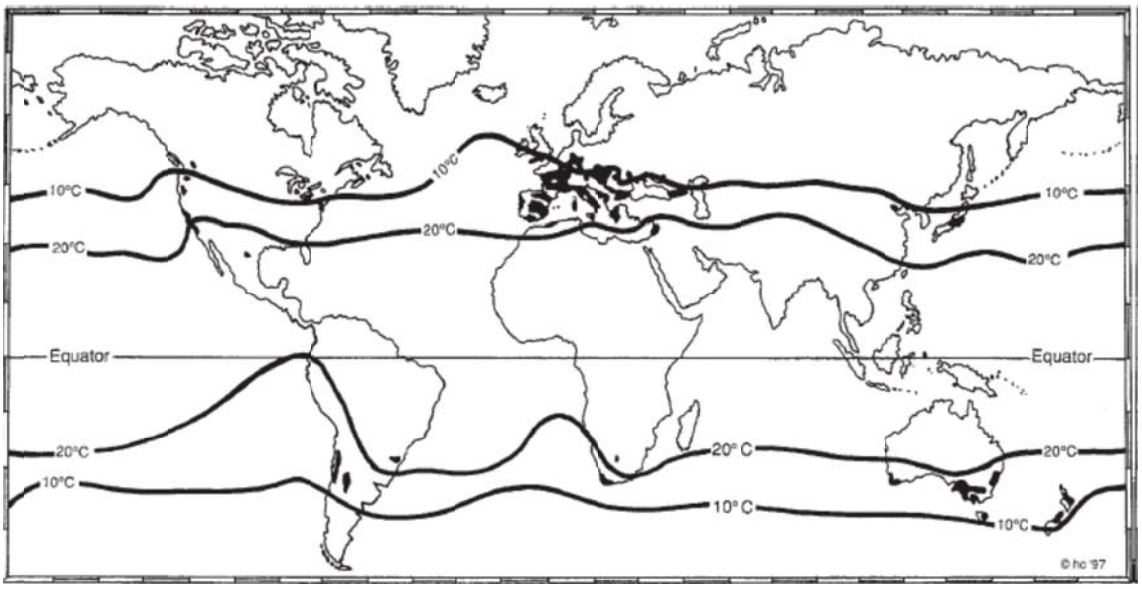

Figure 2.1. The major viticultural regions between the 10 and 20 ^{o}C annual isotherms (Source: Jackson, 2000)

The major viticulture regions in Turkey are located in the Marmara, Aegean, Central Anatolia, and Southeast and East Anatolia regions. Table 2.1 summarizes the grape varieties by these regions (http://www.tcp.gov.tr/).

Table 2.1. Grape varieties by viticulture regions in Turkey
(Source: Aktan & Kalkan, 2000)

Regions	Provinces	White Grapes	Red Grapes
Marmara and Thrace	Tekirdağ Kırklareli Edirne Çanakkale Bursa	Yapıncak Beylerce Riesling Semillion Vasilaki Clairette	Papazkarası Adakarası Karasakız Karalahna Gamay Cinsault Pinot Noir
Aegean	İzmir Manisa Denizli Aydın	Muscat of Bornova Sultaniye Beylerce Semillion Chardonnay	Foça Karası Çalkarası Merlot Cabernet Sauvignon Grenache Syrah
Central Anatolia	Ankara Nevşehir Tokat Amasya	Emir Narince Kalecik Beyazı Hasandede Beyazı Kabarcık	Kalecik Karası Ankara Siyahı Papazkarası Dimrit
South East and East Anatolia	Diyarbakır Elazığ Malatya	Dökülgen Kabarcık	Öküzgözü Boğazkere Horozkarası Sergikarası

According to the survey of Gumus & Gumus (2008), the most widely preferred grape varieties for wine production in Turkey were Boğazkere, Cabernet Sauvignon, Kalecik Karası, Öküzgözü, Çalkarası, Syrah, Merlot for red wine and Sultaniye, Emir, Semillion and Narince for white wine production. Among those, the two native red wine varieties which are widely cultivated in eastern Turkey are the *Vitis vinifera* Öküzgözü and Boğazkere. They are commonly blended together to produce high quality red wine of Turkey. This blended wine can also be blended to other variety wines at 5-10% to increase the quality of other wines. Öküzgözü has high acidity and sugar content at harvest time which makes the wine aromatic and full body (full body: wine with heavy weight). It is a large, round, dark colored and moderately tough-skinned grape. On the other hand, Boğazkere has a lower acidity at harvest with higher phenolic content than Öküzgözü variety. On account of this, its wine is very tannic, strong and heavy. Hence, the grape is not preferred for monovarietal wine and is commonly blended with Öküzgözü. It has medium, round and thick-skinned grapes (Aktan & Kalkan, 2000; Cabaroglu et al., 2002). These two varieties were commonly grown in Elazığ, Diyarbakır and Malatya provinces having characteristics of red colored and highly gravelly soil with calcareous-loam, clay-loam structure. The climate is generally continental in these regions (www.kavaklidere.com). Eastern Anatolia has higher

altitudes and highlands than other regions with continental climate characteristics. The summers are short and dry, and the winter temperatures are generally below 0 ^{o}C with precipitation as snow. Very low temperatures below -20 ^{o}C take place for a short period of time saving the vines from frost. Moreover, the preference of vineyards to hillsides prevents the formation of frost due to the accumulation of cold weather in the valleys (Odabaş, 1980). Besides the eastern regions, these two varieties are recently grown in the Denizli province which has uniform soil, clay-loam, with 34% of silt and 37% of sand in the surface layers. Boğazkere varieties grown in the Denizli province are fruitier and relatively less tannic compared to those grown in other provinces (http://www.tcp.gov.tr/). Moreover, Öküzgözü wines from the Denizli province were richer in anthocyanin, flavan-3-ol and flavonol content than the ones from Elazığ province and according to the sensory analysis, wines from Denizli province were preferred due to better color, body, harmony and astringent properties (Kelebek et al., 2010).

Kalecik Karası which originates from Ankara-Kalecik has recently been cultivated in the Nevşehir, Ürgüp, Kayseri and south of Denizli, as well. It is a round, dark red-black colored, tough-skinned, juicy grape which became a high quality wine grape. Ankara region has a slightly different microclimate than the other central Anatolian wine regions due to the effect of Kızılırmak river. The summers are hot and winters were mild and the soil is clay-loam, loam and calcareous. The vineyards are located about 600-750 m above sea level (www.kavaklidere.com). Papazkarası variety produces high quality red wines and is mainly cultivated in the Kırklareli province. It is also suitable for fresh consumption. Emir is a native white grape variety of *Vitis vinifera* which produces greenish yellow or light yellow colored wines with distinctive aromas and is widely grown in the Kapadokya region. In this region, the soil is mainly composed of lime-rich volcanic ashes and has a tuffaceous character (Aktan & Kalkan, 2000; Cabaroglu et al., 1997). Its calcareous structure with low levels of organic matter and nutritional elements improve wine quality. Moreover, the soil is slightly alkaline, salt-free and has low water absorption characteristics. The region has a continental climate with hot and dry summers and cold and snowy winters (www.kavaklidere.com). Narince is a native grape variety of *Vitis vinifera* which is originating from Tokat province. It is a medium, round and moderately tough-skinned grape variety. The name comes from its thin skin and delicate and fruity aromas. Thus, it can also be used for fresh consumption. It is widely used for the production of semi-dry and dry white

wines. These wines are suitable for aging and could be an important competitor of the European counterparts (Aktan & Kalkan, 2000; Selli et al., 2006a). Tokat region has a continental climate and the vineyards 500 m above sea level are composed of clay and sand with some pebbles (www.kavaklidere.com).

Among the western Anatolia regions, Manisa has a variant soil and climate. The soil is clay-loam, clay-sand and calcareous with both Mediterranean and continental climate characteristics. Moreover, the soil color ranges from pale to deep brown. The Denizli region has hot and dry summers and cool winters with clayey, calcareous and pebbled structured, grey colored soil (www.kavaklidere.com). Çalkarası is mainly grown in the Denizli province with sandy and less fertile soil under Mediterranean climate. Its light red color makes it more suitable for rose wine production (Aktan & Kalkan, 2000; http://www.tcp.gov.tr/). İzmir has a sandy and gravelly soil which is very suitable for the Cabernet Sauvignon variety. Meanwhile, the slopes facing the south, where Merlot grapes are well-suited, have calcareous and clayey structure with stones (http://karya.mu.edu.tr/). Muscat of Bornova is an old native variety of *Vitis vinifera* grown in the Bornova and Bayraklı districts of İzmir. It is a medium, round and green colored variety. Its well-balanced wine has low acidity and fruity and floral aroma. It is used in the production of dry, semi-dry and sweet wines (Aktan & Kalkan, 2000; Selli et al., 2006b). Sultaniye is a widely grown seedless white variety of *Vitis vinifera* in the Aegean region. This variety is cultivated in a fertile, sandy soil and Mediterranean climate. It is preferred both for fresh consumption and raisin production.

Besides these native varieties commonly cultivated in Turkey, there are many others which lose interest of winemakers due to intensive urbanization. For instance, Hasandede is a white cultivar of Ankara province. Yapıncak and Vasilaki are the white cultivars of Marmara region which are commonly cultivated. Kabarcık, Rumi and Dökülgen are white cultivars grown in the Southeast regions and are also used to produce grape molasses. Beylerce is the white cultivar of Bilecik which produces light aromatic wine. Akkemre is originated from Isparta with large, round, thin-skinned grapes. Among the red cultivars, Horozkarası and Sergikarası are cultivated in the Kilis, Gaziantep and Kahramanmaraş provinces. Horozkarası wines with high phenolic compounds require aging for a long time. Sergikarası can also be preferred for raisin production and fresh consumption. It is also used to produce liqueur wines. From the Marmara region, Karalahna, Karasakız and Adakarası cultivars can be used to produce wine and cognac and also for blending purposes (Aktan & Kalkan, 2000).

A lot of high quality varieties have been introduced from Europe since the end of the 19th century. Among those, undoubtedly the most well-known red cultivar from France is Cabernet Sauvignon. The blend of this variety with Merlot or Cabernet Franc, which is a common practice in the Bordeaux province, produces the world's famous Medoc wines. The berries are small, acidic and seedy and its wine possesses a black currant aroma called as violet in France. It is cultivated in the Marmara (Mürefte and Hoşköy), Aegean (Çeşme-Ovacık, Urla, Manisa, Turgutlu, Alaşehir) and Central Anatolia regions. Merlot is another French origin red cultivar grown in the Aegean and Marmara regions. It produces a soft wine and is less tannic than Cabernet Sauvignon. In Turkey, both Cabernet Sauvignon and Merlot are used to produce monovarietal wines. Pinot Noir, another French red cultivar, is the variety of famous Burgundy wines. It is only cultivated in the Marmara region for a limited production due to its sensitivity to environmental conditions. Carignane, which is grown in Spain, France and Algeria for a good quality red wine, is cultivated in the Aegean region in Turkey. Grenache is commonly planted in Spain, South France, South Italy, Sardinia, Sicily, California and Australia. It can be used to make rose wines or blended with other dark red wines. Syrah is thought to be a red cultivar of Iran-Syrah province and then transported by the priests to France in the 13th century. It is grown commonly in France and Australia. It has small, round berries and yields deep-colored flavorful wines (Aktan & Kalkan, 2000; Jackson, 2000). Among the foreign white cultivars, Chardonnay is the finest French cultivar which is grown in Aegean and Marmara regions and Kapadokya province. It can be used both to produce table wines or sparkling wines. Its wine has aromas of apple, peach and melon. Muscat has many varieties grown widely throughout the world with a distinctive and marked aroma (Aktan & Kalkan, 2000; Jackson, 2000).

2.6. The Economy of Wine in Turkey and in the World

The European Union holds the largest global market of wine with annually 17,500 million liters of production (http://ec.europa.eu/). However, in the last fourteen years, the wine trade of five largest E.U. exporters has fallen from 72.2% (1998) to 65.5% (2011) due to the growing wine market. The 25.2% of this market was shared with U.S.A., South Africa, Chile and Argentina. The top ten leading wine producing countries of 2011 vintage are France, Italy, Spain, USA, Argentina, China, Australia,

Chile, South Africa, and Germany. There is an increase in the wine production from 2007 to 2011 in some countries such as: New Zealand, Chile, Australia, France, China and Argentina, while, most of the European countries together with U.S.A., Russian Federation, South Africa and Brazil face a decrease in the growth rate of between -1% to -29% (OIV, 2012b).

According to annual OIV World viticultural statistics, Turkey is the fifth largest country following Spain, France, Italy and China in terms of its surface area for vineyards (508,000 hectares). It is the first largest producer of raisin (409,000 tons) and the second largest grape producer for fresh consumption (1,876,700 tons) following China (http://www.oiv.int/oiv/info/enstatistiquessecteurvitivinicole#secteur). Although, it has a large potential of grape production, only 6% is used for wine production. Approximately, 35% of total grape production is used for fresh consumption, 42% is used to produce raisin, and the rest is used to produce traditional products such as grapefruit pectin and dried fruit pulp (Kızılgöz et al., 2011). The wine production, import and export for the last three years are listed in Table 2.2.

Table 2.2. Wine production, import and export in Turkey (1000 L)
(Source: http://www.ibp.gov.tr/)

	2010	2011	2012
Production	45,299	55,099	31,606
Import	1,693	1,792	2,176
Export	2,708	2,695	2,702

In year 2012, wine production in Turkey was decreased; however, there was an increase in the export values of about 5.9%. The leading wine export in 2012 was to Belgium which was followed by Turkish Republic of Northern Cyprus and Germany. As for the situation in the world for 2012 and 2013, France was the leading country exporting wine with a ratio of 31.3% which was followed by Italy (18.8%) and Spain (10.2%). In terms of import values in 2012, wine was the second most widely imported alcoholic beverage mostly from countries France, Italy and Chile. The leading countries importing wine in the world were U.S.A., England and Germany. Canada, China, Japan and Belgium were the other important wine importing countries in the world (http://www.ibp.gov.tr/; http://www.oiv.int/oiv/info/en_press_conference_may_2014).

Turkey has a high potential to make wine industry one of its important economic sectors based on its suitable climatic and geographic conditions. It has a wide variety of grapes for wine production and has diversity in its microbiological flora. However, the wine industry is not developed due to several problems. These problems were reported as high special consumption taxes, unregistered economy, unfair competition, and lack of state policy, lack of coordination between government and civil institutions, inadequate inspection of market, lack of qualified technical personnel, marketing problems, and weak capital structures of the producers. The beginning of tax-labeling, the improvement of inspection mechanisms and the successful marketing strategies will increase the sales of lower priced table wines and reduce the special consumption taxes. On the other hand, to compete in the growing market with the import of foreign wines, the wineries today are more responsible to produce high quality wines and invest on their facilities. With the contribution of new and modern wineries, it is expected that the growing development of sector will continue in the following years (Gumus & Gumus, 2008).

2.7. Wine Chemistry

Wine has a complex composition of water, alcohols (ethanol and methanol), and a great variety of organic and inorganic compounds such as glycerin, sugars (glucose, fructose), organic (tartaric, malic, citric, lactic, acetic acid) and volatile acids, flavor compounds (esters, aldehydes, terpenes), phenolic compounds (anthocyanins, tannins), vitamins, minerals (anions and cations) and amino acids (Jackson, 2000; Volpe et al., 2009). Its composition is influenced from many factors such as grape variety, soil composition and viticultural applications such as fertilizer or pesticide applications, climate, winemaking practices including yeast culture, aging, storage, quality and hygiene of vinery facilities (Álvarez et al., 2007).

2.7.1. Minerals

Plants consist of minerals at trace levels. These minerals take role in their growth and development and they affect the plant's yield and quality. Among them, the essential minerals such as B, Cu, Fe, Mn, Mo, Ni and Zn take place in metabolic and

cellular mechanisms such as enzyme activation, protein stabilization or chlorophyll synthesis. The deficiency of Fe results in leaf chlorosis, while Zn improves the retention of bunches onto the branches and B affects the number and size of berries. The macronutrients such as N, Mg, Ca, K and P take role in the development of plant and roots, and enhance ripening (Kamsu-Foguem & Flammang, 2014; Yang et al., 2010).

The mineral composition of wine depends on primarily the soil composition of vineyard (fertilizers, pesticides, and pollution), the capacity of grape variety to absorb minerals from soil and the various steps of winemaking practices from the grape to the finished product (Fabani et al., 2010; Marengo & Aceto, 2003; Volpe et al., 2009). Vintage has little or no influence on the metal content of wines (Marengo & Aceto, 2003). Climatic changes may only influence the fungicide treatments that can affect the level of Cu in grapes (Álvarez et al., 2007). It may also affect transpiration. For instance, the grapes at warmer climatic regions have higher K levels than those grown in cooler climatic regions (Jackson, 2000). An indirect effect of climate has been reported as the increased salinity of wines from the arid and semi-arid regions such as parts of Australia or Argentina. Due to the low irrigation, the increased concentrations of Na, K and Cl lead to negative wine consideration such as “brackish”, “sea water like” or “soapy” (Mira de Orduña, 2010).

According to the sources of elements, they can be classified as natural and artificial, while some of them can originate from both sources. The natural elements are primarily influenced from soil and uptake capacity of grape rather than the winemaking practice. These elements include Al, B, Ba, Li, Mg, Mo, Si, Sr, Ti, Mn and Rb as well as lanthanides. On the other hand, those which originate both from natural and winemaking practices can be Ca and Mg (both are soil constituents and added to wine as carbonates to reduce acidity), Cu and Zn (may originate from soil or from fungicidal treatments or from corroded winemaking equipment), Fe (may originate both from soil and artificial sources i.e. corroded winemaking equipment, steel containers), K (the predominant cation of grapes and added as metabisulfite or carbonate), P (soil constituent as phosphites and added as calcium and ammonium salts) or Na (can originate from soil and added as chloride for salting). The metals from artificial sources can be Pb which can be a minor soil constituent or may come from fungicidal treatments, sealed or corroded containers or from pollution (i.e. exhaust gases or industrial fumes).

Moreover, Co, Cr, Ni and V may probably come more from metallic containers than the natural sources. Eventually, Cd is a result of atmospheric pollution (Marengo & Aceto, 2003; Volpe et al., 2009).

The element profile of wine is very often employed in the assessment of geographical origin of wine as well as to determine its quality and safety in terms of the maximum residue limits established by authorities. According to the OIV standard on maximum acceptable limits of various substances in wine, the limits for the following elements in wine are: As (0.2 mg/L), B (80 mg/L), Br (1 mg/L), Cd (0.01 mg/L), Cu (1 mg/L), Pb (0.15 mg/L) and Ag (<0.1 mg/L) (OIV, 2011). According to Turkish Food Codex (2008b), the assigned limit of Pb for wine is 0.20 mg/L. The risk of toxicity from wine consumption may arise from the presence of heavy metals like Cd, Hg, As and Pb. However, they usually precipitate during fermentation. Therefore, their presence at above trace levels indicates contamination after fermentation. There was a decreasing trend of Pb, Cd and Cu in *Podium* white wines of Italy from 1995 to 2010 due to the less use of pesticides with Cd residues, the reduction of Pb emissions from gasoline to the atmosphere and the use of phytoiatric products with lower Cu contents as reported by Illuminati et al. (2014). Elements like Al, Fe, Cu and Zn can also show toxicity when present at high concentrations. On the other hand, the essential minerals to man like Cu, Zn, Fe, K, Ca, Mg, Cr, Co, F, I, Mn, Mo, Ni and Se can be provided with moderate consumption of wine in a balanced diet. Moreover, among the many elements, Cu, Fe, Al, Zn and Ni also influence the aroma and taste of wine and can cause formation of opacity and sometimes affect color of wine. Cu ions can interact with dissolved proteins and cause Cu haziness (casse). Fe can also react with tannins yielding a blue casse. The K level affects the wine stability by precipitating as potassium hydrogen L-(+)-tartarate. High Ca concentrations can delay tartarate precipitation and produce crystals after bottling. In the absence of O_2, primarily Cu and, to a lesser extent, Fe can catalyze oxidative reactions (i.e. oxidizing ascorbic acid to dehydroascorbic acid), while, in the presence of O_2, Fe can catalyze oxidative reactions in browning. Moreover, Fe catalyzes the polymerization of phenolics with acetaldehyde and Mn catalyzes the synthesis of acetaldehyde. The undesired changes in wine taste (metallic or astringent tastes) can be due to the high levels of Fe and Cu. On the other hand, these two at low concentrations play an important role in metabolic activities and fermentation processes as enzyme activators (Jackson, 2000; Stafilov & Karadjova, 2009; Volpe et al., 2009).

There are numerous studies in literature assessing the authenticity of wine samples according to their mineral profiles using several techniques like flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorption spectroscopy (GFAAS), voltammetry, capillary electrophoresis, inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). ICP techniques are the most extensively employed techniques due to their low detection limits, multi element capabilities, and wide dynamic ranges (Aceto et al., 2002; Gonzálvez et al., 2009; Grindlay et al., 2008; Kment et al., 2005; Moreno et al., 2007; Rovio, Sirén, & Sirén, 2011). Wines from countries like France, Spain, Canada, Germany, Italy, Czech Republic, Lithuania, Chile, Argentina, South Africa, Australia, New Zealand, Romania, Slovenia and China have been successfully discriminated according to geographical origin using their elemental profile. Generally, elements like Rb, Ga, Tl, Cr, Be, Si, Mn, Zn, Al, Cs, K, Li, Mg, Ca, Fe and Sr have been found to be useful in the discriminative models (Angus et al., 2006; Coetzee et al., 2005; Etievant et al., 1988; Fabani et al., 2010; Geana et al., 2013; Gomez et al., 2004; Gonzálvez et al., 2009; Kment et al., 2005; Martin et al., 2012; Moreno et al., 2007; Pérez Trujillo et al., 2011; Selih, Sala, & Drgan, 2014; Serapinas et al., 2008; Sperkova & Suchanek, 2005; Thiel et al., 2004; Zou et al., 2012).

Although the mineral profiles of wines have been shown to be successful in the regional discrimination, there aren't any published reports neither on the detailed elemental compositions nor on the classification of Turkish wines using multivariate statistical techniques. Şimşek, Şenol, & Velioğlu (2008) determined the toxic elements (Cu, Pb, As, Cd, Fe) in 50 wines (25 red and 25 white) from Tekirdağ province using atomic absorption spectrophotometer and they have found that seven wine samples exceeded Pb level (0.2 mg/L) and thirty of them exceeded the Cd level (0.01 mg/L).

2.7.2. Phenolic Compounds

Phenolic compounds are the secondary metabolites present in wine grapes. They are synthesized in plants through the phenylpropanoid pathway, while the shikimate pathway being the entry to the biosynthesis of phenylalanine. The shikimate pathway starts with the condensation of phosphoenolpyruvate with erythrose 4-phosphate and ends with the synthesis of chorismate. The aminoacid phenyl alanine is used to produce

4-coumaroyl-CoA which is then combined with malonyl-CoA to produce chalcones. Chalcones are the backbones of flavonoids and yield different types of flavonoids through a series of enzymatic reactions (Figure 2.2). These compounds are highly important in the growth and development of plant and are synthesized in response to environmental stress such as pathogen attack, UV irradiation or wounding (Ververidis et al., 2007). Phenolic compounds are a large and complex group of compounds affecting the characteristics of red and white wines. They occur at lower concentrations in white wines than in red wines. Their major sources are grape stems, seeds and skins. They can also come from yeast metabolism or from wood cooperage. They contribute to color, flavor, astringency, and hardness of wine by the esterification reactions with polysaccharides, organic acids, and other phenolic compounds and make insoluble complexes with proteins. Chemically, they are the cyclic benzene groups with one or more hydroxyl groups. They can be divided as flavonoid and non-flavonoid compounds.

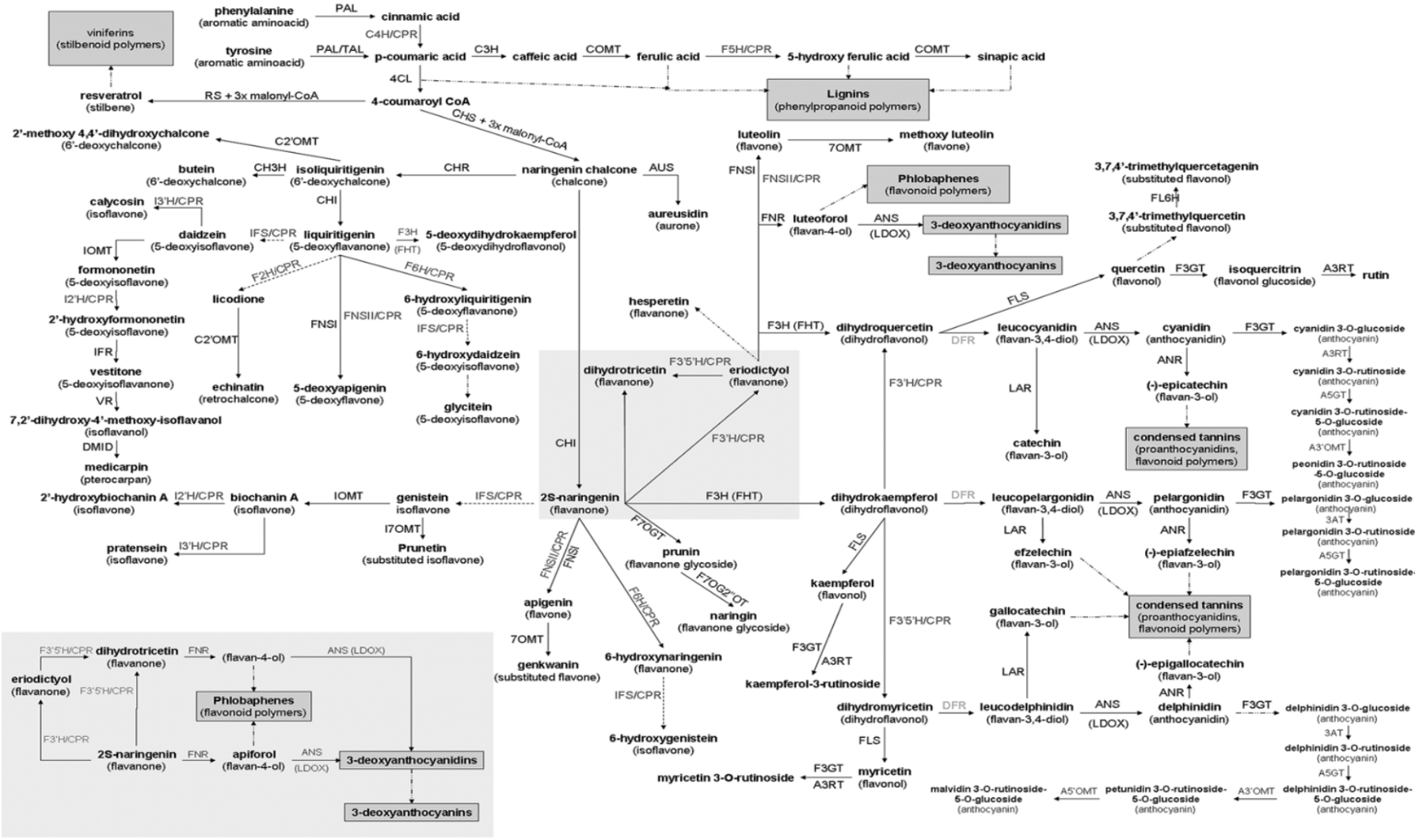

Figure 2.2. The flavonoid biosynthesis pathway
(Source: Ververidis et al., 2007)

The flavonoid ($C_6C_3C_6$) group in wine is the major class of phenolic compounds. It includes anthocyanins (peonidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3-glucoside and their acetyl- and coumaroyl-glycosides), flavan-3-ols (monomers: catechin, epicatechin, gallocatechin, epigallocatechin and their gallates; polymers: proanthocyanidins), and flavonols (quercetin, kaempferol, myricetin, isorhamnetin, and their glycosides such as quercetin-3-rutinoside) (Figure 2.3).

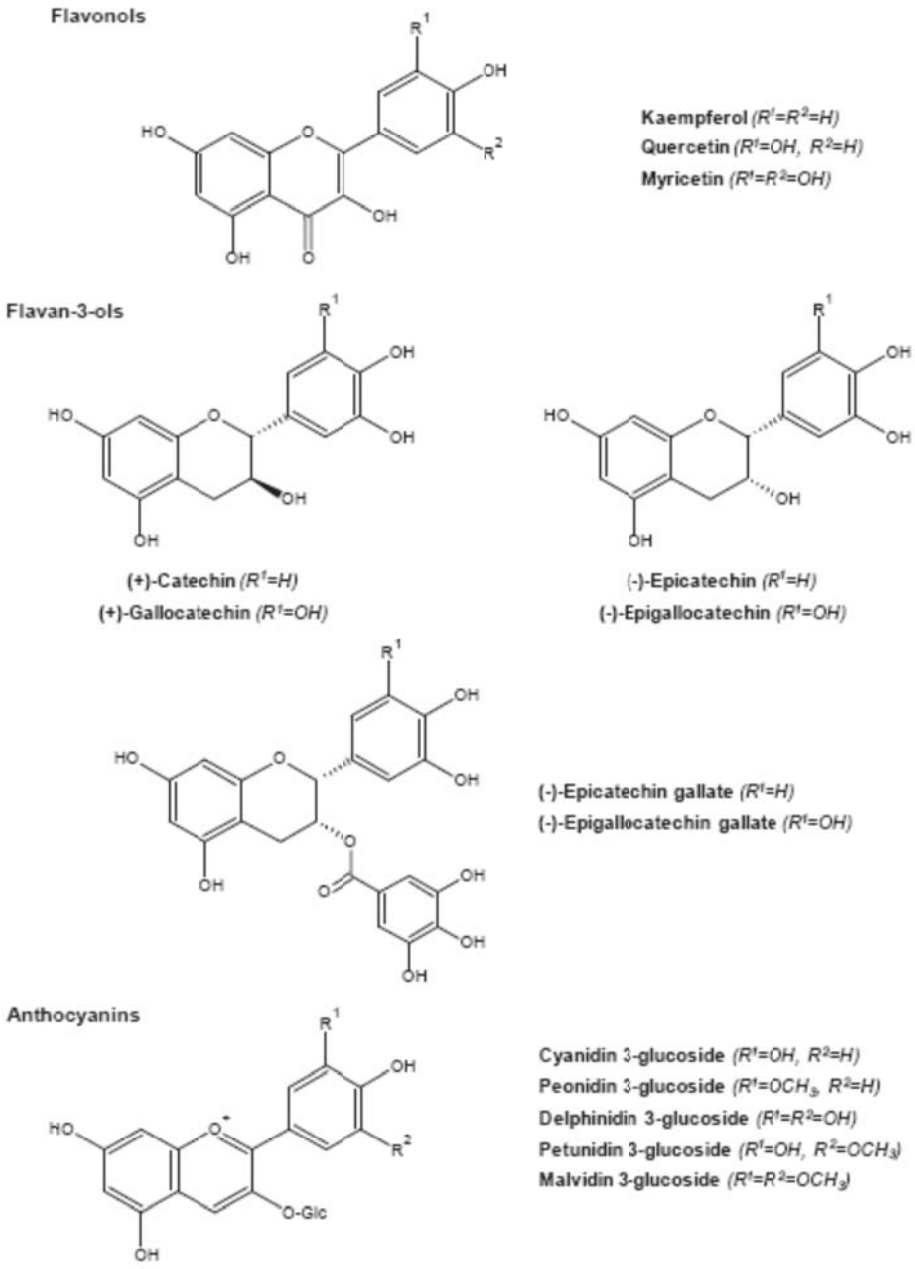

Figure 2.3. The flavonoid compounds in wine
(Source: Oliveira et al., 2011)

They are present in the seeds, skins and stems, therefore their concentration in wine is highly affected from winemaking practices such as pressing or maceration in which extraction takes place. They strongly influence wine sensorial characteristics. For instance, monomeric catechins give bitter taste to wine, whereas polymers cause astringent taste. They can be either in the free form or are polymerized to other flavonoids, sugars or non-flavonoids. Those which are esterified with sugars and non-flavonoids are called glycosides and acyl derivatives, respectively (Jackson, 2000; Oliveira et al., 2011).

Anthocyanins are the main compounds responsible for the color of young red wines and their synthesis is strictly related to the onset of veraison, unlike flavonols. Veraison is the ripening period during which the grapes undergo several changes such as the change of color from green to yellow-green for white grapes or red-blue for red grapes, the change of firmness and size of the berry, rising sugar and decreasing organic acid contents (Ivanova et al., 2011). They are predominantly present in the grapes as glycosides, called as anthocyanins. This form increases their chemical stability and water solubility. The aglycones (anthocyanidins) are rarely present in plants. The common acylation agents are cinnamic acids such as caffeic, p-coumaric, ferulic and sinapic acid, and a range of aliphatic acids such as acetic, malic, malonic, oxalic and succinic acid (Clifford, 2000). The five classes of anthocyanins depend on the position and number of hydroxyl groups on the B ring (R_1 and R_2 positions) and their proportion and amount varies by cultivar and growing conditions (Jackson, 2000). The effect of pH on anthocyanins result in the equilibria of five molecular states: red flavylium cation below pH 2.0, colorless carbinol pseudobase between pH 4.0-5.0, bluish quinoidal base between pH 6.0-7.0 and colorless chalcone between pH 7.0-8.0. Besides pH, temperature, sun-exposure and enzymes affect the stability and modify the regulatory genes that take role in the biosynthesis of anthocyanins (de Andrade et al., 2013).

There are also oligomeric derivatives of anthocyanins that result by the interaction of anthocyanins with other molecules such as, pyruvic acid, vinyl phenol, vinyl catechol, α-ketoglutaric acid, acetone, 4-vinylguaiacol or glyoxylic acid (Pinho et al., 2012). For instance, vitisin-A and vitisin-B, the so-called pyranoanthocyanins are formed by the condensation of grape anthocyanin, malvidin-3-glucoside with pyruvic acid and acetaldehyde, respectively. During maceration/fermentation, the fermenting yeast releases pyruvic acid while anthocyanins are extracted slowly from the grape skins and diffuses into the wine. The vitisin compounds are of great interest to

winemakers as they are more stable and produce deeper color at pH 4.0 than anthocyanins. It was shown that vitisin-A amount was relatively high short after fermentation (5 mg/L) and its content reduces steadily in the following 6-12 months of aging (Morata et al., 2007). Pinotin-A arises from the interaction of malvidin-3-glucoside with caffeic acid through decarboxylation of caffeic acid by the side activities of wine yeast. Due to its slow pathway, this pigment is widely used as an aging indicator in red wines. The concentration of pinotin-A increases exponentially by aging depending on the concentration of caffeic acid rather than on malvidin-3-glucoside (Schwarz, Hofmann, & Winterhalter, 2004).

Flavonols influence red wine coloration by copigmentation and display antioxidant activity. The synthesis of both flavonols and anthocyanins commences with UV exposure. Flavan-3-ols primarily occur in the stems and seeds and are responsible for the astringent and bitter taste of wines. Tannins [$(C_6C_3C_6)_n$] are the polymers of flavonoids. They are divided into gallic tannins and catechic tannins. Gallic tannins are produced during aging in wooden casks. On the other hand, catechic tannins come from the extraction from the grape stems and seeds (Jackson, 2000; Jaitz et al., 2010; Kelebek et al., 2010).

The non-flavonoid group consists of derivatives of hydroxybenzoic and hydroxycinnamic acids, stilbenes (resveratrol) and hydrolysable tannins (Vinyl phenol, guaiacol, syringol, etc.) which are derived from oak barrels during aging (Figure 2.4) (Oliveira et al., 2011). Simple phenols are the phenolic acids including hydroxybenzoic acids (C_6C_1; vanillic, gallic and syringic acids) and hydroxycinnamic acids (C_6C_3; coumaric, ferulic and caffeic acids). Although this group is colorless/yellowish, they significantly affect red wine color through intra- and intermolecular reactions. The most varying composition among cultivars is hydroxycinnamic acids and they commonly esterify with sugars, alcohol or organic acids, mainly tartaric acid (i.e. tartaric acid esters of caffeic, p-coumaric and ferulic acids are caftaric, coutaric and fertaric acids, respectively). Hydroxybenzoic acid derivative levels are higher in wines aged in oak barrels. The hydrolysable tannins (ellagitannins: polymer of ellagic acid and glucose) breakdown into ellagic acid (two molecules of gallic acid). The lignins in wood can also degrade into cinnamaldehyde and benzaldehyde derivatives (Jackson, 2000; Jaitz et al., 2010; Kelebek et al., 2010).

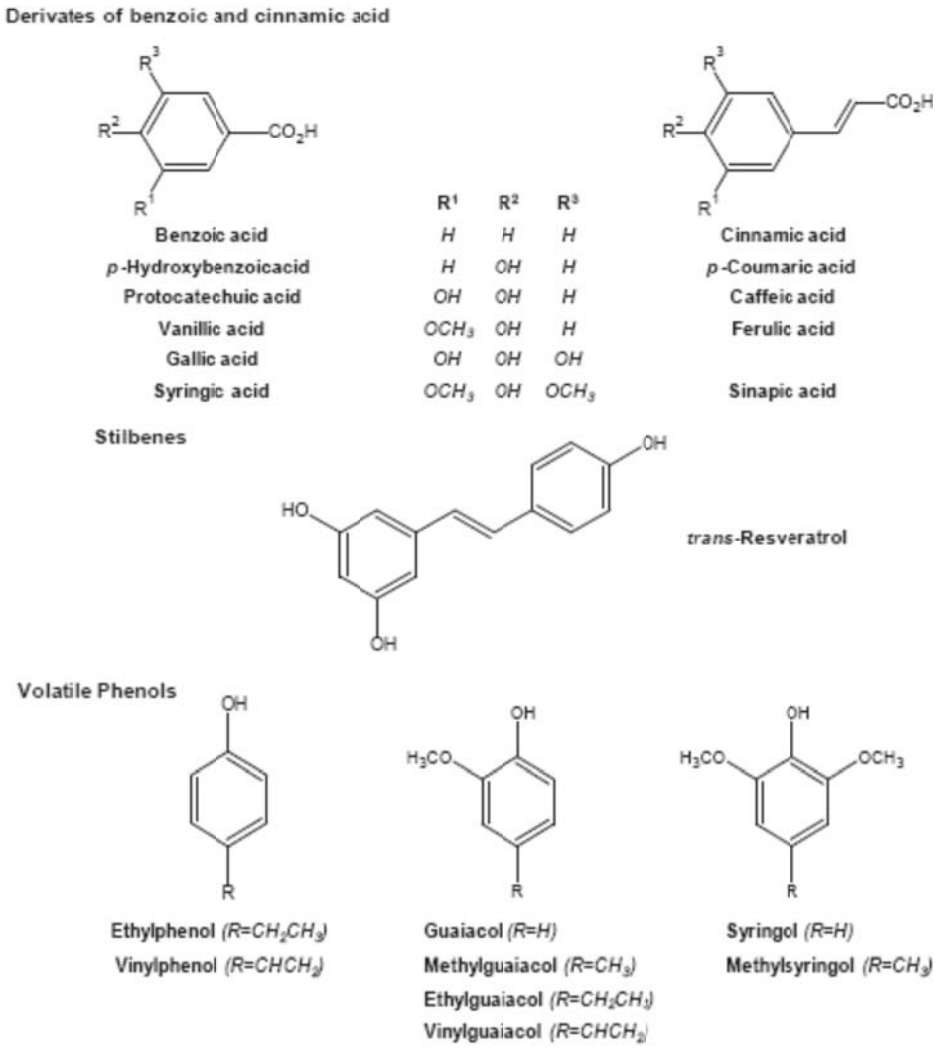

Figure 2.4. The non-flavonoid compounds in wine
(Source: Oliveira et al., 2011)

The stilbene group ($C_6C_2C_6$) includes resveratrol. They are synthesized from p-coumaric acid in response to microbial stress, abiotic stresses such as UV light exposure and herbicide or fungicide applications and their content varies between different cultivars, climatic conditions of the harvest and ecological procedures such as yeast strains, fining agents and aging in oak (Cassidy, Hanley, & Lamuela-Raventos, 2000).

The phenolic composition of wine highly depends on the cultivar, climatic conditions and fruit maturity. Although the main source is the grape, there were differences between them. For instance, the Merlot wines were found to have the highest tannin content, however, for Merlot grapes, the content was significantly higher than that of Cinsault, Carignan, Alicante and Grenache, and significantly lower than that of Mourvedre and Cabernet Sauvignon grapes (Jensen et al., 2008).

Winemaking practices such as temperature and duration of fermentation, pH, ethanol and SO_2 content of juice has influence on the concentration of phenolic compounds, as well. Clarification techniques to obtain limpids and bright wines result in the elimination of colloidal phenolic and suspended substances. Fining agents like gelatin, egg albumin and casein have been demonstrated to reduce phenolic content and lead to changes in color in some wines. Traditional fermentation which has a longer maceration time, extracts more phenolic compounds than the carbonic maceration or thermovinification. On the other hand, the concentration declines with the precipitation of phenolics by polymerization with proteins or by fining or maturation steps. Phenolic compounds are the most varying compounds in wine in terms of quantitative and qualitative changes during aging than any other wine constituent. During aging or storage, the reactions between anthocyanins and other phenolic compounds such as flavan-3-ols are based on the acetaldehyde mediated condensation, co-pigmentation and self-association reactions (Castillo-Sánchez et al., 2008; Jackson, 2000). The copigmentation and polymerization reactions of anthocyanins during bottle storage of young wines lead to change in hue from bluish-red to orangish-red. There is a reduction in flavan-3-ols with time due to condensation with anthocyanins and proteins that produce clouding. The flavonol concentrations may rise during ageing due to the hydrolysis of flavonol glycosides to aglycones (Marquez, Serratosa, & Merida, 2014). The peonidin and petunidin glycoside levels were reported to be decreased during ageing due to the greater impart in the polymerization reactions, while percentage of malvidin, cyanidin and delphinidin glycosides increased. Besides, the gallic and syringic acid levels increased due to the release from the wooden barrels during aging, while ferulic acid level was decreased which might be based on the greater impart in the copigmentation reactions (Lorenzo et al., 2005). All these reactions alter the color of wine at the same time produce a softer and less astringent and bitter tasting wine.

The phenolic compounds have been reported to have several biological activities such as cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral, antibacterial and anti-aging which rely on mainly their antioxidant and antiradical activities (Ivanova et al., 2011; Porgalı & Büyüktuncel, 2012). They have marked influence on the mouth-feel and taste sensations of wine, as well. Among them, anthocyanins have little effect on taste. However, their polymerization with tannins makes them important for the retention of tannins in wine. In addition to bitterness and astringency, phenolic compounds like phenylethanol or methyl anthranilate can contribute to the peppery

sensations, while others can produce a pungent mouth-feel or may influence the perceptions of body, balance, sweetness and acidity (Jackson, 2000).

Oxidation Mechanisms in Wine: The oxidation reactions in wine can be either based on enzymatic or non-enzymatic reactions. Oxidoreductase class enzymes (polyphenol oxidases, peroxidases) are responsible for the enzymatic oxidation of wine by using O_2 as electron acceptor and the browning of grape during processing (as the grapes are crushed). Among the polyphenoloxidase (PPO) group, tyrosinase activities include cresolase and catecholase reactions. The cresolase activity is the hydroxylation of ortho-position next to a hydroxyl group of phenol into ortho-dihydroxybenzene. This is followed by the oxidation of ortho-dihydroxybenzene into ortho-benzoquinone which is called as the catecholase activity. Another member of this group, laccase, catalyzes the oxidation of para-hydroquinones into para-benzoquinones. The ortho-benzoquinone compounds produce brown pigments through polymerization. Or, they are reduced back to catechol form by oxidizing polyphenols, ascorbic acid or SO_2. The peroxidases (POD) in the oxidoreductase class use hydrogen peroxide as electron acceptor to oxidize the donor and produce oxidized donor and water. PPO is a Cu-containing enzyme, while POD is a Fe-containing enzyme. The non-enzymatic or chemical oxidation of wine is mediated by the redox cycle of Fe^{3+}/Fe^{2+} and Cu^{2+}/Cu^{+} and the process is favored by the polyphenols containing an ortho-dihydroxybenzene moiety such as catechin, epicatechin, gallocatechin, gallic acid and caffeic acid (Oliveira et al., 2011).

The enzymatic oxidation of phenolic compounds commences with the rupture of grape cell during crushing with the release of PPO. However, these enzymes are tightly bound to the grape cells which mean that the oxidation drops off after pressing. Thus, oxidation following pressing occurs by non-enzymatic oxidation, called auto oxidation. This process oxidizes phenolic compounds into quinones. Although it is a slow process, it is not substrate specific that can affect a wide variety of phenolic compounds, not only ortho-diphenols as in the enzymatic oxidation. In this process, the O_2 molecule as the electron acceptor is reduced to hydrogen peroxide which is reactive oxygen specie. This in turn can oxidize other compounds in wine such as ethanol, phenolic compounds, SO_2 and amino acids (Jackson, 2000). Reactive oxygen species (ROS) are a group of oxygen radicals including superoxide anion ($O_2^{\bullet -}$), hydroxyperoxyl ($HOO^{\bullet}$), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$) radicals and other non-radicals such as hydrogen peroxide (H_2O_2), ozone (O_3), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$) and lipid

peroxide (LOOH). These oxidative species can be produced by the addition of single electron to O_2 using reduced transition metal ions such as Fe^{2+} (Figure 2.5).

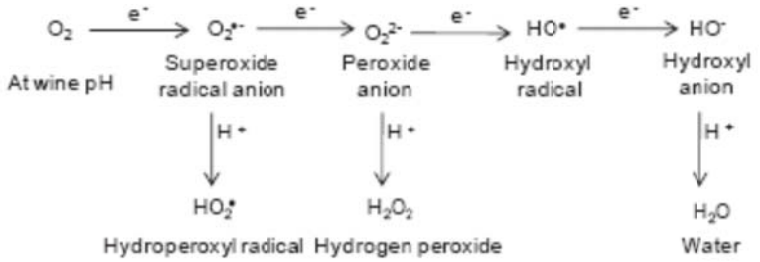

Figure 2.5. Reactive oxygen species
(Source: Oliveira et al., 2011)

The controlled oxidation can be a beneficial mechanism to enhance and stabilize the color and reduce the astringency of red wines, however, for the case of white wines, the quality is adversely affected from browning. Therefore, addition of O_2 to red wine is established to decrease the level of phenolic compounds such as catechin, epicatechin, quercetin, caffeic acid and anthocyanins, while to increase the red polymeric pigments which improve wine color density (Oliveira et al., 2011).

The analysis of phenolic compounds in wine can be either a total index based on spectrophotometric techniques or separation of individual phenolics by chromatographic techniques (High Pressure Liquid Chromatography coupled with Diode Array Detector or Mass Spectroscopy). Besides, new approaches such as cyclic voltammetry, Infrared spectroscopy such as Near Infrared or Fourier Transform Near Infrared spectroscopy techniques are being studied recently (Lorrain et al., 2013).

Recent studies focused on the use of phenolic compounds in the classification of wines by variety, vintage or geographical origin in the concept of authenticity and food safety. Makris, Kallithraka, & Mamalos (2006) differentiated 40 young red wines from five specific geographical regions in Greece according to geographical regions and cultivars using discriminant analysis. They found out that flavonols such as procyanidin B_1 and B_2, caftaric acid, and most anthocyanins excluding peonin and petunin were important variables in terms of discrimination. On the other hand, flavonols were found to be insignificant particularly in the regional discrimination of wines. Jaitz et al. (2010) have also studied the discrimination of 97 authentic red wines produced from six grape varieties grown in eleven specific regions of Austria for five vintages. The samples were classified according to their geographical origin, vintage and grape variety by

using the flavan-3-ol, flavonol, resveratrol and phenolic acid profiles of wine samples by canonical discriminant analysis. Castillo-Muñoz et al. (2010) have characterized 22 white grape varieties from a specific region of Spain using their flavonol profiles according to cultivar with principal component analysis. Li et al. (2011) have discriminated Cabernet Sauvignon wines from five specific regions in China using their polyphenol compositions via hierarchical cluster analysis technique. Another study by Saavedra et al. (2011) employed both anthocyanin and element compositions of Pinot Noir wine samples from different zones of Casablanca valley, Chile, to discriminate them according to geographical region using chemometric tools.

In Turkey, there are a few studies related to the determination of phenolic composition and antioxidant capacities of different variety wines from different regions. However, a comprehensive study about the characterization of Turkish wine samples using phenolic content has not been reported. Kelebek et al. (2010) characterized the phenolic contents of 2005 and 2006 vintage Öküzgözü wines from Denizli and Elazığ provinces. Wines from Denizli were richer in anthocyanin, flavan-3-ol and flavonol contents than those from Elazığ. This was based onto the effects of climate, soil and geographical location. Moreover, 2006 vintage wines contained higher anthocyanin and lower phenolic acid and flavonol contents than 2005 vintage wines. Anli & Vural (2009) determined the antioxidant capacities and phenolic contents of 27 red wines. The wine samples were Kalecik Karası, Merlot and Cabernet Sauvignon collected from four main wine regions, namely, Central Anatolia, Aegean-Denizli, Aegean-Izmir, Aegean-Manisa and Thrace-Mürefte. Particularly, Cabernet Sauvignon wines had higher levels of phenolic antioxidants (catechin, gallic acid and epicatechin) and antioxidant capacities compared to the Merlot and Kalecik Karası wines. On the basis of geographical area, Aegean-Izmir region red wines contained higher levels of biologically important phenolics and showed higher antioxidant capacity than the other regions. This was based on the different environmental and growing conditions and also on the maceration techniques. Another study by Anli et al. (2006) also showed that the East Anatolian wines contained higher levels of catechin, epicatechin and rutin compared to other regions (Thrace, Central Anatolia and Aegean regions). Bozan, Tosun, & Ozcan (2008) have determined the phenolic contents and antioxidant capacities of seeds of five international (Merlot, Cabernet Sauvignon, Cinsault, Hamburg Muscat, Alphonso Lavalle) and six native (Papazkarası, Adakarası, Öküzgözü, Boğazkere, Kalecik Karası, Senso) red grape varieties. They found out that

the flavonol contents of native grape seeds (particularly, Öküzgözü, Papazkarası and Kalecik Karası) except Boğazkere and Senso were significantly greater than the international varieties.

2.7.3. Color

Color is an important property of wine related to its quality and is the first attribute of wine assessed by the consumers during tasting. It is generally caused by the extraction of phenolic compounds in the grape during crushing, maceration, fermentation and evolves through oxidation and aging. The OIV has recommended to characterize the color of wine using CIELab parameters which are red/green chromaticity (a*>0 red, a*<0 green), yellow/blue chromaticity (b*>0 yellow, b*<0 blue) and lightness (L*=0 black, L*=100 colorless). These are the co-ordinates of three dimensional space called as CIE diagram (Figure 2.6) (Meléndez et al., 2001).

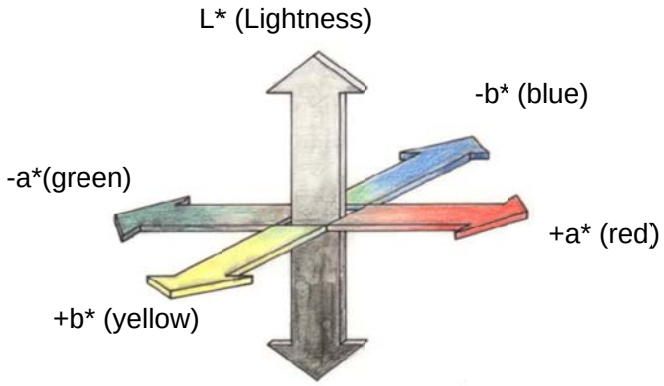

Figure 2.6. CIE colorimetric coordinates
(Source: OIV, 2013)

The non-linear parameters which are hue angle (H*) and chroma (C*) are calculated using L*, a* and b* values. Other color calculations like color density, color intensity, tint, proportion of red coloration, logarithmic color density employ the absorbance at 420 nm, 520 nm and 620 nm (Yildirim, 2006). The chromatic characteristics of must in terms of total phenolic content, color intensity and color tonality or tint enables us to determine whether a must is suitable for wine to be aged in barrels (González-Fernández et al., 2012).

In young red wines, anthocyanins occur at five major molecular states in equilibrium, four free (red flavylium cation, bluish quinoidal base, colorless carbinol pseudobase and pale yellow chalcone) and one bonded to SO_2 (colorless flavene).The color of red young wine comes from the small proportion of anthocyanins that is in the flavylium cation form. This form depends on the pH and sulfur dioxide content of wine. Low pH value enhances red color and color density, whereas increasing pH enhances blue color. Sulfur dioxide has a reversible bleaching effect on anthocyanins. It is known that free anthocyanins and anthocyanidins polymerize with tannins, procyanidins and catechins as well. The polymerization rate is about 25% at the end of fermentation, and increases up to 40% at the end of 1 year storage. These polymerization reactions are important for the color stability of red wine due to the prevention of anthocyanins from oxidation. The differences in the stability of color among red wines have been thought to be based on cultivar variability in tannin content. The anthocyanins also involve in copigmentation reactions with other phenolic compounds (catechins and phenolic acids) in which the formation of stable covalent bonds takes a long time. This is the possible reason for the gradual change of color during aging. The color of aged red wine reflects the proportion of anthocyanin-tannin polymers. These polymers produce yellow, yellow-red, yellow-brown, red, and violet shades in wine. Since most polymers are in brown color, the aged wine color has commonly brickish shades. Color density also decreases with time by the destruction of free anthocyanins (Jackson, 2000). All these reactions take place during aging of wine to produce more stable pigments that stabilize wine color and change it into a more brickish-red color which is characteristic of most aged wines (Pinho et al., 2012).

Castillo-Sánchez et al. (2008) have reported the increase in pH and hue and the reduction in total anthocyanin content, color density and chemical age of Vinhao wines from Portugal. They also presented the effect of winemaking process by the lower color density and higher hue values of wines produced by carbonic maceration than classical fermentation. Moreover, the effect of fining agents was also shown with the lower anthocyanin and color density of fined wines. Lorenzo et al. (2005) reported that the chroma, hue and lightness of red wines rose during aging in barrels.

Rose wines are to some extent similar to red wines in terms of their color; however a certain degree of maceration is necessary to obtain the minimum color with the maximum aroma compounds (Salinas et al., 2005). In contrast to red and rose wines, white wines consist of phenolic compounds at trace levels, particularly

hydroxycinnamates (p-coumaric, ferulic, caftaric acids and their derivatives). It is believed that the yellow color of young white wines results from the limited extraction and oxidation of flavonols, namely quercetin, kaempferol. The yellow golden color of old white wines arise from the oxidation of phenols or galacturonic acid (Jackson, 2000).

In literature, color property of wine has been employed in various studies to investigate their relationship with phenolic compounds and to characterize aging time of wine. For instance, Lorenzo et al. (2005) employed the color intensity, optical density at 620 and 520 nm parameters to characterize the Monastrell, Cabernet Sauvignon and Merlot wines according to their aging time. Yildirim (2006) has evaluated the antioxidant activities, total phenol contents, oenological colorimetric indexes and CieLab parameters (L*, a*, b*, C* and H*) of various fruit wines (black mulberry, blackberry, quince, apple, apricot, melon, red raspberry, bilberry, sour cherry, and strawberry) supplied from local winery in Izmir and assessed their relationship via cluster analysis. Jensen et al. (2008) investigated the prediction of phenolic composition and color of red wines from the detailed phenolic composition of grapes using multivariate techniques. Wines and grape extracts produced from 55 different grapes of 8 *Vitis vinifera* cultivars (Alicante, Merlot, Syrah, Cinsault, Grenache, Carignan, Cabernet Sauvignon, and Mourvedre) were analyzed for their total phenols, anthocyanins by spectroscopy, phenolic composition by HPLC, monomeric and polymeric pigment contents and color by UV-Vis transmission spectra. Principal component analysis (PCA) was performed to demonstrate the groupings and differences of samples. Partial least squares (PLS) was employed to build calibration models by using leave-one-out cross validation. Meléndez et al. (2001) have discriminated the rose, claret and blended wines from each other using the CIELab color parameters (L*, a*, b*, C*, H*, S*) using the soft independent modeling of class analogy technique (SIMCA).

2.7.4. Organic Acids, Sugars and Alcohols

The organic acids in wine are characterized by the ionization of carboxyl group. The carboxyl group releases a hydrogen ion (H^+) and a carboxyl free radical (COO^-). The ionization degree depends on the pH of wine, cation content (primarily K^+) and the

ionizing characteristic of the acid. Generally, the desired pH values for red and white wines are in the ranges of 3.3-3.6 and 3.1-3.4, respectively. Acidity in wine can be volatile or fixed. The fixed acidity which refers to the involatile acids is the primary source of acidity in wine. 90% of fixed acidity in grapes is caused by tartaric and malic acids. On the other hand, volatile acidity such as acetic acid can be removed by steam distillation. The total acidity term is the sum of these two acidity types (Jackson, 2000).

Low molecular weight organic acids are an important group of compounds in wine since they affect the taste and mouth-feel sensations (refreshing taste or sourness), the color stability of red wines by reducing the pH and the microbiological stability of wines. At pH values greater than 3.9, the phenolic compounds can be easily ionized. Their ionized form (phenolate) is very susceptible to oxidation which leads to the loss of fresh aroma and young color. Moreover, acids can precipitate with proteins or pectins that otherwise could produce clouds in a finished wine. And they can also solubilize Cu or Fe that could produce haziness (casse) in wine (Jackson, 2000). Therefore, it is important to quantify organic acids in wine for quality and process control.

The organic acids either originate from the grape or are produced through several processes like alcoholic fermentation, malolactic fermentation or oxidation of ethanol. Tartaric, malic and citric acids originate from grape; while succinic, lactic and acetic acids are produced by the fermentation processes (Mato, Suárez-Luque, & Huidobro, 2005).

Among the volatile acids, acetic acid is the main volatile acid with minor amount of other acids such as formic, butyric and propionic acids. They all have marked odors such as vinegar odor of acetic acid, fatty and rancid butter odors of propionic and butyric acids. Small amount of acetic acid is produced through yeast fermentation which is desired in terms of desired taste and odor (fruity character). However, at high concentrations it gives a sour taste and it is an indication of contamination of grapes, juice or wine with acetic or lactic acid bacteria. Malic acid constitutes almost half of the acidity in grapes and wine. Its concentration decreases during grape maturation particularly at the hot periods of ending season. In cooler climates, it remains at high concentrations in grapes producing a sour taste. Therefore, it is the primary acid that gives an idea about the harvest dates. Tartaric acid is the major grape acid together with malic acid. It is commonly added to wine to increase acidity of high pH wines. During aging, it precipitates by crystallization and crystal deposition in the bottle can be observed. Lactic acid is only produced at very small concentrations by yeast

fermentation. Its main source is the activity of lactic acid bacteria decarboxylating malic acid to lactic acid. This is employed in most red and some of the white wines. The harsh sour taste of malic acid is converted to a smoother taste of lactic acid. Succinic acid, which is a product of yeast metabolism, gives a bitter-salty taste and is resistant to microbial attack and is stable in wine. Another group of acids in wine are sugar acids such as gluconic, glucuronic and galacturonic acids which are produced as a result of grape infection with *Botrytis cinerea* (Jackson, 2000).

The organic acids are analyzed for the determination of maturation of grapes (tartaric and malic acids), and for the control of evolution of acidity during several stages of winemaking such as alcoholic fermentation, malolactic fermentation or aging process (lactic and malic acids). They are also important in the detection of wine alterations such as the increase of acetic or lactic acids or in the determination of spoilage of fruits (Mato et al., 2005).

There are numerous analytical methods to determine organic acid content of wines such as spectrophotometric methods, enzymatic methods, electrophoretic methods and chromatographic methods (thin layer chromatography, gas chromatography, and liquid chromatography). Of all, high pressure liquid chromatography is the most extensively used method. Within this method, ion exchange, ion exclusion or reversed phase chromatography columns can be employed. Among those, ion exchange columns provide narrower peaks without interferences and simple sample preparation (Ding, Koizumi, & Suzuki, 1995; Mato et al., 2005).

Sugars are carbohydrates consisting of several hydroxyl groups and a ketone or aldehyde group. The major grape sugars are glucose and fructose. In mature grape, they are at equal proportions; while at over maturity fructose amount is higher than the glucose. Sucrose, either added before fermentation or rarely naturally present in grapes, is enzymatically split into glucose and fructose during fermentation. Grape sugar content depends on the cultivar, maturity, and health of fruit. It is of great importance for yeast growth and metabolism. The primary wine yeast, *S. cerevisiae*, has the ability to metabolize glucose and fructose. On the other hand, the unfermented sugars which are pentoses (arabinose, rhamnose, xylose, and ribose) are called as residual sugars (Jackson, 2000).

The detection methods for sugars can be based on enzymatic, spectrophotometric techniques, nuclear magnetic resonance spectroscopy and HPLC methods with columns using stationary phases like ion-exchange resins,

octyldecylsilane groups, or amino groups. Ion exchange columns have gained much interest recently. The detection of particularly unfermented residual sugars can be valuable for the determination of cultivar or characterization of different geographical regions (Bernal et al., 1996; Moro et al., 2007).

Alcohols are organic constituents containing one (simple alcohols) or more hydroxyl groups (diols or polyols). Ethanol is the most important alcohol in wine which is primarily produced through yeast fermentation. The factors effecting ethanol production are sugar content, temperature, and yeast strain. Commonly, 14-15 % (vol %) ethanol can be produced through a standard fermentation. Levels above 14 % can be reached by addition of sugar during fermentation or by fortification. Ethanol affects the sensory properties of wine, as well as its stability, and aging. Increasing amount of ethanol during fermentation suppresses the growth of microorganisms that may produce off-odors. Together with the acidity of wine, ethanol content increases the stability of wine during aging in the absence of air. It aids the extraction of pigments and tannins during red wine vinification. It takes role in the formation of volatile and aroma compounds by affecting the yeast metabolism during fermentation and during aging in wooden casks. It affects sweetness by its own sweet taste. High concentration of ethanol in wine is identified with the terms of weight or body. It interacts slowly with organic acids and aldehydes to produce esters and acetals during aging. Methanol is a minor component of wine which is produced by the enzymatic breakdown of pectins. The methyl groups associated with pectin are released as methanol. Thus, the methanol content of wine depends on the pectin content of grape. Pectolytic enzymes added during vinification for clarification can influence the formation of methanol. It has no effect on the sensory properties of wine. However, its importance arises from the oxidation to toxic formaldehyde and formic acid in the body. Through enzymatic reactions, it never reaches to toxic levels for body in wine. Eventually, most of the higher alcohols (the alcohols that have more than two carbon atoms) occur as a result of yeast fermentation and account to almost half percent of aroma compounds of wine (Jackson, 2000).

The most abundant compound after water and ethanol is glycerol. It is a polyol with three hydroxyl groups. In grapes, the amount of glycerol is affected by cultivar, maturity and health of fruit. The amount can increase with an infection of fungus *Botrytis cinerea*. During fermentation, it is synthesized by the reduction of dihydroxyacetone phosphate to glycerol-3-phosphate. Glycerol-3-phosphate is then

enzymatically hydrolyzed to glycerol by glycerol-3-phosphatase. The final concentration in wine is affected by grape maturity, yeast strain, temperature and pH of fermentation, SO_2 amount, and nitrogen source. It affects wine flavor, viscosity, and has a slight sweet taste. It contributes to the body and fullness of wine. It is viewed as a marker of authenticity in several countries, and it can be used as adulterant due to the contribution to the sweetness, body and fullness of wine. Its analytical determination can be based on enzymatic methods, gas chromatography or HPLC with ion exchange columns. HPLC technique has the advantage of determining more than one analyte (for instance glycerol and other alcohols and sugars) in wine, simultaneously (Moro et al., 2007; Sehovic, Petrovic, & Maric, 2004).

In literature, the organic acid, alcohol and sugar contents of wines have been determined for both quality and process control purposes and for the characterization of wines from different geographical regions together with other chemical parameters. For instance, glycerol, D- and L-lactic acids, tartaric acid, malic acid, shikimic acid and methanol were some of the several chemical parameters employed in the multivariate classification of authentic and commercial red and white wines collected from Czech Republic, Hungary, Romania, South Africa, and Australia according to their geographical regions within a European project (Römisch et al., 2009). Rovio et al. (2011) has developed a capillary electrophoresis method to determine inorganic ions, anions of organic acids (acetate, citrate, formate, fumarate, glycolate, lactate, malate, maleate, malonate, oxalate, succinate, tartrate) and carbohydrates (cellobiose, fructose, fucose, galactose, glucose, myo-inositol, mannitol, mannose, rhamnose, ribose, sorbitol, trehalose, xylose) in six Pinot Noir grape wines collected from six different countries (Argentina, New Zealand, Switzerland, France, Australia and Chile). The results were used to evaluate the chemical differences of Pinot Noir wine samples from different geographic origin.

López-Tamames et al. (1996) determined the citric, tartaric, malic, succinic, galacturonic and lactic acid, and glucose, fructose, and glycerol contents of 33 white wines from three grape varieties (Macabeo, Xarello and Parellada) by HPLC. Galacturonic acid content of wines was related to grape variety. Macabeo wines have high amounts of galacturonic acid than the others. Moreover, citric acid and malic acid contents of must were correlated with weather factors such as temperature, atmospheric pressure, and rain, whereas galacturonic acid and glycerol levels in must were affected from humidity.

2.7.5. Nitrogen Containing and Aroma Compounds

There are numerous nitrogen containing compounds in wine such as amines, amides, aminoacids, pyrazines, pyrimidines, proteins and nucleic acids. Amines that have an ($-NH_2$) group can be either volatile or non-volatile in wine. The most studied are the biogenic amines. They are produced as a result of lactic acid bacteria in fermentation by the decarboxylation of free amino acids. Particularly, the most widely studied one, histamine, is known to reduce the sensory quality of wines and induce wine headaches and allergic reactions in people sensitive to this compound (Rupasinghe & Clegg, 2007).

The amides are amines with a carbonyl group. Urea is the simplest amide produced by yeast metabolism at insignificant levels; however, it can be the precursor of ethyl carbamate which is a potential carcinogen. Another class of amine derivatives is aminoacids with carboxyl attached to the amine containing carbon. They are important in terms of enzyme and protein synthesis. They are the substrates of yeasts during fermentation and they can be metabolized to flavor compounds such as higher alcohols, aldehydes, phenols, lactones and organic acids (Jackson, 2000). Their variation in must depends on cultivar, viticultural and oenological practices and environmental conditions. On the other hand, their presence in wine relies on the yeast strain, temperature, time of storage over yeast and the added NH_4^+ amount. The free amino acid content of wine has been employed in the determination of authenticity, geographical origin and fermentation kinetics. Moreover, they are determined to assess the toxicological or oenological substances in wine (Bouloumpasi et al., 2002). For instance, Etievant et al. (1988) have found out that proline, hydroxyproline and ethanolamine were effective in the differentiation of 34 French red wines from three regions. Also, Soufleros et al. (2003) have managed to classify 42 Greek white wines from six grape varieties according to variety, origin and vintage. The close relationship between amino acid content and volatile profile of wine was explained with the influence of grape variety according to a study by Hernandez-Orte, Cacho, & Ferreira (2002).

Aroma compounds are important constituents regarding the final quality of wine. They can be either in the form of free volatile compounds affecting odor of wine, or as non-volatile sugar bound glycosidic conjugates. Alcohols, esters, organic acids,

volatile phenols, aldehydes, ketones and monoterpenes can be listed as the aroma compounds present in wine. The aroma composition of wine depends on several factors such as climatic conditions, geographical region, viticultural practices, cultivar, yeast and winemaking practices. For instance, skin contact in white winemaking can improve fruity and flowery attributes of wine (Selli et al., 2006a). Fermentation taking place in new oak gives vanilla aromas to wine (van Leeuwen & Seguin, 2006).

The common instrumental analysis of wine aroma compounds includes gas chromatography coupled to flame ionization detector, olfactometry or mass spectroscopy. And among many extraction methods, solid phase microextraction is the most rapid and simple technique eliminating the use of headspace sampling device coupled to gas chromatography (Cabaroglu et al., 1997; Demyttenaere et al., 2003; Selli et al., 2006a; 2006b).

In literature the aroma composition of native Narince and Bornova Muscat wines were characterized by Selli et al. (2006a; 2006b), while the Emir wines were characterized by Cabaroglu et al. (1997). Yıldırım et al. (2007) have characterized the Cinsault, Cabernet Sauvignon, Gamay, Syrah, and Çalkarası, Boğazkere Kalecik Karası and Karasakız variety wines and associated some specific attributes to grape varieties. For instance, astringency was related to Boğazkere sample, sweetness was related to Karasakız and bitterness and metallic attributes were related to Cabernet Sauvignon and Kalecik Karası wine samples, respectively.

2.8. Principles of Winemaking Process

Since Louis Pasteur demonstrated that wines were produced by alcohol fermentation of grape juice by yeasts, winemaking process has become a modern industry with scientific research activities in the viticulture and oenology. Viticulture is the study of grapes and grape cultivation whereas oenology is the study of processing of grapes after harvest until the retailing of wine (Doyle, Beuchat, & Montville, 2001). The main purpose of juice preparation is the prevention of undesired reactions in juices which may lead to defects in wines. The basic steps of process are shown in Figure 2.7.

Vinification generally starts with the introduction of grape after harvest into the winery. The extraneous material such as leaves and stems are removed. Destemming is commonly employed to avoid from their bitter taste in wine. However, partial or

complete dry stem addition (20-50%) to fermentation can be employed in some red wine styles to provide some woody aromas and to improve the tannin content in wines.

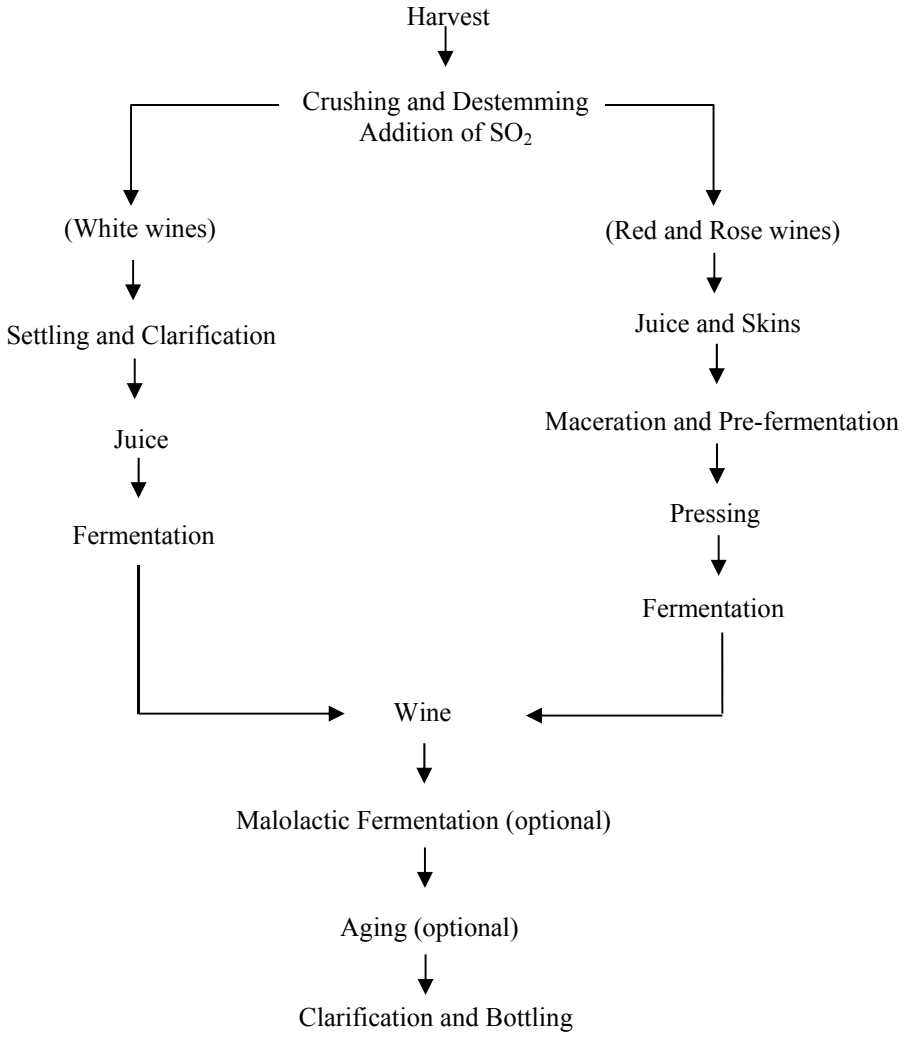

Figure 2.7. The basic steps of winemaking process
(Source: Boulton et al.,1996)

Crushing aims to break the berry so that the juice can be extracted from the grapes. It is the initial step of the juice, skin, pulp, and seed in contact that influences the extent of extraction of grape component. In some cases, the crushing process can be modified. For instance, carbonic maceration requires whole berries for the occurrence of internal cellular fermentation. The saved amount of whole berries by partial crushing will release their aroma during pressing and give the character of carbonic maceration to wine. Another condition that avoids crushing is the production of sparkling wines in which whole pressing process is widely employed to lower the level of flavonoid and

suspended solid content in wine. This approach is commonly used in the production of white juice from Pinot Noir and Pinot Meunier red grapes for sparkling wines and from Zinfandel and Grenache red grapes for blush white wines (Boulton et al., 1996).

The obtained must should be carefully handled to produce a healthy wine without defects such as low titratable acidity, high pH, lower intensity of fruity aromas, and the formation of H_2S or volatile acidity. The maceration step is the contact of skin and juice to release the phenolic and flavor compounds from the seeds, skins and pulp. It is induced by the activity of hydrolytic and pectic enzymes which are released by the rupture of cells during crushing. For white wines, the maceration time is minimum, rarely reaches more than a few hours. The best white wines are produced from free-run juices that run freely from the crushed grapes (Jackson, 2000). With red musts, the extraction and time of skin contact are much greater. Thermovinification (thermal extraction) process may be employed for a rapid extraction which includes heating of juice to 45-55 ^{o}C for 10-30 minutes and mixing of skins. During the handling of must as well as crushing, transport and harvesting steps, the pickup of oxygen from air should be prevented to avoid the activity of oxidases as they meet up with their substrates in the juice. The best approach is the use of inert gas (CO_2) blankets (Boulton et al., 1996).

The next steps for white wine are the removal of skins from the juice and clarification. The skins are removed for lower phenol extraction. The common practice is the natural settling which uses the gravity settling for the separation of skins from the juice in vertical tanks. On the other hand, drainer equipment can also be used immediately after crushing or after a period of juice and skin contact in the production of distinctive varietal white wines. Clarification is the reduction of suspended grape solid down to levels of 1-2% in white juice before fermentation and is a common practice for fruity white table wines. It is performed due to several reasons: 1- The removal of oxidative enzymes related to the pulp and skin, 2- The removal of wild mold flora primarily found on the skins, 3- The removal of laccase enzyme from the botrytis or other molds, 4- The removal of sulfur and other chemicals of vineyard practices which are related to pulp and skins, 5- The removal of some amount of esterase activity within the grape tissue that prevents the accumulation of esters during fermentation.

The clarification process can be applied by several methods like natural settling by gravity, centrifugation, and filtration by the aid of diatomaceous earth or cross flow filters or cold settling. Cold settling is induced by the addition of pectolytic enzymes to break down grape material at 5-10 ^{o}C for 24-48 hours (Boulton et al., 1996; Doyle et al., 2001).

For red wines, during the maceration step, fermentation may begin naturally or by yeast inoculation. The skins rise to the top of the juice and form a cap. The juice is regularly pumped over the cap to extract anthocyanins and other phenolic compounds to give color and bitter and astringent character to wine. This extraction can be promoted with the production of ethanol by preliminary fermentation. Some alternative practices can be employed such as thermovinification or carbonic maceration (the maceration of uncrushed or partially crushed grapes under the gas CO_2 to remove O_2 at 25-35 ^{o}C for 8-10 days) (Doyle et al., 2001).

Rose wines are produced from red grapes with a relatively shorter maceration time. Generally, the duration depends on the desired rose color and maceration ends before alcoholic fermentation starts (Jackson, 2000). The European Community has stated that the blending of red and white wines to produce rose wines is fraudulent production (Meléndez et al., 2001).

Prior to alcoholic fermentation, several treatments can be employed to the juice and must. Nutrients such as nitrogen sources (ammonium salts) or vitamins (biotin, thiamin, pantothenic acid or inositol) can be added to assist yeast growth and inflate fermentation. The nutrient level of white juice is generally less than that of red juice which is in contact with the skin for a longer period. The settling and clarification processes cause deficiencies in the nutrient level of white juice. The deficiencies in nutrients may yield in incomplete fermentation and the production of by products such as acetic acid, pyruvic acid or H_2S. The pH can be adjusted with approved acids such as tartaric acid or with calcium carbonate salts (Doyle et al., 2001).

SO_2 addition at levels between 50-200 mg/L is a common application for decades as an antioxidant and antimicrobial agent and to inhibit phenol oxidase activity. The inhibition of enzyme is based on the competition between the sulfite and oxygen for the oxygen binding site of enzyme. It can reverse the first step of oxidation reaction, converting the quinone back to its phenol. This leads to a delay in the browning reaction rather than a complete enzyme inactivation or antimicrobial activity. It is a good substrate for laccase enzyme, thereby preventing the formation of peroxide.

It also reacts with carbonyl compounds and produces nonvolatile bisulfite adducts and thereby, prevents unpleasant sensory properties (Doyle et al., 2001; Oliveira et al., 2011).

Ascorbic acid is naturally present in grapes; however, it is quickly depleted following crushing of grapes by either scavenging oxygen or reducing ortho-quinone derivatives. It can be an additive for antioxidant purposes at a concentration of 50 to 150 mg/L, especially to white wine. Presence of SO_2 is important when ascorbic acid is added due to the rapid oxidation to dehydroascorbic acid and hydrogen peroxide (Oliveira et al., 2011).

The alcoholic fermentation is the transformation of grape juice into wine by the yeast of genus *Saccharomyces*, commonly *S. cerevisiae*. Although *S. cerevisiae* is the main wine yeast, it occurs at very low populations (<50 CFU/mL) and is rarely isolated from sound grapes. The main yeasts are the non-*Saccharomyces* yeasts or wild yeasts, such as species of *Kloeckera, Hanseniaspora* and to a lesser extent the species of *Candida, Metschnikowia, Pichia, Kluyveromyces*, and *Hansenula*. The fermentation process can commence by this natural microflora present on the grapes; however, the non-*Saccharomyces* yeasts grow in the first 2-4 days of fermentation, then they die off. The fermentation can also occur by the inoculation of known strain of *Saccharomyces*. This practice produces more accurate and rapid results however it lacks the advantage of producing wine with varied and unique characteristics (Doyle et al., 2001).

The yeast metabolizes the grape sugars, glucose and fructose, and produce ethanol and carbon dioxide (Figure 2.8). The fermentation parameters can be 10-18 ^{o}C for 7-14 days for white wines and 20-30 ^{o}C for 7 days for red wines. Higher temperature for red wine is necessary for the extraction of color from grape skins. Alcoholic fermentation is ended when the juice sugars, glucose and fructose, are completely utilized (Boulton et al., 1996; Doyle et al., 2001).

The alcoholic fermentation can immediately be followed by malolactic fermentation which commences naturally with the activity of lactic acid bacteria in wine or by the inoculation of *Oenococcus oeni*. It is the decarboxylation of L-malic acid to L-lactic acid. The benefits are the reduction of acidity in high acid wines which is particularly favored in cool climatic regions [the grapes have higher acidity (pH<3.5) in cool regions than those of in warm regions], enhancement of sensory characteristics through bacterial activity and enhancement of microbiological stability. Most red wines and few white wines undergo malolactic fermentation. The flavor changes through this

process can be undesirable for white wines that have a mild fragrance. The undesired effects of malolactic fermentation can be the excessive reduction of acidity in high pH wines which increases the potential of spoilage, production of undesirable flavors, color changes and production of amines. It is particularly prevented by wineries in warm viticultural regions. The control methods are addition of SO_2 and cold storage (Davis et al., 1985; Doyle et al., 2001).

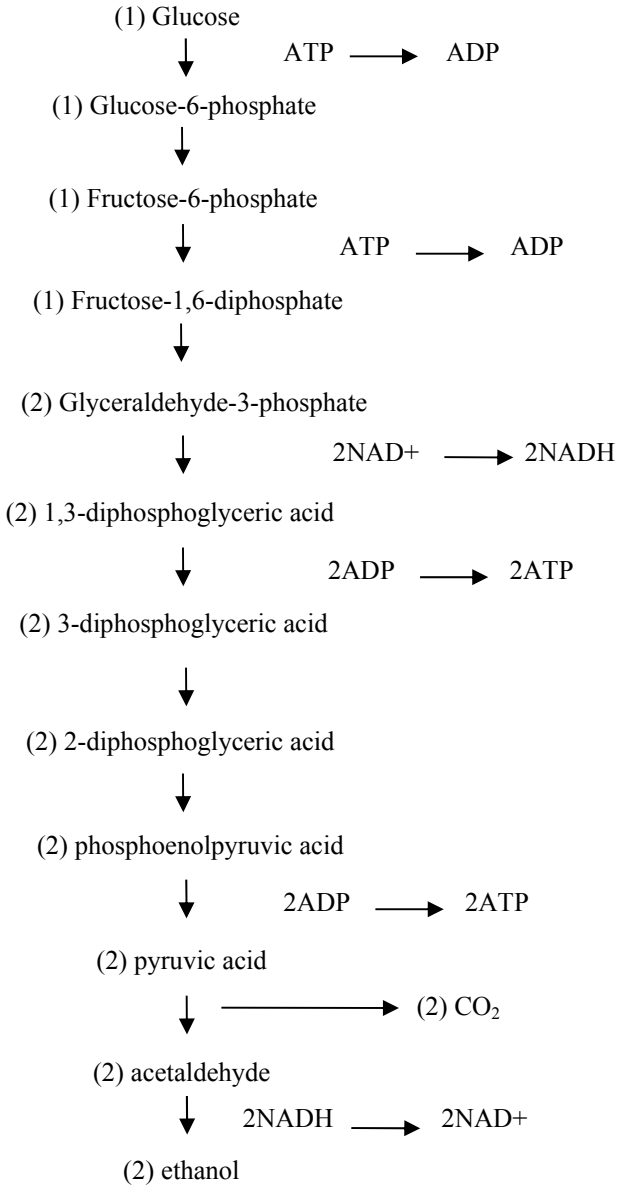

Figure 2.8. Fermentation steps
(Source: Boulton et al.,1996)

2.9. Health Related Aspects of Wine

Wine has the potential to take place in the Mediterranean diet with its rich composition containing various bioactive compounds. Among those, the most widely spelled compound is resveratrol which is from the stilbene family of phenolic compounds. It is synthesized in the leaf tissues of many plants against fungal infection or exposure to ultraviolet light. In grapes, it is present in the skin and seed of grapes which increases its concentration in red wine due to the longer contact of skin and must during the fermentation process. Its concentration depends on such factors as grape variety, geographic region, agronomic factors, climatic factors, plant stress conditions and oenological practices. Based on its antioxidant activity, it suppresses peroxidation of lipids which is related to the coronary heart diseases and myocardial infarction. It protects the cardiovascular system and has neuroprotective and anti-aging activities (Fernández-Mar et al., 2012). However, there are other studies suggesting that the health benefits of wine consumption could be due to the various phenolic compounds present in wine rather than the effects of dietary resveratrol intake (Vitaglione et al., 2005; Xiang et al., 2014).

Hydroxytyrosol, which is a phenyl ethyl alcohol, is mainly present in virgin olive oil. However, wine seems to be another source of hydroxytyrosol in our diet. Although, hydroxytyrosol has toxic effect, it is considered that a daily consumption of up to 2 g/kg body weight is safe according to the available studies (Fernández-Mar et al., 2012). The majority of studies related to the health benefits of hydroxytyrosol have been developed on the olive oil matrix being the main source of this compound. It has been shown that it has antioxidant activity by scavenging peroxyl, hydroxyl and other free radicals. Moreover, the antimicrobial activity of hydroxytyrosol has been shown against gram positive bacteria being higher than gram negative bacteria. Melatonin, which is a neurohormone, is also synthesized in plants as a secondary metabolite. It has been recently reported that grapes and wines are sources of this compound with a lower amount than the other compounds. It has antioxidant activity by scavenging free radicals. Its ability to inhibit tumour cells has been partially defined. Resveratrol, hydroxytyrosol and melatonin present in wine could act synergically for a protection against oxidative stress and this supports the health benefits of wine to be an important component of Mediterranean diet. However, there are still issues to be addressed about

the health benefits of these compounds since the related studies are in the initial stages and there is too much variability in the studies (Fernández-Mar et al., 2012).

The “French paradox” term is associated with the low mortality levels of French people due to coronary artery diseases although the consumption of saturated fats, smoking are no lower and the blood cholesterol levels are generally higher than other countries. This low risk of mortality was related to the moderate consumption of beer and particularly wine (180 mL/person/day). The inhibition of low density lipoproteins (LDL) by wine polyphenols was attributed to the presence of catechin group. Catechins and procyanidins were found to inhibit LDL oxidation in vitro, and increase the antioxidant capacities of human plasma and reduce myocardial postichemic damage in rats with an increase in ascorbic levels of plasma (Landrault et al., 2001).

In addition to the commonly publicized benefit to cardiovascular system, moderate wine consumption also reduces the effects of stress, enhances sociability and lowers the rate of depression, improves self-esteem and appetite in the elderly (Jackson, 2000). On the other hand, the undesired influence is related to the induction of headaches based on the histamine intolerance of consumers (Jarisch & Wantke, 1996).

The nutritional safety of wine is also under control with the related regulations in Europe such as the EC Regulation No. 2676/90 which dictates the methods to be used by the wineries. Yet, there is still the risk of metal toxicity in wine resulting from pollution of soil and air through industrial wastes, use of pesticides and from the use of metal equipments in the process. Among those, Cd, Hg, As and Pb are the most toxic to human which can cause damage to kidneys, nervous and immune systems, and in some cases cancerous effects. The symptoms of heavy metal intoxication are irritability, mood changes, depression, headaches, tremor, and loss of memory and reduced capacity of sight (Volpe et al., 2009).

On the other hand, other metals such as Cu, Fe and Zn are both essential to human and can be toxic when present at high concentrations. For instance, Cu is important in respiration because it is necessary for the synthesis of hemoglobin which transports O_2 in the blood stream. It also participates in the synthesis of collagen and the neurotransmitter noradrenalin and takes place in the breakdown of polyunsaturated fatty acids. Fe interacting with Cu and proteins produce hemoglobin. Moreover, it is necessary in the synthesis of collagen and acts as a co-factor in the synthesis of serotonin, dopamine and neurotransmitter noradrenalin. Zn plays part in absorption and interacts with vitamins. It is a component of over 200 enzymes necessary for digestion

and metabolism. At sufficient concentrations, it competes with Cd, reducing its absorption by the metabolism. The presence of many other essential minerals such as K, Ca, Mg, Cr, Co, F, I, Mn, Mo, Ni and Se make wine a beneficial drink when combined to a balanced diet at moderate consumptions for human (Volpe et al., 2009).

2.10. The Effect of *Terroir* on Wine Chemistry

The French term "terroir" is an interactive ecosystem including climate, soil and vine. These factors related to the geographic origin affect the quality, taste and style characteristics of wine. Moreover, it includes human factors such as socioeconomics and history, and viticultural and oenological practices of a given vine area (van Leeuwen & Seguin, 2006; Zhao, 2005).

There are different phenological periods during the berry growth. The first stage "bud break" takes place between late March and late May. It is followed with "flowering" (bloom) which ends by late July. The final stage is the "veraison" that ends by September (harvest) (Nicholas et al., 2011). During these phenological stages, berry develops and its composition changes. Tannins, including the monomers of flavan-3-ol [(±)-catechin, (-)-epicatechin and (-)-epicatechin gallate] and polymers of proanthocyanidins (procyanidin B_1, B_2, B_3 and B_4), were biosynthesized at the initial stages of berry growth earlier than veraison and reaches maximum around veraison. Between veraison and harvest, their biosynthesis stops or slows. In the same way, quercetin being the main component, flavonoid synthesis starts before veraison and increases during berry ripening. Veraison is the ripening stage of grapes. The changes include accumulation of color (anthocyanins in red grapes), aroma compounds, amino acids, tannins, sugar and minerals as well as color changes from green to red or yellow-green and organic acid content particularly malic acid decreases. The firmness of berry reduces and berry size increases. Essentially, anthocyanin accumulation commences at veraison and proceeds throughout the ripening period. In the post veraison period, the anthocyanin content may decrease due to berry shrinkage (Ivanova et al., 2011; Koundouras et al., 2009). The harvest decision is made according to the sugar content and the balance of sugar with the acidity and pH of berry. For instance, the production of sweet and strong wine is associated with maximum sugar concentration which is

maintained with late harvest, while the production of softer wine requires early harvest with higher acidity and lower sugar content of berries (Ivanova et al., 2011).

Vine phenological stages are triggered by suitable weather conditions such as adequate temperature, precipitation and sun exposure. The temperature should reach 10^{o}C for the onset of growing season and a relatively cool weather should follow. The ideal temperature range should be between 12-22 ^{o}C during the growing season and 3^{o}C in winters. Lower temperatures during winter will cause crop injuries while temperatures above 35^{o}C will lead to heat stress and damage the vine. Moreover, the grapevine requires around 600-700 mm of water during the growing season (de Andrade et al., 2013; Ferrandino & Lovisolo, 2014; Fraga et al., 2014; Lorenzo et al., 2012).

The potential effects of climate on grape production and its consequences on wine production and quality are well-established. Rising temperatures can advance the harvest dates. Therefore, new viticultural regions in the world become suitable for grape production (i.e. Peru, Thailand, Cambodia, India, Brazil and Venezuela) and join the traditional regions of "new world" (Canada, U.S.A., Chile, Argentina, South Africa, Australia, New Zealand, China) and the "old world" (Mira de Orduña, 2010).

It was reported that warmer temperatures increase metabolic rates and affect metabolite accumulation within the grape. For instance, the photosynthesis level decreases at temperatures above 25^{o}C and metabolic processes and sugar accumulation may stop above 30^{o}C. Although the temperature effect on sugar accumulation is small, high sugar concentrations can be reached at harvest days due to concentration by evaporative loss. Therefore, high wine alcohol levels can be obtained. With increasing temperature, malic acid levels decrease and K level increases due to the accumulation from other above-ground vine organs during grape maturation. The low total acidity levels and increased K levels together result in increased pH levels (Mira de Orduña, 2010). The increased formation of fermentation by-products such as glycerol and acetic acid was also reported due to the effect of high sugar concentration of grape on the glycolytic and pentose phosphate genes (Montealegre et al., 2006). High temperatures also affect wine aroma and color. In hot seasons, the phenolic maturation is not completely reached and unbalanced soft wines are obtained (de Andrade et al., 2013). It was suggested that high temperature (≥ 30^{o}C) of grape berry reduces the synthesis of anthocyanins and hence reduce grape color (Mira de Orduña, 2010). Moreover, increased formation of malvidin, petunidin and delphinidin coumaroylated derivatives

have also been described with increased temperatures (Montealegre et al., 2006). Flavonoids are known as photo-protectants which explain their dependency on sun exposure (Mira de Orduña, 2010). Tarara et al. (2008) have reported the synergistic effect of sun-exposure and temperature on Merlot berries and found out the decrease in anthocyanins except malvidin derivatives with increased temperatures of sun-exposed Merlot berries. Nicholas et al. (2011) have found a positive correlation between tannins, anthocyanins and iron reactive phenolics and the warm temperatures between bud break-flowering and cool temperatures between flowering-veraison. Tannins were increased by warm nights before bud break and warm days between bud break-flowering, while anthocyanins were increased by temperatures between 16-22^{o}C from veraison to harvest (Nicholas et al., 2011).

Another climate parameter affecting berry composition is the water deficit of plant. Water regime of a vine involves both soil characteristics and climate conditions. A high water deficiency with high water retention capacity soil did not affected the carotenoid accumulation, while a severe water stress in a low water retention capacity soil yielded higher carotenoid (Ferrandino & Lovisolo, 2014). The winter and spring rainfalls are highly beneficial for vine development, while rainfalls in the beginning of summer can lead to viticultural problems like the formation of fungal disease (www.gap.gov.tr). Severe water stress in the early stages will delay the development of grapevine, in the same way; excessive water may increase the risk of pests, diseases, excessive vigor (Fraga et al., 2014). If water status of the plant is good, the plant will use its energy more on the production of leaves and stems rather than berries. However, under mild water stress, the sugar formed by photosynthesis will be used to feed berries. A floor providing mild water deficiency is necessary to produce high quality wines that will lead to early accumulation of sugar in the berries (Kamsu-Foguem & Flammang, 2014). It is well known that in unirrigated soils, berry size decreases, on the other hand, total phenolic and anthocyanin contents increase and high quality wines with low yield are produced. As berry size reduces, the surface area: volume ratio of grape will increase which results in the concentration of phenolic compounds located at the skin of grape (Bucchetti et al., 2011; Ojeda et al., 2002; Montealegre et al., 2006; van Leeuwen & Seguin, 2006). It was also reported that water deficiency may directly affect the biosynthesis of phenolic compounds either positively or negatively depending on the type of phenolic compound, period and severity of water deficit (Ojeda et al., 2002; Montealegre et al., 2006). Strong water deficiency at veraison increased the

biosynthesis of flavonols, anthocyanins, and proanthocyanins but did not affect flavan-3-ols, as reported by Ojeda et al. (2002). Other studies have also reported increased anthocyanin concentrations with water deficiency during veraison but tannins were not affected. They were rather affected from early water deficits. The high tannin content was related to the higher skin/berry weight rather than the increased biosynthesis of tannins (Bucchetti et al., 2011).

It is still unclear whether the effect of vine water status on grape phenolics relies on the flavonoid biosynthesis or on the water availability related changes in berry growth and vine vigor. Moreover, water effects on berry composition are conflicting due to different irrigation applications and environmental conditions (Koundouras et al., 2009). It is hypothesized the role of hormonal signals, thereby the synthesis of abscisic acid which induces the up-regulation of genes contributing flavonoid biosynthesis under water-deficiency conditions. The plant's secondary metabolism is a defense mechanism in response to abiotic stress (water deficiency, elevated light and temperature) and yields phenolic compounds and volatile compounds. Correspondingly, higher quality grapes with better taste, color, and aroma can be produced and the secondary metabolites involve in the stabilization and aging processes, as well. Abscisic acid (ABA) is the main compound acting on the response of plant to abiotic stresses, especially drought. There is an increase of ABA concentration in the berry during veraison and it is accompanied with sugar accumulation. It was shown to enhance several processes during veraison, i.e. accumulation of soluble solids and anthocyanins and decrease in the level of organic acids. However, the causes of accumulation of ABA still remain unclear (Ferrandino & Lovisolo, 2014).

High quality and well-balanced wines are related to mild water stress during ripening and cool and moderately wet weather before ripening, in the early stages. And these weather requirements are fulfilled by the Mediterranean region (Fraga et al., 2014). In the Mediterranean region, wine is considered as an essential element of diet (van Leeuwen & Seguin, 2006). The wine producing regions within the Mediterranean climate have long growing seasons with moderate to warm temperatures during the crucial phenological stages of grape. Winters are generally warmer than that of in continental or maritime climates. The change in temperature between seasons is little. On the other hand, there is very little rainfall during the ripening period of grape especially in sandy and gravelly soils that have no capacity to retain water. Therefore, the vineyards may require additional irrigation to prevent water deficit stress

(Ferrandino & Lovisolo, 2014). Fraga et al. (2014) have reported that the best climate conditions favoring the wine production in the Minho wine region of Portuguese were: moderate cool weather in February-March, warm weather in May, warm and moist weather in June, moderate dry weather during ripening and cool weather in September.

Cooler seasons result in earlier ripening with fresher and more acidic and green colored grapes. Therefore, at high latitudes with cool climates, it is better to cultivate early ripening grapes such as Pinot Noir, Chardonnay or Gewürztraminer to produce high quality wines. In cooler regions where the ripening of grape is difficult, it is generally preferred to locate vineyards on steep and south-facing slopes as in the case of Mosel Valley in Germany. Warm and dry summers enhance grape ripening and produce grapes with higher sugar and lower acidity. The early ripening of grapes in the warmer climate of lower latitude regions reduces the aromatic expression of wines (de Andrade et al., 2013; van Leeuwen & Seguin, 2006).

In terms of soil characteristics, vines have the potential to grow on a wide variety of soils with well-drainage. The pebbles and stones in the soil increase soil drainage. Therefore the soil warms up quickly and enables early ripening (van Leeuwen & Seguin, 2006). There are various types of soil such as sandy, loam, gravelly, and calcareous. Sandy soils have low water retaining capacities, therefore require irrigation. Clayey soils have poor drainage and produce lower quality wines but high yield. They are not preferred for viticulture. On the other hand, loamy soils, which consist of 20-50% clay and 80-50% sand, are highly suitable for viticulture. The gravelly soils exist mainly along the riversides and have well-drainage characteristics. They warm up due to pebbles but have low water retaining capacities. Finally, calcareous soils have 20% or more clay in the structure and have high water retaining capacities. Yield in this type of soil is low but the quality of grapes is high (Uzun, 2004). The soil type depends on the macronutrient and trace element composition. For instance, a sandy soil will be deficient in B and Cu. Moreover, soil is the main source of nutrients to vine which are mostly found in minerals (Kamsu-Foguem & Flammang, 2014). Infertile soil being more composite and with more inorganic ions enhances the synthesis of flavonoids rather than a fertile soil (Li et al., 2011). High quality wines generally come from poor soils. Limestone is known to produce high quality wines. However, even in the Bordeaux region, the high quality wine yielding soils are of different type (acidic gravelly, neutral gravelly, alkaline limestone, heavy clay etc.). Dry soils (i.e. stony soils) warm up quickly that leads to early ripening (van Leeuwen & Seguin, 2006).

2.11. Principles of Wine Instrumental Analysis

2.11.1. Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) and Optic Emission Spectroscopy (ICP-OES)

The coupling of plasma based spectrometry techniques with important detectors enables the characterization and quantification of multielement composition of samples simultaneously. This field has attracted much attention recently starting with the environmental analysis which is followed by the elemental speciation of biological systems. Among the most widely used plasma based techniques are ICP-MS and ICP-OES. ICP-MS is a sensitive and selective detector that detects all available elements in the periodic table except the atmospheric gases (O, C and N) and those restricted for Ar ionization (He, Ne and F) (Meija, Mounicou, & Caruso, 2004). The basic components of an ICP-MS system are demonstrated in Figure 2.9. The instrument generally consists of a nebulizer, spray chamber, plasma torch, interface cones, vacuum chamber, ion optics, mass analyzer and a detector. The liquid sample is pumped into the nebulizer. In the nebulizer the sample is converted into a fine aerosol with the aid of argon gas. The large aerosol droplets are eliminated in the spray chamber and transferred to the waste. The small aerosol droplets are carried to the plasma torch. The plasma torch is a concentric quartz tube in which plasma is produced at very high temperatures (7.000-10.000K). The plasma is formed by the interaction of magnetic field produced by radio frequency passing through a copper coil, on a tangential flow of argon gas through the torch. This has the effect of ionizing the gas. In ICP-MS instruments, the torch is positioned horizontally and is used to generate positively charged ions. However, in ICP-OES instrument, the torch is vertically positioned to produce photons of light by exciting the electrons from ground state to higher energy levels. As the excited electron emits the energy at a specific wavelength, it is the characteristic of the analyte. The ICP-MS instrument avoids the formation of photons which makes it a sensitive instrument with low detection values (ppt).

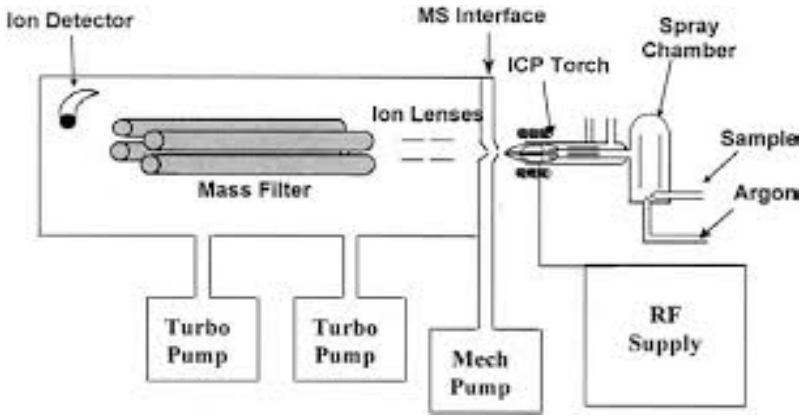

Figure 2.9. Basic components of ICP-MS
(Source: Thomas, 2004)

In the ICP-MS instrument, the ions pass through the interface which is maintained at vacuum (1-2 torr) into the mass spectrometer. The vacuum at mass spectrometer is approximately 10^{-6} torr. The mass separation in the spectrometer can be based on different technologies such as quadrupole, magnetic sector and time of flight. Their aim is to allow particular ions with determined mass-to-charge ratios to the detector and eliminate all others (interfering and matrix ions). Most quadrupole instruments are designed with collision/reaction cells (ORS) which feed a gas into the cell or interface to eliminate polyatomic spectral or ionic interferences by using ion-molecule collision mechanisms.

The final step is the conversion of ions into an electrical signal with an ion detector. The commonly used one is the dynode detector which contains a series of metal dynodes. The ions emerging from the mass detector hit the first dynode and are converted into electrons. These electrons are attracted by the second and further dynodes where multiplication of electrons takes place. The streams of electrons are processed by data handling (Thomas, 2013). Compared with OES, MS allows lower detection limits and avoids interferences. On the other hand, OES offers higher sample throughput, higher tolerance to the dissolved particles and a lower price (Meija et al., 2004).

2.11.2. High Pressure Liquid Chromatography (HPLC)

Chromatography is the science of separation of mixtures into individual fractions. It involves the mass transfer of analyte between the stationary phase and mobile phase. Stationary phase is the column packing material (adsorbent). It can be either liquid adsorbed on a solid or a solid material. Mobile phase is the gas or liquid media flowing continuously through the system. It carries the analyte through the system. HPLC is one mode of chromatographic separation technique, which is the most widely used one in food industry. It is a reliable, easy to use, powerful and versatile instrument with wide applicability, sensitivity and ready adaptability to accurate quantitative analysis.

The HPLC system is composed of a mobile phase reservoir, a degasser, a pump, an injection port, a column with an oven, a detector and a software program which is used for data analysis. The mobile phase is pumped through the system and carries the sample injected into the system. The sample entering the column is separated according to its affinity to column packing material or solubility in the mobile phase. The detector at the end of the column creates signals for each analyte which we call peaks and the series of peaks is called a chromatogram (Hışıl, 1994; Skoog, Holler, & Nieman, 1998).

According to the separation techniques, the types of liquid chromatography can be listed as size exclusion (analytes having molecular weights > 10000), ion exchange (lower molecular weight, ionic analytes), partition (small, polar and non-ionic analytes) and adsorption chromatography (non-polar analytes) (Figure 2.10) (Skoog et al., 1998).

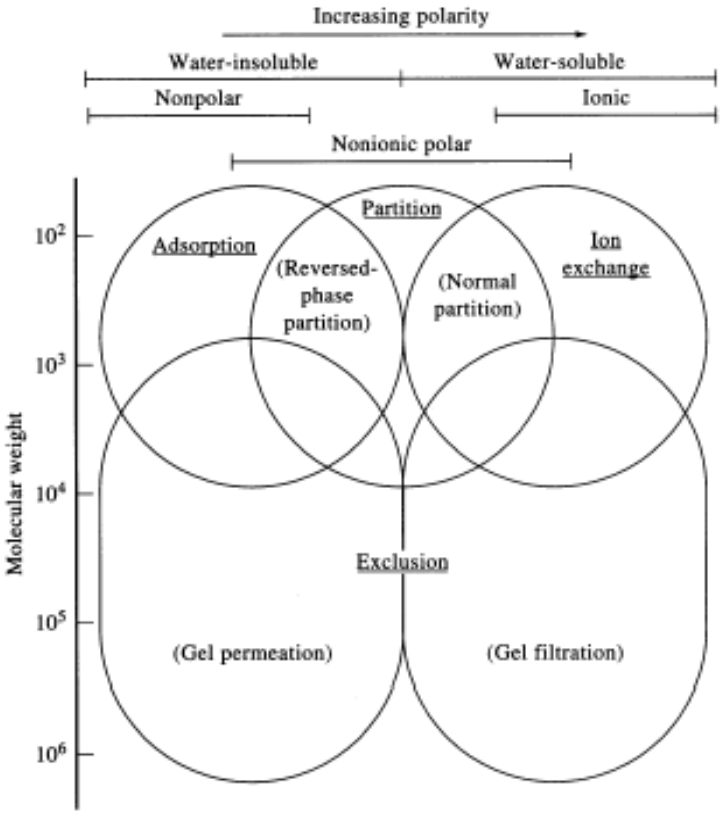

Figure 2.10. Different modes of liquid chromatography
(Source: Skoog et al., 1998)

The most widely used of all in food industry is partition chromatography, particularly reversed phase. In this method, the solid support (silica) is coated with a thin layer of liquid either by physical or chemical bonding. This liquid bonded solid support is the stationary phase, which is immiscible with the mobile phase. Based on the relative polarities of two phases, we have normal and reversed phase chromatographies. In normal phase chromatography, the polarity of stationary phase (the solid support contains polar cyano, amino, diol groups) is greater than that of mobile phase (n-hexane, ethyl ether, chloroform, etc.). In reversed phase chromatography, the mobile phase (water with varying concentrations of methanol, acetonitrile, THF, isopropanol) is more polar than the stationary phase (non-polar octylsilyl (C_8), octyldecylsilyl (C_{18}), phenyl, trimethylsilyl or polymer based material). The separation depends on the polarity of analytes like polar likes polar and a nonpolar likes nonpolar. Reversed phase chromatography has a wide range of applicability including pharmaceuticals (antibiotics, steroids etc.), amino acids, proteins, carbohydrates, lipids, artificial sweeteners, antioxidants, aflatoxins, food additives, dyes, pesticides, herbicides, drugs and their metabolites (Hışıl, 1994; Skoog et al., 1998). Another separation technique

employed in food analysis is the ion exchange chromatography which is based on the exchange equilibrium of like sign ions between the mobile phase and the surface of an insoluble, high molecular weight solid stationary phase (ion exchange resin). The ions in the mobile phase compete with the ions of analyte for the active sites of ion exchange resin. It has been applied for the determination of drugs and their metabolites, vitamins, sugars, and pharmaceutical preparations. In adsorption chromatography, the stationary phase is solid with surface sites retaining the analyte. This technique is preferred to separate isomeric mixtures and analytes that are soluble in non-polar solvents. Eventually, size exclusion chromatography separates the analytes based on their ability to penetrate into the pores of the stationary phase material. The larger analytes will be eluted first whereas the smaller ones will penetrate more into the pores of column (Hışıl, 1994; Skoog et al., 1998). The detector systems in HPLC respond either to changes in bulk property of mobile phase such as refractive index detector (RID) or to some property of analyte that is not possessed by the mobile phase such as UV absorbance, fluorescence or diffusion current. Some examples to this type of detectors are UV-Vis absorbance detectors (UVD, DAD), fluorescence detector (FLD) or electrochemical detector (ECD). The absorbance detectors are the most widely used ones in food industry with sensitivity. The theory is based on Lambert Beer's law. The mobile phase flowing through the sample cell creates the maximum signal which is reduced by the flow of sample through the cell. Photodiode array detector (DAD) passes all wavelengths of light through the sample cell, than focuses each wavelength on a single sensor element. This type is the most versatile and expensive of all. They are preferred frequently in method development and are very useful. On the other hand, the samples which do not absorb UV radiation are detected by refractive index detector. It is based on the difference in the refractive index of mobile phase including the sample. It is capable of responding to almost all analytes and is a reliable system. However, it is extremely temperature sensitive and does not have low detection limits as other types of detectors (Skoog et al., 1998).

CHAPTER 3

MULTIVARIATE STATISTICAL TECHNIQUES

The people in food industry should perform several quality control tasks during production. The chemical composition and physical properties of raw materials and final products are required to be determined. The possible adulteration or geographical origin of raw materials should also be identified. The process parameters should be monitored for changes that can affect final product quality. These tasks may require the examination of more than one property of the food sample. With the introduction of modern instrumental analysis and availability of computers, multiple responses are collected for each sample at a time. This causes the accumulation of large data sets during process. Eventually, the necessity of examining all data points in a multivariate sense arises. Multivariate statistical analyses (chemometrics) gives information about the relationship among variables in the whole sample matrix (Cozzolino et al., 2009).

Exploratory data analysis deals with the questions like "can a chromatogram be used to decide on the origin of wine, and if so, what main properties can be used to differentiate wines?" It can expose hidden patterns, trends or possible outliers in complex data by reducing the data to a more comprehensible form (Brereton, 2003).

3.1. Unsupervised Statistical Techniques

The unsupervised statistical techniques investigate the similarity or relationship within the data set without the information of any class membership or how many clusters exist in the data. In use of unsupervised techniques, no information or grouping is required before the model development. The two common techniques are principal component analysis and hierarchical cluster analysis.

3.1.1. Principal Component Analysis (PCA)

PCA is the most widely used multivariate statistical technique. It is used for screening, extracting and compressing multivariate data. It builds a mathematical model that transforms a matrix of correlated response variables into a new set of non-correlated variables called principal components (PC). By means of principal components, the numbers of variables are reduced to a smaller number of scores which describes the particular structure of original data matrix. For the case of non-correlated original variables, one cannot make use of PCA. It produces linear combinations of variables that describe the structure in the original data matrix (Brereton, 2003; Cozzolino et al., 2009). For the case of wine characterization, it reduces the dimension of data into smaller number of principal components and demonstrates the possible grouping of samples according to grape variety, geographical origin or harvest year (Álvarez et al., 2007; Saavedra et al., 2011; Serapinas et al., 2008). The mathematical representation of original data matrix in PCA can be written as:

$$\mathbf{X} = \mathbf{T}\,\mathbf{P} + \mathbf{E} \qquad (3.1)$$

where **X** is the original matrix with I rows (samples) and J columns (variables), **T** is the scores matrix with I rows (samples) and A columns (scores), **P** is the loadings matrix with J columns (variables) and A rows (loadings), and **E** is the error matrix with I rows and J columns. A is the number of principal components (it is less than or equal to J). The score matrix contains column vectors and the loading matrix contains row vectors for each principal component (Figure 3.1) (Brereton, 2003; Eriksson et al., 2001).

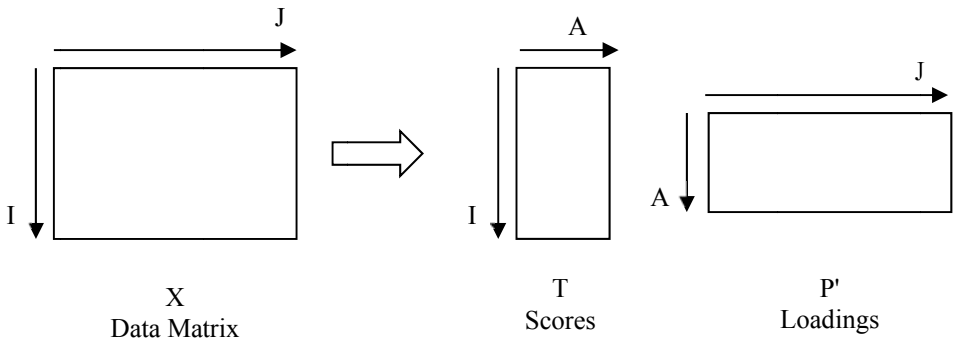

Figure 3.1. Graphical representation of PCA modeling
(Source: Brereton, 2003)

The geometric interpretation of PCA can be simply demonstrated by the three variable axes of a J-dimensional variable space (Figure 3.2). Each observation (i) in the data matrix is placed in the 3-dimensional variable space. Then, the first PC is positioned as the line that best approximates the data in the least squares sense. Each data can be projected on this new line to get a co-ordinate which is called as the score. The second PC is the line orthogonal to the first PC. Hence, PCA reduces residual variance while maximizing score variance and produces linear combinations of variables as lines or planes (Eriksson et al., 2001).

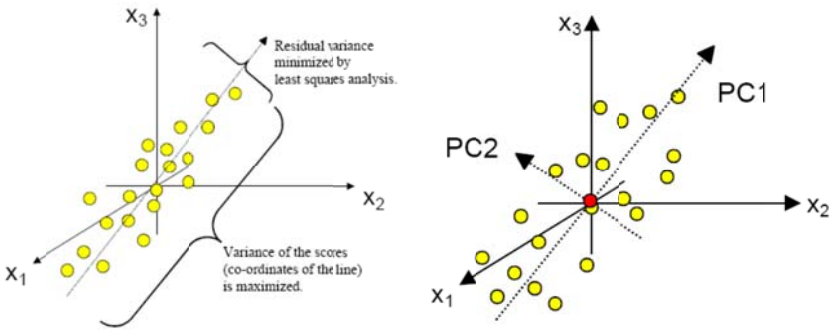

Figure 3.2. Geometric projection of PCA
(Source: Eriksson et al., 2001)

After PCA, the significance of each component can be determined from the eigenvalues of the correlation matrix of data set. The more significant the components are, the larger their eigenvalues are. The eigenvectors of the correlation matrix determines the weights of the principal components or loadings. The Nonlinear Iterative Partial Least Squares (NIPALS) and Singular Value Decomposition (SVD) algorithms are used in the calculation of principal components.

The NIPALS algorithm is also capable of working with matrices having moderate amounts of randomly distributed missing observations. It has the following steps following scaling and mean-centering of X data (Wold, 1987):

-Set t vector as the score vector for the column, e.g. having the largest variance in X.

-Calculate the p' loading vector as $p' = t' \mathbf{X}/t' t$.

-Normalize p to 1 by multiplying with $c = 1 / (p' p)^{0.5}$

-Calculate a new score vector $t = \mathbf{X}\, p / p' p$. The i^{th} element in t is the slope in the linear regression of p' on the i^{th} row in $\mathbf{X}$.

-Check for convergence. If the difference between the sums of squares of each element in two consecutive score vectors is larger than a predefined threshold, then go to the second step.

-Calculate residuals $\mathbf{E} = \mathbf{X} - \mathbf{T}\,\mathbf{P}$. To estimate the next dimension, use $\mathbf{E}$ as $\mathbf{X}$.

The geometric pattern of observations in the J-dimensional space is influenced by the variables. To understand which variables are the most influential and to observe the correlation between them, the loadings vectors are displayed in a plot which is called as the loadings plot. The positively or negatively correlated variables are grouped together or they are positioned on opposite sides of the plot origin, respectively. Moreover, the more influential the variables are, the further away they lie from the plot origin (Eriksson et al., 2001).

The performance of PCA models were evaluated by the goodness of fit (R^2) and the goodness of prediction (R^2_{pred}) coefficients. R^2 is the explained variance, a quantity ranging from 0 (no explanation) to 1 (complete explanation). It describes the extent of each variable explained by the model. However, the problem with R^2 is that it can be inflationary and can approach to unity as the number of components in the model increases. Therefore, it is not sufficient to have high R^2 but also get good R^2_{pred} values. R^2_{pred} value indicates the predictive ability of the model and can be estimated by how accurately the X data can be predicted either internally from the existing data or externally by the use of an independent validation set. Cross validated R^2 value is calculated by leave-one-out cross validation. The technique keeps an observation out of the model development and builds a model with the reduced data. This procedure is repeated until each observation is kept out of model development once. Each time, the omitted data point is predicted with the developed models. Then, the predictive residual sum of squares (PRESS) value is calculated:

$$PRESS = \sum_{i} \sum_{m} (y_{im} - \hat{y}_{im})^2 \quad (3.2)$$

where y_{im} is the actual and $\hat{y}_{im}$ is the predicted data. For each model dimension, cross validation is employed and a PRESS value is calculated. This is compared with the residual sum of squares (RSS) of previous dimension. If the PRESS value is significantly greater than the RSS value, that model dimension is insignificant. And model building is stopped. In other words, a component is considered as significant if the PRESS/ RSS value is smaller than around 0.9. For all the significant components, an overall $PRESS/SS_{TOT}$ value is computed and used to calculate the goodness of fit value (cross validated R^2 or R^2_{pred}). SS_{TOT} is the total variation in **X** matrix after standardization.

$$R^2 = 1 - RSS / SS_{TOT} \quad (3.3)$$

$$R^2_{pred} = 1 - PRESS/SS_{TOT} \quad (3.4)$$

A model with $R^2_{pred}>0.5$ is generally considered as good and $R^2_{pred}>0.9$ is excellent. Moreover, without a high R^2, it is not possible to obtain a high R^2_{pred} value and the difference between these two should not be big, generally less than 0.2-0.3. Before PCA, the data can be pre-processed by scaling (mean-centering and standardization). Mean-centering is the subtraction of average value of each variable from the data to re-position the co-ordinate system to pass through origin. The most common scaling method is unit variance and it is the multiplication of each column of X data with its inverse standard deviation (1/s). Hence, each variable has equal variances avoiding the domination of one variable over another due to larger lengths (Eriksson et al., 2001).

3.1.2. Hierarchical Cluster Analysis (HCA)

Another unsupervised pattern recognition technique is HCA that groups samples according to their similarities and demonstrates the groups in the form of a 2-dimensional plot called dendrogram. The first step is to calculate the similarities

between objects and then use linkage methods to link the objects. The dendrogram or sometimes called tree diagram has the vertical axis showing similarity of or distance between samples while horizontal axis includes organized objects according to their similarities in a row. For instance, according to Figure 3.3, sample 1 is very different from the others. The similarity is small, hence the distance is large. Similar samples have smaller distances (Brereton, 2003).

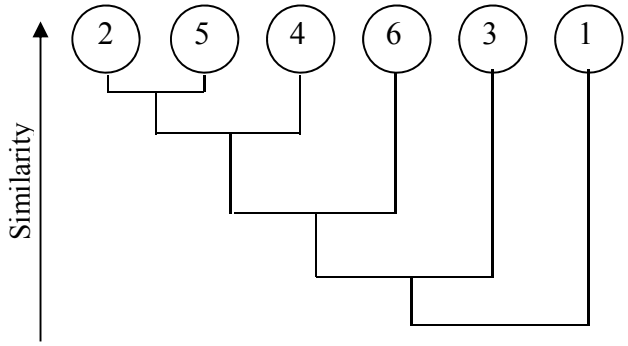

Figure 3.3. Dendrogram of a cluster analysis
(Source: Brereton, 2003)

The similarity matrix is prepared between samples using a numerical indicator which can be correlation coefficient, Euclidean, Manhattan (city block) or Mahalanobis distances. The correlation coefficient method produces distances between 0 and 1 for positively correlated observations. The value 1 indicates that the observations are identical. Euclidean method measures the distance by the square root of the sum of squared differences, whereas Manhattan method is the sum of absolute distances. Mahalanobis technique is similar to Euclidean distance with the exception of inverse of variance-covariance matrix inserted as a scaling factor (Brereton, 2003). After all the similarities have been calculated, it is then determined how close the clusters are located by using linkage techniques. There are several linkage techniques such as single, complete, centroid, average, median and Ward's methods. The single linkage technique employs the minimum distance between two variables in different clusters. This technique works best when clusters are clearly separated. It tends to form one large cluster with the other clusters containing few samples. To the contrary, complete linkage technique takes the maximum distance between two variables in two different clusters. The results are highly sensitive to outliers. The clusters produced with this method are rather compact and tight. Average linkage is the mean distance between two

variables in different clusters. Centroid linkage takes the distance between the centroids or means of two different clusters. This linkage is another averaging technique. The average and centroid methods tend to produce clusters of similar sizes (Figure 3.4) (Johnson & Wichern, 2007; Mooi & Sarstedt, 2011). The median linkage is the median distance between the two variables in different clusters. This is also an averaging technique that uses median rather than the mean. Eventually, Ward's linkage is the distance taking the sum of squared deviations from points to centroids. The aim is to minimize the within-cluster sum of squares. In other words, it combines samples whose merger minimizes the within-cluster variance (the smallest increase in the error sum of squares), rather than their similarity. It is sensitive to outliers and tends to produce clusters having similar number of samples (Johnson & Wichern, 2007; Mooi & Sarstedt, 2011).

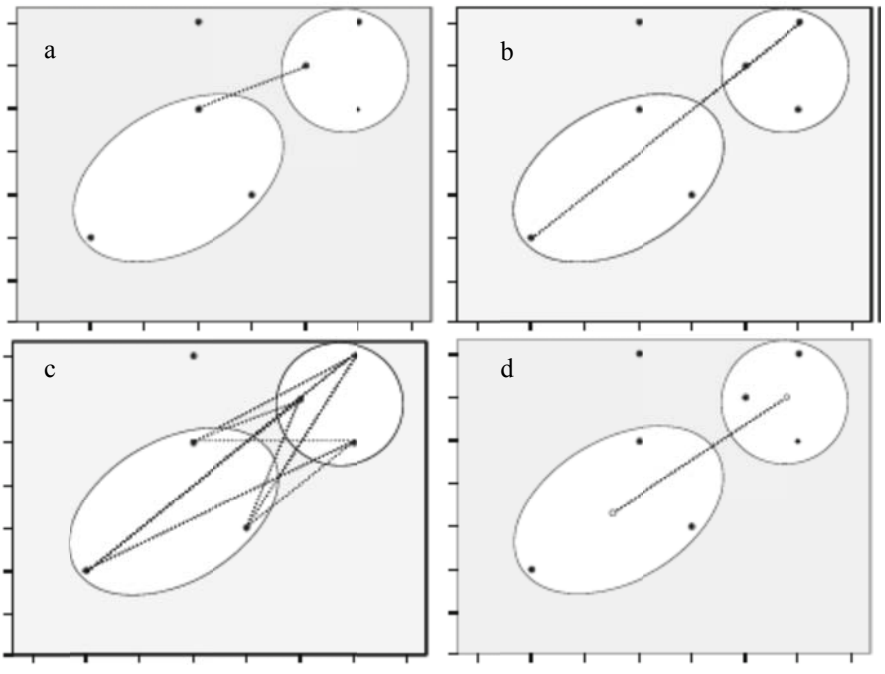

Figure 3.4. Graphical representation of linkage methods: (a) single, (b) complete, (c) average, (d) centroid (Source: Mooi & Sarstedt, 2011)

3.2. Supervised Statistical Techniques

Supervised statistical techniques are used to group samples using variables with predefined groups. The method has a training set which is used to produce a mathematical model between measurements of samples and their known groups. These

samples are called as training set. Then the model is employed to predict groups on a set of different observations called test or validation set (Brereton, 2003). Among the supervised techniques, partial least squares-discriminant analysis and soft independent modeling of class analogy techniques are discussed in this section.

3.2.1. Partial Least Squares-Discriminant Analysis (PLS-DA)

The partial least squares-discriminant analysis (PLS-DA) is a supervised pattern recognition technique that has the advantage of applying partial least squares (PLS) algorithm onto the discrimination problem. Therefore before further explaining the discriminant-PLS, the algorithm of PLS will be explained here. PLS is a quantitative analysis technique that is used for relating two data matrices, **X** (predictor variables i.e. spectral data measured on chemical samples) and **Y** (dependent variables i.e. analyte concentration), by a linear multivariate model. **Y** data are predicted with **X** data by using the correlation structure between two. For this aim, two models are set:

$$\mathbf{X} = \mathbf{T}\,\mathbf{P} + \mathbf{E} \tag{3.5}$$

$$\mathbf{Y} = \mathbf{T}\,\mathbf{C} + \mathbf{F} \tag{3.6}$$

X is the matrix of predictors and **Y** is the matrix of response variables. **T** is the score matrix of **X** which is used both to model ***X*** and to predict **Y**. The information related to the variables are stored in loading matrix of **X** (**P′**), and weights matrix of **Y** (**C′**). The residual matrices are **E** and **F**. The X-scores **T** can be computed by the regression of **X** and X-weight matrix **W***. **W*** gives information about how the X-variables combine to form the scores **T** (the covariance structure between predictor and response variables). As the **T** matrix is put into Equation 3.6, the regression model will be equal to equation 3.8 where **CW*** are the PLS coefficients (Equation 3.9).

$$\mathbf{T} = \mathbf{X}\,\mathbf{W}^* \tag{3.7}$$

$$\mathbf{Y} = \mathbf{X}\,\mathbf{W}^*\,\mathbf{C} + \mathbf{F} \tag{3.8}$$

$$\mathbf{B} = \mathbf{C}\,\mathbf{W}^{*} \tag{3.9}$$

The difference between PCA and PLS is that PCA is a maximum variance least squares projection of **X**, while PLS is the maximum covariance model of relationship between **X** and **Y**. The graphical representation is shown in Figure 3.5.

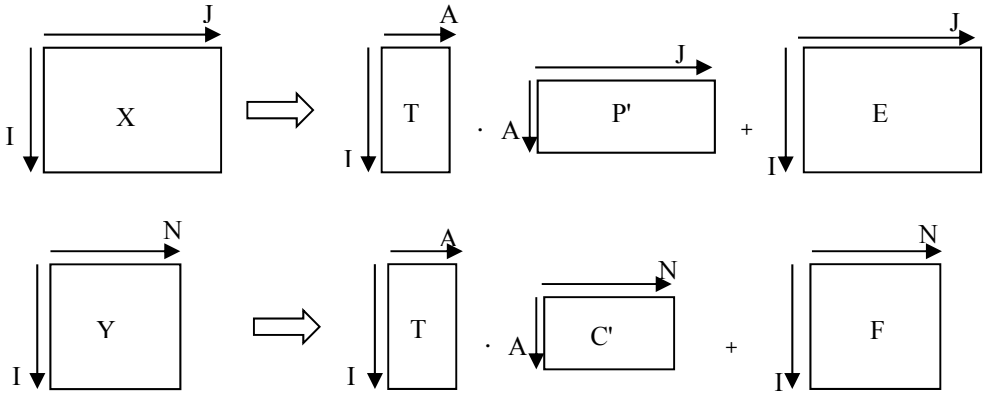

Figure 3.5. Graphical representation of PLS modeling
(Source: Eriksson et al., 2001)

The **X** matrix is divided into a calibration and validation set. The calibration set builds the model and the validation tests the predictive ability of the calibration model. The aim is to have good correlation between the scores of predictor and dependent variables for the prediction of **Y** variables. The significant number of PLS components is determined by leave-one-out cross validation to avoid overfitting. Overfitting is producing a well-fitting model with no predictive power due to the high number of variables or components (greater than the number of samples). The aim is to produce a model with optimal fit and good predictive ability (Eriksson et al., 2001).

The power of PLS models are evaluated by the R^2 and R^2_{pred} coefficients as in PCA. A value for R^2 between 0.66-0.81 is considered as approximate quantitative prediction, and a value between 0.82-0.90 indicates good prediction. Values above 0.91 indicate excellent prediction (Saeys, Mouazen, & Ramon, 2005). In addition to R^2 and R^2_{pred} parameters, root mean square error of calibration (RMSEC) and validation (RMSEP) and residual predictive deviation (RPD) parameters can also be calculated. RMSEC indicates the mean error of calibration model and RMSEP is calculated to test the predictive ability of calibration model.

$$\text{RMSEC} = \sqrt{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2 \Big/ n - p - 2} \quad (3.10)$$

where n is the number of samples in the calibration set, and n-p-2 is the degrees of freedom for the calibration set, p is the number of principal components, y_i is the reference value for sample *i*, and $\hat{y}_i$ is the predicted value for sample *i*.

$$\text{RMSEP} = \sqrt{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2 \Big/ n} \quad (3.11)$$

where n is the number of samples in the validation set, and n-1 is the degrees of freedom for the validation set, y_i is the reference value for sample *i*, and $\hat{y}_i$ is the predicted value for sample *i*. RPD is calculated to standardize predictive accuracy by taking the ratio of standard deviation of validation set and RMSEP (Versari, Parpinello, & Laghi, 2012). The RPD value below 1.5 indicates that the calibration is not usable for prediction, whereas, values between 1.5 and 2.0 indicate that the high and low levels can be distinguished. RPD value between 2.0 and 2.5 is considered as approximate prediction, and it is good and excellent prediction between 2.5 and 3.0 and >3.0, respectively (Saeys et al., 2005).

Discriminant analysis (DA) or sometimes called hard modeling technique is a supervised technique that aims to discriminate two or more groups with respect to a set of variables. It can be considered as a qualitative method in which samples are calibrated for class membership instead of variable. Therefore, in PLS-DA the regression is done to a matrix of dummy variables representing group membership. If an observation belongs to a class, then it is assigned with the dummy variable 1. Otherwise, it is assigned with 0 (Eriksson et al., 2001). The observations in the model is developed from a calibration set of known class membership and a validation or test set is used to validate the calibration model. Leave-one-out cross validation technique is employed to determine the significant components.

3.2.2. Soft Independent Modeling of Class Analogy (SIMCA)

SIMCA is a soft modeling technique. The idea in soft modeling is that the observations may belong to either one of the two classes. However, there are two more alternatives that the observation may belong to both classes simultaneously or neither class. For instance, a chemical compound may have both ester and alkene groups, thus will have spectroscopic functionalities of both groups. Independent modeling of classes enables the addition of new class models without changing the other class models (Brereton, 2003). The initial step of SIMCA is the modeling of each group separately with PCA. Then, the distance of each observation (s_0) to the model is computed using the residual observation variance. It is the geometric mean orthogonal distance of each observation to the model.

$$s_0 = \sqrt{\sum_{i=1}^{N} \sum_{j=1}^{K} e_{ij}^{\,2} \Big/ (N - A - A_0) * (K - A)} \tag{3.12}$$

where A is the number of PC, A_0 is 1 if the model is centered and 0 otherwise, j index is for variables and i is for observations. If the residual distance from the model is larger than the critical distance of the class, then the observation is an outlier. The critical distance bounds a region of space for each class and is based on the F distribution at the 95% confidence.

$$s_{crit} = \sqrt{F_{crit} {s_0}^2} \tag{3.13}$$

where F_{crit} is the value for (K-A) and (K-A)(N-A-A_0) degrees of freedom at significance level α. Similar to most of the supervised statistical techniques, the SIMCA class models should also be tested with a validation set. New observations (x_{new}) are predicted for a class membership with the built models. The residual vector, e_{new}, is calculated:

$$e_{new} = x_{new} - \hat{x}_{new} \tag{3.14}$$

where $\hat{x}_{new}$ is the predicted X_{new} in the space of a particular class. Then, the distance can be calculated from Equation 3.15:

$$s_{new} = \sqrt{\sum_{j=A+1}^{K} e_{new_j}{}^2 \Big/ (K-A)} \tag{3.15}$$

If s_{new} is smaller than s_{crit}, then the new observation belongs to the specified class (De Maesschalck et al., 1999; Eriksson et al., 2001). The common graphical representation of SIMCA is the Cooman's plots. If data belong to a class, they should fall within the class membership limit and should locate at the left side of the vertical limit or below the horizontal limit (Cozzolino et al., 2011). A graphical representation of Cooman's plot is shown in Figure 3.6.

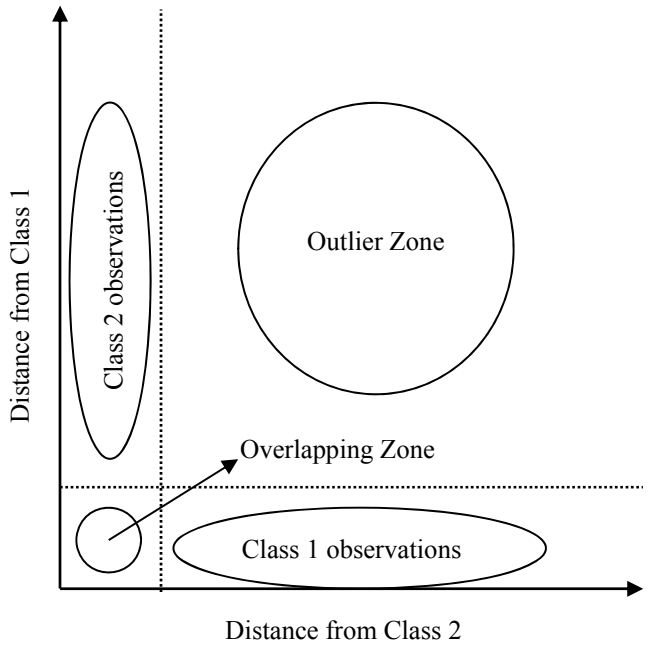

Figure 3.6. Graphical representation of cooman's plot

The multivariate statistical tools considered in this chapter (PCA, PLS-DA, SIMCA and HCA) has been widely employed in the classification of wine and grape samples by scientists (Álvarez et al., 2007; Castillo-Muñoz et al., 2010; Coetzee et al., 2005; González-Fernández et al., 2012; Gonzálvez et al., 2009; Li et al., 2011; Marengo & Aceto, 2003; Meléndez et al., 2001; Saavedra et al., 2011; Serapinas et al., 2008; Thiel et al., 2004; Yildirim, 2006).

CHAPTER 4

MATERIALS AND METHODS

4.1. Sample Treatment Schedule

The dates of the purchases and analyses for each year were reported in Table 4.1.

Table 4.1. Purchase and analysis dates of wine samples annually

Harvest Year	Purchase Date	ICP Analyses	Polyphenol Analyses	Acid-Sugar-Alcohol Analyses	Total Phenol-Chemical Analyses	Color Analyses
2006	10.2008	06.2009	12.2009	03.2010	12.2009	02.2010
2007	09.2009	09.2009	01.2010	03.2010	12.2009	02.2010
2008	09.2010	01.2011	02.2011	04.2011	01.2011	01.2011
2009	09.2011	10.2011	10.2011	10.2011	10.2011	10.2011

4.2. Wine Samples

The commercial wine samples were purchased from market and the information of the samples in this study was based on the data given on the label of wine bottles. A total number of 136 wine samples were collected from the vintage years of 2006, 2007, 2008 and 2009 and from 12 geographical regions. The geographic regions are shown in a Turkey map in Appendix A. These wines were produced from 13 different grape varieties. Among these grape varieties, 8 were characteristic to Turkey and the other 5 were commercially valuable grapes. The total numbers of red, rose and white wines were 80, 7 and 49, respectively and they were purchased from the market during four years belonged to 19 wine production companies. Their ethanol content given on the labels ranged between 10-15 % (v/v). The samples were coded in 4 or 5 characters (letters and numbers) starting with the grape variety and followed by the harvest year, production area and wine producing company at the end part. The wine samples from the same variety, harvest year, producer and geographical origin were coded as “a” and “b” as the final letters. Additionally, semi-sweet wines were coded with the letter “s” at

the end of the code. The codes are listed in Table 4.2. The list of wine samples classified based on their color, grape variety and viticulture regions are listed in Table 4.3.

Table 4.2. Coding of wine samples

Variety	Code	Harvest Year	Code	Region	Code
Boğazkere	B	2006	6	Ankara	A
Cabernet Sauvignon	C	2007	7	Bozcaada	B
Çalkarası	L	2008	8	Denizli	D
Chardonnay	H	2009	9	Denizli-Ankara	X
Emir	E			Denizli-Manisa	Y
Kalecik Karası	K			Diyarbakır	C
Merlot	M			Diyarbakır-Ankara	Z
Muscat	T			Diyarbakır-Denizli	W
Narince	N			Edirne	F
Öküzgözü	O			Elazığ	E
Papazkarası	P			İzmir	I
Syrah	S			Kapadokya	K
Sultaniye	U			Kapadokya-Diyarbakır	Q
				Kırklareli	L
				Manisa	M
				Tekirdağ	T
				Tokat	R
				Denizli-Urla-Trakya	H

Table 4.3. Classification of wine samples based on the grape varieties and viticulture regions

Grape Variety	Wine Type	Viticulture Region	City	Vintage Year	Amount
Boğazkere	Dry Red	East Anatolia	Diyarbakır	6-8-9	1-1-1
Boğazkere	Dry Red	Central Anatolia	Kapadokya	6-8	1-1
Boğazkere	Dry Red	Central-East Anatolia	Diyarbakır-Ankara	7	1
Boğazkere	Dry Red	Southeast Anatolia- Aegean	Diyarbakır-Denizli	7-8-9	1-1-1
Boğazkere	Dry Red	Black Sea	Tokat	7	1
Cab. Sauvignon	Dry Red	Aegean	İzmir	6-7	2-1
Cab. Sauvignon	Dry Red	Aegean	Bozcaada	7	1
Cab. Sauvignon	Dry Red	Aegean-Central Anatolia	Denizli-Ankara	7	1
Cab. Sauvignon	Dry Red	Marmara	Tekirdag	8	1
Cab. Sauvignon	Dry Red	Marmara	Edirne	6-8	1-1
Cab. Sauvignon	Dry Red	Central Anatolia	Kapadokya	7-8	1-1
Cab. Sauvignon	Dry Red	Black Sea	Tokat	7	1
Çalkarası	Dry Rose	Aegean	Denizli	6-8	2-1
Çalkarası	S-S* Rose	Aegean	Denizli	6-9	1-1

(cont. on next page)

Table 4.3. (Cont.)

Grape Variety	Wine Type	Viticulture Region	City	Vintage Year	Amount
Çalkarası	Sweet Red	Aegean	Denizli	8	1
Kalecik Karası	Dry Red	Aegean	Denizli	6-7-8	3-5-2
Kalecik Karası	Dry Red	Aegean	İzmir	6	1
Kalecik Karası	Dry Red	Marmara	Tekirdag	6	1
Kalecik Karası	Dry Red	Central Anatolia	Ankara	6-7-8	1-1-2
Kalecik Karası	Dry Red	Central Anatolia	Kapadokya	7	1
Merlot	Dry Red	Aegean	Denizli	6-7-8	1-1-1
Merlot	Dry Red	Aegean	Denizli-Manisa	7	1
Merlot	Dry Red	Aegean	İzmir	6-7-9	2-1-1
Merlot	Dry Red	Marmara	Tekirdağ	6-7-8	1-1-1
Merlot	Dry Red	Marmara	Edirne	8	1
Merlot	Dry Red	-	Denizli-Urla-Trakya	9	1
Öküzgözü	Dry Red	East Anatolia	Elazığ	6-7-8-9	2-2-3-3
Öküzgözü	Dry Rose	East Anatolia	Elazığ	6-7	1-1
Öküzgözü	Dry Red	Central Anatolia	Kapadokya	6	1
Öküzgözü	Dry Red	Central Anatolia	Tokat	7	1
Papazkarası	Dry Red	Marmara	Tekirdağ	6	2
Papazkarası	Dry Red	Marmara	Kırklareli	6	1
Syrah	Dry Red	Aegean	Denizli	6-7-8-9	3-4-2-1
Syrah	Dry Red	Aegean	İzmir	8	1
Syrah	Dry Red	Aegean	Manisa	8-9	1-1
Emir	Dry White	Central Anatolia	Kapadokya	6-7-8-9	2-3-3-3
Muscat	S-S* White	Aegean	Denizli	6-7-8-9	1-2-1-1
Muscat	S-S* White	Aegean	İzmir	6-8-9	1-1-1
Muscat	Dry White	Aegean	İzmir	6	1
Muscat	Dry White	Marmara	Tekirdağ	6	1
Muscat	Dry White	Aegean	Manisa	8	1
Narince	Dry White	Black Sea	Tokat	6-7-8	1-3-2
Narince	Dry White	Aegean	Denizli	6	1
Narince	Dry White	Aegean	Manisa	8-9	1-1
Sultaniye	Dry White	Aegean	Denizli	6-7-8	2-2-1
Sultaniye	Dry White	Aegean	İzmir	6-7	1-1
Sultaniye	Dry White	Aegean	Manisa	6	1
Chardonnay	Dry White	Aegean	Denizli	6-7-9	1-1-1
Chardonnay	Dry White	Aegean	İzmir	6-8-9	1-1-2
Chardonnay	Dry White	Marmara	Tekirdağ	6-7	1-1
Chardonnay	Dry White	Central Anatolia	Kapadokya	8	1

*S-S: Semi-sweet

The instrumental and chemical analyses used for the characterization of the wine samples in this study are given in Table 4.4. The details are given in the following sections.

Table 4.4. Chemical analyses and instruments

Analysis	Instrument
Elemental Analysis	ICP-MS, ICP-OES
Polyphenol Analysis	HPLC-DAD
Color Analysis	UV-Vis. Spectrophotometer
Organic acid - Sugar - Alcohol Analyses	HPLC-RID-DAD
Total Phenol Content	UV-Vis. Spectrophotometer
Titratable Acidity	-
pH	pH meter
Brix	Refractometer

4.3. ICP Analysis

4.3.1. Reagents

Ultra-pure water with a maximum resistivity of 18.2 MΩ/cm obtained from a Sartorius Arium 611 VF system (Sartorius AG, Goettingen, Germany) was used for the preparation of samples and standard solutions. HNO_3 (suprapur, 65%) and H_2O_2 (suprapur, 30%) were purchased from Merck (Merck Co., Darmstadt, Germany). Multielement standard solution of Al, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr, Te, Tl, Zn (100 mg/L, Merck Co., Darmstadt, Germany) was dissolved in 1% HNO_3 (v/v) for external calibration. For ICP-MS analyses, Rh (10 mg/L, Merck Co., Darmstadt, Germany) was used to prepare the internal standard solution. A solution of 1 µg/L Li, Y, Co, Tl, Ce (10 µg/L, Agilent Technologies, Santa Clara, CA, USA) was used for the optimization of ICP-MS. A certified reference wine sample containing 69.3 µg/L Cd and 260 µg/L Pb was used for the accuracy of ICP-MS analysis (T0777, FAPAS, York, UK). Plastic material was used for the preparation and storage of solutions.

4.3.2. Instrumentation

ICP-MS measurements were carried out with an Agilent 7500ce ORS instrument, equipped with a concentric nebulizer, nickel sampling cone and peristaltic pump (Agilent Technologies, Santa Clara, CA, USA). Measurements by ICP-OES were carried out using a Varian Liberty Series II instrument with axial viewing plasma type (Varian Inc., Palo Alto, CA, USA). The optimization parameters and operating conditions of ICP-MS and ICP-OES are listed in Table 4.5 and Table 4.6, respectively.

Table 4.5. ICP-MS parameters

ICP-MS Parameters	Value
RF Power	1550 W
Sampling Depth	8-9 mm
Gas	Argon
Carrier Gas Flow	0.9 L/ min
Make-up Gas Flow	0.15-0.19 L/min
Nebulizer pump	0.1 rps
ORS	FOODORS
Interference Equation	$^{208}Pb= {}^{208}Pb+{}^{206}Pb+{}^{207}Pb$
Sample and Skimmer Cones	Nickel
Nebulizer	Concentric
Reaction/ Collision Parameters	**Value**
He gas flow	4 mL/min
Signal Measurement Parameters	**Value**
Acquisition Mode	Spectrum Multi Tune
Acquisition Time	174 sec
Calibration	External
Internal Standard	^{103}Rh
Repetition	3
Stabilization Time	30 sec
Optimization Parameters	**Value**
Standard Mode	
Oxide Ratio (CeO/ Ce - 156:140)	< 2% cps
Doubly charged (Ce++/ Ce+ - 70:140)	< 3% cps
^{7}Li counts	> 2000 cps
^{89}Y counts	> 3000 cps
^{205}Tl counts	> 3000 cps
He Mode	
^{59}Co counts	> 1000 cps
H_2 Mode	
^{89}Y counts	> 2000 cps
Integration Time (all modes)	0.1 sec

The low oxide ratios indicate that the ICP-MS instrument is capable of reaching high plasma temperature, which is necessary to dissociate the strong CeO bond. The appropriate ORS was FOODORS for the wine samples, and the spray chamber temperature was 2^{o}C to remove the excessive amount of water in the matrix which can lead to the formation of oxides. No interference equation was employed except for ^{208}Pb due to the variability of the isotope ratios in the samples. He and no gas ORS modes have been employed. He as collision gas was preferred for most of the elements to remove polyatomic interferences. Polyatomic ions collide with He ions in the octopole reaction cell, and loose energy. They therefore cannot pass the potential barrier that blocks the entrance to the detector.

Table 4.6. ICP-OES parameters

ICP-AES parameters	Value
Power	1.2 kW
PMT Voltage	650 V
Gas	Argon
Plasma Gas	15 L/min
Auxiliary Gas	1.5 L/min
Nebulizer	Concentric
Pump Rate	15 rpm
Fast Pump	On
Rinse Time	10 sec
Sample Uptake	30 sec
Integration Time	2 sec
Replicates	3
Calibration	External
Internal Standard	None

The major elements such as Na, Mg, K, Ca and Fe were quantified via ICP-OES. The isotopes and modes employed in ICP-MS measurements, and the wavelengths employed in ICP-OES measurements are listed in Table 4.7.

Table 4.7. ICP parameters

	ICP-MS			ICP-OES
Elements	Isotopes	ORS Mode	Integration Time/ Point (sec)	Wavelength (nm)
Li	7	He	0.10	-
Be	9	Ar	0.10	-
B	11	He	0.05	-
Na	-	-	-	589.592
Mg	-	-	-	279.553
Al	27	He	0.05	-
K	-	-	-	766.490
Ca	-	-	-	393.366
Cr	53	He	0.10	-
Mn	55	He	0.05	
Fe	-	-	-	239.562
Co	59	He	0.10	-
Ni	60	He	0.10	-
Cu	63	He	0.10	-
Zn	66	He	0.30	-
Ga	71	Ar	0.10	-
Sr	88	He	0.10	-
Cd	114	He	0.10	-
Ba	138	He	0.10	-
Tl	205	Ar	0.10	-
Pb	208	He	0.10	-

4.3.3. Standards and spikes

For ICP-MS calibration, fresh stock solutions of 1000 µg/L and 10 µg/L were prepared daily from multielement standard with 1% nitric acid solution. A total number of 20 calibration solutions were prepared (500, 400, 300, 200, 100, 80, 50, 30, 10 µg/L were prepared from 1000 µg/L stock and 7.5, 5, 2.5, 1, 0.5, 0.1, 0.08, 0.05, 0.025, 0.01 µg/L were prepared from 10 µg/L stock solution). Rh was added as internal standard to each calibration solution, wine sample and spiked sample at a concentration of 10 µg/L of final solution. The 8 calibration solutions of ICP-OES (0.3, 0.6, 1, 3, 6, 10, 30 and 60 mg/L) were prepared from the multielement standard. Spike studies were also performed for red, rose and white wines daily. The spike levels and the calibration standard ranges for each element are given in Table 4.8.

Table 4.8. Calibration standard ranges and spike concentrations

Element	ICP-MS (µg/L)	Spike concentrations (µg/L)	ICP-OES (mg/L)	Spike concentrations (mg/L)
Li	0.01 - 30	10 – 100	-	-
Be	0.01-0.5	2	-	-
B	80-500	1000	-	-
Na	-	-	1-10	10
Mg	-	-	3-30	10
Al	10-200	100 – 1000	-	-
K	-	-	6-60	10
Ca	-	-	0.3-10	10
Cr	0.1-5	10-100	-	-
Mn	10-500	100-1000	-	-
Fe	-	-	0.3-3	1-10
Co	0.01-0.5	2	-	-
Ni	0.252-30	10-100	-	-
Cu	0.252-25	100	-	-
Zn	2.5-30	100	-	-
Ga	0.01-0.1	2	-	-
Sr	10-80	100	-	-
Cd	0.01-1	2	-	-
Ba	1-30	100	-	-
Tl	0.01-0.5	2	-	-
Pb	0.01-10	10	-	-

4.3.4. Sample Treatment

All the plastic materials used for diluting and storing the samples and standards were cleaned to avoid contamination by trace metals. They were soaked in 10 % HNO_3 (v/v) for at least 24 h and rinsed with ultrapure water several times, before use. The outer layer of neck of commercial wine bottles was cleaned with 2 % nitric acid solution to remove dust and avoid contamination by trace metals.

Once the bottles were opened, the samples were treated according to the Turkish Standard 3606 procedure (TSE, 1981). This procedure was based on the wet digestion of organic material in an open vessel using convective thermal energy. 5 mL of wine sample was taken into 100 mL erlenmeyer with wide neck. Rh was added as internal standard (ISTD) at the final concentration 10 µg/L to eliminate matrix interferences. Three minutes were given to dissolve the ISTD in the sample and then 10 mL HNO_3 was added. Initially, brown fume and effervescence were observed due to the oxidation of organic compounds by the acid. The solution was then heated up to 150 ^{o}C until it

evaporated to a volume of 5 mL. Following cooling, 10 mL HNO_3 and 4 mL H_2O_2 were added. The heating process at 150 ^oC proceeded to a final volume of 5 mL. Following cooling, the solution was heated at 150 ^oC with 5 mL HNO_3 and 2 mL H_2O_2 until white fume was observed. The last step was the addition of 5 mL HNO_3, 2 mL H_2O_2 and 10 mL ultrapure water and digesting the solution until white fume was diminished. Eventually, the solution was diluted to a final volume of 100 mL with ultrapure water. A colorless or pale yellow colored, transparent solution should be obtained.

The acid digestion procedure should dissolve the solid content of sample. Thus, the plasma energy of instrument can be expended for the ionization of atoms instead of decomposition of sample matrix. The solution should neither be evaporated to dryness nor heated up to boiling temperature at any of the steps. The samples were stable at 4 ^oC for 48 hours. The FAPAS certified reference wine sample was treated in the same way as the wine samples.

4.4. Color Analysis

Spectrophotometric measurements were performed according to OIV method (OIV, 2013) by a UV2450 model Shimadzu instrument (Shimadzu Inc., Kyoto, Japan). Transmittance scans between 400-700 nm, with 2 nm sampling intervals were recorded with a quartz cuvette of 10 mm path length for white and rose wines, and 1 mm path length for red wines. The measurements were repeated three times. The colorimetric coordinates (L*, a*, b*) and their derivatives (C* and H*) were calculated by the Shimadzu UVPC optional color analysis software version 2.7 (Shimadzu Inc., Kyoto, Japan) using illuminant D65 and observer placed at 10^o. The transmittance measurements taken by 1 mm path length cell must be transformed to 10 mm before calculations. The colorimetric coordinates are listed as in Table 4.9.

Table 4.9. Colorimetric coordinates

Colorimetric Coordinates	Symbol	Unit	Interval	Calculations
Lightness	L*	-	0-100 0 black 100 colorless	-
Red/Green Chromaticity	a*	-	a*<0 green a*>0 red	-
Yellow/Blue Chromaticity	b*	-	b*<0 blue b*>0 yellow	-
Chroma	C*	-	-	$\sqrt{(a^{*2}+ b^{*2})}$
Tint	H*	^{o}	0-360^{o}	arctan(b*/ a*)

The other calculated color parameters are listed in Table 4.10 (Kelebek et al., 2010; Yildirim, 2006).

Table 4.10. Color variables

Color Parameter	Symbol	Calculations
Color Density	CD	$Abs_{420nm}+Abs_{520nm}$
Tint	T	Abs_{420nm}/ Abs_{520nm}
Color Intensity	CI	$Abs_{420nm}+Abs_{520nm}+Abs_{620nm}$
Proportion of red coloration	dA(%)	$[Abs_{520nm}-(Abs_{420nm}-Abs_{620nm})/2]*100/Abs_{520nm}$
Logarithmic Color Density	K-K	$\log(Abs_{420nm}+Abs_{520nm})$
Red%	R%	$Abs_{520nm}*100/CI$
Yellow%	Y%	$Abs_{420nm}*100/CI$
Blue%	Bl%	$Abs_{620nm}*100/CI$

4.5. Polyphenol Analysis

4.5.1. Reagents

$NH_4H_2PO_4$ and H_3PO_4 (85%) were purchased from Merck (Merck Co., Darmstadt, Germany), and HPLC grade acetonitrile was purchased from Sigma-Aldrich (Sigma-Aldrich GmbH, Seelze, Germany). HPLC grade pure standards were used in the study: (±)-catechin hydrate, malvidin-3-glucoside (90%), quercetin (95%), quercetin-3-rutinoside (95%), quercetin-3-glucoside (90%), and quercetin-3-galactoside (97%) were purchased from Sigma-Aldrich (Sigma-Aldrich GmbH, Seelze, Germany). (-)-epicatechin (95%), caffeic acid (95%), ferulic acid (99%), gallic acid (98%), kaempferol (96%), myricetin (96%), o-coumaric acid (97%), p-coumaric acid (98%), procyanidin B_2 (90%), and vanillic acid (97%) were purchased from Fluka (Sigma-Aldrich GmbH,

Seelze, Germany). Resveratrol (99%), procyanidin B_1 (80%) were purchased from Extrasynthese (Extrasynthese Chemical S.A.S., Genay, France). Deionized water was obtained from Sartorius Arium 611 VF system (Sartorius AG, Goettingen, Germany). All the wine samples were filtered through 0.45-µm pore size membrane filters (Sartorius AG, Goettingen, Germany).

4.5.2. Instrumentation

The HPLC method was developed by Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez (2007). Chromatographic analyses were performed on an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) containing a G1322A degasser, G1311 quat pump, G1329 autosampler, G1316B column oven, and G1315D diode array detector. An Ace 5 C_{18} (250 x 4.6 mm) column was employed for the separation (AC Technologies, Aberdeen, Scotland). 20 µl of filtered sample was injected to the system, column oven was set to 20 ^{o}C, and the chromatograms were recorded at 280, 320, 360 and 520 nm. Table 4.11 shows the gradient mobile phase program.

Table 4.11. Mobile phase gradient of the HPLC method

Time (min)	Flow rate (ml/min)	%of mobile phase A[a]	%of mobile phase B[b]	%of mobile phase C[c]
Initial	1.0	100	0	0
2.00	1.0	100	0	0
5.00	1.0	92	8	0
17.00	1.0	0	14	86
22.00	1.0	0	18	82
29.50	1.0	0	21	79
55.00	1.0	0	33	67
70.00	1.0	0	50	50
75.00	1.0	0	50	50
78.00	1.0	20	80	0
81.00	1.0	20	80	0
86.00	1.0	100	0	0

[a]Mobile phase A: $NH_4H_2PO_4$ 50mM, pH= 2.6.
[b]Mobile phase B: 20% mobile phase A and 80% acetonitrile.
[c]Mobile phase C: H_3PO_4 200mM, pH= 1.5.

Identification and quantification of phenolic compounds were performed according to the retention times of pure standards and external standard method, respectively. The non-commercial phenolic compounds were quantified by using the

calibration curves of most similar compounds: the anthocyanins, their derivatives, vitisin-A and pinotin-A, were quantified by using the malvidin-3-glucoside curve; myricetin-3-glucoside and quercetin-3-glucuronide were quantified by using the quercetin-3-glucoside curve, respectively. These non-commercial phenolic compounds were identified from the chromatograms of wine samples studied by Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez (2007) and from the control of UV-Vis spectrums. Data acquisition and peak processing were performed using Chemstation Rev. B.03.02 software (Agilent Technologies, Santa Clara, CA, USA). The measurements were repeated two times.

4.5.3. Standards

The stock solution was prepared by dissolving the pure standards in HPLC grade methanol. Calibration standards were prepared by diluting the stock solution with methanol: water mixture (50:50). The calibration concentrations and quantification wavelengths are listed in Table 4.12.

Table 4.12. Polyphenol standard concentrations

Standard Name	Quantification wavelength	Calibration standards (mg/L)
Gallic acid	280	10-30-70-100-150-200
(-)-epicatechin	280	10-25-50-75-100-150-200
(+)-catechin	280	10-25-50-75-100-150-200
o-coumaric acid	280	0.5-0.75-1-2.5-5-7.5-10
Vanillic acid	280	1-5-10-25-50-75
Procyanidin B_1	280	0.5-1-10-50-100-150
Caffeic acid	320	1-5-10-25-50-75
Ferulic acid	320	0.25-0.5-1-2.5-5-7.5-10
p-coumaric acid	320	1-5-10-25-50-75
Resveratrol	320	0.12-0.3-0.6-0.9-1.2-3-6-12
Rutin	360	1-2.5-10-20-50-100-200
Myricetin	360	1-5-10-25-50-100
Quercetin	360	0.75-1-5-10-25-50-100
Kaempferol	360	0.75-1-5-10-25-50-100
Quercetin-3-glucoside	360	1-5-10-25-50-100
Quercetin-3-galactoside	360	1-10-20-50-100
Malvidin-3-glucoside	520	5-25-50-100-250

4.6. Organic Acid, Sugar, Alcohol Analyses

4.6.1. Reagents

HPLC grade acetonitrile, methanol, ethanol, L-tartaric acid (99.5%), pyruvic acid (98%), citric acid (99.5%), succinic acid (99%), D-(+)-glucose, D-(-)-fructose (99%), glycerol (99.5%) and shikimic acid (99%) were purchased from Sigma-Aldrich (Sigma-Aldrich GmbH, Seelze, Germany). H_2SO_4 (95-97%), L-(+)-lactic acid (99%), L-(-)-malic acid (99.5%) were purchased from Fluka (Sigma-Aldrich GmbH, Seelze, Germany). Acetic acid (99.9%) was purchased from Supelco (Sigma-Aldrich GmbH, Seelze, Germany). Sucrose was purchased from Panreac (Panreac Química, Barcelona, Spain). Deionized water was obtained from Sartorius Arium 611 VF system (Sartorius AG, Goettingen, Germany). Octadecyl silica cartridges (C_{18}, 500 mg/ 3 ml) were purchased from Agilent (Agilent Technologies, Santa Clara, CA, USA). 0.45-µm pore size membrane filters were used in the study (Sartorius AG, Goettingen, Germany).

4.6.2. Instrumentation

The HPLC method was based on the study of Castellari et al. (2000). Chromatographic analyses were performed on a Perkin Elmer HPLC (Perkin Elmer Inc., Norwalk, CT, USA) containing PE series 200 ternary pump, autosampler, vacuum degasser, and column oven, NCI 900 interface, PE series 200a diode array detector, and PE series 200EP refractive index detector. An HPX-87H (300 x 7.8 mm, 9 µm) column attached to the guard column (30 x 4.6 mm) was employed for separation (Bio-Rad Laboratories, Hercules, CA, USA). 20 µL of sample was injected to the system, and the column oven was set to 45^{o}C. Mobile phase was 0.045 N H_2SO_4 with 6% acetonitrile flowing at a rate of 0.5 mL/min within the system. The detection of sugars, ethanol and methanol were performed by refractive index detector, whereas organic acids were detected by diode array detector (210 nm). Data acquisition and peak processing were performed with Totalchrome version 6.3.1 (Perkin Elmer Inc., Norwalk, CT, USA).

Original malic acid value was calculated as: 1.0 x malic acid + 1.489 x lactic acid (Schlesier et al., 2009). It represents the malic acid amount prior to malolactic fermentation.

4.6.3. Standards

The calibration standards and stock solutions were dissolved with the mobile phase at the following concentrations as listed in Table 4.13.

Table 4.13. Organic acid, sugar, alcohol standard concentrations

Standard	Stock solution (mg/L)	Calibration Standards (mg/L)
Glucose	50000	500-2000-4000-6000-8000-10000-15000-20000-25000
Fructose	50000	500-2000-4000-6000-8000-10000-15000-20000-25000
Sucrose	50000	500-1000-2500-5000-7500-10000-15000
Glycerol	50000	500-1000-2500-5000-7500-10000-15000
Ethanol	-	% (1-2-5-10-15-20)
Tartaric Acid	1000	10-50-100-250-350-500
Malic Acid	1000	10-50-100-250-350-500
Lactic Acid	1000	10-50-100-250-350-500
Pyruvic Acid	1000	5-10-15-20-25-50-75-100
Succinic Acid	1000	5-10-15-20-25-50-75-100
Citric Acid	1000	5-10-15-20-25-50-75-100
Acetic Acid	1000	10-25-50-75-100-150-200

4.6.4. Sample Preparation

For the determination of organic acids, to eliminate the phenolic components in red and rose wines, the samples were cleaned through C_{18} cartridges according to the OIV method (OIV, 2013). The cartridges were conditioned with 10 mL methanol, and then with 10 mL water. Then, 8 mL of red or rose wine samples were passed through the column. The first 3 mL was discarded, and the rest 5 mL was collected. This was followed by dilution with the mobile phase by 10-fold and filtering prior to injection. White wines were only diluted with the mobile phase by tenfold and then filtered. Sample preparation was repeated two times. The sugar components were measured by direct injection.

4.7. Total Phenol Content Analysis

Total phenolic content was determined according to the Folin Ciocalteu method modified as a micro-scale protocol to reduce the assay volume from the standard method of Singleton & Rossi (1965) (Arnous, Makris, & Kefalas, 2001). 40 µL of rose and white wine samples was pipetted into 3.16 mL water, 200 µL of 2 N Folin Ciocalteu reagent was added to the wine sample (Sigma-Aldrich GmbH, Seelze, Germany). The mixture was mixed with vortex. After 5 minutes, 600 µL of saturated sodium carbonate solution was added to the solution (20 g Na_2CO_3 in 100 mL water) (Sigma-Aldrich GmbH, Seelze, Germany). This was followed by mixing with vortex. After leaving the solution for 2 hr. at 20 ^oC, the absorbance was read at 765 nm against blank (Shimadzu UV 2450, Shimadzu Corp., Kyoto, Japan). For the case of red wines, the samples were diluted by one half with water. The measurements were repeated three times. The results were expressed in terms of gallic acid equivalent (mg GAE/L). Gallic acid stock solution was prepared by dissolving 0.5 g of dry gallic acid (Sigma-Aldrich GmbH, Seelze, Germany) in 10 mL ethanol, and filling the volume to 100 mL with water. Gallic acid calibration curve was prepared by diluting the stock solution to 400, 700, 1000, 1300, 1700 and 2000 mg/L.

4.8. Chemical Analyses

The pH value of wine samples was determined by WTW Inolab Series 720 pH meter (WTW GmbH, Weilheim, Germany). Brix was determined by Mettler Toledo RE 50 refractometer (MT GmbH, Schwerzenbach, Switzerland). Total acidity was determined according to OIV method (OIV, 2013). The total acidity of wine is the sum of its titratable acidities when it is titrated to pH 7.0 using an alkaline solution. Bromothymol blue (95%) was the color indicator (Sigma-Aldrich GmbH, Seelze, Germany). The solution of bromothymol was prepared by dissolving 4 g of bromothymol blue in 200 mL ethanol (96% v/v) (Sigma-Aldrich GmbH, Seelze, Germany), then 200 mL of water free of CO_2 was added. This was followed by addition of 7.5 mL 1 mol/L NaOH solution (40 g NaOH/ 1000 mL water) (Merck Co., Darmstadt, Germany) to produce blue-green color (pH 7.0) and the final volume was made up to 1000 mL by water. A preliminary test was employed to determine the end

point color of titration. Preliminary test: 1 mL of bromothymol blue solution was added into 25 mL of boiled distilled water. Then 10 mL of wine free of CO_2 was added which was prepared by applying vacuum to 50 mL of wine for one or two minutes. 0.1 mol/L NaOH solution was added to produce blue-green color. Finally, 5 mL of pH 7.0 buffer solution was added. The buffer solution was prepared by dissolving 107.3 g of KH_2PO_4 (Merck Co., Darmstadt, Germany) in 500 ml of 1 mol/L NaOH solution. And the final volume was made up to 1000 mL by water. The total acidity measurements were performed by adding 1 mL bromothymol solution into 30 mL of boiled distilled water. 10 mL wine sample was added to this solution and titration was performed with n ml of 0.1 mol/L NaOH solution until the same color was obtained in the preliminary test. The total acidity in terms of g tartaric acid/L wine was calculated by using the formula 10 x n x 0.075.

4.9. Statistical Analyses

The results were analyzed by one-way analysis of variance (ANOVA) using Tukey's test with Minitab statistical software (ver. 16, Minitab Inc., State College, PA, USA). The data were statistically significant if $p<0.05$. The correlation matrix of data was checked by Minitab using Pearson correlation test with 99.5% confidence interval (ver. 16, Minitab Inc., State College, PA, USA). The ICP and HPLC data were statistically evaluated with Simca-P software (ver. 13, Umetrics Inc., Umea, Sweden). Principal component analysis (PCA), Cooman's plot technique and partial least square-discriminant analysis (PLS-DA) were used to evaluate the effect of grape variety, harvesting year and growing region on wine chemical characteristics. Hierarchical cluster analysis (HCA) was employed using Minitab. The visible spectral data were evaluated using PCA, PLS-DA, Cooman's plot and PLS techniques, as well.

Prior to modeling, the data were standardized by subtracting the averages and dividing them to the standard deviations and transformations were applied to minimize skewness and at the same time to normalize the data. For the evaluation of visible spectral data, standardization was followed by data pre-processing using spectral filtering techniques including second order derivative filtering and wavelet compression spectra technique (WCS). The aim of pre-processing is to remove the undesired systematic variations in X data that are unrelated to Y. This procedure will improve the

predictive ability of model. Such variations in the spectra can be due to baseline drift or wavelength regions of low information. WCS can probe a signal according to a scale. It uses a mother wavelet function which is Daubechies-4 with 99.5% confidence interval in this study. This is one of the most commonly applied wavelet function for fluorescence and NIR spectra (Eriksson et al., 2001). The first and second order derivation technique was applied with quadratic polynomial order. The significant variables in the models were chosen by the use of variable importance in the projection (VIP) plots generated by Simca-P software. VIP is a parameter that reflects the importance of each X-variable both for the X- and Y- models when interpreting a PLS model. The weighted sum of squares of the PLS weights ($w^*_{al}{}^2$) are multiplied by the explained sum of squares of that PLS dimension and the number of terms in the model (n). This is divided by the sum of squares of the PLS model. Finally, the VIP value is calculated by taking the square root of that number. It is a positive value and VIP values larger than 1 are the most influential to the model. Values around 0.7-0.8 also work well for variable selection (Eriksson et al., 2001).

$$VIP_{ak} = \sqrt{\frac{n \times \sum_{l=1}^{k} w^{*2}_{al} \times SS_l(Y)}{\sum_{l=1}^{k} SS_l(Y)}} \qquad (4.1)$$

In the PLS-DA and Cooman's plot techniques, approximately 80% of the data set was chosen for model development and the remaining 20% constituted the validation set. The validation set were discussed with respect to the membership probability of each observation given by the Simca-P software. This is the probability that the observation belongs to the model within the 95% confidence interval. The ones with membership probabilities less than 5% were considered to be outliers and they do not belong to the model. For the PLS models of visible spectra, approximately 20% of the data set was kept for validation and 80% of it was employed for the calibration model.

CHAPTER 5

RESULTS AND DISCUSSION

In this chapter, chemical and spectroscopic analyses were discussed in the order of element and phenolic profiles, color properties, organic acid and sugar compositions and other quality parameters. The results in the tables are given as the minimum, maximum and median of four harvest years. The average of repeated measurements was employed in the statistical models. The statistical analysis of data by multivariate techniques with respect to grape variety, geographical origin and harvest year were given in two sections as unsupervised analysis [Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)] and supervised analysis [Partial Least Squares-Discriminant Analysis (PLS-DA) and Soft Independent Modeling of Class Analogy (SIMCA)]. In the final part, the visible spectra of samples were used for the classification of wines and then for the prediction of their phenolic compounds.

5.1. Element Analysis via ICP

The detection limits were calculated as three times the standard deviation of signal of blank sample (prepared ten times). The quantification limits were ten times the standard deviation of signal of blank sample. Recoveries were calculated based on the difference of spiked and un-spiked sample and taking the ratio of this value to the assigned value. The detection limits, recovery values and accuracy studies with the certified reference material were calculated for the validation of the proposed method. The limit of detection (LOD) and recovery values for red, rose and white wines are reported in Table 5.1. The highly volatile elements Se and Te were not quantified due to the loss during open vessel acid digestion procedure (Recovery < 50%). The recovery values of spiked samples ranged from 77 to 120% for all elements except K and Zn (<60% in red wines). Mg in red and rose wines and Ga, Cd, and Tl in white wines produced recoveries >120%. Bi concentrations were found to be below the detection limits for the majority of samples. In general, relative standard deviation <15% was obtained for the most variables. The trace elements with high relative standard

deviations such as Be, Cd, Tl and Ga were eliminated from data analysis (although reported in the tables).

Table 5.1. The analytical conditions of elements in wine samples (µg/L)

Element	LOD	Recovery (%) of red wines	Recovery (%) of rose wines	Recovery (%) of white wines
Ca*	14.96	104[e]	>120[e]	102[e]
Fe*	0.12	104[d] 98±2[e]	101±13[d] 103±7[e]	104±21[d] 100±1[e]
K*	0.15	<60[e]	112[e]	108±3[e]
Mg*	0.24	>120[e]	>120[e]	95[e]
Na*	0.71	85±5[e]	84±11[e]	86±11[e]
B	6.13	91±14[d]	107[d]	94±2[d]
Al	1.20	90±28[c] 115±3[d]	125[c] 116±4[d]	85±4[c] 119[d]
Sr	0.01	94±8[c]	107±16[c]	78±11[c]
Li	0.06	109±1[b] 102±8[c]	96[b] 109±6[c]	91±28[b] 105±3[c]
Be	0.0005	101±12[a]	99±6[a]	96[a]
Cr	0.04	98±6[b] 102±15[c]	102[b] 109±6[c]	102±10[b] 102±5[c]
Mn	0.02	90±19[c] 116±4[d]	103[c] 112[d]	90±14[c] 115±6[d]
Co	0.003	93±9[a]	101±13[a]	120[a]
Ni	0.10	89±9[b] 96±10[c]	90[b] 92[c]	106±13[b] 99±7[c]
Cu	0.02	92±5[c]	96±2[c]	97±3[c]
Zn	1.03	<60[c]	109[c]	<60[c]
Ga	0.003	115±2[a]	112[a]	>120[a]
Cd	0.01	107±8[a]	96±8[a]	>120[a]
Ba	0.09	95±3[c]	95[c]	93±3[c]
Tl	0.001	111±11[a]	105[a]	>120[a]
Pb	0.05	90±15[b]	85[b]	77[b]

[a]2 µg/Lspike, [b]10 µg/Lspike, [c]100 µg/Lspike, [d]1 mg/Lspike, [e]10 mg/Lspike
*Concentrations in mg/L

The results and recovery values of certified reference wine sample (FAPAS), which contained 69.3 µg/L of Cd and 260 µg/L of Pb element, are reported in Table 5.2. The mean recovery values for Cd and Pb were 89% ± 14 and 108% ± 11, respectively. However, Cd results were eliminated from statistical analysis due to the high relative standard deviations.

Table 5.2. Quantitative results of FAPAS certified reference wine sample

Element	Mode	Isotope	Result (µg/L)	Recovery (%)
Cd	He	114	62.71	90
Cd	He	114	49.15	71
Cd	He	114	72.93	105
Cd	He	114	63.25	91
Pb	He	208	262.25	101
Pb	He	208	264.09	102
Pb	He	208	323.98	125
Pb	He	208	270.85	104

The element concentrations of monovarietal red, rose and white wine samples are reported in Tables 5.3 and 5.4. The following elements were quantified in the samples: Al, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, and Zn. The correlation coefficients and analytical conditions of calibration models for ICP-MS and ICP-OES instruments are reported in Appendix Table B.1 and B.2. The samples were below the OIV limits for Zn (5 mg/L) and Pb (0.15 mg/L). According to Turkish Food Codex (2008b), the assigned limit of Pb for wine is 0.20 mg/L. Unlike other alcoholic beverages, due to the acidic nature, the heavy metal concentration of wine samples varies widely (Alkış et al., 2014). The median content of Ca and Fe were consistent with the data from European viticulture areas and South Africa (Coetzee et al., 2005; Smeyers-Verbeke et al., 2009). The Fe contents of red and white wines were also in agreement with data observed elsewhere (Alkış et al., 2014; Şimşek et al., 2008). The median of Na and Mg contents were slightly greater than European wines, although the minimum-maximum ranges were consistent, and median levels were consistent with the Argentinean and Spanish wines. K levels in Turkish wines were lower than the levels in European wines but consistent with Argentinean wines (Fabani et al., 2010; Gonzálvez et al., 2009; Smeyers-Verbeke et al., 2009). The minor elements were similar to those in literature (Coetzee et al., 2005; Gonzálvez et al., 2009; Smeyers-Verbeke et al., 2009). However, Pb Cd and Cu levels were found lower than the data observed elsewhere (Şimşek et al., 2008). The highest variation in wine samples was observed in Cu similar to the study of Geana et al. (2013). They suggested that the high variability may originate from Cu accumulation in soil due to the old practices of copper sulphate or other copper-based fungicides to cope with vine downy mildew. They also concluded that frequent use of agrochemicals like pesticides results in soil contamination (Cu, Ni, Zn, Cd) which also shows up in the wine bottle.

The element profiles of red and white wines differed from each other with higher amounts of K and Ba, and lower levels of Li and Zn in red wines. The slightly higher levels of minerals in red wines can be explained by the prolonged leaching of minerals from the grape during maceration process (Coetzee et al., 2005; Martin et al., 2012). Greenough, Longerich, & Jackson (1997) have found that Li and Zn were higher in the white wines, whereas Mo, Mg, Ba, Ca and P were higher in the red wines from Okanagan valley. They reported that the red and white wine discriminating elements were water soluble litophile (Li, Na, K, Mg, Ca, Sr, Ba, Al, B) or chalcophile (Cu, Zn, Pb) elements. Moreover, the correlation between the amount of Cu, Ni and Cr in wines

and in grapes were found to be weak due to the influence of winemaking processes like pre-concentration, use of metallic containers and filtering agents (Vystavna et al., 2014; Zou et al., 2012). From the so-called natural minerals like Ba, B, Li, Al or Sr (independent of productive or agricultural activities), Li and Sr were significantly high in Emir white wines, and Ba and B were significantly low in Boğazkere and Öküzgözü red wines ($p<0.05$). Zn, like other minerals such as Ca, Mg, K, Fe, Na or Cu, can arise in wine originating either from soil or from productive activities like fungicidal treatments. Pb originates from atmospheric pollution or from fungicidal treatment (Volpe et al., 2009). Both Zn and Pb levels were high in Muscat white wines. Muscat wines also contained significantly higher Mn and Mg than the other varieties ($p<0.05$).

Table 5.3. Element concentrations of red and rose wines (µg/L)

	Element	Ca*	Fe*	K*	Mg*	Na*	Sr*	B*	Al*	Ba	Li	Cr	Mn	Co	Ni	Cu	Pb	Zn	Ga	Cd	Be	Tl
	min	36	0.35	127	66	8.7	0.19	2.67	0.16	32	8.93	7.29	0.81	<LOD	16.56	2.18	0.23	116	0.10	0.31	0.06	<LOD
Boğazkere	max	86	6.67	528	152	82.9	0.65	6.83	2.35	191	24.16	50.37	1.53	14.28	61.27	84.48	27.38	464	0.77	18.33	0.40	0.47
	med	61	1.49	299	104	21.2	0.39	4.31	0.83	109	14.99	21.20	1.10	5.13	37.15	22.61	9.03	249	0.37	0.61	0.15	0.19
	min	15	0.68	425	108	6.7	0.27	4.93	0.24	60	5.17	14.30	0.73	2.42	29.69	2.67	7.97	157	0.24	0.16	0.06	0.16
Cabernet	max	64	3.46	992	167	44.6	1.65	8.64	2.80	307	33.58	113.53	2.49	9.47	62.47	250.75	47.03	640	0.81	15.21	5.60	2.46
Sauvignon	med	52	1.81	546	125	24.5	0.48	6.69	0.67	189	14.02	24.75	1.30	4.87	49.16	68.49	15.04	358	0.41	0.46	0.19	0.35
	min	20	0.84	182	74	0.7	0.23	4.53	0.17	92	5.63	7.67	0.61	1.57	10.58	13.47	1.11	115	0.11	0.04	<LOD	0.08
Kalecik	max	62	4.61	604	227	52.3	1.64	10.81	1.47	277	70.91	43.15	1.20	7.66	60.64	426.34	33.35	333	0.63	21.77	0.33	0.52
Karası	med	47	1.27	416	110	21.2	0.43	6.24	0.46	131	15.69	17.03	0.97	3.33	31.60	70.01	7.03	227	0.31	0.28	0.10	0.21
Çalkarası	single sample	70	0.56	543	152	4.0	0.22	5.62	0.40	86	8.26	16.02	0.73	1.58	20.29	75.34	4.63	328	0.30	8.23	0.05	0.27
	min	15	0.48	97	97	4.3	0.22	4.21	0.29	67	6.55	10.59	0.94	1.22	23.81	15.25	2.12	185	0.19	0.07	0.02	<LOD
Merlot	max	61	4.53	612	198	35.2	0.85	8.57	1.81	389	31.76	40.93	5.28	14.96	130.86	304.64	40.84	591	0.95	17.29	5.21	1.45
	med	49	1.30	338	127	20.2	0.48	6.66	0.57	116	10.19	19.82	1.27	4.59	45.05	152.85	13.05	446	0.38	0.35	0.14	0.21
	min	23	0.43	190	83	8.6	0.40	4.31	0.33	54	8.85	8.92	0.83	<LOD	10.07	9.70	1.28	81	0.09	0.11	0.02	<LOD
Öküzgözü	max	89	2.98	716	154	58.9	0.97	12.98	1.16	163	26.07	28.84	1.13	5.79	43.42	306.72	27.70	560	0.62	16.82	0.33	0.26
	med	65	1.13	259	106	19.0	0.60	5.64	0.70	107	15.32	17.34	1.04	3.23	24.74	36.81	8.90	246	0.30	0.53	0.13	0.13
	min	38	1.40	325	106	20.1	0.59	4.88	0.34	151	19.25	12.57	1.08	3.92	29.28	35.92	11.28	212	0.29	0.27	0.20	0.17
Papazkarası	max	75	5.73	377	116	41.4	0.86	6.83	2.28	197	22.48	43.77	1.60	10.18	63.11	348.27	22.52	324	0.79	0.35	0.25	0.28
	med	56	2.59	367	112	31.4	0.82	6.05	1.08	186	21.83	22.89	1.27	9.00	59.94	151.87	20.89	303	0.62	0.33	0.22	0.19
	min	41	0.50	242	108	7.6	0.23	4.53	0.29	60	5.31	9.01	0.85	0.68	22.66	19.80	2.00	262	0.16	0.18	<LOD	<LOD
Syrah	max	70	5.14	815	201	140.1	0.95	9.95	1.28	223	45.82	41.01	1.25	6.53	83.05	656.50	30.35	792	0.59	20.36	0.24	0.97
	med	57	1.24	405	129	17.9	0.39	7.04	0.46	119	8.93	21.79	1.11	4.71	34.79	115.75	8.65	378	0.33	0.38	0.11	0.15
	min	48	0.76	162	85	3.3	0.21	3.76	0.39	64	8.91	9.59	0.73	2.37	6.92	15.61	3.90	177	0.35	0.14	0.06	0.06
Çalkarası	max	79	1.18	334	112	27.7	0.27	5.75	1.21	87	18.63	15.98	1.26	6.05	63.76	38.58	11.29	419	0.99	21.18	0.25	0.36
Rose	med	65	0.88	235	108	8.8	0.25	4.54	0.45	68	15.55	10.72	1.09	2.70	16.77	27.78	10.18	211	0.41	0.31	0.11	0.22
	min	15	2.12	<LOD	106	20.3	0.60	2.95	1.37	98	20.06	24.05	1.41	8.54	74.94	51.84	6.92	361	0.65	0.39	0.39	0.27
Öküzgözü	max	54	5.59	281	108	35.5	1.99	5.79	1.91	107	57.76	36.79	2.55	13.08	121.35	101.44	13.25	380	0.73	0.40	0.46	0.37
Rose	med	35	3.86	141	107	27.9	1.30	4.37	1.64	103	38.91	30.42	1.98	10.81	98.15	76.64	10.08	370	0.68	0.39	0.43	0.32

*Concentrations in (mg/L). min: minimum, max: maximum, med: median, <lod:below detection limit

Table 5.4. Element concentrations of white wines (µg/L)

	Element	Ca*	Fe*	K*	Mg*	Na*	Sr*	B*	Al*	Ba	Li	Cr	Mn	Co	Ni	Cu	Pb	Zn	Ga	Cd	Be	Tl
	min	43	0.40	60	77	9.8	0.56	3.93	0.24	38	25.76	7.65	0.46	<LOD	7.44	<LOD	5.24	185	0.09	0.12	0.18	<LOD
Emir	max	93	2.39	416	148	100.1	1.26	6.70	1.66	135	386.37	28.16	1.15	7.57	117.79	195.20	33.84	648	0.83	31.40	2.06	0.27
	med	67	0.69	174	96	21.5	0.89	5.02	0.46	69	146.57	11.94	0.68	2.47	17.76	23.49	9.20	308	0.36	0.43	0.32	0.09
	min	15	0.12	108	54	0.7	0.18	4.10	0.28	35	3.51	8.37	0.44	0.98	16.94	18.41	1.85	175	0.15	0.20	0.03	<LOD
Chardonnay	max	94	52.77	468	153	29.8	1.20	9.89	1.37	104	119.22	27.11	0.99	5.84	84.91	467.65	33.76	764	0.41	36.22	4.19	1.77
	med	67	0.67	297	110	16.6	0.27	5.22	0.48	63	13.81	14.09	0.81	3.92	45.23	91.37	14.34	379	0.30	0.55	0.29	0.32
	min	39	0.33	185	70	13.9	0.16	3.36	0.23	36	9.97	5.81	0.54	0.70	9.55	6.94	2.02	115	0.11	0.13	0.04	<LOD
Narince	max	100	8.83	507	140	44.4	1.03	6.00	2.43	116	121.62	50.65	1.28	11.46	70.92	1055.5	27.33	808	1.02	8.00	0.70	0.53
	med	70	0.66	298	93	25.0	0.70	4.52	0.62	83	26.92	14.56	0.77	2.18	16.98	47.56	11.39	315	0.36	0.47	0.33	0.12
	min	15	0.49	26	107	14.7	0.34	3.54	0.40	46	7.02	7.80	0.68	1.93	18.78	23.67	18.41	246	0.15	0.30	0.28	<LOD
Muscat	max	93	2.78	445	181	65.3	0.61	5.97	2.71	194	42.75	93.55	2.20	13.06	115.16	300.50	72.43	663	2.09	31.65	3.63	0.88
	med	64	0.82	178	135	28.7	0.43	4.96	0.67	75	29.40	13.65	1.02	4.73	43.87	49.95	29.06	417	0.32	0.69	0.89	0.44
	min	43	0.32	53	89	10.9	0.23	3.11	0.23	52	9.69	8.11	0.24	1.14	8.39	16.13	2.94	75	0.17	0.10	0.07	0.02
Sultaniye	max	112	3.47	566	153	47.8	2.00	10.27	2.35	135	81.24	57.57	1.13	16.75	190.71	211.09	13.75	509	0.81	8.50	0.71	0.38
	med	64	1.05	180	112	39.7	0.49	4.40	0.51	82	31.88	17.42	0.71	2.73	24.60	71.31	10.20	262	0.40	0.32	0.34	0.11

*Concentrations in (mg/L).min: minimum, max: maximum, med: median, <lod:below detection limit

5.2. Polyphenol Analysis via HPLC

The detection limits (LOD) and recovery values of wine samples are reported in Table 5.5. The correlation coefficients, retention times, analytical conditions of calibration models of HPLC instrument are reported in Appendix Tables B.3 and B.4. The HPLC chromatograms of red and white wine samples are shown in Appendix Figures C.1 and C.2. The detection limits were calculated according to the OIV method (2013) using the graphical approach based on the background noise of a blank sample. The following formulas were employed:

$$\text{LOD: } 3 \times h_{max} \times RF \tag{5.1}$$

$$\text{LOQ: } 10 \times h_{max} \times RF \tag{5.2}$$

The response factor (RF) of the instrument is the quantity/signal ratio. The quantity is the concentration of the analyte and signal is the height of the analyte peak. h_{max} is the greatest variation in absorbance unit on the y-axis of chromatogram of between two points. The distance between two points is twenty times the width at mid-height of the analyte peak.

Table 5.5. Analytical conditions of polyphenols in wine samples (mg/L)

Standard Name	LOD	Recovery of Red wines (%)	Recovery of Rose wines (%)	Recovery of White wines (%)
Malvidin-3-glucoside	0.0270	83±7[d]	89±11[a]	81[a]
Rutin	0.0289	87±10[b]	91±13[a]	97±2[a]
Kaempferol	0.0279	95±10[b]	93±14[a]	98±6[a]
Quercetin	0.0196	95±11[b]	87±6[a]	87±7[a]
Myricetin	0.0192	86±4[b]	107±5[a]	100±14[a]
Resveratrol	0.0011	93±1[b]	87±5[a]	81±5[a]
p-coumaric acid	0.0109	80±1[b]	87±10[a]	95±11[a]
Ferulic acid	0.0149	88±9[b]	83±1[a]	84±2[a]
Caffeic acid	0.0194	82±6[c]	85±1[a]	80[a]
Gallic acid	2.1351	86±8[c]	95±7[b]	101±1[b]
(+)-catechin	1.3166	97±1[c]	92±11[b]	94±6[b]
Vanillic acid	0.0352	85±5[b]	89±1[a]	86±3[a]
(-)-epicatechin	0.2774	86±5[c]	101±4[b]	100±6[b]
o-coumaric acid	0.0525	84±3[b]	79±3[a]	80±4[a]
Quercetin-3-glucoside	0.0245	93±8[b]	89[a]	84±5[a]
Quercetin-3-galactoside	0.0612	89±1[b]	94±5[a]	97±1[a]
Procyanidin B_1	0.2113	88[c]	83[b]	85[b]

[a]1 mg/Lspike, [b]5 mg/L spike, [c]10 mg/L spike

The polyphenol concentrations of red, rose and white wine samples are reported in Tables 5.6, 5.7 and 5.8 (The abbreviations used in these tables are explained at the bottom of Table 5.8 and the chemical formulas of measured phenolic compounds are demonstrated in Figure 2.3 and 2.4). The most abundant anthocyanin was malvidin-3-glucoside in red wine samples due to its high stability (Saavedra et al., 2011). Other main anthocyanin-glycosides were peonidin-, petunidin- and delphinidin-3-glucosides. Cyanidin-3-glucoside was overlapped by interfering peaks in the chromatogram and therefore was not quantified. The anthocyanin concentrations were consistent with the data in literature (Garcia Falcon et al., 2007; Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007). The malvidin-3-glucoside concentrations of Syrah, Cabernet Sauvignon and Merlot wines were in good agreement with the same cultivars of southern France (Landrault et al., 2001).

There was no statistical significance among wines in terms of total coumaroylated (Tcoum) and acetylated (Tace) anthocyanin derivatives, however the following observations could be made: Total amount of coumaroylated anthocyanins was the highest in Öküzgözü, Boğazkere, and Merlot wines, and the lowest in Cabernet Sauvignon and Kalecik Karası wines ($p<0.05$). The total amount of acetylated anthocyanins was higher for Syrah, Merlot and Cabernet Sauvignon wines. Although the total amounts were found statistically insignificant, the ratio of coumaroylated to acetylated anthocyanins was found significantly different ($p<0.05$). It was the lowest in Boğazkere and Öküzgözü wines and the highest in Cabernet Sauvignon wines. Similarly, the same property was observed for Tempranillo variety wines in Spain, as opposed to those from Merlot and Cabernet Sauvignon (Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007). The median results of red wines indicated that the anthocyanin concentrations of Syrah, Cabernet Sauvignon and Öküzgözü were higher than the other varieties. In rose wines, the anthocyanins were detected at trace amounts.

It was reported that young wines contained the maximum concentrations of vitisin-A and trace amounts of pinotin-A (Rentzsch et al., 2010). The median value of the ratio of vitisin-A to pinotin-A (vitA/pinA) of red wine samples was greater than 1.0 for all red wine varieties except Kalecik Karası wines (0.53). Öküzgözü wines had significantly high vitA/pinA ($p<0.05$). Vitisin-A results were similar to the data observed in the literature (Morata et al., 2007). Syrah wines were the richest in vitisin-A and pinotin-A content of all red wines.

The detected flavonol compounds were rutin (quercetin-3-rutinoside), quercetin, myricetin, kaempferol and their glycosides which display maximum absorbance at 360 nm. The results were in agreement with the data reported elsewhere (Anli et al., 2006; Garcia Falcon et al., 2007). The myricetin and rutin concentrations of Öküzgözü and Kalecik Karası wines were similar to the results reported by Porgalı & Büyüktuncel (2012). Meanwhile, the quercetin amounts of Öküzgözü, Boğazkere, Papazkarası and Kalecik Karası wines were similar to those studied by Özkan & Baydar (2006). The rutin concentrations in this study were the highest for Öküzgözü wines which was followed by Cabernet Sauvignon, Syrah and Boğazkere wines. Anli et al. (2006) have also found that Boğazkere, Öküzgözü and Cabernet Sauvignon wines were richer in rutin concentration than the other varieties (Kalecik Karası, Merlot, Syrah, Çalkarası, Adakarası, Papazkarası, Carignan, Cinsault, Pinot Noir, Gamay). Moreover, the flavonol concentrations of Merlot, Cabernet Sauvignon and Syrah wines were lower than those from Greece (Makris et al., 2006).

Among the flavonol-glycosides, quercetin-3-glucoside and myricetin-3-glucoside were the most abundant in red wines. Syrah wines were significantly the richest of all red wines in terms of flavonol content, similar to the study of Makris et al. (2006) ($p<0.05$). The quercetin-3-glucuronide concentrations of Boğazkere, Öküzgözü and Papazkarası wines were higher than the quercetin-3-glucoside concentrations, unlike other varieties (Merlot, Syrah, Kalecik Karası, Çalkarası). The most abundant flavonol was myricetin-3-glucoside for most of the varieties except Kalecik Karası, Merlot, Papazkarası and Çalkarası wines. Rose wines were richer in flavonol contents than the white wines.

The resveratrol from the stilbenes group and hydroxycinnamic acids such as caffeic acid, ferulic acid and p-coumaric acid were detected by their high absorbance at 320 nm. On the other hand, hydroxybenzoic acids (vanillic and gallic acids) and flavan-3-ols such as (-)-epicatechin, (+)-catechin, and procyanidins were detected in the chromatogram at 280 nm. Procyanidin B_2 was not quantified since it was overlapped by another peak.

The gallic acid and (-)-epicatechin values of Cabernet Sauvignon wines were higher than those of Kalecik Karası and Merlot wines, similar to the data reported in the literature (Anli & Vural, 2009; Ünsal, 2007). Unlike the results of these studies, the (+)-catechin concentrations of Cabernet Sauvignon wines were found higher than those of Kalecik Karası wines. Gallic acid and (+)-catechin were the dominant phenolic acids in

red wines in accordance with the results of Porgalı & Büyüktuncel (2012). They stated that the high concentration of gallic acid in red wines was due to the hydrolysis of flavonoid gallate esters, which was absent in white wines by the lack of skin contact. The native varieties, Öküzgözü and Boğazkere wines were the richest in gallic acid and o-coumaric acid than the non-native varieties: Merlot, Syrah and Cabernet Sauvignon wines. To the contrary, the non-native wines had significantly higher (-)-epicatechin than Öküzgözü, Boğazkere and Kalecik Karası wines ($p<0.05$). Anli et al. (2006) have also reported that Syrah and Cabernet Sauvignon wines had higher (-)-epicatechin than Öküzgözü, Boğazkere and other native variety wines.

The procyanidin B_1, (-)-epicatechin and (+)-catechin concentrations of Merlot and Cabernet Sauvignon wines in this study were higher than those from Greece whereas they were lower for Syrah wines (Makris et al., 2006). The procyanidin B_1, (-)-epicatechin and caffeic acid concentrations of Syrah, Merlot and Cabernet Sauvignon wines were found greater than those from southern France. On the other hand, the gallic acid and (+)-catechin concentrations were similar (Landrault et al., 2001). The concentrations of caffeic acid, p-coumaric acid, (-)-epicatechin, (+)-catechin in Boğazkere, Öküzgözü and Kalecik Karası wines were higher than those reported by Özkan & Baydar (2006) except for Papazkarası wines. The resveratrol results of Öküzgözü and Kalecik Karası were in good agreement with the data observed by Porgalı & Büyüktuncel (2012) and Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez (2007). On the other hand they were lower than the results observed by Anli et al. (2006). The highest amounts were observed in Papazkarası, Öküzgözü and Syrah wines. Gurbuz et al. (2007) have also quantified the highest resveratrol in Öküzgözü wines among others (Merlot, Cabernet Sauvignon, Kalecik Karası, Boğazkere and Çalkarası).

In white wine samples, anthocyanins were not detected. Quercetin-3-galactoside was the most abundant flavonol in white wines. Jaitz et al. (2010) have found that the dominant flavonol was quercetin-type flavonols, and, quercetin-3-*O*-glucuronide was the main flavonol derivative in all of the white grape varieties of Spain. Sultaniye white wines were the poorest of all white wines in terms of polyphenol contents. Muscat white wines had significantly higher hydroxycinnamic acids and lower hydroxybenzoic acids (gallic and vanillic acids) than the other varieties ($p<0.05$). Narince white wines were rich in flavan-3-ol and Emir wines were rich in resveratrol. The flavan-3-ol contents of Narince and Emir wines were higher and resveratrol contents were lower than those

reported elsewhere (Gurbuz et al. (2007). It should be mentioned again that some of the native wines have been found with their higher content of bioactive phenolic compounds such as gallic acid and resveratrol. Boğazkere and Öküzgözü wines had higher gallic acid, and Öküzgözü and Emir wines had higher resveratrol contents that the other variety wines.

Table 5.6. The concentrations of malvidin compounds and its derivatives in red and rose wines (mg/L)

		mal3G	peo3G	pet3G	del3G	vitA	del3Ga	pet3Ga	peo3Ga	mal3Ga	del3Gc	pinA	mal3Gc	Tace	Tcoum	Tace/ Tcoum	vitA/pinA
Boğazkere	min	1.32	n.d.	0.23	0.24	0.76	n.d.	n.d.	0.06	0.15	n.d.	0.37	0.14	0.29	0.14	0.84	0.78
	max	69.03	8.57	15.12	12.96	4.61	5.64	6.17	5.97	15.48	6.36	5.00	16.78	32.81	23.14	2.59	2.67
	med	19.86	1.10	2.67	2.21	1.24	0.14	0.33	0.57	2.51	0.37	0.76	3.19	3.77	3.56	1.23	1.25
Cabernet	min	0.96	0.04	0.08	0.08	0.39	n.d.	n.d.	0.04	0.11	n.d.	0.21	0.07	0.28	0.07	3.73	0.66
Sauvignon	max	32.06	2.04	4.00	3.13	4.05	0.85	1.07	1.16	13.06	0.38	1.91	3.32	16.14	3.65	7.08	6.50
	med	19.90	0.91	1.71	1.37	1.37	0.26	0.44	0.55	8.09	0.15	0.77	1.17	9.73	1.37	4.88	1.84
Kalecik	min	1.07	0.06	0.23	0.09	0.33	n.d.	0.03	0.04	0.21	n.d.	0.41	0.10	0.28	0.10	1.39	0.11
Karası	max	48.15	2.64	4.28	2.79	1.10	0.78	1.07	1.70	16.45	0.87	2.89	11.37	19.74	12.24	4.12	1.68
	med	18.43	0.91	1.39	1.10	0.58	0.21	0.33	0.68	5.00	0.24	1.19	2.44	6.18	2.68	2.93	0.59
Çalkarası	single sample	14.53	1.36	1.54	1.01	0.32	0.28	0.39	0.81	4.45	0.20	0.31	2.70	5.94	2.90	2.05	1.02
Merlot	min	0.54	0.06	0.08	0.15	0.55	n.d.	n.d.	n.d.	n.d.	n.d.	0.19	0.06	0.04	0.06	0.12	0.90
	max	56.12	6.75	7.88	6.88	4.66	6.03	5.93	5.82	19.71	4.82	5.19	7.88	32.52	12.70	5.47	7.08
	med	16.81	2.41	3.04	2.83	1.28	0.60	0.74	1.11	5.36	0.36	0.60	1.37	7.78	1.64	2.75	1.69
Öküzgözü	min	4.50	0.34	0.93	0.79	0.78	0.07	0.14	n.d.	0.35	n.d.	0.27	0.36	0.56	0.40	0.85	0.60
	max	47.07	7.79	12.34	10.79	4.71	6.66	5.88	5.50	10.94	5.30	5.15	10.61	28.51	15.91	2.57	6.86
	med	30.26	1.93	5.23	4.05	1.26	0.39	0.69	0.70	4.37	0.63	0.99	3.99	6.36	4.53	1.57	1.36
Papazkarası	min	1.14	0.08	0.22	0.06	0.39	0.02	n.d.	0.04	0.11	n.d.	0.49	0.08	0.17	0.08	2.15	0.35
	max	16.65	2.42	2.29	1.93	0.94	0.29	0.42	0.73	2.75	0.22	1.12	1.73	4.19	1.95	4.28	1.60
	med	2.43	0.20	0.28	0.09	0.78	0.10	0.07	0.11	0.73	0.02	0.76	0.21	1.01	0.24	2.15	1.23
Syrah	min	10.62	1.10	1.83	1.27	1.23	0.09	n.d.	n.d.	n.d.	n.d.	0.31	0.90	0.09	1.17	0.05	0.43
	max	72.01	10.91	13.37	10.43	4.84	6.25	6.13	8.21	28.36	5.70	5.37	12.55	48.71	18.25	4.46	5.15
	med	33.70	2.13	3.39	2.26	1.69	0.45	0.84	1.16	9.81	0.36	1.23	2.78	12.42	3.12	3.30	1.02
Çalkarası	min	0.26	0.08	0.08	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
Rose	max	2.09	0.99	0.47	0.43	0.02	n.d.	n.d.	n.d.	0.03	n.d.	0.10	0.23	0.03	0.23	-	-
	med	0.83	0.21	0.14	0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05	0.13	n.d.	0.13	-	-
Ökzügözü	min	<lod	n.d.	0.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
Rose	max	0.17	0.02	1.21	n.d.	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04	n.d.	0.04	-	-
	med	0.17	0.01	0.88	n.d.	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02	n.d.	0.02	-	-

min: minimum, max: maximum, med: median, n.d.: not detected, <lod:below detection limit

Table 5.7. The concentrations of phenolic compounds in red and rose wines (mg/L)

		rutn	quer	myric	kaemp	Q3glucosi	Q3galact	Q3glucuron	myric3G	caffe	p-coum	ferul	tresv	gallic	DLcatec	vanill	(-)-epicat	o-coum	PB1
Boğazkere	min	1.30	0.75	0.35	0.38	0.98	0.11	3.62	0.39	11.69	1.64	0.73	0.18	16.95	12.79	5.33	5.69	0.46	19.10
	max	3.99	7.71	7.00	1.35	11.33	10.54	11.42	18.96	38.33	9.66	3.02	1.02	84.15	41.04	9.74	15.04	3.82	39.32
	med	3.07	3.21	2.34	0.61	5.32	2.46	8.71	9.87	19.16	6.26	1.43	0.24	51.79	32.31	6.38	10.44	3.00	31.98
Cabernet	min	0.78	1.14	0.80	0.03	0.18	2.12	0.69	0.00	4.19	3.75	0.63	0.17	38.56	17.07	4.48	8.16	0.84	9.20
Sauvignon	max	5.31	11.41	6.81	1.07	33.93	8.35	19.16	32.02	33.78	12.33	2.93	0.60	120.64	99.55	11.00	53.21	2.01	79.77
	med	3.37	5.05	4.46	0.67	9.37	5.05	9.46	10.67	10.40	7.87	1.19	0.33	44.73	40.67	6.15	23.58	1.37	41.06
Kalecik	min	0.92	0.48	0.04	<lod	1.94	1.42	4.33	2.58	7.05	2.92	0.73	0.13	13.69	12.41	5.12	5.38	0.57	8.16
Karası	max	3.92	10.05	6.08	2.73	45.84	11.24	17.91	39.56	37.84	11.08	1.71	0.56	45.51	48.45	10.75	28.40	1.64	50.90
	med	2.17	3.93	1.85	0.79	12.55	7.23	8.76	11.95	20.20	5.86	1.14	0.38	26.70	38.28	6.74	16.83	1.15	42.14
Çalkarası	single sample	0.98	3.49	1.10	1.03	8.70	7.82	5.10	7.83	4.98	2.02	0.63	0.14	20.11	28.81	5.98	10.84	0.80	28.57
Merlot	min	0.82	0.96	0.72	0.03	0.21	0.20	0.65	n.d.	4.20	2.68	0.56	0.15	23.47	28.28	4.08	17.73	0.23	22.06
	max	6.03	8.85	5.76	1.18	29.01	9.24	26.43	27.72	36.33	10.36	1.96	0.78	103.88	153.61	9.97	81.88	2.11	99.16
	med	2.52	5.28	3.33	0.59	16.84	4.11	11.53	12.38	9.94	6.84	1.00	0.41	49.15	49.84	6.59	31.48	1.36	43.14
Öküzgözü	min	2.57	1.20	1.04	0.53	3.19	1.56	3.24	5.49	8.19	3.19	0.49	0.21	45.56	28.53	4.64	7.20	0.25	27.06
	max	5.87	6.73	5.73	1.32	6.56	12.52	12.85	12.20	44.11	16.50	1.82	1.14	109.59	90.85	8.55	26.86	3.71	70.62
	med	3.90	3.64	2.18	0.69	4.67	2.34	9.52	9.86	13.04	4.89	1.23	0.48	61.52	51.29	5.92	14.39	2.12	43.95
Papazkarası	min	1.85	1.21	0.75	0.52	2.30	1.55	8.09	4.26	9.29	3.16	0.79	0.47	38.98	15.75	5.84	5.54	0.87	4.99
	max	3.47	15.68	6.73	0.98	10.33	6.36	11.19	13.04	22.63	6.78	1.34	1.52	51.33	43.78	7.38	25.81	1.60	43.08
	med	2.04	6.09	2.53	0.82	2.70	3.42	10.09	4.72	17.29	6.19	1.25	0.56	40.18	20.49	7.14	7.45	1.56	9.94
Syrah	min	0.94	3.15	2.40	0.56	2.19	2.17	9.92	10.83	4.61	2.17	0.31	0.17	18.56	30.65	4.15	18.59	0.59	24.22
	max	5.31	15.77	16.58	1.75	47.01	12.52	19.94	40.38	26.90	13.81	1.46	0.75	68.70	70.93	8.95	41.98	1.54	62.36
	med	3.09	7.87	4.59	1.00	28.62	8.29	14.52	30.24	17.32	8.27	1.06	0.48	36.96	37.96	7.02	23.33	1.02	31.78
Çalkarası	min	0.13	0.14	0.02	0.03	0.11	1.42	1.57	n.d.	3.11	1.11	0.72	0.16	10.43	17.90	2.58	6.18	1.14	10.18
Rose	max	0.62	1.65	0.14	0.43	1.91	2.45	5.02	0.43	6.11	2.80	1.44	1.01	36.13	25.29	4.71	14.22	2.42	19.78
	med	0.28	0.68	0.06	0.03	0.17	1.72	3.58	0.35	3.67	1.47	0.99	0.45	19.59	20.57	4.46	10.02	1.75	16.90
Ökzügözü	min	0.04	0.24	0.02	0.03	0.05	0.11	0.22	n.d.	0.73	0.26	0.15	<lod	6.12	3.23	0.82	1.14	0.42	1.99
Rose	max	0.12	0.32	0.14	0.03	0.06	0.20	0.64	0.07	2.65	0.51	0.49	0.08	11.42	4.14	0.95	3.18	1.29	4.76
	med	0.08	0.28	0.08	0.03	0.06	0.15	0.43	0.04	1.69	0.39	0.32	0.04	8.77	3.69	0.88	2.16	0.86	3.37

min: minimum, max: maximum, med: median, n.d.: not detected, <lod:below detection limit

Table 5.8. The concentrations of phenolic compounds in white wines (mg/L)

		rutn	quer	myric	kaemp	Q3glucosi	Q3galact	Q3glucuron	myric3G	caffe	p-coum	ferul	tresv	gallic	DLcatec	vanill	(-)-epicat	o-coum	PB1
Emir	min	<lod	<lod	<lod	<lod	<lod	<lod	0.11	n.d.	0.94	0.22	0.23	0.05	3.71	2.61	0.33	0.94	<lod	<lod
	max	1.26	2.81	2.56	0.92	1.39	12.11	4.97	0.21	8.32	4.65	1.12	0.71	15.10	9.63	1.96	4.90	2.88	6.46
	med	0.06	0.79	0.02	0.03	0.08	1.58	0.49	n.d.	4.39	1.99	0.66	0.18	9.77	5.74	1.30	2.20	1.48	4.06
Chardonnay	min	<lod	<lod	<lod	<lod	<lod	<lod	n.d.	n.d.	1.10	0.43	0.33	0.04	5.81	1.71	0.48	0.68	0.11	<lod
	max	0.79	3.01	1.21	1.04	2.17	17.11	2.53	0.30	5.96	3.06	0.93	0.14	17.20	12.76	1.69	6.09	0.99	7.98
	med	0.12	0.33	0.02	0.03	0.13	1.54	0.10	n.d.	2.94	1.52	0.68	0.07	10.02	5.04	0.94	2.23	0.19	3.84
Narince	min	<lod	0.57	<lod	<lod	<lod	<lod	n.d.	n.d.	2.65	1.05	0.50	0.03	5.63	2.61	0.70	1.40	0.14	<lod
	max	0.27	6.47	0.58	0.94	3.43	15.11	3.05	0.18	20.98	6.32	1.44	0.29	33.07	13.05	2.47	4.37	1.84	6.63
	med	0.11	1.65	0.02	0.03	0.04	0.80	0.90	n.d.	4.94	1.48	0.75	0.13	12.35	10.04	1.30	2.71	0.83	4.26
Muscat	min	<lod	<lod	<lod	<lod	<lod	<lod	n.d.	n.d.	1.90	2.00	0.63	<lod	<lod	2.19	0.06	0.85	0.16	<lod
	max	0.22	8.07	0.29	0.93	3.21	14.11	7.97	0.22	22.35	13.61	1.43	0.78	12.65	20.05	1.31	4.51	1.33	9.08
	med	0.12	0.87	0.02	0.03	0.05	1.64	0.33	0.00	15.09	9.38	0.98	0.12	7.95	5.44	0.67	1.84	0.34	2.04
Sultaniye	min	<lod	<lod	<lod	<lod	<lod	<lod	n.d.	n.d.	0.37	0.08	0.06	<lod	6.46	1.61	0.37	0.55	0.24	<lod
	max	0.25	0.99	0.38	0.10	2.18	1.72	0.61	0.11	1.99	1.19	0.70	0.08	17.21	4.46	1.06	2.22	0.62	5.29
	med	0.06	0.29	0.02	0.03	0.02	0.06	n.d.	n.d.	1.38	0.60	0.47	0.03	10.67	2.72	0.62	1.35	0.40	2.40

mal3G: malvidin-3-glucoside, peo3G: peonidin-3-glucoside, pet3G: petunidin-3-glucoside, del3G: delphinidin-3-glucoside, vitA: vitisin-A, del3Ga: delphinidin-3-glucoside acetate, pet3Ga: petunidin-3-glucoside acetate, peo3Ga: peonidin-3-glucoside acetate, mal3Ga: malvidin-3-glucoside acetate, del3Gc: delphinidin-3-glucoside coumarate, pinA: pinotin-A, mal3Gc: malvidin-3-glucoside coumarate, Tcoum: Total of coumaroylated malvidins, Tace: Total of acetylated malvidins, rutn: rutin, quer: quercetin, myric: myricetin, kaemp: kaempferol, Q3glucosi: Quercetin-3-glucoside, Q3galact: Quercetin-3-galactoside, Q3glucuron: Quercetin-3-glucuronide, myric3G: myricetin-3-glucoside, caffe: caffeic acid, p-coum: p-coumaric acid, ferul: ferulic acid, tresv: resveratrol, gallic: gallic acid, DLcatec: (+)-catechin, vanill: vanillic acid, (-)-epicat: (-)-epicatchin, o-coum: o-coumaric acid, PB1: Procyanidin B_1.
min: minimum, max: maximum, med: median, n.d.: not detected, <lod:below detection limit

5.3. Color Analysis via Spectrophotometer

A representative transmittance plot including red, rose and white wine samples is shown in Appendix D. The color parameters of red, rose and white wine samples are reported in Tables 5.9 and 5.10. According to the results, Cabernet Sauvignon and Syrah wines had significantly the highest blue% and they had the lowest lightness. Moreover, Syrah wines had significantly the lowest hue and the highest color density, color intensity and logarithmic color density which was followed by Cabernet Sauvignon wines ($p<0.05$). These two varieties, at the same time, had the lowest CIElab color parameters. Fanzone et al. (2012) related the high color intensity of Malbec and Cabernet Sauvignon wines of Argentina to their high blue% and red%, respectively.

Boğazkere, Öküzgözü and Syrah wines had significantly higher red% and tint and lower yellow% results than other varieties. Kalecik Karası wines had significantly the highest lightness and lowest color density, color intensity, logarithmic color density and blue% of all. Cabernet Sauvignon and Kalecik Karası wines were significantly high in yellow% and tint, and low in red% ($p<0.05$).

The chroma and blue% values of Cabernet Sauvignon wines were similar to those reported by Fanzone et al. (2012). On the other hand, the lightness, red/green chromaticity, red% and color intensity values were lower and the hue, yellow/blue chromaticity and yellow% values were higher than Cabernet Sauvignon wines from Argentina. Meanwhile, Malbec wines of Argentina showed some similarity in terms of lightness, red/green chromaticity, red% and yellow% to Boğazkere and Öküzgözü wines.

The color density values of Monastrell wines from Spain were similar to that of Kalecik Karası and Papazkarası wines and the tint values were also in good agreement (Gomez-Plaza, et al. 1999). The red%, yellow%, blue% and color intensity of Merlot wines were in good agreement with Merlot wines from southern Spain, while Syrah wines had higher color intensity and red% and lower yellow% than Syrah wines of southern Spain (Marquez et al., 2014).

Among the white wines, Muscat and Sultaniye wines showed opposite color characteristics. For instance, lightness, hue, tint and yellow% values were the highest for Muscat wines, whereas they were the lowest for Sultaniye wines. On the other hand, yellow/blue chromaticity, chroma, color density, color intensity, proportion of red coloration, logarithmic color density and red% values were the lowest for Muscat wines, and the highest for Sultaniye wines. This pattern may be related to the high and low levels of hydroxycinnamic acids in Muscat and Sultaniye wines, respectively. Besides, Chardonnay wines produced the lowest red/green chromaticity and blue%.

Table 5.9. Color parameters of red and rose wine samples

		L*	a*	b*	C*	H*	CD	T	CI	dA(%)	K-K	%R	%Y	%B
Boğazkere	min	9.97	38.53	16.95	42.10	23.75	3.49	0.58	3.85	63.66	0.54	47.28	32.95	8.95
	max	40.31	56.03	44.58	70.01	40.10	8.52	0.92	9.84	80.17	0.93	56.52	43.54	13.43
	med	21.68	53.55	34.38	64.23	32.96	6.41	0.75	7.12	74.44	0.81	51.69	37.81	10.07
Cabernet Sauvignon	min	2.35	15.81	4.05	16.33	14.38	3.69	0.76	4.05	52.19	0.57	40.37	36.81	8.92
	max	40.57	53.68	53.12	74.23	45.69	12.97	1.22	15.21	77.24	1.11	49.17	49.12	14.76
	med	18.06	47.01	30.97	56.29	33.15	7.78	0.92	8.69	65.88	0.89	46.22	42.66	10.74
Kalecik Karası	min	13.03	41.98	21.97	47.52	27.10	2.39	0.76	2.61	50.87	0.38	42.15	37.81	8.21
	max	55.23	60.20	50.74	75.71	47.89	7.91	1.18	9.07	74.66	0.90	51.22	49.64	12.76
	med	36.49	53.71	42.05	67.61	38.46	4.03	0.94	4.42	63.34	0.60	46.74	43.77	9.44
Çalkarası	single sample	53.44	43.87	24.09	50.05	28.77	2.11	0.88	2.34	66.37	0.32	47.81	42.18	10.01
Merlot	min	2.66	17.52	4.57	18.11	14.62	4.03	0.70	4.42	54.41	0.60	42.28	35.97	8.72
	max	37.81	55.02	51.58	75.42	43.14	11.89	1.14	14.00	77.42	1.08	51.18	48.14	15.06
	med	22.48	51.33	37.46	64.06	35.35	6.39	0.89	7.12	67.45	0.81	47.07	41.79	10.90
Öküzgözü	min	13.37	42.99	22.61	48.58	27.73	5.07	0.62	5.60	66.66	0.71	48.89	34.38	8.40
	max	30.82	58.31	48.28	75.70	39.62	7.95	0.84	9.10	78.79	0.90	55.79	41.68	12.60
	med	23.79	54.69	36.29	65.68	34.09	5.92	0.74	6.55	73.43	0.77	51.49	38.03	10.23
Papazkarası	min	30.14	47.61	45.18	65.63	38.82	2.95	0.87	3.24	55.54	0.47	43.40	42.12	9.00
	max	46.72	56.44	46.86	72.45	43.50	4.92	1.10	5.42	66.21	0.69	48.63	47.60	10.25
	med	34.98	51.40	45.43	69.55	42.35	4.07	0.98	4.53	62.38	0.61	45.37	44.38	9.25
Syrah	min	3.23	22.48	5.57	23.16	13.91	5.83	0.69	6.47	65.99	0.77	46.61	34.93	9.86
	max	25.65	54.37	42.11	68.78	37.75	14.11	0.89	16.56	79.98	1.15	52.03	42.37	14.81
	med	15.78	47.97	27.16	55.12	29.52	8.34	0.77	9.52	72.48	0.92	50.26	38.69	10.69
Çalkarası rose	min	78.04	4.98	8.48	9.84	59.60	0.24	1.62	0.25	9.39	-0.62	31.27	59.24	2.83
	max	95.03	18.70	45.20	48.92	72.81	1.22	2.01	1.30	23.15	0.08	37.07	62.75	6.34
	med	88.94	7.19	23.26	24.35	67.54	0.59	1.74	0.63	22.13	-0.23	34.14	60.10	6.03
Öküzgözü rose	min	89.93	7.71	9.92	12.85	44.82	0.36	1.23	0.40	41.88	-0.44	38.99	51.26	7.13
	max	91.16	9.98	10.28	14.07	53.13	0.40	1.36	0.44	46.96	-0.39	41.61	53.16	7.85
	med	90.55	8.85	10.10	13.46	48.98	0.38	1.30	0.42	44.42	-0.42	40.30	52.21	7.49

L: lightness, a*: red/green chromaticity, b*: yellow/blue chromaticity, C*: chroma, H*: hue, CD: color density, T: tint,
CI: color intensity, dA%: proportion of red coloration, K-K: logarithmic color density, R%: redness, Y%: yellowness, B%: blueness
min: minimum, max: maximum, med: median.

Table 5.10. Color parameters of white wine samples

		L*	a*	b*	C*	H*	CD	T	CI	dA(%)	K-K	%R	%Y	%B
Emir	min	94.77	-1.40	4.13	4.18	80.54	0.08	2.05	0.08	-173.75	-1.11	14.04	59.95	2.70
	max	99.10	1.67	10.68	10.72	101.23	0.26	5.80	0.29	16.02	-0.59	29.28	81.40	10.77
	med	98.24	-0.64	6.59	6.62	96.57	0.12	4.30	0.13	-102.70	-0.91	18.05	77.56	4.65
Chardonnay	min	96.93	-1.41	5.25	5.36	94.77	0.09	3.99	0.09	-253.49	-1.05	11.98	74.29	1.67
	max	99.24	-0.84	12.41	12.46	102.01	0.24	7.21	0.25	-80.37	-0.63	18.63	86.35	7.09
	med	98.30	-1.13	8.29	8.40	98.52	0.14	4.96	0.15	-133.00	-0.85	16.01	79.24	3.90
Narince	min	97.38	-1.42	4.41	4.46	91.83	0.08	3.62	0.08	-223.33	-1.10	12.93	74.60	1.72
	max	99.13	-0.29	9.56	9.62	100.70	0.18	6.60	0.19	-69.23	-0.74	20.63	85.34	6.35
	med	97.96	-0.84	8.21	8.24	96.80	0.15	4.48	0.16	-110.87	-0.83	17.42	78.03	4.73
Muscat	min	95.99	-1.27	3.51	3.58	91.55	0.06	3.58	0.06	-260.98	-1.20	11.88	73.85	1.16
	max	99.43	-0.45	16.70	16.71	102.95	0.28	7.32	0.30	-67.74	-0.55	20.81	86.96	8.46
	med	99.12	-0.84	5.02	5.14	100.34	0.09	5.49	0.09	-161.76	-1.07	14.83	80.95	4.35
Sultaniye	min	96.45	-1.88	6.77	6.78	89.66	0.13	3.34	0.14	-148.61	-0.87	15.19	70.91	3.75
	max	98.12	0.07	12.59	12.72	98.49	0.23	5.28	0.24	-48.57	-0.64	21.99	80.17	7.88
	med	97.50	-0.53	9.55	9.58	93.41	0.18	3.59	0.19	-67.43	-0.75	20.54	74.31	4.77

L: lightness, a*: red/green chromaticity, b*: yellow/blue chromaticity, C*: chroma, H*: hue, CD: color density, T: tint,
CI: color intensity, dA%: proportion of red coloration, K-K: logarithmic color density, R%: redness, Y%: yellowness, B%: blueness
min: minimum, max: maximum, med: median.

5.4. Organic Acid, Sugar, Alcohol Analyses via HPLC

Shikimic acid was not quantified due to the overlap of its peak with succinic acid. In the same way, sucrose was not quantified due to some unknown interfering peaks in the chromatogram. The limit of detection (LOD) and recovery values for red, rose and white wines are reported in Table 5.11. The correlation coefficients, retention times, analytical conditions of calibration models of HPLC instrument are reported in Appendix B.5. The chromatograms of a red and white wine sample are shown in Appendix Figures C.3 and C.4.

Table 5.11. Analytical conditions of organic acid, sugar, alcohol analysis in wine samples (mg/L)

Standard Name	LOD	Recovery of Red wines (%)	Recovery of Rose wines (%)	Recovery of White wines (%)
Glucose	267.12	91[a]	104[a]	101[a]
Fructose	263.27	94[a]	87[a]	112[a]
Glycerol	237.15	94[a]	93[a]	92[a]
Ethanol	%0.03	75[a]	75[a]	73[a]
Citric acid	0.59	77[a]	103[a]	100[a]
Tartaric acid	3.57	80[a]	95[a]	83[a]
Malic acid	4.58	76[a]	83[a]	90[a]
Pyruvic acid	1.08	98[a]	106[a]	105[a]
Succinic acid	0.88	83[a]	82[a]	110[a]
Lactic acid	3.35	86[a]	85[a]	88[a]
Acetic acid	1.84	75[a]	73[a]	71[a]

[a]1000 mg/L spike

The detection limits were calculated using the graphical approach method explained in section 5.2. The organic acid-sugar-alcohol concentrations of red and rose-white wine samples are reported in Tables 5.12 and 5.13. Çalkarası was the sole sweet red wine. Çalkarası rose and some of Muscat white wines were semi-sweet wines, thereby they contained higher glucose, fructose and lower glycerol levels than the dry wines. Öküzgözü wines were recognized with significantly higher glucose and glucose/glycerol ratio ($p<0.05$) than the other red wines. Meanwhile, the lowest glucose content was observed in Merlot wines. In terms of glycerol content, the highest levels were observed in Syrah red and Sultaniye white wines. Glucose, fructose and glycerol concentrations were in agreement with the literature (Castellari et al., 2000; Moro et al., 2007; Smeyers-Verbeke et al., 2009).

Table 5.12. The organic acid, sugar, alcohol concentrations in red and rose wines (mg/L)

		Glucose	Fructose	Glycerol	Ethanol (%)	Citric A.	Tartaric A.	Malic A.	Pyruvic A.	Succinic A.	Lactic A.	Acetic A.	Original Malic A.
	min	267	263	6062	10	36.9	1375	154	<LOD	1532	534	146	1151
Boğazkere	max	1897	2043	9637	13	127.5	3009	570	13.90	3285	1015	1322	1832
	med	631	333	8255	13	67.8	2185	227	1.08	2278	808	348	1375
Cabernet	min	275	263	3202	12	5.1	876	16	<LOD	2464	1217	212	1975
Sauvignon	max	827	4871	10717	14	88.7	1659	443	21.00	8478	3530	921	5272
	med	472	293	9061	13	73.5	1281	196	1.08	4339	1342	361	2407
Kalecik	min	267	263	3194	11	14.9	1486	122	<LOD	1850	995	265	1737
Karası	max	858	1685	10907	14	109.7	2794	453	9.28	5384	2979	1308	4672
	med	386	324	8936	13	35.2	1916	271	1.08	2640	1324	588	2167
Çalkarası	single sample	15687	31955	7150	10	100.5	2659	1527	21.32	3827	898	152	2864
	min	267	263	7269	12	0.6	1381	121	<LOD	1738	711	268	1349
Merlot	max	741	938	14199	15	127.6	2106	891	34.97	4832	1838	738	3166
	med	371	299	9031	14	68.4	1811	290	1.08	2646	1034	422	1842
	min	267	263	7657	12	13.9	1848	151	<LOD	1050	631	145	1091
Öküzgözü	max	2729	2299	9711	14	151.9	2552	346	19.52	2659	2129	796	3452
	med	765	324	8334	12	67.9	2106	245	1.08	1822	814	251	1493
	min	284	263	1777	11	12.5	1568	162	<LOD	1499	940	664	1682
Papazkarası	max	499	1176	9056	13	75.5	1818	282	1.08	3923	3396	1071	5294
	med	452	324	7925	13	31.2	1707	237	1.08	2569	1560	905	2485
	min	289	263	7438	11	14.2	1248	238	<LOD	1843	950	198	1686
Syrah	max	2236	6774	10989	15	255.0	2334	444	5.32	6792	2884	990	4739
	med	538	462	9150	13	123.3	1857	369	1.08	3573	1521	365	2634
Çalkarası	min	513	471	5030	11	73.3	2024	1077	1.078	86	450	178	1747
Rose	max	4192	19420	7229	14	131.9	3049	1820	1.078	5522	4135	581	7447
	med	574	1805	6096	13	82.8	2317	1290	1.078	4052	566	376	2147
Öküzgözü	min	365	263	6644	10	161.7	1699	449	1.078	986	1517	577	2708
Rose	max	404	263	7269	12	166.7	2285	473	3.413	1401	2139	806	3658
	med	385	263	6957	11	164.2	1992	461	2.245	1194	1828	692	3183

min: minimum, max: maximum, med: median, <lod: below detection limit

Table 5.13. The organic acid, sugar, alcohol concentrations in white wines (mg/L)

		Glucose	Fructose	Glycerol	Ethanol (%)	Citric A.	Tartaric A.	Malic A.	Pyruvic A.	Succinic A.	Lactic A.	Acetic A.	Original Malic A.
	min	271	263	4346	11	52.3	856	699	1.078	688	233	75	1354
Emir	max	3245	4116	6803	13	607.6	3420	2235	169.980	2012	2057	329	3762
	med	467	285	5688	12	145.0	2291	1448	1.078	1243	344	131	2064
	min	267	330	3839	9	20.9	1145	737	1.078	1267	341	114	1950
Chardonnay	max	1219	6003	7702	14	538.3	3301	4262	13.805	3501	1310	305	4957
	med	472	1418	6204	13	196.3	1923	2768	1.078	2085	493	204	3519
	min	267	263	4282	10	78.5	871	686	1.078	1153	330	41	2033
Narince	max	2729	2707	8016	13	740.0	2592	2589	45.951	2911	1878	305	3483
	med	421	337	5669	13	218.1	1598	1881	1.078	1649	475	179	2940
	min	267	292	3149	9	14.5	1555	451	1.078	666	246	82	1759
Muscat	max	16741	33056	7837	13	1074.4	3707	1884	22.707	1601	1214	231	2784
	med	5395	13435	5324	12	279.1	2559	1491	1.078	1043	480	97	2259
	min	337	263	2185	11	48.2	1275	315	1.078	635	275	8	1164
Sultaniye	max	576	1321	8134	14	387.8	3063	1254	1.078	2100	4444	1095	6931
	med	420	306	6480	13	144.5	1871	942	1.078	1100	308	233	1510

min: minimum, max: maximum, med: median.

Pyruvic acid was found at trace amounts for the majority of samples. Citric acid content for Muscat wines was greater than the other varieties which might be due to fortification of this wine with citric acid. Citric acid and acetic acid results were similar to the data in the literature. However succinic acid results were greater than those reported by Calull, Mar, & Borrull (1992) and Ding et al. (1995). This overestimation of succinic acid may be due to the overlap of its signal with shikimic acid. Tartaric acid content was significantly low in Cabernet Sauvignon red, and Chardonnay and Narince white wines ($p<0.05$). It was the highest in Boğazkere and Öküzgözü red wines, and Muscat white wines. The malic acid, original malic acid and lactic acid contents were the highest in Chardonnay wines and the lowest in Sultaniye white wines, while they were the highest in Syrah red wines. Boğazkere and Öküzgözü wines had significantly the lowest lactic acid content of all ($p<0.05$). Tartaric acid, original malic acid and malic acid and the minimum and maximum range of lactic acid results were consistent with the data by Smeyers-Verbeke et al. (2009). Citric acid, tartaric acid, malic acid and acetic acid results were also in good agreement with Ding et al. (1995).

5.5. Total Phenol Content and Chemical Analyses

The total phenol, total acidity, pH and refractive index measurements are reported in Table 5.14. The calibration curves of total phenol content analysis are presented in Appendix E. The lowest pH values were observed in Boğazkere and Öküzgözü red wines, and in Muscat and Emir white wines; the high pH values were observed in Cabernet Sauvignon, Kalecik Karası and Chardonnay wines. The highest total phenol content was observed in Boğazkere red and Narince white wines. The Cabernet Sauvignon wines were higher in total phenol content than the Merlot and Kalecik Karası wines, similar to the study of Anli & Vural (2009). Porgalı & Büyüktuncel (2012) have reported that large differences were observed in total phenol content between wines from different countries which was likely to be the result of different grape varieties, geographic regions and winemaking techniques. They have also found out that the total content of polyphenols and antioxidant activity were much higher in Buzbağı wines of Boğazkere-Öküzgözü grapes than other variety wines (Kalecik Karası, Karaoğlan and Öküzgözü-Kalecik Karası, Öküzgözü-Karaoğlan). The refractive index values were higher in the wines of non-native grapes (Cabernet

Sauvignon, Merlot and Syrah). The results were consistent with the data in literature (Kallithraka et al., 2001; Kelebek et al., 2010).

Table 5.14. The results of chemical analyses of wine samples

		Total Phenol (mg/L)	Refractive Index (brix)	pH	Total Acidity (mg tartaric acid/L)
Boğazkere	min	2370	6.68	3.31	3.64
	max	3904	8.52	3.74	4.99
	med	2990	7.69	3.46	4.58
Cabernet Sauvignon	min	2284	6.92	3.51	3.64
	max	3569	9.19	4.08	6.04
	med	2590	7.86	3.73	4.13
Kalecik Karası	min	1269	6.69	3.33	3.56
	max	2487	8.44	3.93	5.18
	med	1819	7.72	3.71	4.20
Çalkarası	single sample	1476	10.87	3.51	4.95
Merlot	min	1646	7.08	3.31	3.15
	max	3579	8.67	3.85	4.65
	med	2439	8.11	3.67	4.20
Öküzgözü	min	926	6.92	3.33	3.79
	max	2849	8.00	3.70	4.84
	med	1988	7.45	3.45	4.56
Papazkarası	min	1765	7.02	3.45	4.61
	max	2651	7.60	3.51	6.60
	med	1843	7.13	3.50	5.10
Syrah	min	1962	7.58	3.49	2.21
	max	3150	9.05	3.98	5.81
	med	2214	8.17	3.65	4.88
Çalkarası Rose	min	693	6.99	3.12	3.94
	max	1017	8.37	3.37	5.55
	med	715	7.08	3.24	5.12
Öküzgözü Rose	min	292	6.13	3.41	3.69
	max	303	6.28	3.73	4.50
	med	297	6.20	3.57	4.10
Emir	min	238	5.09	2.97	3.53
	max	527	6.67	3.95	5.63
	med	306	6.11	3.21	4.39
Chardonnay	min	194	6.14	3.03	2.96
	max	414	7.34	3.62	6.90
	med	288	6.67	3.43	5.34
Narince	min	236	5.23	3.06	3.56
	max	416	6.73	3.83	5.59
	med	345	6.41	3.37	4.24
Muscat	min	264	6.51	2.96	3.45
	max	417	9.27	3.43	6.04
	med	317	8.37	3.20	5.10
Sultaniye	min	178	6.35	3.06	2.40
	max	317	6.95	4.01	6.00
	med	212	6.45	3.40	4.29

min: minimum, max: maximum, med: median.

5.6. Climate Parameters of Four Harvest Years

Meteorological data including rainfall, temperature and hours of sunshine-exposure have been collected from Meteorological Service of Turkish State for the four harvest years (Table 5.15) (www.dmi.gov.tr). The highest temperature, sunshine-exposure and rainfall values were recorded in İzmir region. The average temperatures of four harvest years of wine producing regions indicated that the İzmir, Manisa and Denizli were the hottest, while Tokat and Nevşehir were the coldest regions. The average of sunshine-exposure values for the four harvest years was high for Denizli and İzmir. Although the temperatures were not as high as in İzmir, the sunshine-exposure in Diyarbakır, Elazığ, Çanakkale and Nevşehir regions are also high. It was the lowest in Tekirdağ and Manisa. In terms of rainfall, the western regions were wet (Denizli, İzmir, Manisa, Tekirdağ, Çanakkale) while Nevşehir was the driest. Moreover, 2009 vintage was recognized with its highest rainfall amounts for most of the regions.

The climate data from April to September was reported to be the interval of berry growth. Within this interval, the chemical composition of berry develops and the berry maturates. For this reason, the climate data for this period was reported in detail in Table 5.16. According to these climate parameters, Kapadokya was recognized with its low rainfall and temperature unlike the İzmir region, but has sunshine values as high as in İzmir. Tekirdağ region was recognized with its high rainfall and low sunshine and temperature values.

Table 5.15. The climate parameters of four harvest years

	Harvest Years	Ankara	Tokat	Nevşehir	Çanakkale	Tekirdağ	İzmir	Denizli	Manisa	Diyarbakır	Elazığ
Average Temperature (°C)	2006	12.2	12.5	11.2	14.7	14.0	17.9	16.4	16.9	15.9	13.9
	2007	14.0	13.5	11.5	16.7	14.6	18.8	17.7	17.7	15.2	13.4
	2008	12.8	12.0	11.0	15.6	14.9	18.9	17.6	17.7	15.9	13.4
	2009	13.0	13.1	11.5	15.9	14.9	18.7	17.4	17.6	16.2	13.6
	Average of Four Harvest Years	13.0	12.8	11.3	15.7	14.6	18.6	17.3	17.4	15.8	13.6
Average of Total Daily Sunshine Exposure (hr)	2006	7.0	6.3	7.2	6.9	6.1	7.9	6.9	6.5	7.5	7.8
	2007	6.4	7.0	7.5	7.5	6.3	8.1	7.4	4.2	7.1	7.4
	2008	5.8	6.2	7.6	7.3	4.9	7.9	7.3	6.7	7.7	7.4
	2009	5.8	6.1	7.0	6.7	5.7	7.7	6.8	6.1	-	6.9
	Average of Four Harvest Years	6.3	6.4	7.3	7.1	5.8	7.9	7.1	5.9	7.4	7.4
Total Rainfall (mm)	2006	372	377	25.9	483	492	745	511	630	593	393
	2007	305	426	40.3	588	547	487	534	479	397	324
	2008	323	471	27	344	338	427	323	406	371	315
	2009	460	593	45.3	686	815	1072	801	970	457	431
	Total of Four Harvest Years	1460	1867	34.6	2100	2191	2732	2169	2484	1818	1463

Table 5.16. The climate parameters during the berry growth period

		Average Temperature/Month (°C) (T)						Average of Total Daily Sunshine-Exposure/Month (hr) (S)						Total Rainfall/Month (mm) (R)					
Region	Year	T4	T5	T6	T7	T8	T9	S4	S5	S6	S7	S8	S9	R4	R5	R6	R7	R8	R9
Ankara	2006	13.0	16.5	21.6	23.1	27.2	18.1	8.0	8.7	10.8	11.7	11.5	7.6	29.4	29.5	31.8	2.2	0.1	78.3
	2007	9.1	20.5	22.6	26.8	26.4	20.9	6.6	8.5	8.6	10.1	8.9	8.0	23.8	17.9	31.7	3.9	9.8	0.0
	2008	13.8	15.5	22.1	24.9	26.7	19.9	3.3	7.2	9.9	11.0	10.4	7.3	32.7	45.4	10.3	0.0	0.7	61.6
	2009	11.1	15.9	22.0	23.6	23.3	18.3	6.5	7.4	9.5	9.0	10.2	7.7	71.0	24.8	28.0	13.9	0.4	10.3
Tokat	2006	13.1	15.9	21.6	20.9	26.3	19.0	6.3	7.3	10.0	7.8	11.0	8.0	48.5	91.4	5.8	0.0	0.0	15.8
	2007	9.4	20.3	21.7	24.1	25.0	20.8	7.3	9.1	10.1	10.3	10.0	9.7	43.2	31.7	33.8	0.2	0.1	38.5
	2008	14.7	15.1	19.5	23.1	23.9	19.2	5.3	8.4	9.2	10.1	8.3	7.4	51.6	34.2	53.7	0.0	13.3	52.7
	2009	11.2	15.5	21.3	22.4	20.6	17.7	7.7	7.9	9.0	8.3	10.6	7.3	45.5	60.1	20.0	73.9	0.5	29.2
Nevşehir	2006	11.4	15.3	21.1	20.9	25.9	16.8	6.5	9.4	11.6	12.0	11.5	8.9	70.2	29.6	5.2	0.7	0.8	17.1
	2007	6.5	18.1	20.7	23.9	23.7	18.0	6.9	9.2	11.4	12.4	11.3	10.1	73.5	89.4	46.2	0.4	14.2	3.1
	2008	13.6	14.1	19.7	23.0	23.4	18.5	6.8	9.3	12.0	13.3	11.7	8.4	22.0	46.1	15.2	0.0	1.1	31.2
	2009	9.0	13.9	20.3	21.6	20.5	15.8	7.2	9.9	11.6	10.9	12.5	8.2	51.5	63.3	25.7	52.7	0.0	12.2
Çanakkale	2006	13.1	17.6	22.1	24.8	26.4	21.2	6.9	10.6	10.4	11.4	11.5	7.4	3.8	16.7	23.0	8.2	1.2	70.6
	2007	12.4	18.7	24.5	27.0	26.3	21.0	10.1	8.6	10.8	12.3	11.6	9.0	18.1	44.7	35.2	0.0	0.1	3.2
	2008	13.8	17.8	23.5	25.8	26.2	20.6	4.9	10.5	12.2	12.6	11.0	7.1	48.0	0.2	6.3	0.6	34.1	32.2
	2009	12.2	18.4	22.7	26.4	25.3	20.6	6.6	10.0	11.0	12.0	11.1	7.0	40.3	17.9	16.1	1.2	0.0	39.8
Tekirdağ	2006	12.4	17.2	21.6	23.8	25.8	20.2	6.8	9.2	9.5	9.9	10.0	6.5	9.5	14.1	29.0	4.0	10.8	108.9
	2007	10.8	18.3	24.0	25.5	25.6	-	8.2	8.0	9.9	11.0	9.0	7.1	17.4	45.9	9.1	0.0	3.1	33.1
	2008	14.0	17.2	22.4	24.4	25.4	20.0	3.1	6.9	7.3	8.3	8.4	3.3	20.1	18.9	42.8	12.0	1.2	29.5
	2009	11.5	17.5	22.0	25.1	24.1	19.8	6.7	8.3	9.5	10.0	9.8	6.1	32.2	13.4	11.5	66.3	0.0	132.8

4: April, 5: May, 6: June, 7: July, 8: August, 9: September

(cont. on next page)

Table 5.16. (Cont.)

		Average Temperature/Month (°C) (T)						Average of Total Daily Sunshine-Exposure/Month (hr) (S)						Total Rainfall/Month (mm) (R)					
Region	Year	T4	T5	T6	T7	T8	T9	S4	S5	S6	S7	S8	S9	R4	R5	R6	R7	R8	R9
İzmir	2006	17.3	21.0	25.6	28.0	29.2	23.8	7.0	10.7	11.2	11.9	11.6	9.4	29.4	0.2	10.0	0.0	0.0	167.2
	2007	16.1	22.4	27.4	30.0	29.1	24.4	9.3	9.2	10.6	12.3	11.6	10.2	19.3	44.1	0.3	0.0	0.0	0.0
	2008	18.0	21.0	26.9	28.6	29.2	23.9	5.9	10.8	11.7	12.2	11.0	8.6	62.3	4.9	0.4	0.0	0.0	55.0
	2009	16.0	21.4	26.2	29.0	27.8	23.2	7.0	10.4	11.5	12.1	12.1	9.3	83.8	44.3	9.2	0.0	0.0	51.2
Denizli	2006	15.8	20.0	24.6	27.6	29.5	22.8	6.1	9.6	10.9	11.3	10.9	7.7	38.6	40.4	45.0	20.0	0.0	15.7
	2007	14.4	22.5	27.2	30.7	29.7	24.1	7.5	9.5	10.4	11.8	10.8	9.3	38.5	16.6	13.7	1.6	3.4	14.6
	2008	16.4	20.7	27.2	29.3	30.1	23.5	6.2	10.1	11.7	11.9	10.1	7.8	47.9	0.1	2.5	6.0	17.4	19.3
	2009	15.2	20.3	26.4	28.9	28.2	22.6	6.6	9.9	11.1	11.7	10.6	7.9	57.8	65.6	3.2	0.0	0.3	14.5
Manisa	2006	16.8	21.3	25.7	28.0	29.8	23.7	6.1	9.8	10.2	11.1	10.8	8.1	35.7	3.1	31.2	5.3	0.0	56.5
	2007		20.8	27.0	29.5	29.5	23.9	-	1.4	6.0	4.7	9.0	5.3	18.6	37.2	26.6	0.0	0.0	0.0
	2008	16.6	20.7	26.9	28.8	29.7	23.4	5.3	8.9	10.6	11.0	10.3	7.8	40.8	9.8	15.8	0.0	0.4	45.4
	2009	15.4	21.1	26.3	28.9	27.8	22.8	6.2	9.4	10.4	10.7	10.6	7.7	68.2	25.6	7.2	0.0	0.0	36.2
Diyarbakır	2006	14.5	19.4	28.4	31.3	32.6	25.0	5.3	10.5	12.0	12.0	10.4	9.1	77.9	38.4	0.0	6.1	0.0	3.5
	2007	10.3	20.6	27.2	31.8	31.4	25.1	5.1	6.5	11.5	11.7	10.2	10.1	88.2	45.5	19.5	0.0	0.2	0.0
	2008	16.8	18.6	27.3	31.7	31.9	24.0	6.3	9.7	12.4	12.3	9.6	7.8	19.0	34.9	2.2	0.0	2.0	68.2
	2009	12.9	18.7	26.5	30.5	28.3	22.8	-	-	-	-	-	-	43.7	9.1	25.8	1.4	0.0	25.2
Elazığ	2006	13.0	17.5	26.3	27.4	29.5	21.3	5.8	10.5	12.3	11.8	10.7	10.2	72.4	29.1	1.8	0.1	1.6	5.1
	2007	8.4	20.0	24.0	28.0	27.7	22.8	6.2	8.5	11.8	12.2	11.2	10.6	77.1	24.7	6.0	0.8	2.4	0.0
	2008	15.3	16.5	23.2	28.2	28.5	21.8	6.4	9.5	12.2	12.7	9.8	9.0	11.0	35.4	22.0	0.0	0.2	30.8
	2009	11.1	17.1	24.3	26.3	25.0	19.6	7.6	10.2	9.7	11.6	11.8	9.7	48.8	15.0	5.5	16.3	0.3	31.7

4: April, 5: May, 6: June, 7: July, 8: August, 9: September

5.7. Statistical Analyses

In this section, the potential of the chemical parameters as means of discriminating the monovarietal wines was investigated. The red and white wines were evaluated separately due to colorimetric, chromatographic and spectrometric differences. Rose wines were excluded from the data set except for the models produced by the mineral data. The statistical analyses for some samples having values below the limit of detection were performed by assigning the corresponding limit of detection value. Data sets were standardized by subtracting the averages and dividing them to the standard deviations. Transformation to normality was employed on the variables to minimize skewness if necessary. The results obtained from the unsupervised statistical techniques (PCA and HCA) were followed by the results of supervised techniques (PLS-DA and SIMCA). The Pearson correlation coefficients are given in Appendix Tables F.1 and F.2 (only the highly correlated data are presented).

5.7.1. Unsupervised Techniques

5.7.1.1. Principal Component Analysis (PCA)

The discrimination of red and white wines was investigated through PCA using the variables: elements, polyphenols, color parameters, organic acid and sugar contents. 65 red and 43 white wines were employed in the models. The red wine model produced by the elemental variables, included 65 red and 5 rose wines due to the similarity of these two types in terms of element content. Generally in all models, it was observed that some of the red and white wine samples from particular wine producing companies were always outliers and their clustering was not based on varietal, regional or harvest year distribution. Therefore, the following wine samples were excluded from the models: B6C14, K6D14, S8I14, P6L14, C6I2, M6I2, C8F16, M8F16, K8A12, O8E12, P6T11, M6T13, C8T13, M8T13, B7Z15, U6I14, U7I14, N8R14, E6K14, T6I2, T6T9. In the PCA score plots, the colors were given for each grape variety, while the letters were in the order of grape variety-vintage-geographic region. This was performed to clearly observe the effects of grape variety, geographic region and vintage on the discrimination. According to Table 5.17, the PCA models with the highest R^2_{pred} values

were obtained with the color parameters for both red and white wines, while the element variables produced the lowest values.

Table 5.17. PCA model parameters of red and white wines

	Variables	# of Principal Component	R^2	R^2_{pred}
RED WINES	Elements	2	0.410	0.089
	Polyphenols	11	0.935	0.520
	Color Parameters	5	0.995	0.971
	Organic acid-Sugar	2	0.435	0.145
WHITE WINES	Elements	2	0.348	<0
	Polyphenols	2	0.427	0.134
	Color Parameters	4	0.993	0.968
	Organic acid-Sugar	2	0.488	0.102

Discrimination with element compositions: In the literature, the direct relation between soil and wine element concentrations does not exist. This implies the complex soil-plant interaction and the influence of environmental, climatic and process applications in viticulture and wine making (Serapinas et al., 2008). Nevertheless, the mineral content has been widely used to assess the geographic origin of wine rather than discrimination based on grape variety or harvest year (Fabani et al., 2010; Martin et al., 2012; Saavedra et al., 2011). Zou et al. (2012) reported that the differences in multielement composition of Chinese wines were mainly due to the differences in soil geochemistry and the remaining variation could be based on the differences in grape physiology, vine variety, vineyard management (application of fertilizers, pesticides, fungicides) and winemaking practices (fermentation, aging, fining etc.). The climatic changes may also influence either directly affecting the use of fungicides or indirectly by increased salinity in the arid regions (Mira de Orduña, 2010).

In the score plot of red and rose wines, Öküzgözü and Boğazkere red wines, and Çalkarası rose wines were clustered together due to their higher Ca and lower B levels (Figure 5.1A). Öküzgözü and Boğazkere wines were also recognized with their low Cu contents. The majority of Boğazkere and Öküzgözü red wines were from the eastern regions (Diyarbakır and Elazığ). There were only 8 from Tokat, Kapadokya and Diyarbakır-Denizli regions. Despite of the regional differences, these two native varieties were clustered together. The possible reasons might be the influence of genetic properties of these two native grape varieties or the influence of blending of these two varieties with each other. It is well-known that they are commonly used as blends. Another reason might be the close geographic origin of these two native varieties. To be

certain, whether the difference of these two native varieties comes from geographic origin or cultivar, Boğazkere and Öküzgözü wine samples from different regions should be higher in number. Limited number of samples made it difficult to fully evaluate the effect of grape variety and vineyard location. However, the score plot indicated that these two native varieties of mainly eastern origin discriminated themselves from the other regions. The mineral profile of Çalkarası rose wines resembled Öküzgözü and Boğazkere red wines even though they were from Denizli region. Below the horizontal axis, the other variety wines were located without showing any particular discrimination. In the study of Villagra et al., (2012), a clear differentiation could not be observed for Cabernet Sauvignon, Syrah and Carménere red wines, as well as Chardonnay and Sauvignon Blanc white wines. They commented that, although commercial wines were labeled under a specific variety name, they could be blended with one or more of other variety wines of similar aroma at a rate of maximum 25% in Chile.

In this study, the data on the wine bottles were the basis of the information for the wine samples. Only one of the wine producing companies demonstrated on the wine label that their wine products were blended by 15% with other variety wines (Boğazkere wine blended with Kalecik Karası and Cabernet Sauvignon, and Cabernet Sauvignon wine blended with Syrah and Boğazkere, and Öküzgözü wine blended with Cabernet Sauvignon). Moreover, 5 of the wine samples did not contain the geographic origin of grape variety on the labels. This implied that grapes of a single variety coming from different geographic regions were blended during the process. It is also necessary to emphasize that these commercial wine samples were produced under different process conditions. The expected variability in their chemical composition due to different vineyards, vintages or cultivars might also be influenced from different winemaking processes. Despite of these various sources of variations, the wines of some varieties and some geographical origins separated themselves from the others. In the loadings plot, the upper left-hand corner was occupied with Fe, Co, Cr, Al, Sr and Li. The Pearson coefficients indicated significant correlations among them: Fe-Co (0.62), Fe-Al (0.58), Cr-Al (0.62), Co-Al (0.61), Li-Sr (0.73), Sr-Ba (0.61) ($p<0.05$).

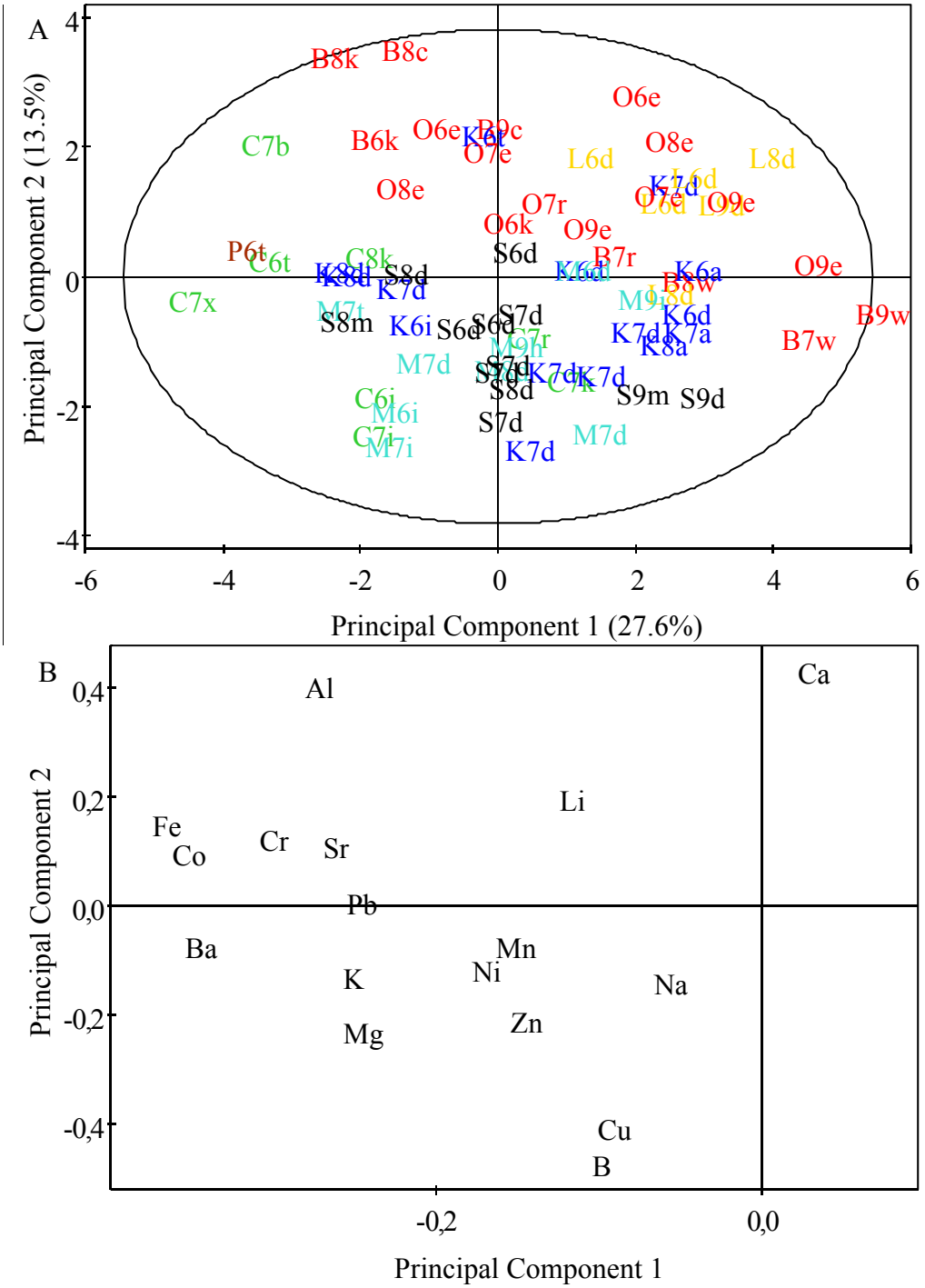

Figure 5.1. PCA score (A) and loading (B) plots of red and rose wines based on mineral content: PC1 vs PC2. Coloring: **Boğazkere**, **Öküzgözü**, **Çalkarası**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Papazkarası**, **Syrah**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, b: Bozcaada, a: Ankara, k: Kapadokya, r: Tokat, e: Elazığ, c: Diyarbakır, h: Denizli-Tekirdağ-İzmir, x: Denizli-Ankara, w: Denizli-Diyarbakır

The score plot of white wines demonstrated the discrimination between Muscat and Emir wines (Figure 5.2A). The discrimination was based on the higher Li, Sr and lower Cu contents of Emir wines, and the higher Pb, Co and Mn levels of Muscat wines (Figure 5.2A). Emir is a native grape variety of Kapadokya region and all Emir wines in this study were from this region. For the discrimination of Emir wines, the high Li, Sr and low Cu results might be influenced either by the Emir cultivar or the soil characteristics of Kapadokya region, or the influence of both. It should be noted here that the Chardonnay wines from Denizli, İzmir and Manisa regions were recognized with their low Li and Sr levels except one from Kapadokya. This sample was close to the Emir variety of Kapadokya cluster with its high Li and Sr contents. Cabaroglu et al. (1997) reported that the Kapadokya region was mainly formed from lime-rich volcanic ashes and has a tuffaceous character. Sr element is highly related to Ca and the high concentrations is an indication of calcareous rocks (lime-rich soil) (weppi.gtk.fi). From the loadings plot, the close location of Sr and Ca elements confirms this information (Figure 5.2B). On the other hand, Muscat wines which the grapes are widely grown in West Anatolia were from the Denizli, İzmir and Manisa regions. Among the different cultivars (Narince, Chardonnay, Sultaniye and Muscat) from West Anatolia (Denizli, İzmir and Manisa), Muscat wines had the highest Pb, Co and Mn levels. On the upper right-hand side of the score plot, the Manisa region wines of Muscat, Narince and Sultaniye varieties were clustered due to their high Li contents. The so-called natural minerals that do not depend on agricultural and processing activities such as Li and Sr played an important role on the regional discrimination of white wine samples. In the loadings plot, Sr and Li were close to each other indicating their relationship with each other. The Pearson coefficients indicated significant correlations between Li-Sr (0.63) and Co-Al (0.69), Cr-Al (0.59), Co-Ba (0.67) ($p<0.05$). For both red and white wines, Li and Sr were highly correlated to each other. Moreover, they were negatively correlated to the temperature parameters of April, August and September (correlation coefficients> 0.59 with $p<0.05$). Co and Al were significantly correlated to the total rainfall in September ($p<0.05$).

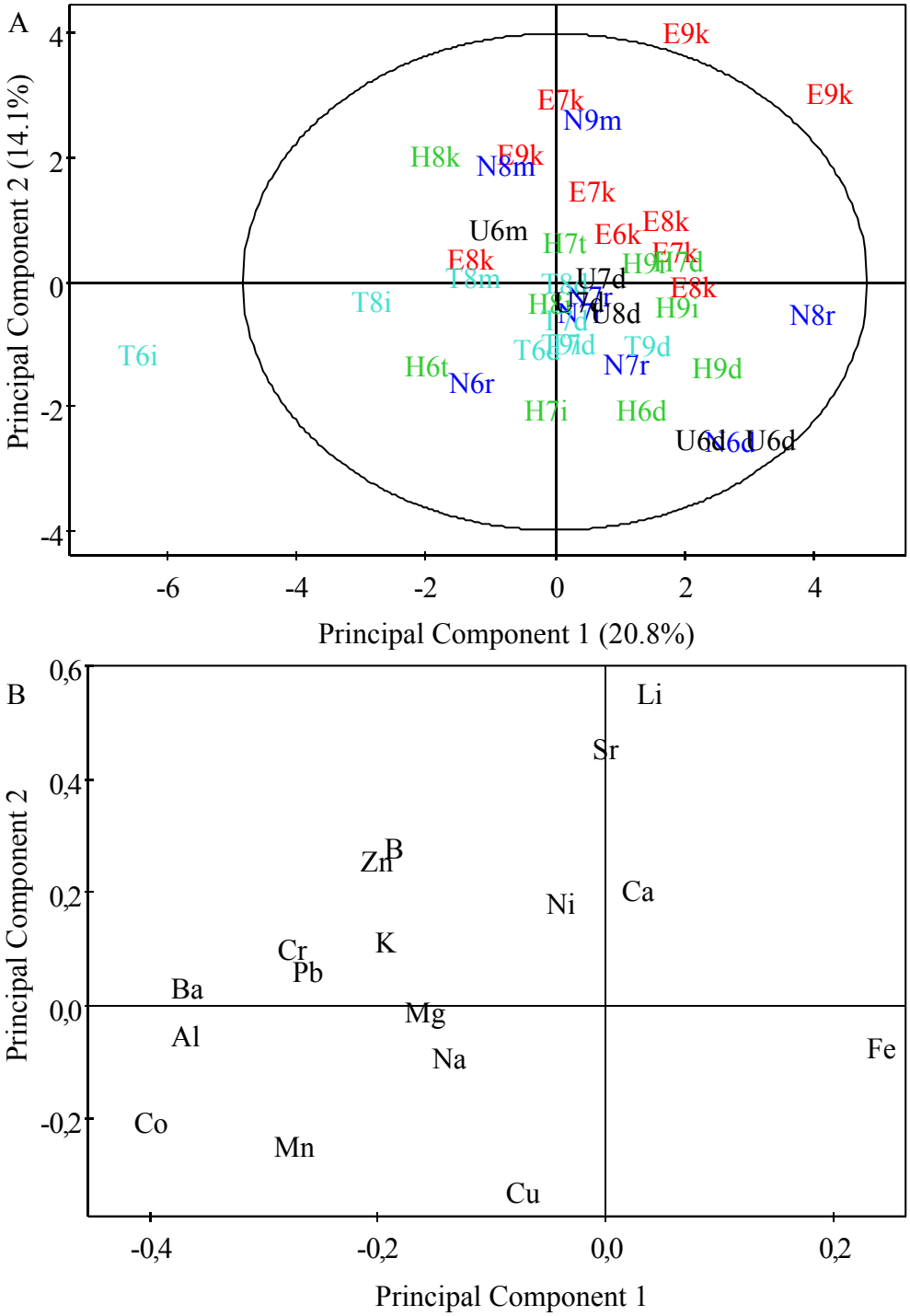

Figure 5.2. PCA score (A) and loading (B) plots of white wines based on mineral content: PC1 vs PC2. Coloring: **Emir**, **Chardonnay**, **Narince**, **Muscat**, **Sultaniye**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, k: Kapadokya, r: Tokat

Discrimination with polyphenol compositions: In the literature, the polyphenol content has been used to assess the geographic origin, vintage and cultivar of wine (Castillo-Muñoz et al., 2010; de Andrade et al., 2013; Jaitz et al., 2010; Li et al., 2011; Makris et al., 2006; Rastija, Srečnik, & Marica Medić, 2009). The polyphenol composition of a cultivar indicates its genetic potential due to the enzymatic reactions involved in the biosynthesis. The enzymatic activity depends on the environmental factors, such as sun-exposure, temperature water deficiency of the plant, degree of grape ripeness, berry size or vegetative vigour of the plant, varying at different geographical regions. Therefore, the polyphenol composition of wines even from the same cultivars may vary based on their geographic regions or vice versa. The ageing of wine and technological influences are other factors that could alter the polyphenol composition (Ivanova et al., 2011; Makris et al., 2006; Montealegre et al., 2006). Fanzone et al. (2012) have also reported that fungal infections in addition to grape variety, winemaking procedures and weather conditions could explain the differences in the polyphenol concentrations.

According to the score plot of red wines 2009 vintage wines were predominantly discriminated from the other vintages due to their high flavonol (kaempferol, myricetin, quercetin) and vitisin-A, pinotin-A and anthocyanin contents (Figure 5.3). Moreover, it was the only vintage with vitA/pinA ratio lower than 1.0. The high anthocyanin contents of 2009 vintage red wines might be based on the increased biosynthesis of anthocyanins due to the water deficiency during the veraison period (August). The anthocyanins along with the flavonols were synthesized via the phenylpropanoid pathway during the veraison period. In addition, it was reported that the vine water stress significantly affected the berry development and composition. The more the vine water stress, the higher the concentrations of berry sugar and anthocyanin (van Leeuwen et al., 2004). Although the highest precipitation before flowering (March and April) was observed in 2009, it was the minimum during veraison (1.68 mm in August) among other years. The precipitation amounts of 2006, 2007 and 2008 during veraison were 5.04 mm, 3.85 mm and 7.14 mm, respectively.

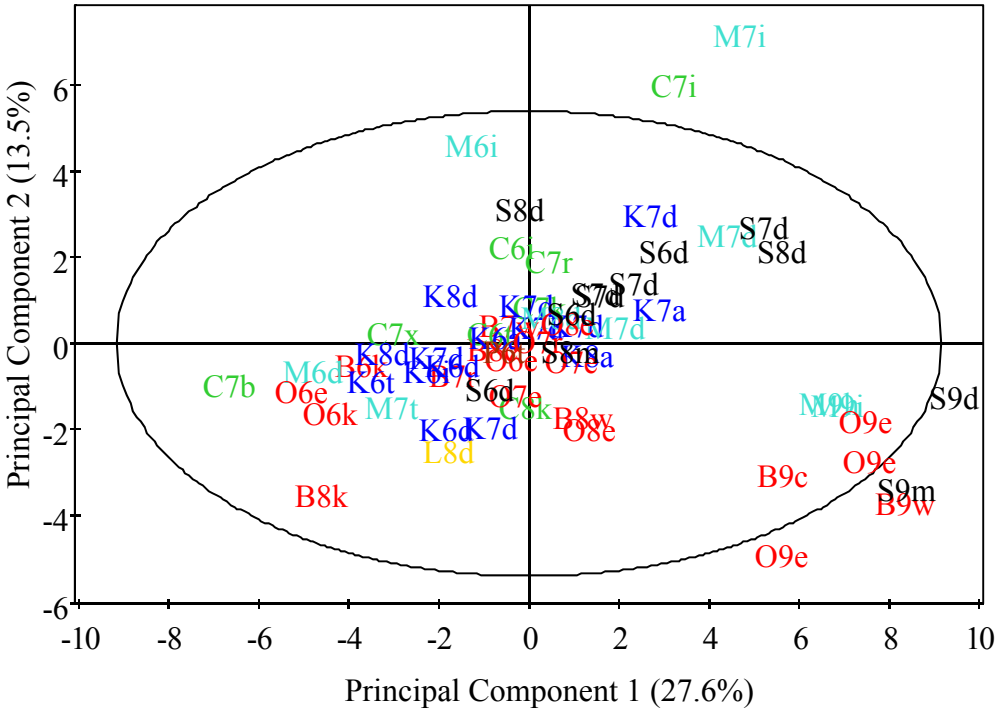

Figure 5.3. PCA score plot of red wines based on polyphenol contents: PC1 vs PC2. Coloring: **Boğazkere**, **Öküzgözü**, **Çalkarası**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Papazkarası**, **Syrah**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, b: Bozcaada, a: Ankara, k: Kapadokya, r: Tokat, e: Elazığ, c: Diyarbakır, h: Denizli-Tekirdağ-İzmir, x: Denizli-Ankara, w: Denizli-Diyarbakır

In the loading plot, the clusters of anthocyanins, flavan-3-ols, flavonols and flavonol glycosides can be observed (Figure 5.4). Some degrees of correlations were found between malvidin-3-glucoside and rutin (0.60), malvidin-3-glucoside acetate and quercetin-3-glucoside (0.58), procyanidin B_1 and quercetin-3-glucoside (0.59) and procyanidin B_1 and vanillic acid (0.60).

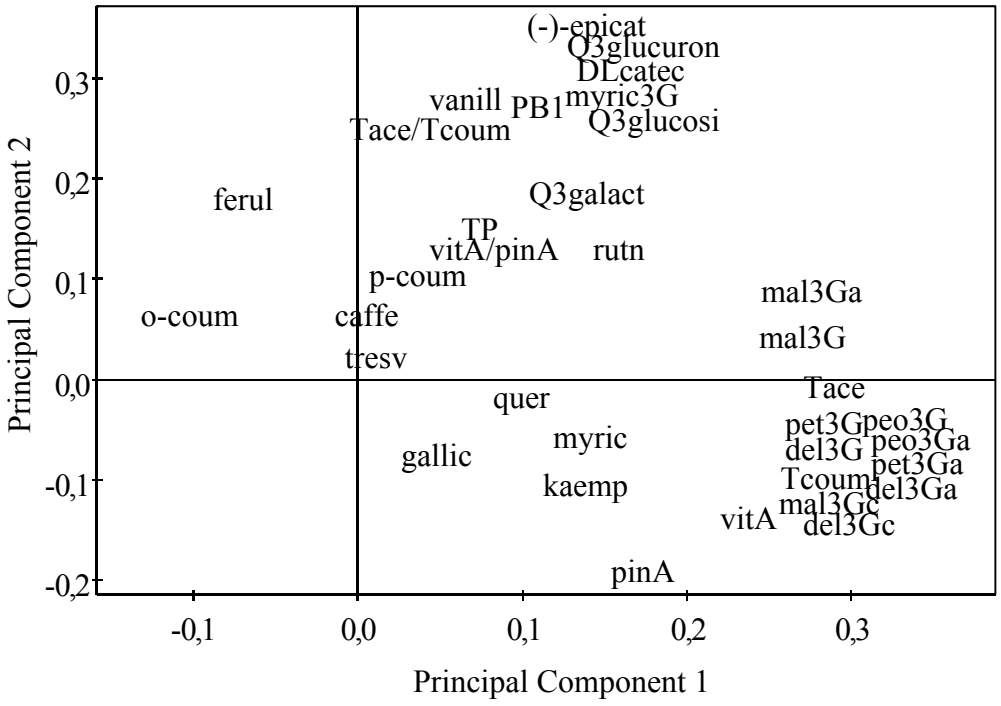

Figure 5.4. PCA loading plot of red wines based on polyphenol contents. Loadings: TP: total phenol content, mal3G: malvidin-3-glucoside, peo3G: peonidin-3-glucoside, pet3G: petunidin-3-glucoside, del3G: delphinidin-3-glucoside, mal3Ga: malvidin-3-glucoside acetate, peo3Ga: peonidin-3-glucoside acetate, pet3Ga: petunidin-3-glucoside acetate, del3Ga: delphinidin-3-glucoside acetate, mal3Gc: malvidin-3-glucoside coumarate, del3Gc: delphinidin-3-glucoside coumarate, vitA: vitisin-A, pinA: pinotin-A, Tace: Total Acetates, Tcoum: Total Coumarates, (-)-epicat: (-)-epicatechin, Dlcatec: (+)-catechin, PB1: procyanidin B_1,o-coum: o-coumaric acid, p-coum: p-coumaric acid, caffe: caffeic acid, gallic: gallic acid, vanill: vanillic acid, ferul: ferulic acid, rutn: rutin, myric: myricetin, quer: quercetin, kaemp: kaempferol, Q3galact: quercetin-3-galactoside, myric3G: myricetin-3-glucoside, Q3glucosi: quercetin-3-glucoside, Q3glucuron: quercetin-3-glucuronide

In the score plot of white wines, the polyphenol variables discriminated Sultaniye and Muscat wines from each other, even though they all originated from the western regions (Denizli-Manisa-İzmir). This was based on the high and low amounts of hydroxycinnamic acids (ferulic, p-coumaric and caffeic acids) in Muscat and Sultaniye wines, respectively (Figure 5.5). The score plot also presented a discrimination based on harvest year. 2009 vintage wines of Emir, Narince, Chardonnay and Muscat were clustered on the upper right-corner of the score plot based on their higher flavonol (quercetin, kaempferol, quercetin-3-glucoside, quercetin-3-galactoside, quercetin-3-glucuronide) and flavan-3-ol [(+)-catechin and (-)-epicatechin] contents. The high flavonol content might be related to the up-regulation of genes contributing flavonol synthesis due to strong water deficit observed in 2009 during veraison. According to Ojeda et al. (2002), mild water deficiency before veraison and strong water deficiency during veraison positively affected the flavonol biosynthesis in growing Syrah berries. On the other hand, 2008 vintage wines were on the lower right-corner with high phenolic acids (vanillic, ferulic, caffeic, and coumaric acids) and total phenol content. 2006 and 2007 vintage wines were located on the left-hand side of the score plot with their lower total phenol contents, (+)-catechin, quercetin-3-glucoside and quercetin-3-galactoside levels than 2008 and 2009 vintage wines. In literature, the discrimination of red wines according to vintage using their anthocyanin contents was commonly reported (Lorrain, Chira, & Teissedre, 2011; van Leeuwen et al., 2004). However, in this study, the polyphenol composition of wine samples was highly efficient to discriminate 2009 vintage, both for red and white wines.

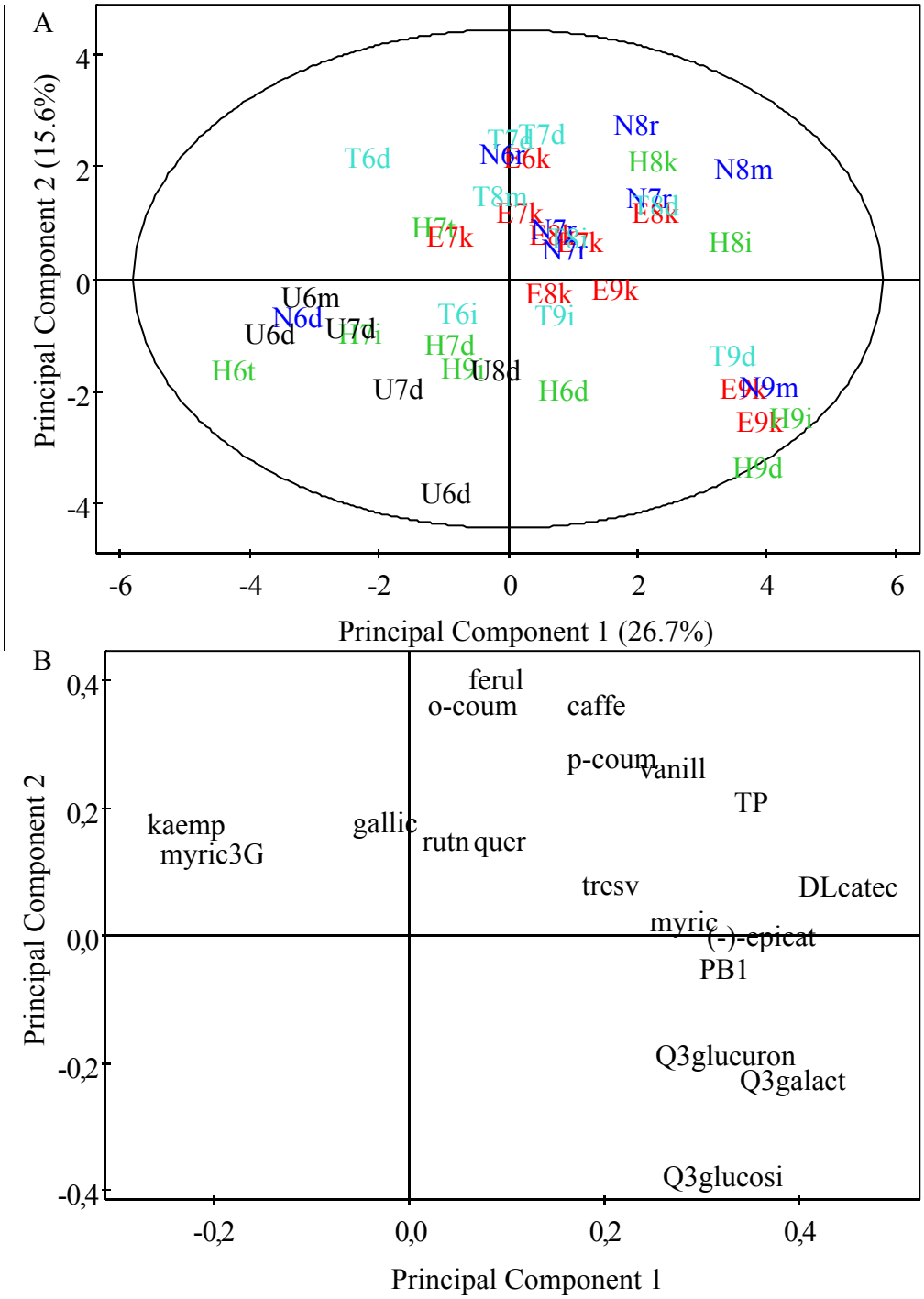

Figure 5.5. PCA score (A) and loading (B) plots of white wines based on polyphenol content: PC1 vs PC2. Coloring: **Emir**, **Chardonnay**, **Narince**, **Muscat**, **Sultaniye**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, k: Kapadokya, r: Tokat. Loadings: TP: total phenol content, (-)-epicat: (-)-epicatechin, Dlcatec: (+)-catechin, PB1: procyanidin B_1, o-coum: o-coumaric acid, p-coum: p-coumaric acid, caffe: caffeic acid, gallic: gallic acid, vanill: vanillic acid, ferul: ferulic acid, rutn: rutin, myric: myricetin, quer: quercetin, kaemp: kaempferol, Q3galact: quercetin-3-galactoside, myric3G: myricetin-3-glucoside, Q3glucosi: quercetin-3-glucoside, Q3glucuron: quercetin-3-glucuronide

Discrimination with color parameters: The color characteristics of wine are directly related to the polyphenol composition as well as vinification techniques, polymerization or oxidation reactions that take place during storage or ageing. The formation of color in grape generally takes place during the veraison period with the biosynthesis of phenolic compounds (Ivanova et al., 2011; Yildirim, 2006).

The color parameters were the most powerful variables to discriminate red wines in terms of cultivar (R^2_{pred}: 0.971). The clusters of each cultivar could be observed in the score plot (Figure 5.6A). The first PC was responsible for the discrimination of Kalecik Karası wines from Syrah wines although the majority of these cultivars originate from Denizli. Syrah wines had high logarithmic color density, color density and color intensity values, while Kalecik Karası wines had higher CIELab color parameters (L*, b*, C*, H*), tint and yellow% and lower red% and proportion of red coloration. Kalecik Karası had the lowest vitisin-A among the red wines and it was reported that the pyranoanthocyanins were more stable than anthocyanins and gave more color than other pigments (Fanzone et al., 2012).

According to the Pearson coefficients, significant correlations were found between the color parameters and phenolic compounds of red wines. Tint and yellow% were negatively, and red% was positively correlated to the petunidin- and delphinidin-3-glucoside compounds (correlation coefficients> 0.59). The median results of Kalecik Karası and Cabernet Sauvignon indicated that these two varieties were the lowest in petunidin- and delphinidin-3-glucosides and relatively, they had the lowest red%, and highest tint and yellow%. The high color density, color intensity and logarithmic color density and low lightness values of Syrah wines might be due to their rich anthocyanin and flavonol contents. Fanzone et al. (2012) have reported that high quality wines rich in anthocyanin and total phenol contents tend to have higher color intensity, chroma, red/green chromaticity, yellow/blue chromaticity and lower lightness.

The second PC discriminated Boğazkere-Öküzgözü and Cabernet Sauvignon wines from the other cultivars. Boğazkere and Öküzgözü wines had higher red%, red/green chromaticity, chroma and proportion of red coloration values, while Cabernet Sauvignon wines had higher yellow%, color density, color intensity and tint values. The high red% and proportion of red coloration values of Boğazkere and Öküzgözü wines might be related to their lower pH values. It is well-known that low pH enhances red color and color density (Jackson, 2000).

In addition to varietal discrimination, the color parameters also discriminated 2006-2007 and 2008-2009 vintage wines. 2006-2007 vintage wines were mainly located at the center and on the lower right-hand side of the score plot, whereas the last two vintages were located on the left and upper-hand side. The CIElab colorimetric parameters (L*, b*, C*, H*), yellow% values were lower while color intensity, proportion of red coloration and red% values were higher for 2008-2009 vintage wines. It should be reminded that 2009 vintage wines had high anthocyanin contents. It is well-known that the color characteristics of wine samples rely on their polyphenol compositions, and the color parameters were able to classify the red wine samples in this study according to their grape variety and to a lesser extent harvest year. The higher potential of color parameters to discriminate wine samples than the polyphenol variables might be due to the interference free characteristic of spectrophotometric technique.

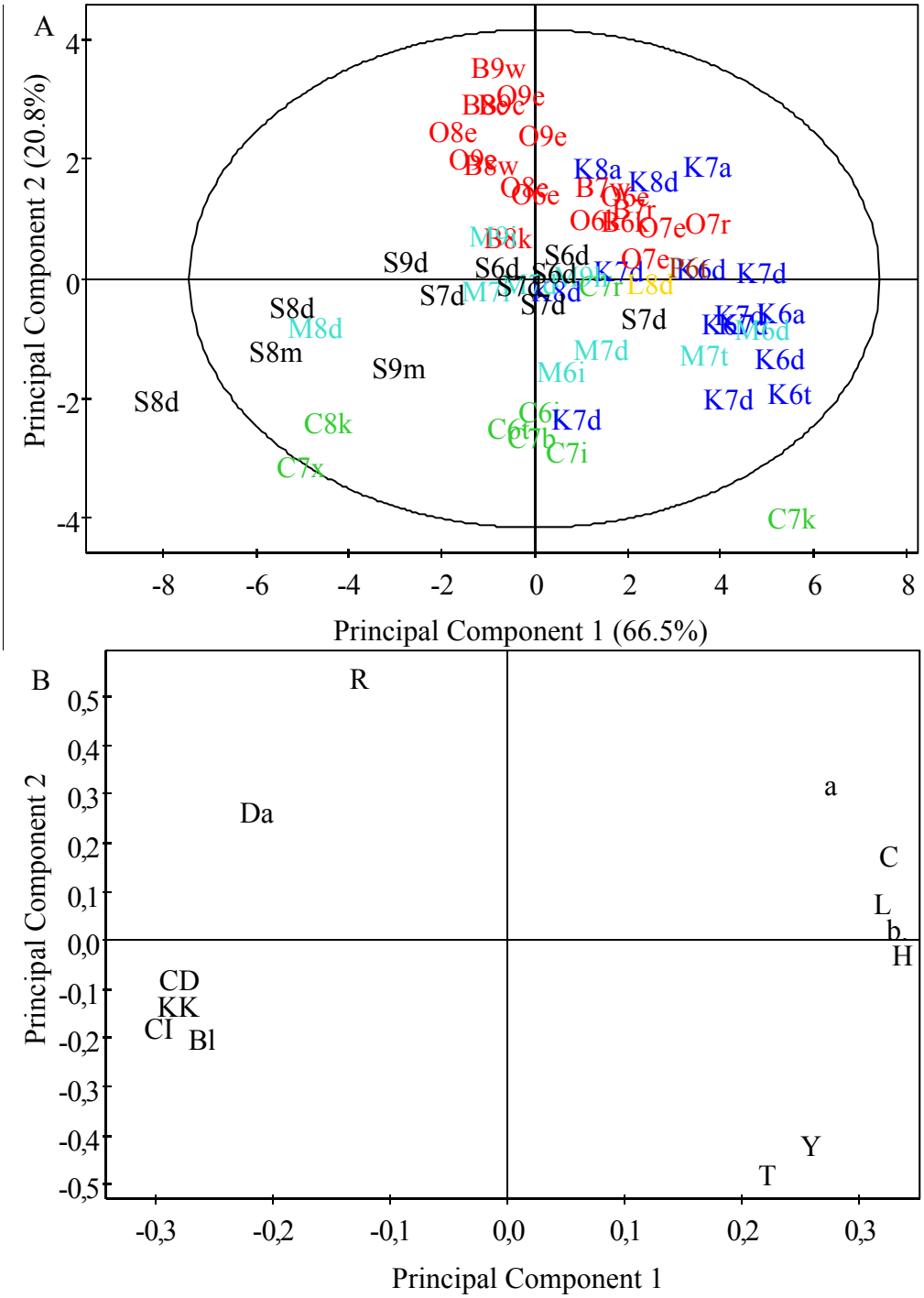

Figure 5.6. PCA score (A) and loading (B) plots of red wines based on color parameters: PC1 vs PC2. Coloring: **Boğazkere**, **Öküzgözü**, **Çalkarası**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Papazkarası**, **Syrah**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, b: Bozcaada, a: Ankara, k: Kapadokya, r: Tokat, e: Elazığ, c: Diyarbakır, h: Denizli-Tekirdağ-İzmir, x: Denizli-Ankara, w: Denizli-Diyarbakır. Loadings: L: lightness, a: red/green chromaticity, b.: yellow/blue chromaticity, C: chroma, H: hue, CD: color density, T: tint, CI: color intensity, Da: proportion of red coloration, KK: logarithmic color density, R: red%, Y: yellow%, Bl: blue%

For the case of white wines, although the color parameters produced a very powerful PCA model with R^2_{pred} of 0.968, the score plot was not effective to discriminate wines by cultivar as opposed to red wines (Figure 5.7A). Muscat, Chardonnay and Sultaniye clusters could be observed in the score plot, however Emir and Narince were scattered. The first PC discriminated Sultaniye and Muscat wines with higher hue, yellow%, tint and lower yellow/blue chromaticity, chroma, color intensity, proportion of red coloration, logarithmic color intensity and red% values of Muscat wines (Figure 5.7B). Muscat wines were recognized with their high hydroxycinnamic acid levels (caffeic, p-coumaric and ferulic acid) while Sultaniye wines were the poorest in terms of polyphenols. This was also supported by the Pearson coefficients (0.58-0.62) that indicated a significantly negative correlation between the color parameters (yellow/blue chromaticity, chroma and logarithmic color density) and hydroxycinnamic acid contents (p-coumaric and caffeic acids) ($p<0.05$). Chardonnay wines with the highest red/green chromaticity values were discriminated by the second PC. They also have the highest yellow/blue chromaticity and chroma values following Sultaniye wines.

The score plot also indicated a vintage discrimination, similar to the red wines. 2009 vintage wines were on the right-hand side of the plot due to higher yellow%, hue and tint and lower proportion of red coloration and red% values. It should be remembered that 2009 vintage wines were rich in flavonols and flavan-3-ols. Flavonols are yellow pigments that have direct impact on the color of white wines (Castillo-Muñoz et al., 2010). 2008 vintage wines were also mainly clustered on the right-hand corner of the score plot by their low yellow/blue chromaticity, chroma and proportion of red coloration and high yellow%, and tint values. On the other hand, 2006 and 2007 vintage wines mainly on the left side of the score plot with high proportion of red coloration had lower total phenol contents than 2008-2009 vintage wines.

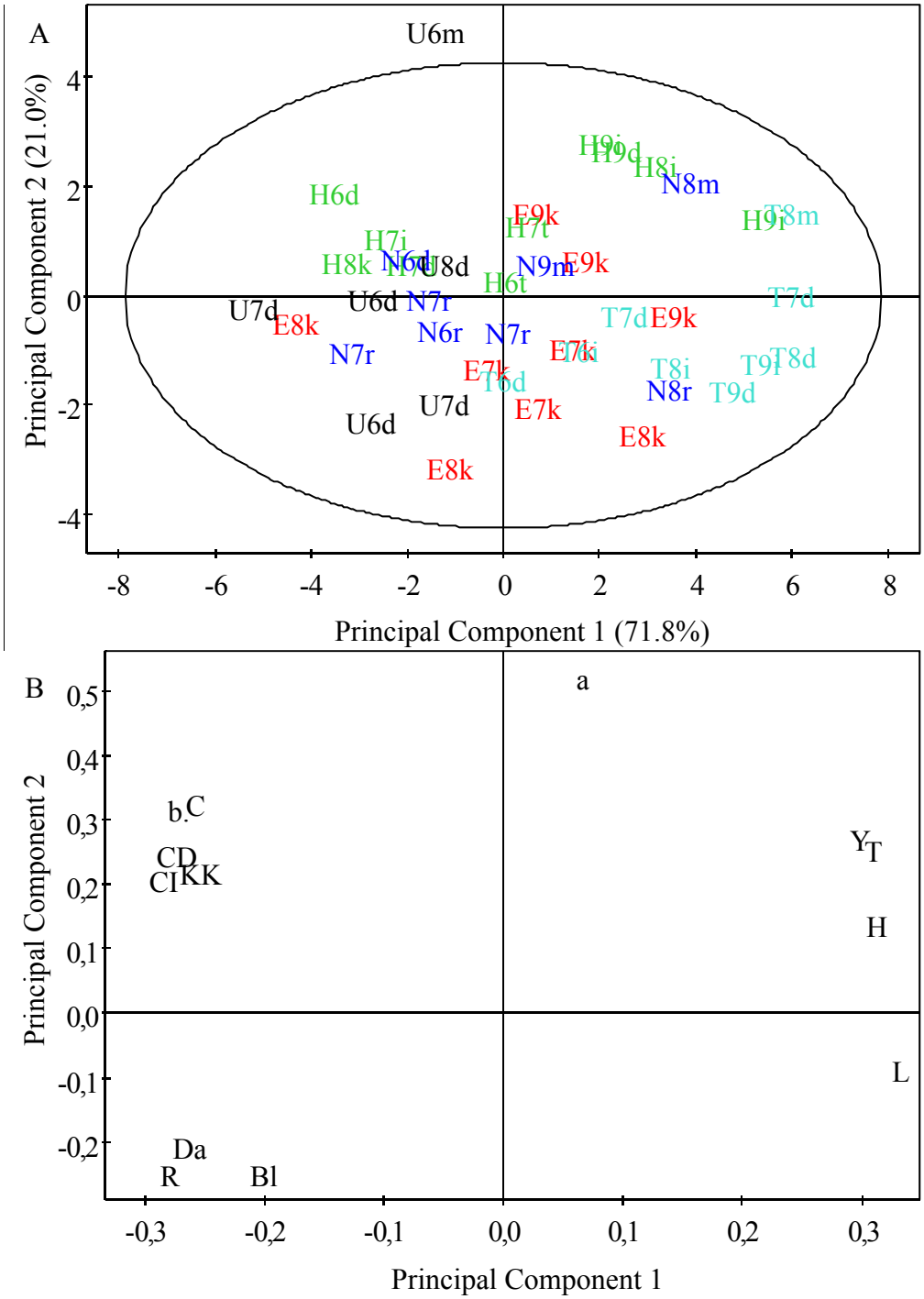

Figure 5.7. PCA score (A) and loading (B) plots of white wines based on color parameters: PC1 vs PC2. Coloring: **Emir**, **Chardonnay**, **Narince**, **Muscat**, **Sultaniye**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, k: Kapadokya, r: Tokat. Loadings: L: lightness, a: red/green chromaticity, b.: yellow/blue chromaticity, C: chroma, H: hue, CD: color density, T: tint, CI: color intensity, Da: proportion of red coloration, KK: logarithmic color density, R: red%, Y: yellow%, Bl: blue

Discrimination with organic acid-sugar contents: Regardless of geographic region and different vintages, Boğazkere and Öküzgözü varieties discriminated themselves from the other varieties due to lower pH, glycerol, original malic acid, lactic acid and higher sugar and tartaric acid (Figure 5.8). Discrimination among other varieties was poor. Cabernet Sauvignon and Syrah wines were recognized with their high original malic acid contents. These two cultivars together with Kalecik Karası wines were rich in lactic acid and glycerol as well. Citric acid was the highest in Syrah wines and the lowest in Kalecik Karası wines, while tartaric acid was the lowest in Cabernet Sauvignon wines. Acetic acid content of Kalecik Karası wines was the highest among other varieties.

In terms of white wines, the clusters of Muscat, Chardonnay and Sultaniye were observed in the score plot (Figure 5.9). According to the second PC, Chardonnay wines were the richest and Sultaniye wines were the poorest in malic, lactic and original malic acid contents, respectively. Chardonnay wines together with Muscat wines had the highest total acidity levels while Sultaniye wines were the lowest. On the other hand, according to the first PC, Muscat wines were recognized with their high sugar level which was based on the presence of semi-sweet wines in this cluster. Although the high sugar content of Muscat wines was a result of winemaking process, it was reported that Muscat accumulated more sugar than Chardonnay (Vystavna et al., 2014). Having the lowest pH, acetic acid and glycerol content and the highest tartaric acid content differentiated Muscat wines from Chardonnay and Sultaniye wines. This variety was the most acidic wine among the other red and white wines with an average pH of 3.16 and an average total acidity of 5138 mg tartaric acid/L.

The sugar and organic acid content of wine samples can originate either from the grape or are produced through winemaking processes. Tartaric, malic and citric acids originate from grape; while succinic, lactic and acetic acids are produced by the fermentation processes (Mato et al., 2005). In literature, organic acids and sugars have been employed in the characterization of wines from different geographical regions together with other chemical parameters (López-Tamames et al., 1996; Römisch et al., 2009). In addition, they have considerable influence on the taste and mouth-feel sensations of wine.

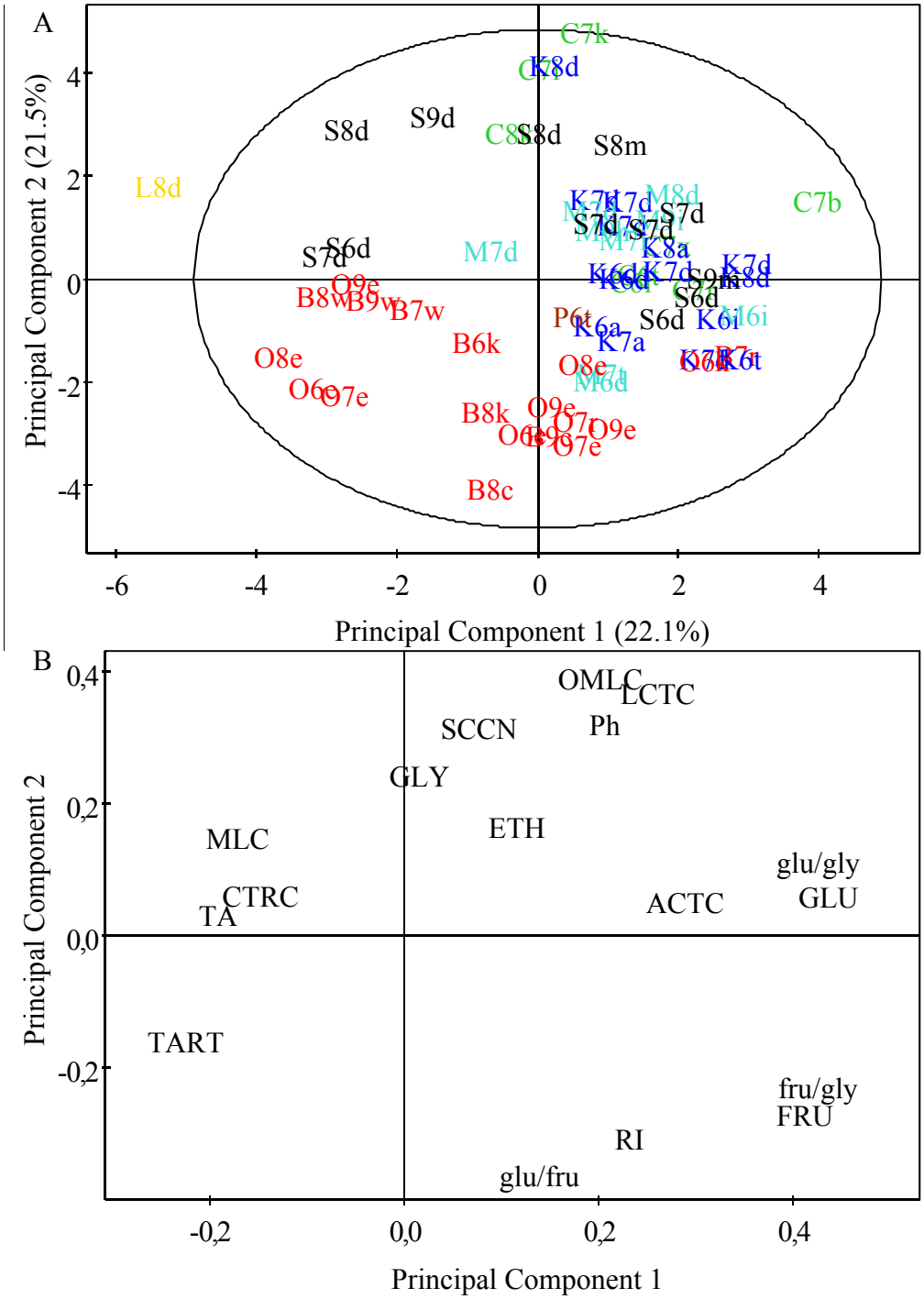

Figure 5.8. PCA score (A) and loading (B) plots of red wines based on organic acid and sugar contents: PC1 vs PC2. Coloring: **Boğazkere**, **Öküzgözü**, **Çalkarası**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Papazkarası**, **Syrah**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, b: Bozcaada, a: Ankara, k: Kapadokya, r: Tokat, e: Elazığ, c: Diyarbakır, h: Denizli-Tekirdağ-İzmir, x: Denizli-Ankara, w: Denizli-Diyarbakır. (Loading codes were shown below Figure 5.9)

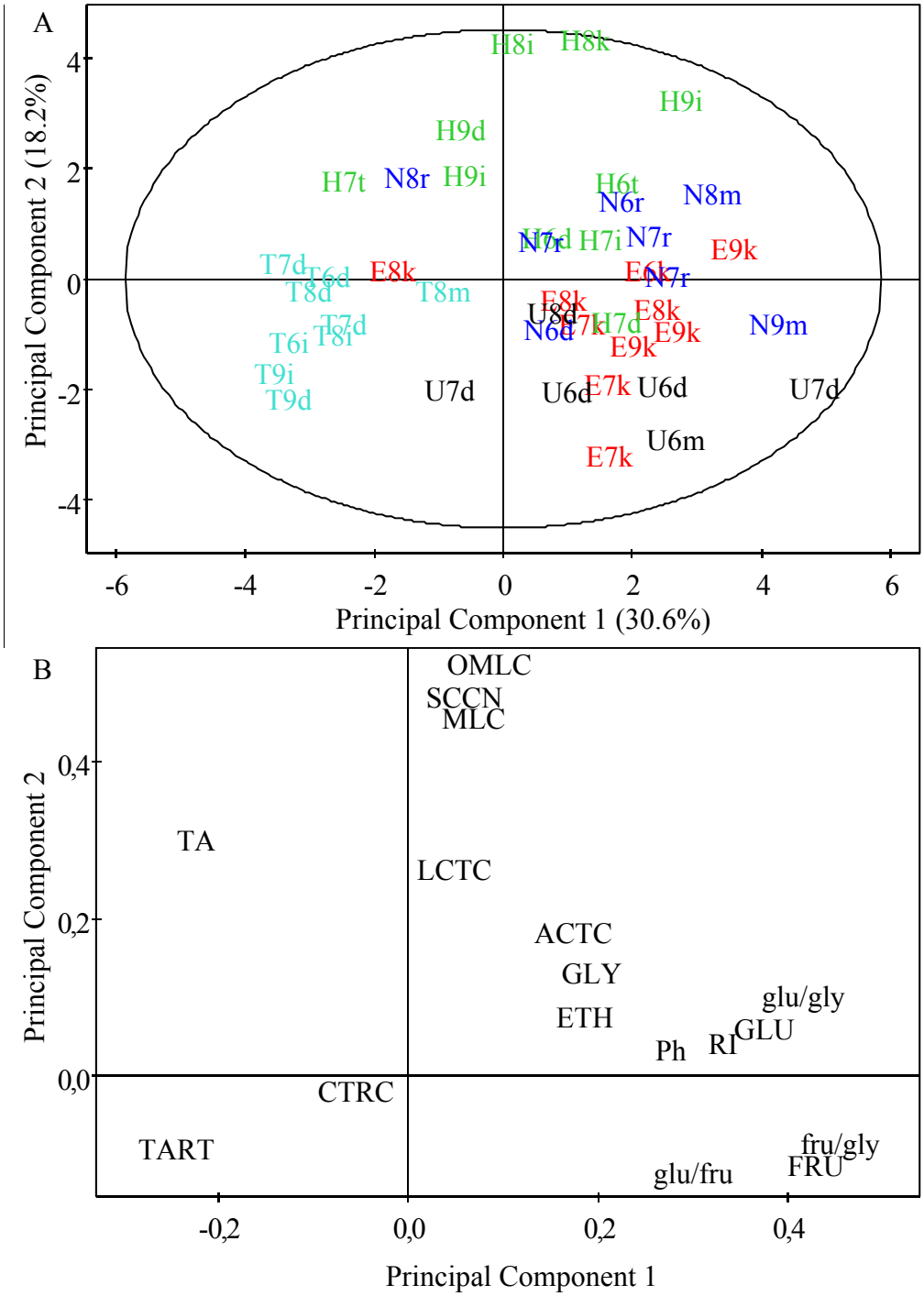

Figure 5.9. PCA score (A) and loading (B) plots of white wines based on organic acid and sugar contents: PC1 vs PC2. Coloring: **Emir**, **Chardonnay**, **Narince**, **Muscat**, **Sultaniye**. Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, k: Kapadokya, r: Tokat. Loadings: RI: refractive index, Ph: pH, TA: total acidity, GLU: glucose, FRU: fructose, GLY: glycerol, ETH: ethanol, CTRC: citric acid, TART: tartaric acid, MLC: malic acid, SCCN: succinic acid, LCTC: lactic acid, ACTC: acetic acid, glu/gly: glucose/glycerol ratio, fru/gly: fructose/glycerol ratio, glu/fru: glucose/fructose ratio, OMLC: original malic acid

The wine samples were collected from various wine producers in this study, and each of them has different process conditions. For instance Boğazkere and Öküzgözü wines were collected from 8 different wine producers, while Muscat wines were from 6 wine producers. Regardless of their producers, these varieties produced very distinct clusters. The remarkable variables were organic acids at most: tartaric, original malic, acetic, malic and lactic acids, and pH, glycerol and sugars.

The PCA technique provided useful information about the general pattern of discrimination. The main variable sets employed so far were elements, polyphenol contents, color parameters and organic acid-sugar contents. The combination of the variables was either investigated to improve the discrimination ability of established PCA models. For instance, models employing all variables or combination of polyphenol content and color parameters were established. Neither of these trials produced PCA models with higher R^2_{pred} values nor did they improve the discrimination. For this reason, results regarding these models were not given in this section.

5.7.1.2. Hierarchical Cluster Analysis (HCA)

HCA was the other unsupervised technique that was employed in the classification study. The color parameters and organic acid and sugar contents were not effective to discriminate both red and white wines therefore their dendrograms were not demonstrated. With the element contents, standardized variables were employed in the model development with Euclidean technique and ward linkage method. The colors were automatically given by the program representing the number of clusters for each cultivar. Therefore, 6 clusters were given for red wines and 5 clusters were given for white wines.

In the dendrogram of red wines, the dark blue colored cluster belonged to 2009 vintage wines of Boğazkere, Öküzgözü, Merlot and Syrah from Elazığ, Diyarbakır, Manisa, Denizli and İzmir regions (Figure 5.10A). The dendrogram did not provide information regarding the varietal or geographic discrimination of red wines. In the dendrogram of white wines, most of the Emir wines from Kapadokya region were clustered together (red cluster), except those from 2009 vintage (Figure 5.10B). On the other hand, all 2009 vintage wines were located in the dark blue cluster except Muscat

wines. Muscat wines were in the green cluster except the ones from İzmir region. Thiel et al. (2004) have visualized the structure of their data using Euclidean distance and ward linkage method with the element profile of white wines (3 grape varieties) from Pfalz and Rheinhessen regions and were able to discriminate the two regions from each other. However, instead of the 33 rare earth elements they have measured, only 13 relevant elements (As, B, Be, Cs, Li, Mg, Pb, Si, Sn, Sr, Ti, W, Y) were employed in their models.

The HCA dendrograms produced with the polyphenol variables were superior to those of element contents. The standardized polyphenol variables were employed in the model with Euclidean technique to measure the distance and ward linkage method. Boğazkere and Öküzgözü wines were in the red cluster indicating the similarity of these two varieties (Figure 5.11A). Similar to the dendrogram of red wines with elements, 2009 vintage wines of Merlot, Syrah, Boğazkere and Öküzgözü were clustered together in green color. In the dendrogram of white wines, 2009 vintage wines of Emir, Chardonnay and Narince were clustered as green and blue (Figure 5.11B). However, 2009 vintage of Muscat wines were not included in this cluster, they were rather grouped with the other vintage Muscat wines in pink color. The influence of Muscat grape variety on the discrimination was clear. Emir and Narince wines were located in the red cluster except N6d (Narince of 2006 vintage from Denizli). This cluster includes three Chardonnay and one Muscat from different regions but any Sultaniye wines. The PCA score plot established with the polyphenol variables also indicated that Emir and Narince variety wines overlapped with each other. On the other hand, the orange cluster included Chardonnay and all Sultaniye wines with T6i (Muscat of 2006 vintage from İzmir) and N6d.

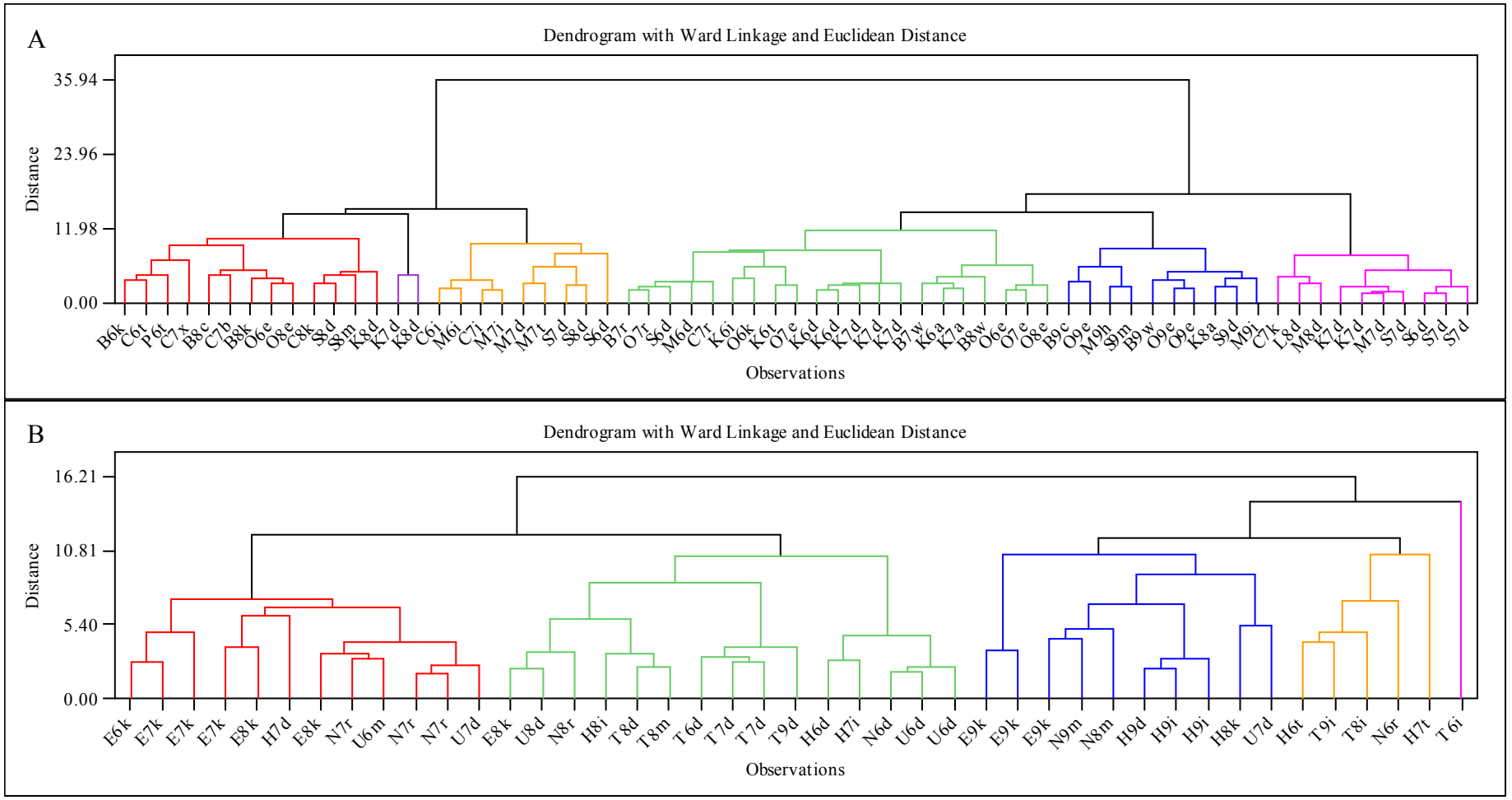

Figure 5.10. Dendrograms of red (A) and white (B) wines based on element content. Varieties: B: Boğazkere, O: Öküzgözü, C: Cabernet Sauvignon, K: Kalecik Karası, M: Merlot, S: Syrah, P: Papazkarası, L: Çalkarası, E: Emir, H: Chardonnay, N: Narince, T: Muscat, U: Sultaniye, Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, b: Bozcaada, e: Elazığ, r: Tokat, k: Kapadokya, a: Ankara, h: Denizli-Tekirdağ-Urla, x: Denizli-Ankara

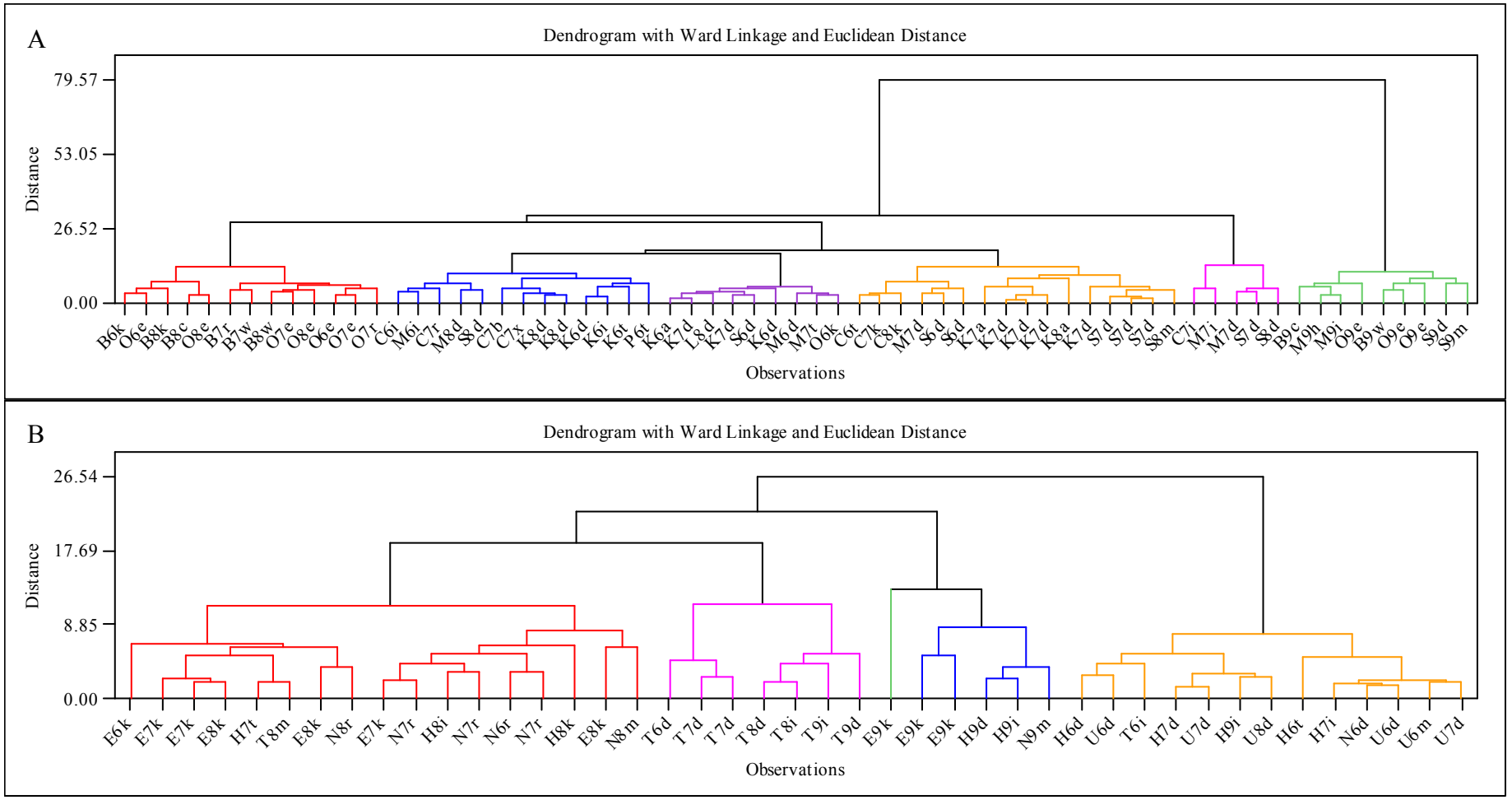

Figure 5.11. Dendrograms of red (A) and white (B) wines based on polyphenol content. Varieties: B: Boğazkere, O: Öküzgözü, C: Cabernet Sauvignon, K: Kalecik Karası, M: Merlot, S: Syrah, P: Papazkarası, L: Çalkarası, E: Emir, H: Chardonnay, N: Narince, T: Muscat, U: Sultaniye, Regions: d: Denizli, i: İzmir, m: Manisa, t: Tekirdağ, b: Bozcaada, e: Elazığ, r: Tokat, k: Kapadokya, a: Ankara, h: Denizli-Tekirdağ-Urla, x: Denizli-Ankara

5.7.2. Supervised Statistical Techniques

In this section, the two supervised statistical techniques which were partial least squares-discriminant analysis (PLS-DA) and soft independent modeling of class analogy (SIMCA) were employed for the varietal, regional and vintage discrimination of red and white wines using the variables: elements, polyphenols, color parameters and organic acid and sugar contents.

5.7.2.1. Partial Least Squares-Discriminant Analysis (PLS-DA)

5.7.2.1.1. Varietal Discrimination

In PLS-DA models, the classes to which an observation belonged were assigned previously. Boğazkere and Öküzgözü were put in the same class based on their similarity observed in the PCA results. Therefore, 5 classes were established for red wines; Boğazkere and Öküzgözü (BO) with 15 samples, Cabernet Sauvignon (C) with 6 samples, Kalecik Karası (K) with 12 samples, Merlot (M) with 8 samples, and Syrah (S) with 9 samples. Çalkarası red (1 sample) and Papazkarası red wines (1 samples) were set as classless in the models since the number of observations was not sufficient to build a class. For white wines, 5 classes were established; Emir (E) with 8 samples, Chardonnay (H) with 8 samples, Narince (N) with 7 samples, Muscat (T) with 7 samples and Sultaniye (U) with 5 samples. In PLS-DA models, the VIP feature of Simca-P software identified the significant variables that had the most significant impact on the model. Therefore, unlike PCA models based on all variables, PLS-DA models were established with the significant variables. PLS-DA model parameters are summarized in Table 5.18.

Table 5.18. PLS-DA model parameters of varietal discrimination of red and white wines

		Variables	# of PC	R^2_X	R^2_Y	R^2_{pred}	Calib. set	Valid. set	Observations in the validation set
Red Wines	Elements	10: Ca, K, B, Al, Ba, Li, Mn, Cu, Zn, Mg	3	0.556	0.353	0.160	52	13	B8c6, B8k8, C7i11, C7r3, K6d6, K7d6, K8d6, M7d7, O6k8, O9e5, S7d4, S8m5, S9d4
	Phenols	19: TP, pet3G, peo3G, del3G, del3Gc, mal3Gc, vitA, Tcoum, Tace/Tcoum, vitA/ pinA, rutn, quer, myric, Q3glucosi, Q3galact, myric3G, gallic, (-)-epicat, o-coum	3	0.667	0.463	0.329	52	13	B7r3, B9w4, C7i11, K7a4, K7d7, K8d6, M7d7, M9h6, O6k8, O9e4, S6d6, S7d7, S8m5
	Color	6: a, T, Da, KK, R, Y	3	0.992	0.338	0.253	52	13	B8c6, B8k8, C6t1, C7r3, K7d10, K7d6, K8d6, M7d7, O6k8, O8e6, S7d4, S8m5
	Acid-Sugar	6: Ph, GLU, TART, LCTC, glu/gly, OMLC	3	0.878	0.280	0.182	52	13	B8c6, B8k8, C6t1, C7r3, K7d10, K7d6, K8d6, M7d7, O6k8, O8e6, S7d4, S8m5
	All Significant Parameters	41 variables	7	0.739	0.769	0.446	52	13	B8c6, B8w4, C7i11, C7r3, K6t9, K7d6, K8a4, M7d7, O6k8, O9e5, S7d4, S8m5, S9d4
White Wines	Elements	9: K, Mg, Na, Sr, Li, Mn, Co, Ni, Cu	2	0.405	0.304	0.203	35	8	E7k5, E9k8, H6d10, H9i11, N7r3, T8m5, T9d4, U6d5
	Phenols	11: TP, Q3glucuron, caffe, p-coum, ferul, tresv, gallic, Dlcatec, vanill, (-)-epicat, PB1	3	0.681	0.371	0.169	35	8	E7k5, E9k5, H6d10, H9i11, N7r3, T8m5, T9d4, U6d5
	Color	11: L, b, C, H, CD, T, CI, KK, R, Y, Bl	2	0.938	0.204	0.118	35	8	E7k5, E8k8, H6d10, H9i11, N7r3, T8m5, T6i6s, U6d5
	Acid-Sugar	14: RI, TA, GLU, FRU, TART, MLC, LCTC, ACTC, glu/gly, fru/gly, glu/fru, OMLC	4	0.829	0.521	0.324	35	8	E7k5, E9k8, H7i11, H9d10, N7r3, T8m5, T9d4, U6d5
	All Significant Parameters	45 variables	3	0.543	0.529	0.402	35	8	E7k5, E9k8, H7i11, H9d10, N7r3, T8m5, T9d4, U6d5

Discrimination with element composition: PLS-DA technique established with the element content produced results similar to that of PCA. The model parameters were not improved and the same discrimination pattern was observed with this technique. The cluster of Boğazkere and Öküzgözü wines from the remaining red wines was based on the significant variables: Ca, Cu and B. While for white wines, the discrimination between Emir and Muscat wines was based on the significant variables: Sr, Li, Cu, Co, Mn and Pb. Thus, the scores and loadings plots regarding PLS-DA technique were not presented.

Discrimination with polyphenol composition: PCA score plot showed a very dominant effect of 2009 vintage wines and the discrimination among the remaining wines was not clear. PLS-DA technique produced a superior model by eliminating the variables which were significant for vintage discrimination rather than grape variety. Boğazkere and Öküzgözü cluster was clearly discriminated from the other cultivars according to the first PC (Figure 5.12A). Although Öküzgözü wines were higher in anthocyanin content than Boğazkere wines, these two varieties appeared in the same cluster based on their high gallic and o-coumaric acids, delphinidin-3-glucoside and coumaroylated malvidin derivatives, and low Tace/Tcoum, quercetin-3-glucoside, quercetin-3-galactoside and (-)-epicatechin contents.

Among the red wines, Boğazkere and Öküzgözü had the lowest acylated anthocyanins (total of coumaroylated and acetylated anthocyanin derivatives), and the acylation with coumaric acid was more preferred to acylation with acetic acid. This might be due to the high o-coumaric acid and low acetic acid contents of these two native varieties. It was reported that the acylated malvidin derivatives were more stable than the non-acylated malvidins, however, it is still unclear what controls the acylation of malvidins with acetic or coumaric acids. Berry temperature may influence the enzymes responsible for the acylation step of phenylpropanoid pathway which are aliphatic and aromatic acyltransferases. The temperature stress might direct more anthocyanin compounds towards acylation due to the stability of these compounds (Tarara et al., 2008). de Andrade et al. (2013) have reported that Cabernet Sauvignon wines from Sao Francisco valley had higher acetylated and coumaroylated anthocyanin derivatives than those from other regions (Brazil, Chile). This was related to the warm climate and high exposure of grape berries to sunlight increasing the activity of anthocyanin acyltransferase that converts glycoside derivatives into acetylated forms.

Boğazkere and Öküzgözü are the two native grape varieties widely grown in East Anatolia (Diyarbakır and Elazığ), which are used as blends for high quality wine production in Turkey (Kelebek et al., 2010). In this study, they were from the Diyarbakır, Elazığ, Kapadokya and Tokat regions which have different soil and climate characteristics. The statistical analysis showed the discrimination of these two native varieties from the remaining wines; therefore, it can be concluded that Boğazkere and Öküzgözü wines define the characteristics of red wines from East Anatolia.

While Boğazkere and Öküzgözü had the highest coumaroylated anthocyanin derivatives, the foreign varieties, Merlot and Syrah had the highest acetylated anthocyanin derivatives. Syrah wines collected from West Anatolia (Denizli and Manisa) were rich in anthocyanins. Moreover, they were rich in flavonol-glycosides unlike Boğazkere and Öküzgözü wines. The cluster on the upper left-corner of the score plot was mainly due to the high content of flavonol-glycosides (quercetin-3-glucoside, quercetin-3-galactoside, quercetin and myricetin-3-glucoside). Makris et al. (2006) have also reported higher anthocyanin and flavonol content of Syrah wines than Cabernet Sauvignon, Merlot and other native Greek wines.

The discrimination between Kalecik Karası and Syrah wines was achieved with the second PC and was influenced by the significant variables: flavonols, vitisin-A, vitA/pinA ratio and (-)-epicatechin. Kalecik Karası wines collected from different regions (Denizli, İzmir, Tekirdağ and Ankara), were the only variety having vitA/pinA ratios lower than 1.0 and they had lower anthocyanin (peonidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside), flavonol and vitisin-A contents than Syrah wines. Vitisin-A and pinotin-A, the so-called pyranoanthocyanins are formed by the interaction of malvidin-3-glucoside with pyruvic acid and caffeic acid through yeast metabolism (Schwarz et al., 2004). Rentzsch et al. (2010) reported that young wines contained maximum concentrations of vitisin-A and trace amounts of pinotin-A. Since, all the wine samples in this study were young wines the low vitA/pinA ratio might be based on the yeast metabolism and on the concentrations of caffeic acid and malvidin-3-glucoside.

In addition to the varietal discrimination, the influence of 2009 vintage could be observed in the score plot even though the classes were established for each cultivar. 2009 vintage wines of Merlot, Syrah, Boğazkere and Öküzgözü were all located on the upper right-hand side of the score plot based on the higher vitisin-A and anthocyanin variables (Figure 5.12A). van Leeuwen et al. (2004) have stated that the vintage and to a

lesser extent soil were more effective on anthocyanin concentrations of berries. On the other hand, Makris et al. (2006) have discriminated Syrah, Merlot, Cabernet Sauvignon and various native Greek wines (Mandilaria, Xinomavro, Agiorgitiko) using the anthocyanin contents (main anthocyanins except peonidin- and petunidin glucosides and coumaroylated and acetylated malvidin derivatives), flavan-3-ols (epicatechin, catechin), procyanidin B_1 and B_2, as well as the hydroxycinnamic acids (caffeic, coutaric, caftaric, coumaric acid). Unlike our findings, flavonols had no impact on the varietal discrimination of red wines. However, their wine samples were all from the same vintage (2004). Our findings indicated that both the vintage and cultivar had effect on the phenolic composition of red wines and their classification.

The discrimination of Kalecik Karası wines from Cabernet Sauvignon and Merlot wines was achieved with the first and third PCs (Figure 5.12C). The significant variables total phenol content, gallic acid, quercetin, myricetin, vitisin-A and vitA/pinA ratio were lower in Kalecik Karası wines than Cabernet Sauvignon and Merlot wines. The membership probability values of prediction set were between 0.12-0.99 indicating correct classification (Figure 5.12D).

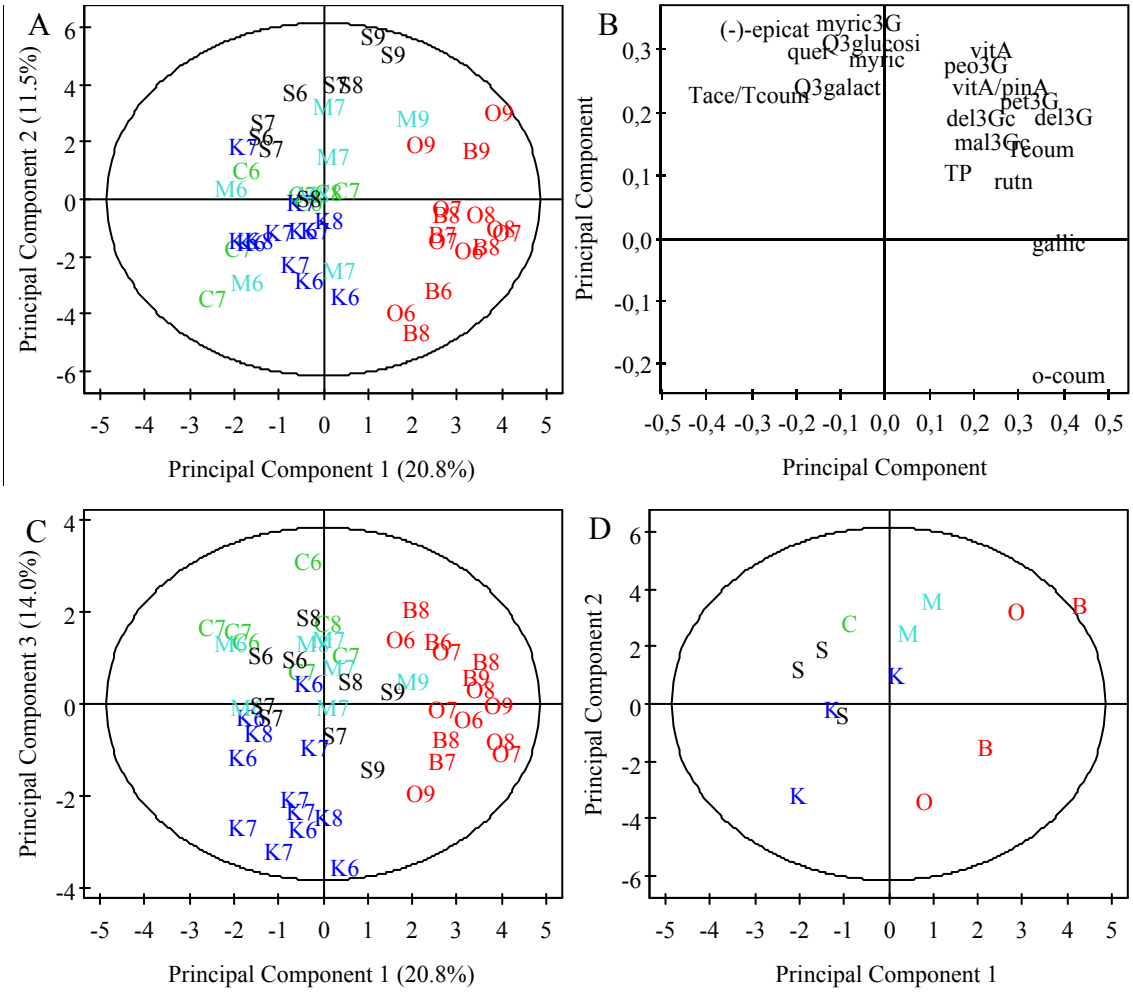

Figure 5.12. PLS-DA scores (A, C), loading (B) and validation (D) plots of red wines based on polyphenol contents discriminated according to grape variety: (A) PC1 vs PC2, (C) PC1 vs PC3. Coloring: **Boğazkere**, **Öküzgözü**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Syrah**. Loadings: TP: total phenol content, del3G: delphinidin-3-glucoside, peo3G: peonidin-3-glucoside, pet3G: petunidin-3-glucoside, del3Gc: delphinidin-3-glucoside coumarate, mal3Gc: malvidin-3-glucoside coumarate, vitA: vitisin-A, Tace: Total Acetates, Tcoum: Total Coumarates, (-)-epicat: (-)-epicatechin, o-coum: o-coumaric acid, gallic: gallic acid, rutn: rutin, myric: myricetin, quer: quercetin, Q3galact: Quercetin-3-galactoside, myric3G: myricetin-3-glucoside, Q3glucosi: Quercetin-3-glucoside

In terms of white wines, flavonols exerted no impact on the varietal discrimination, unlike red wines. They rather have influence on the discrimination of white wines according to harvest year. According to the score plot, Emir wines of Kapadokya and Narince wines of Tokat regions were discriminated from Muscat and Sultaniye wines of West Anatolia (Figure 5.13A). As it was explained in the PCA discrimination, the Sultaniye and Muscat wines were discriminated from each other due to the contents of hydroxycinnamic acids (ferulic, p-coumaric and caffeic acids). Their

concentration was significantly high in Muscat wines ($p<0.05$), and low in Sultaniye wines although both varieties originated from the Denizli, Manisa and İzmir regions. With the first and second PCs, the overlap of Emir and Narince wines could be explained with their higher procyanidin B_1 and vanillic acid concentrations than the other wines. On the other hand, the score plot between the first and third PC showed the discrimination between Emir and Narince wines based on the significantly higher resveratrol content of Emir wines ($p<0.05$), and higher (+)-catechin content of Narince wines (Figure 5.13C). The samples in the validation set had membership probability values of between 0.07-0.99 indicating correct predictions (Figure 5.13D).

Similar to Sr and Li contents of white wines, resveratrol was also negatively correlated to the temperature parameters of April, August and September according to the Pearson coefficients (correlation coefficients > 0.58, $p<0.05$). Cassidy, Hanley, & Lamuela-Raventos (2000) have reported that resveratrol in wines was dependent upon grape variety, climatic conditions of the harvest, and ecological procedures such as yeast strains, fining agents and aging in oak. It was synthesized from p-coumaric acid and in response to microbial stress, abiotic stresses such as UV light exposure and herbicide or fungicide applications. In addition to Sr and Li contents, the resveratrol content was also significantly high in Emir wines of Kapadokya region ($p<0.05$). This region was recognized with its low rainfalls and temperature values, but has sunshine values as high as in İzmir (Table 5.15). It has the lowest temperature values in April, August and September among all regions, as well. Therefore, high sunshine-exposure and low temperature characteristics of Kapadokya region and the influence of Emir cultivar might have yielded high concentrations of resveratrol. Fanzone et al. (2012) explained the higher resveratrol content of Malbec wines than the Cabernet Sauvignon wines due to the Malbec grapes grown at high altitudes at which agroecological conditions favored the synthesis of stilbenes.

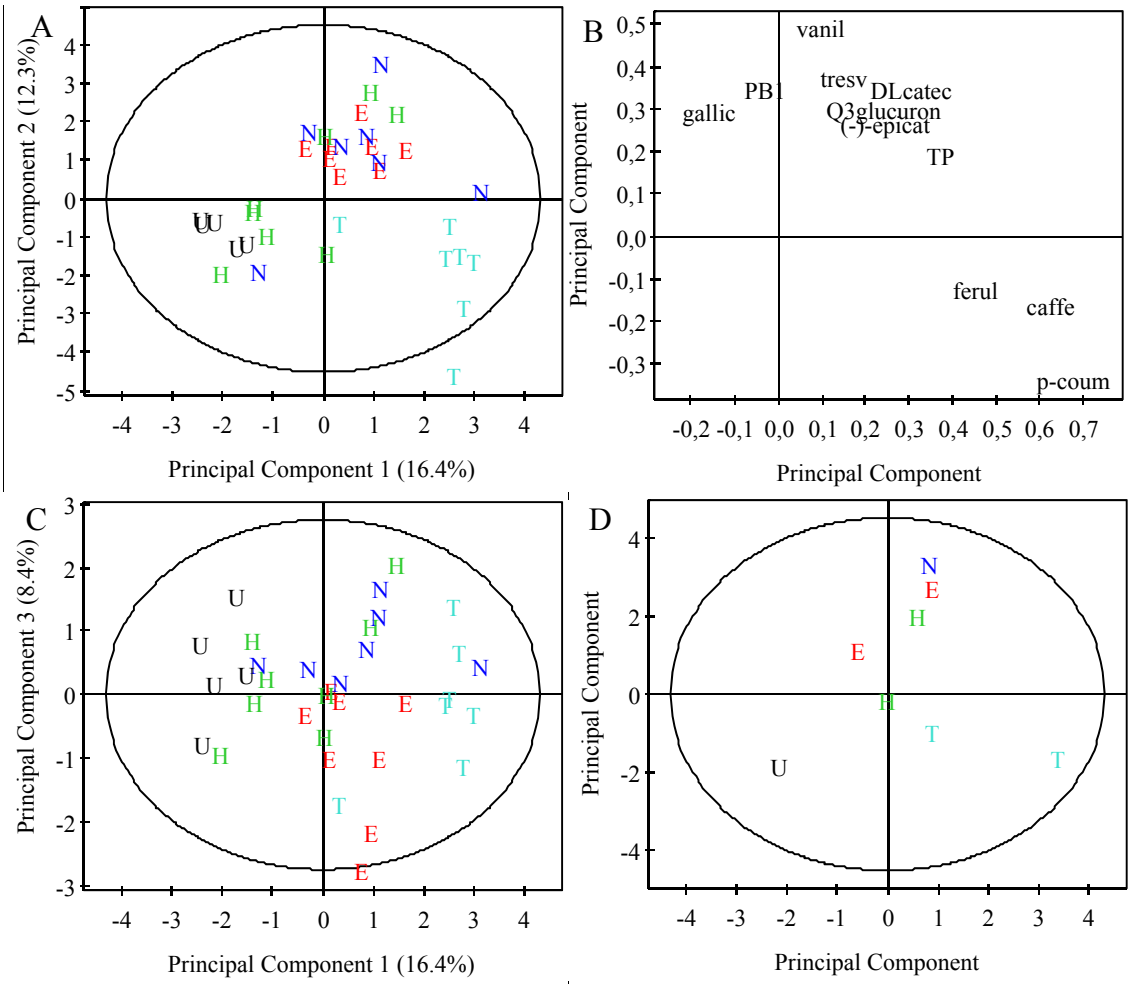

Figure 5.13. PLS-DA scores (A, C), loading (B) and validation (D) plots of white wines based on polyphenol contents discriminated according to grape variety: (A) PC1 vs PC2, (C) PC1 vs PC3. Coloring: **Emir**, **Chardonnay**, **Narince**, **Muscat**, **Sultaniye**. Loadings: TP: total phenol content, Q3glucuron: quercetin-3-glucuronide, caffe: caffeic acid, p-coum: p-coumaric acid, ferul: ferulic acid, tresv: resveratrol, gallic: gallic acid, Dlcatec: (+)-catechin, (-)-epicat: (-)-epicatechin, vanill: vanillic acid, PB1: Procyanidin B_1

Discrimination with color parameters: Although the PCA model of red wines was very powerful with R^2_{pred} value of 0.972, the PLS-DA model had less predictive ability (R^2_{pred}: 0.253). However, the discrimination was clear for all the grape varieties similar to the PCA score plot. This might be based on the supervised technique of PLS-DA forcing the samples into classes for each grape variety. Similar to the previous PLS-DA models established with polyphenol and element contents, Boğazkere and Öküzgözü wines were classed together and they were discriminated from Cabernet Sauvignon and Kalecik Karası wines according to the first PC due to higher red%, proportion of red coloration and lower yellow% and tint values of Boğazkere and

Öküzgözü wines (Figure 5.14). The first PC also discriminated Syrah wines from Kalecik Karası and Cabernet Sauvignon wines although the majority of these cultivars originate from the western regions. The significant variables were tint and yellow% which were lower and logarithmic color density which was higher in Syrah wines. Kalecik Karası cluster was recognized with lower logarithmic color density, proportion of red coloration and red%, and higher tint and yellow% values. It should be reminded that the low red%, and high yellow% and tint of Kalecik Karası wines was found to be correlated to the low petunidin- and delphinidin-3-glucoside concentrations according to the Pearson correlation coefficients. According to the second PC, Syrah and Cabernet Sauvignon wines were discriminated from Kalecik Karası and Boğazkere-Öküzgözü classes. Syrah and Cabernet Sauvignon wines had lower red/green chromaticity and higher logarithmic color density values than the other classes. The membership probability values of red wine samples in the validation set were between 0.06-0.98 indicating correct predictions (Figure 5.14D).

The score plot between the first and third PC indicated the discrimination with respect to vintage rather than variety (Figure 5.14C). This was also observed in the PCA results. 2008 and 2009 vintage wines appeared on the right hand-side of the score plot, respectively. They had higher red/green chromaticity and proportion of red coloration values.

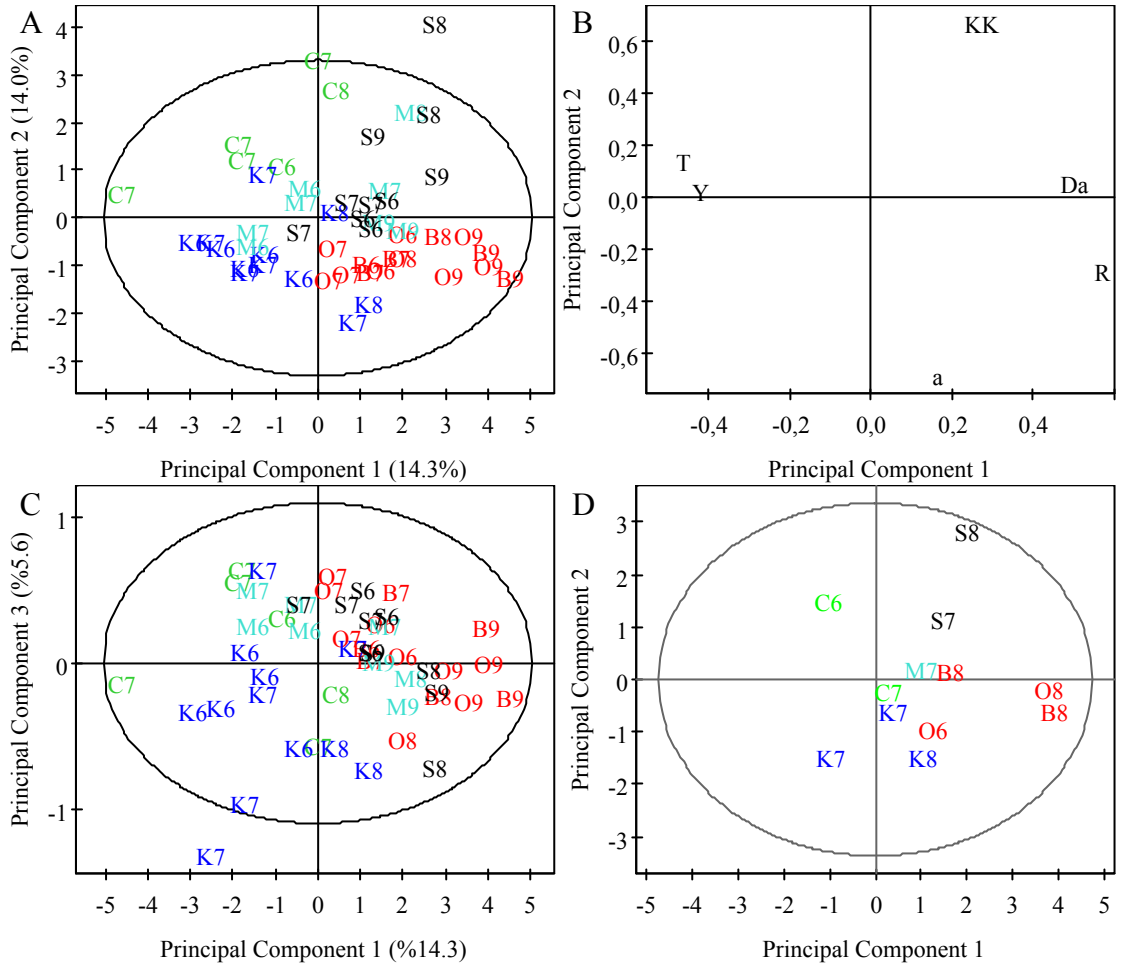

Figure 5.14. PLS-DA scores (A, C), loading (B) and validation (D) plots of red wines based on color parameters discriminated according to grape variety: (A) PC1 vs PC2, (C) PC1 vs PC3. Coloring: **Boğazkere**, **Öküzgözü**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Syrah**. Loadings: a: red/green chromaticity, T: tint, Da: proportion of red coloration, KK: logarithmic color density, R: red%, Y: yellow%

In the discrimination of white wines using PLS-DA technique, all color parameters were found significant except proportion of red coloration and red/blue chromaticity, and almost the same discrimination in the PCA model was observed. For this reason, the PLS-DA plots were not shown.

Discrimination with organic acid and sugar compositions: Similar to the PCA score plot, Öküzgözü and Boğazkere cluster could be discriminated from the remaining varieties with their higher tartaric acid, glucose and glucose/glycerol ratios, and lower pH values, lactic acid and original malic acid contents according to the first PC (Figure 5.15A). To the contrary, Cabernet Sauvignon and Syrah wines had high original malic acid and lactic acid contents. The high level of lactic acid is a precursor of high malic acid in wine, since malic acid is decarboxylated to lactic acid through malolactic fermentation. And, in grape berries grown at higher temperatures and longer sun-exposure, malic acid level reduces (Lee et al., 2009). In our study, the lactic and original malic acid levels of Boğazkere, Öküzgözü and Cabernet Sauvignon wines from the temperate regions such as Kapadokya and Tokat were examined, and Cabernet Sauvignon wines had higher acid contents (lactic acid: 1455.81 mg/L, original malic acid: 2481.72 mg/L) compared to Öküzgözü (lactic acid: 855.35 mg/L, original malic acid: 1504.88 mg/L) and Boğazkere (lactic acid: 712.32 mg/L, original malic acid: 1391.65 mg/L). Therefore, it can be concluded that the cultivar effect was more obvious on the discrimination than the vintage and geographic origin. The second PC discriminated Kalecik Karası and Merlot wines from the other cultivars with their low glucose content and glucose/glycerol ratio. Cabernet Sauvignon wines were the lowest in tartaric acid. The membership probability values of validation set were between 0.48-0.99 (Figure 5.15C).

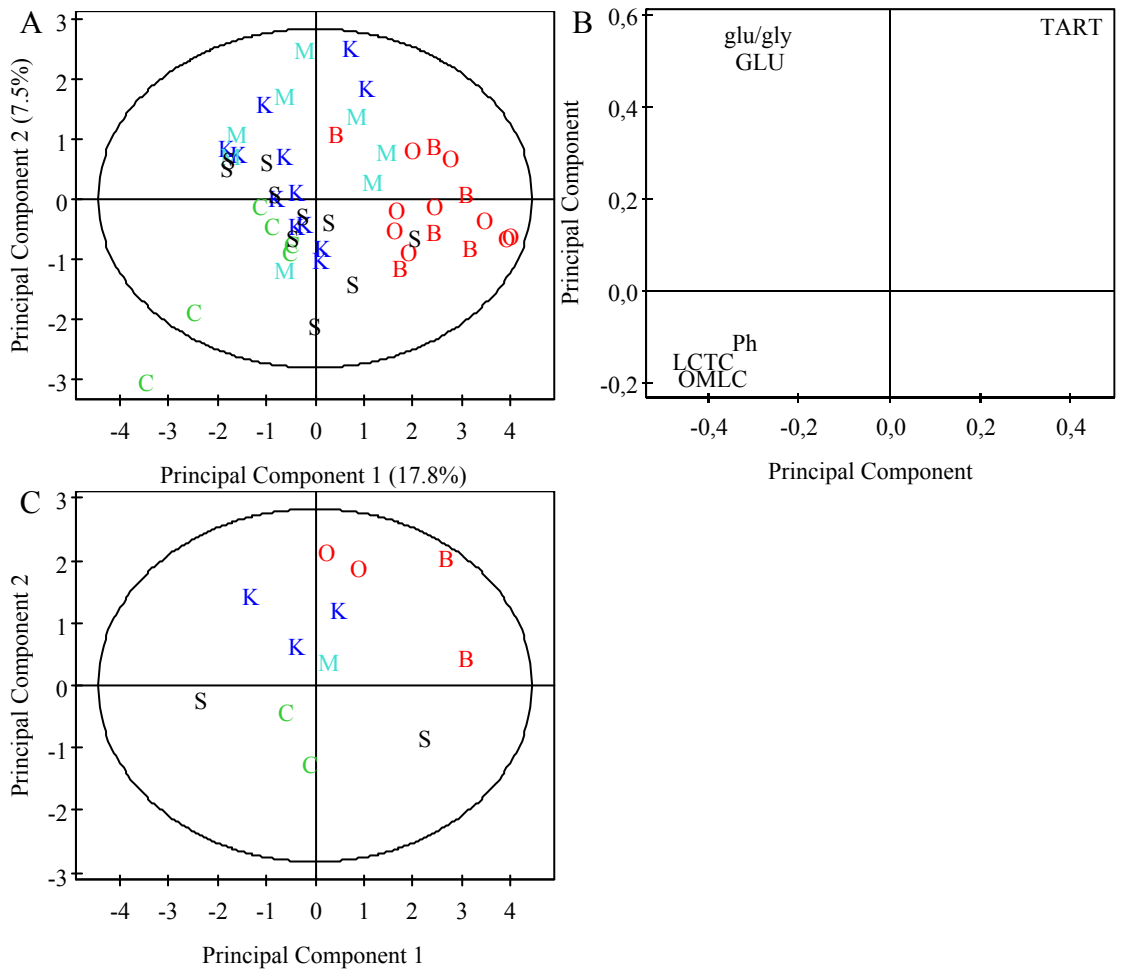

Figure 5.15. PLS-DA score (A), loading (B) and validation (C) plots of red wines based on organic acid and sugar content discriminated according to grape variety: PC1 vs PC2. Coloring: **Boğazkere**, **Öküzgözü**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Syrah**. Loadings: Ph: pH, GLU: glucose, glu/gly: glucose/glycerol ratio, TART: tartaric acid, LCTC: lactic acid, OMLC: original malic acid

The PLS-DA technique produced the best varietal discrimination of white wines (Figure 5.16A). The significant variables malic, lactic and original malic acid discriminated Chardonnay and Sultaniye wines according to the second PC. Chardonnay wines were the richest and Sultaniye wines were the poorest in these organic acids. Moreover, Chardonnay and Muscat wines had the highest total acidity, while Sultaniye wines had the lowest. According to the first PC, Muscat wines were discriminated with their high sugar levels, tartaric and acetic acid contents from the other varieties. Of the 9 Muscat samples, 6 were semi-sweet wines. Their cluster was influenced from the winemaking techniques. On the other hand, they were the most acidic wines among the white wines. The membership probability values of validation set were between 0.38-0.99 indicating correct classification (Figure 5.16C).

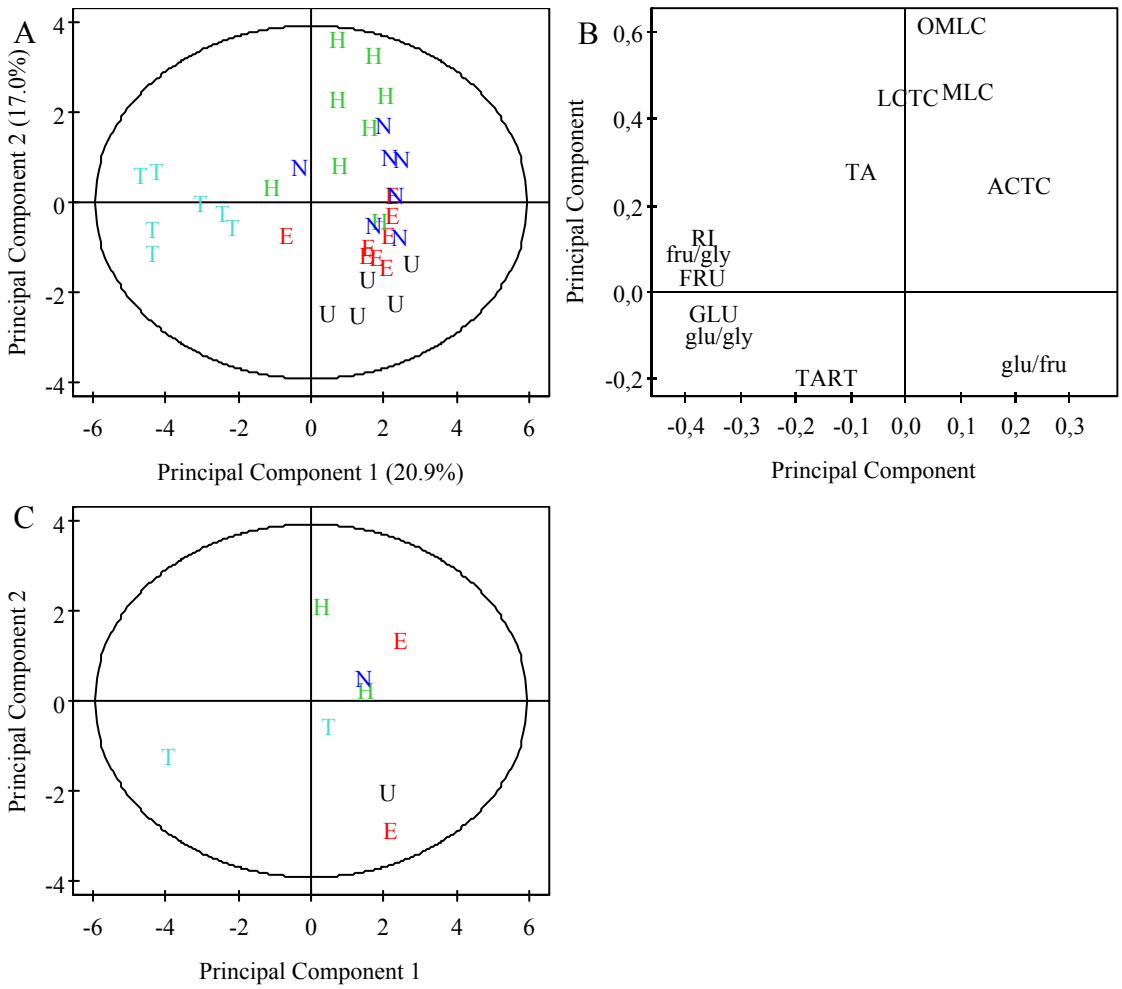

Figure 5.16. PLS-DA score (A), loading (B) and validation (C) plots of white wines based on organic acid and sugar content discriminated according to grape variety: PC1 vs PC2. Coloring: **Emir**, **Chardonnay**, **Narince**, **Muscat**, **Sultaniye**. Loadings: RI: refractive index, TA: total acidity, GLU: glucose, FRU: fructose, TART: tartaric acid, MLC: malic acid, LCTC: lactic acid, ACTC: acetic acid, OMLC: original malic acid

The final PLS-DA models include combination of all the significant variables employed so far, and their discriminative powers were investigated. The most powerful PLS-DA models with the highest R^2_{pred} values were produced by using all significant parameters (R^2_{pred}: 0.446 for red and R^2_{pred}: 0.402 for white wines). The discrimination among different cultivars was clearer. The distinct discrimination of Boğazkere and Öküzgözü cluster from the other varieties was observed in all of the PCA and PLS-DA models. They were discriminated with the first PC due to their higher red%, proportion of red coloration, tartaric acid, o-coumaric acid, gallic acid, coumaroylated malvidin derivatives, Ca, and lower Tace/Tcoum, pH, lactic acid, original malic acid, yellow%, tint, Cu, B, quercetin-3-glucoside, quercetin-3-galactoside and (-)-epicatechin contents

(Figure 5.17A). The second PC discriminated Kalecik Karası wines from Syrah, Cabernet Sauvignon and Merlot wines with lower vitisin-A, vitA/pinA ratio, quercetin, myricetin contents of Kalecik Karası wines. Syrah wines were recognized with their high anthocyanin, flavonol-glycoside contents and logarithmic color density values. The third PC was responsible for the discrimination of Cabernet Sauvignon wines with low total coumaroylated malvidin contents, tint, yellow%, glucose and low tartaric acid (Figure 5.17C). The membership probability values of validation set were between 0.06-0.96 (Figure 5.17D). As the number of significant variables was increased, both the model parameters and the discrimination were improved.

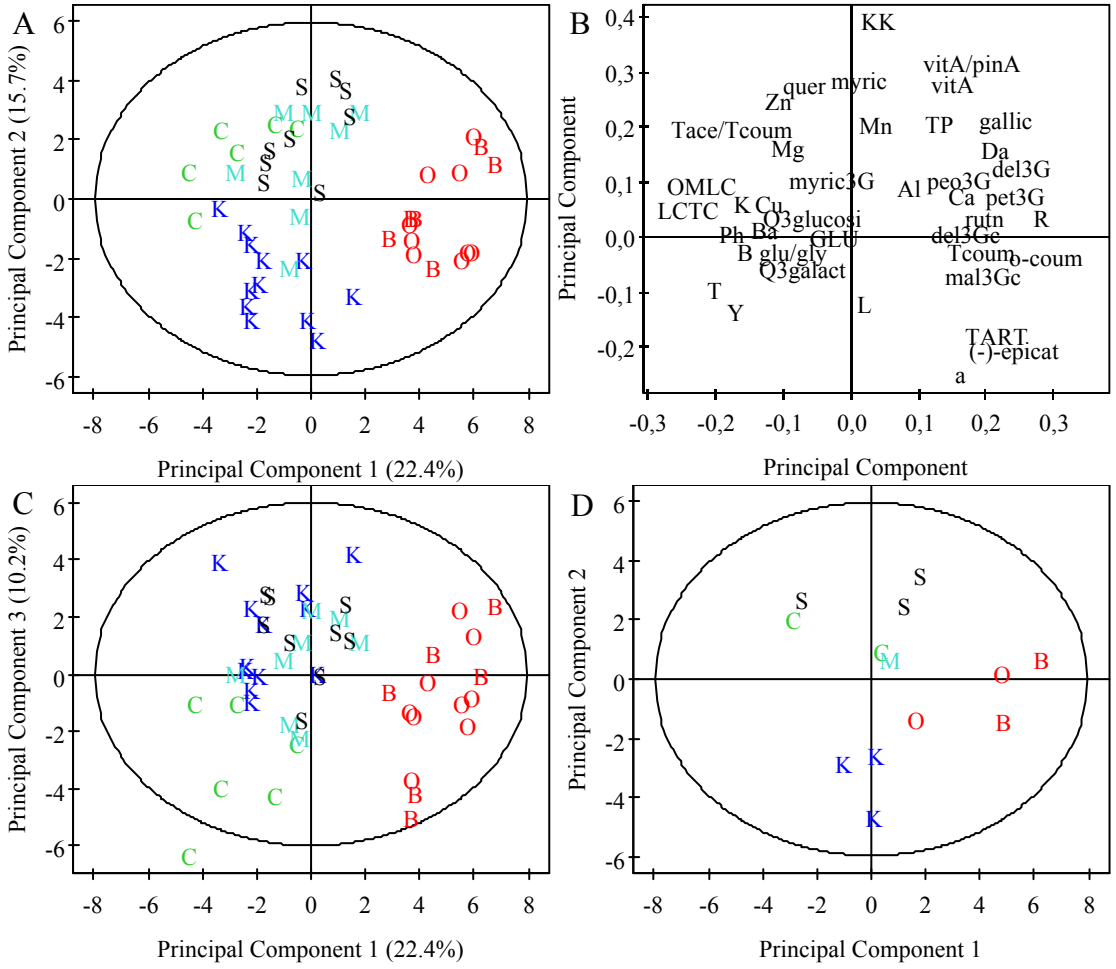

Figure 5.17. PLS-DA scores (A, C), loading (B) and validation (D) plots of red wines based on all significant variables discriminated according to grape variety: (A) PC1 vs PC2, (C) PC1 vs PC3. Coloring: **Boğazkere**, **Öküzgözü**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Syrah**

For white wines, the high sugar content of Muscat wines was due to the winemaking technique (Figure 5.18A). All the semi-sweet white wines belonged to

Muscat class. Moreover, the high contents of Pb, Co, Mn, hydroxycinnamic acids, tartaric acid and low procyanidin B_1, gallic acid and color parameters (yellow/blue chromaticity, chroma, color density and color intensity). The second PC was responsible for the discrimination of Emir and Narince wines from the other classes. This was based on the higher concentrations of Sr, procyanidin B_1 and vanillic acid of Emir and Narince wines. On the other hand, in Figure 5.18C, Emir and Narince wines were discriminated from each other due to the higher resveratrol content of Emir wines and higher (+)-catechin content of Narince wines. Moreover, the organic acids such as malic, lactic and original malic acid were able to discriminate Chardonnay and Sultaniye wines. Chardonnay wines were the richest and Sultaniye wines were the poorest in malic, lactic and original malic acid contents, respectively. The membership probability values of validation set were between 0.09-0.98 (Figure 5.18D).

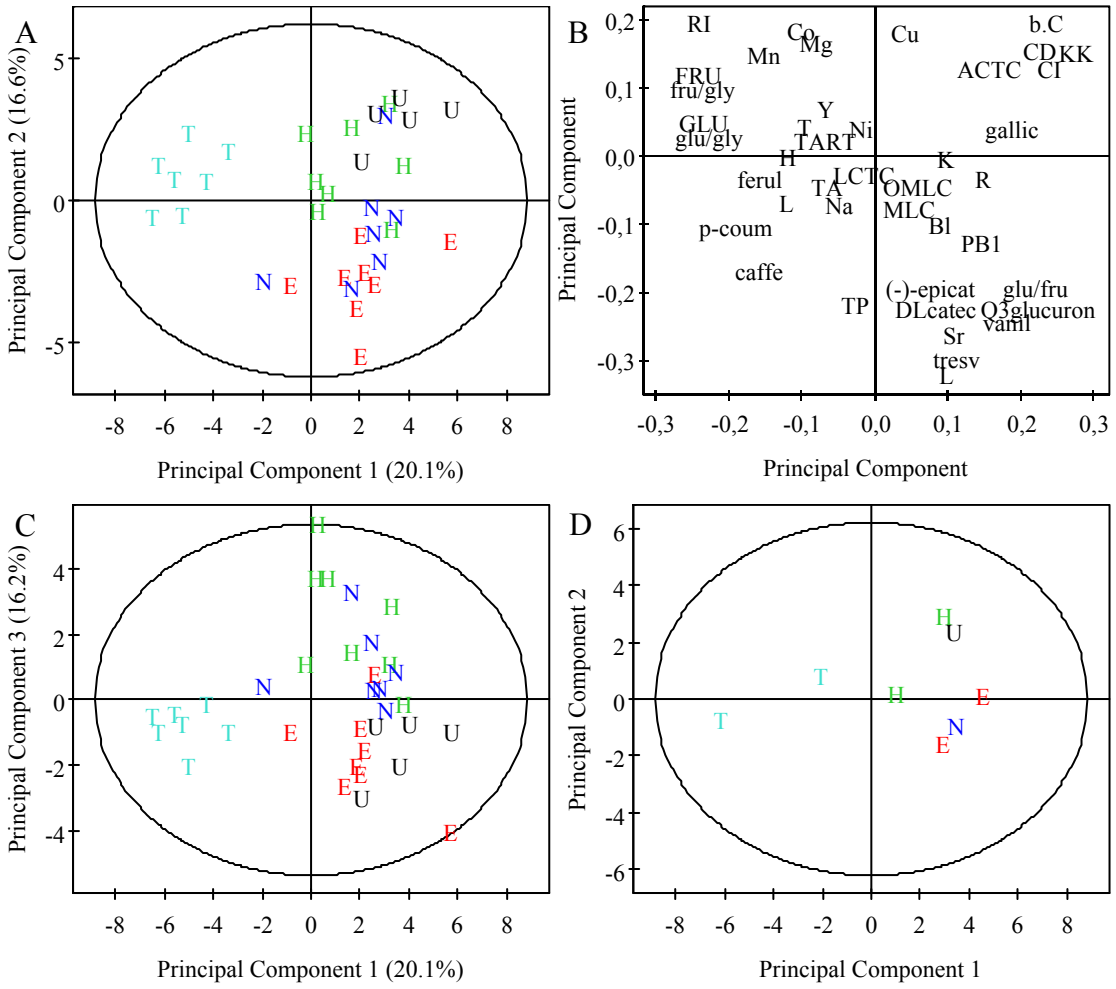

Figure 5.18. PLS-DA scores (A, C), loading (B) and validation (D) plots of white wines based on all significant variables discriminated according to grape variety: (A) PC1 vs PC2, (C) PC1 vs PC3. Coloring: **Emir**, **Chardonnay**, **Narince**, **Muscat**, **Sultaniye**

5.7.2.1.2. Geographical Discrimination

In this section of the study, the PLS-DA technique was employed for the geographic discrimination of wine samples using the significant variables determined according to the VIP feature of Simca-P software. The element and polyphenol compositions were effective in the geographical discrimination of both red and white wine samples. The PLS-DA model parameters are listed in Table 5.19.

In the PLS-DA classes the closer regions were grouped in the same class to increase the number of observations. For this aim, in the model of red wines, Denizli (d), İzmir (i) and Manisa (m) were grouped in a class as Western Anatolia with 34 samples, whereas Bozcaada (b) and Tekirdağ (t) class represented the North-West Turkey with 5 samples. Diyarbakır (c) and Elazığ (e) were grouped in a class as East Anatolia with 11 samples and Kapadokya (k) represented Central Anatolia with 5 samples. The individual classes for Ankara (3 sample), Tokat (3 sample), Tekirdağ-İzmir-Denizli (1 sample), Diyarbakır-Denizli (3 sample) and Denizli-Ankara (1 sample) regions could not be established due to the insufficient number of observations. Therefore wines from those regions were set as classless in the PLS-DA models.

Similar to the red wines, the white wines were grouped in 3 classes including Denizli (d), İzmir (i) and Manisa (m) in a class with 25 observations, and Kapadokya (k) and Tokat (r) as the other two classes with 11 and 5 observations. The two Tekirdağ wines were set as classless due to the insufficient number of samples to build a class.

The variables different than those used in varietal discrimination were employed in the geographic discrimination of wines, since the importance of a particular variable in the discrimination of grape variety may not be significant in the case of geographic region or vice versa (Villagra et al., 2012). According to the VIP feature of Simca-P, almost the same elements were found significant in the geographic discrimination of both red and white wines using the PLS-DA technique (additionally K and Ca were employed in the model of red wines).

Table 5.19. PLS-DA model parameters of geographic discrimination of red and white wines

		Variables	# of PC	R^2_X	R^2_Y	R^2_{pred}	Calib. set	Valid. set	Observations in the validation set
Red Wines	Elements	12: Ca, K, Mg, Sr, B, Al, Ba, Li, Co, Ni, Cu, Pb	2	0.453	0.443	0.317	43	11	B8c6, C6t1, C7i11, C7k8, K7d6, K8d11, M7d7, O8e6, S6d6, S8d4, S8m5
	Polyphenols	22: peo3G, pet3G, del3G, mal3Ga, del3Gc, mal3Gc, vitA, Tcoum, Tace/Tcoum, rutn, quer, myric, Q3glucosi, Q3galact, Q3glucuron, myric3G, caffe, tresv, gallic, vanill, (-)-epicat, o-coum	4	0.711	0.543	0.159	43	11	B8c6, C6t1, C7i11, C7k8, K7d6, K8d11, M7d7, O8e6, S6d6, S8d4, S8m5
	Element - Polyphenols	34 variables	3	0.559	0.532	0.338	43	11	C6t1, C7i11, C7k8, K7d6, K8d11, M7d7, O8e6, O9e6, S6d6, S8d4, S8m5
White Wines	Elements	10: Mg, Sr, B, Al, Ba, Li, Co, Ni, Cu, Pb	2	0.358	0.524	0.350	33	8	E7k12, E8k5, H8i11, N7r3, T8m5, T9d4, U6d10, U7d5
	Polyphenols	9: TP, kaemp, Q3glucuron, tresv, DLcatec, vanill, (-)-epicat, o-coum, PB1	2	0.595	0.377	0.247	33	8	E7k12, E8k5, H8i11, N7r3, T8m5, T9d4, U6d10, U7d5
	Element-Polyphenols	19 variables	2	0.329	0.600	0.410	33	8	E7k12, E8k5, H8i11, N7r3, T8m5, T9d4, U6d10, U7d5

Red wines of grapes cultivated in West Anatolia (Denizli, Izmir, Manisa, and Tekirdağ) could clearly be discriminated from those in East (Elazığ and Diyarbakır) (Figure 5.19A). The wines originating from West Anatolia had higher Pb, Cu, B and lower Ca levels than the wines from east. According to the ANOVA results, red wines from Diyarbakır, Elazığ, Ankara and Denizli had significantly lower Pb content than those from Kapadokya, Manisa, İzmir, Tekirdağ and Tokat ($p< 0.05$). The high Pb content of western region wines may be explained by the high industrial development of western Turkey. Alkış et al. (2014) have also reported that the Pb and Cd levels of grapes from the Aegean and Marmara regions were more than those from East Anatolia. They concluded that Marmara was a highly industrialized region with vast amounts of thermal power plants. The large amount of thermal power plants in the East and Central Anatolia regions may affect the heavy metal content of wines, especially for Pb and Cd. On the other hand, according to another study, the major source of Pb contamination in table wines was the vinification processes (Almeida & Vasconcelos, 2003). Pb can also originate from environmental factors such as soil contamination, atmospheric pollution, and fungicidal treatment (Volpe et al., 2009). In our study, the wine samples were from different producers. Regardless of producer, the wines of western regions such as Izmir, Manisa, and Tekirdağ had higher Pb levels than the wines of other regions, but still had less than the legal limit set by the OIV (0.15 mg/L).

The Kapadokya region wines had high Li and Sr contents, whereas Denizli, Manisa and Tokat wines were poor in terms of these elements. Wines from İzmir region were rich in Ba and those from Tekirdağ were rich in Pb, Co and Ni. Mainly, the litophile elements with a few chalcophile (Cu, Pb) and siderophile (Co, Ni) elements were employed in the model. Those, which were dependent on the winemaking technology, such as Cr, Fe, Mn and Zn (Thiel et al., 2004) were not found significant in the geographic discrimination of red wines except Cu. In literature, similar elements were employed in the geographic discrimination of wines from different countries. For instance, Geana et al. (2013), have found Pb, Cu, Zn, Co, Ni, Sr, Mn, Ag, Cr, Rb and V as significant variables to discriminate wines from three important wine regions in Romania. Fabani et al. (2010) have classified Argentinean wines using the following variables: K, Fe, Ca, Cr, Mg, Zn and Mn. All wines in the prediction set were classified correct by the developed calibration model except one sample (B8c). The membership probability values of the validation set were between 0.16-0.99 (Figure 5.19C).

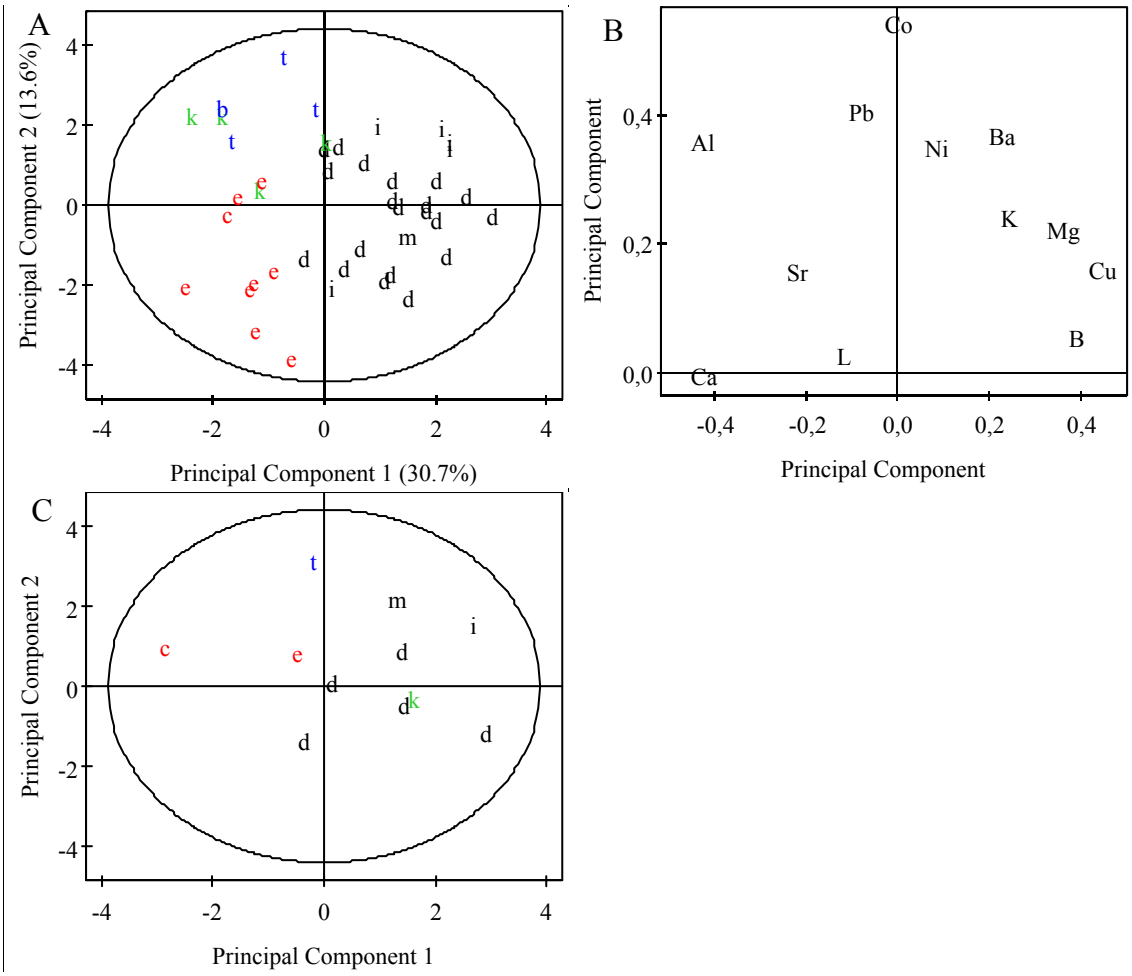

Figure 5.19. PLS-DA score (A), loading (B) and validation (C) plots of red wines based on element contents discriminated according to geographic region: PC1 vs PC2. Coloring: **Elazığ(e)-Diyarbakır(c)**, **Kapadokya(k)**, **Tekirdağ(t)-Bozcaada(m)**, **Denizli(d)-İzmir(i)-Manisa(m)**

White wines of grapes from Kapadokya (Emir, Chardonnay) and Manisa (Sultaniye, Narince and Muscat) regions were the richest in Li contents, despite of their different classes (Figure 5.20A). The clusters of Kapadokya and Tokat regions were based on their lower Mg and Ni, and higher Sr levels than the other regions. On the other hand, the West Anatolia (İzmir, Tekirdağ and Manisa) wines were rich in Pb. It should be mentioned again that western Turkey is a highly industrialized area. Wines of Denizli origin were poor in Sr, Li, Ba, and Pb contents. The concentrations of natural minerals such as Ba, B, Li, Al or Sr do not depend on agricultural and processing activities, and they played a role on the regional discrimination of wine samples. Martin et al. (2012) have also employed mainly the litophile elements together with some of the siderophile and rare earth elements as the most reliable elements (Sr, Li, Na, Mg, K, Ca, Fe, Ba, Ni, Zn, Mn, Si, P, Rb, Cs) to discriminate the red and white wine samples from

Australia. Cugnetto et al. (2014) have employed Sr, Ti, Ba, Mn and Si to discriminate wine samples from Alpine and Langhe areas. For this study, it was recognized that the longer the distances among the vine growing regions were, the better the discrimination was. Similar results were reported by Capron, Smeyersverbeke, and Massart (2007). Marengo and Aceto (2003) have also poorly classified Nebbiolo grape red wines from north Italy due to the narrow region of provenances which was less than 1600 km^2. In the same way, Martin et al. (2012) came across with mis-classification difficulties in the closer regions (150 km). They concluded that the mis-classification might arise from the similar pedology and climate characteristics of closer regions. Selih et al. (2014) have also failed to discriminate the geographic origin of Slovenian white wines due to the close location of regions (approximately 300 km) using the PCA technique. However, they managed to discriminate the regions using the counter-propagation artificial neural network modeling method instead of PCA. All white wines in the validation set were classified correct with membership probability values between 0.37-0.98 (Figure 5.20C).

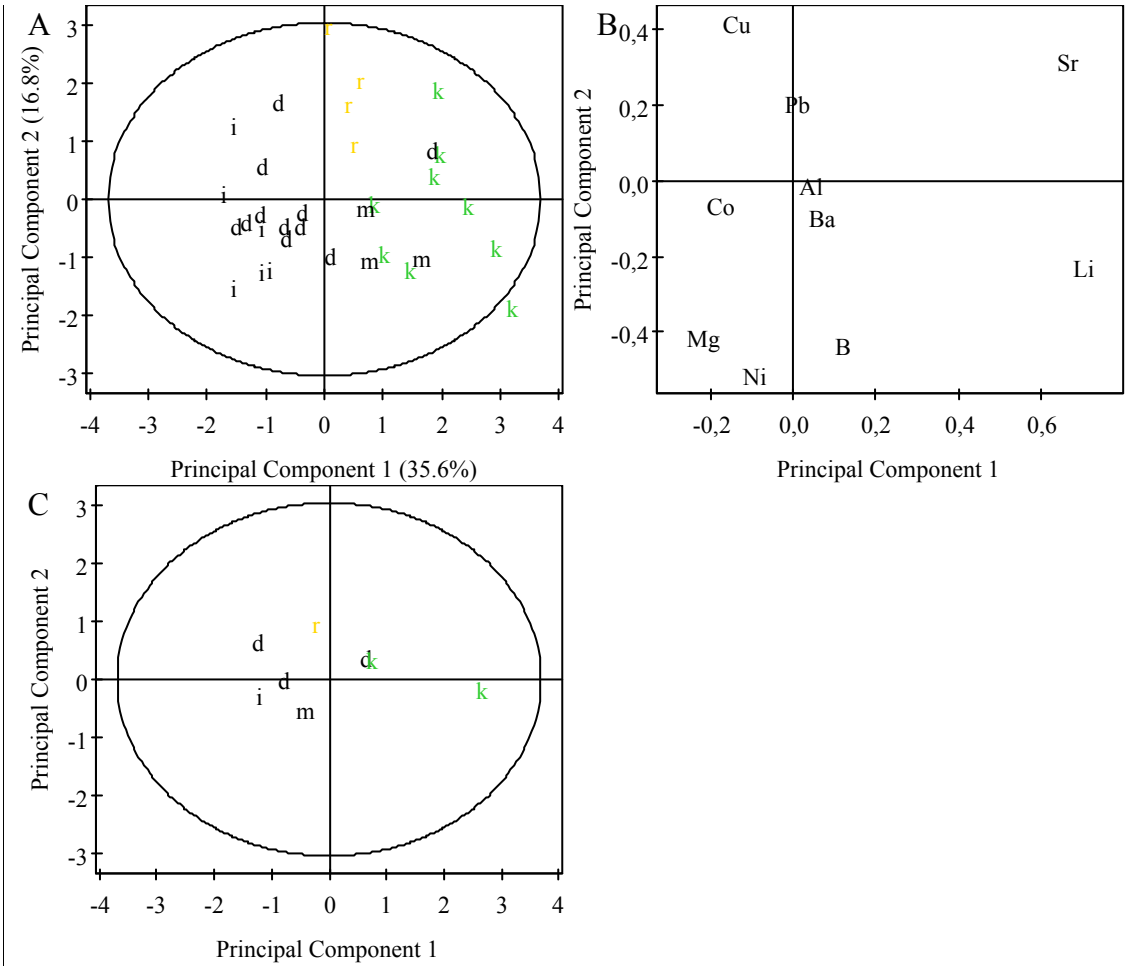

Figure 5.20. PLS-DA score (A), loading (B) and validation (C) plots of white wines based on element contents discriminated according to geographic region: PC1 vs PC2. Coloring: **Kapadokya(k)**, **Tokat(r)**, **Denizli(d)-İzmir(i)-Manisa(m)**

The ability of polyphenol variables to discriminate wines by their geographic origin was not superior to that of element contents. Similar to the PLS-DA plot of element contents, a clear discrimination of wines from eastern and western regions could be observed and this discrimination relied more on the significant variables: Tace/Tcoum, gallic acid and (-)-epicatechin (Figure 5.21A). The eastern region wines on the left-hand side of the score plot were from Boğazkere and Öküzgözü cultivars and they were rich in gallic acid and coumaroylated malvidin derivatives and poor in (-)-epicatechin. On the other hand, the right-hand side of the score plot was occupied with western region wines of 4 different cultivars (Syrah, Merlot, Cabernet Sauvignon, Kalecik Karası).

Wines from red grapes of Tekirdağ region were the poorest in terms of flavonol-glycosides (quercetin-3-glucoside, quercetin-3-glucuronide and myricetin-3-glucoside). They were from 4 different cultivars: Kalecik Karası, Cabernet Sauvignon, Papazkarası and Merlot. Among the all vineyard regions, Tekirdağ had the lowest average of total daily sunshine-exposure (hr) at all vintages particularly during ripening period of grape and generally had high rainfalls (Table 5.15). Fanzone et al. (2012) related the elevated flavonol concentrations of Malbec and Cabernet Sauvignon to high sunlight radiation of grapes during the ripening period. Therefore, the low flavonol-glycoside content of Tekirdağ region might be influenced from the low sunshine-exposure of grape in this region. According to another study by Ünsal (2007), Kalecik Karası wines from Tekirdağ region had higher (+)-catechin and (-)-epicatechin concentrations than those from Ankara region and they concluded that the differences relied mainly on geographic regions. In this study, the number of Kalecik Karası wines from Tekirdağ (n=1) and Ankara (n=3) were not sufficient for a reliable comparison. However, Kalecik Karası wine from Tekirdağ had higher (-)-epicatechin and lower (+)-catechin than those from Ankara, Denizli and İzmir. Among the 11 samples in the validation set, only one sample was classified as outlier (C6t) and the remaining samples had membership probability values between 0.21-0.98 indicating correct classification (Figure 5.21C).

The polyphenol variables employed in the models of varietal and regional discrimination were almost similar. There were only total phenol and vitA/pinA ratio missing and quercetin-3-glucuronide, caffeic and vanillic acids and resveratrol added to the model of regional discrimination. In fact, in the cluster of western region wines in Figure 5.21A, there was discrimination between Kalecik Karası and Syrah wines. Moreover, the model discriminating grape varieties had higher R^2_{pred} (0.329) values and no mis-classified samples. It is convenient to conclude that the polyphenol variables are more effective to discriminate wine samples in terms of grape variety rather than geographic origins. Nevertheless, there are various studies discriminating wines according to their geographic origins in literature. Makris, et al. (2006) have discriminated Syrah, Cabernet Sauvignon, Merlot and native wines of Greece all from 2004 vintage according to their geographic origins using anthocyanin-glycosides, flavan-3-ols, flavonols, procyanidin B_1 and B_2, and hydroxycinnamic acids. Li et al. (2011) have discriminated Cabernet Sauvignon wines from five specific regions in China using their polyphenol compositions, however, all the wine samples were of the same vintage (2007). They suggested that though similar polyphenol composition was

observed between the regional wines, there were some differences in various phenolic compounds depending on the strong effect of terroir. Rastija et al. (2009) have reported that flavonols and resveratrol were the main variables discriminating the Croatian wines according to geographic region and grape variety using the PCA and cluster analysis techniques.

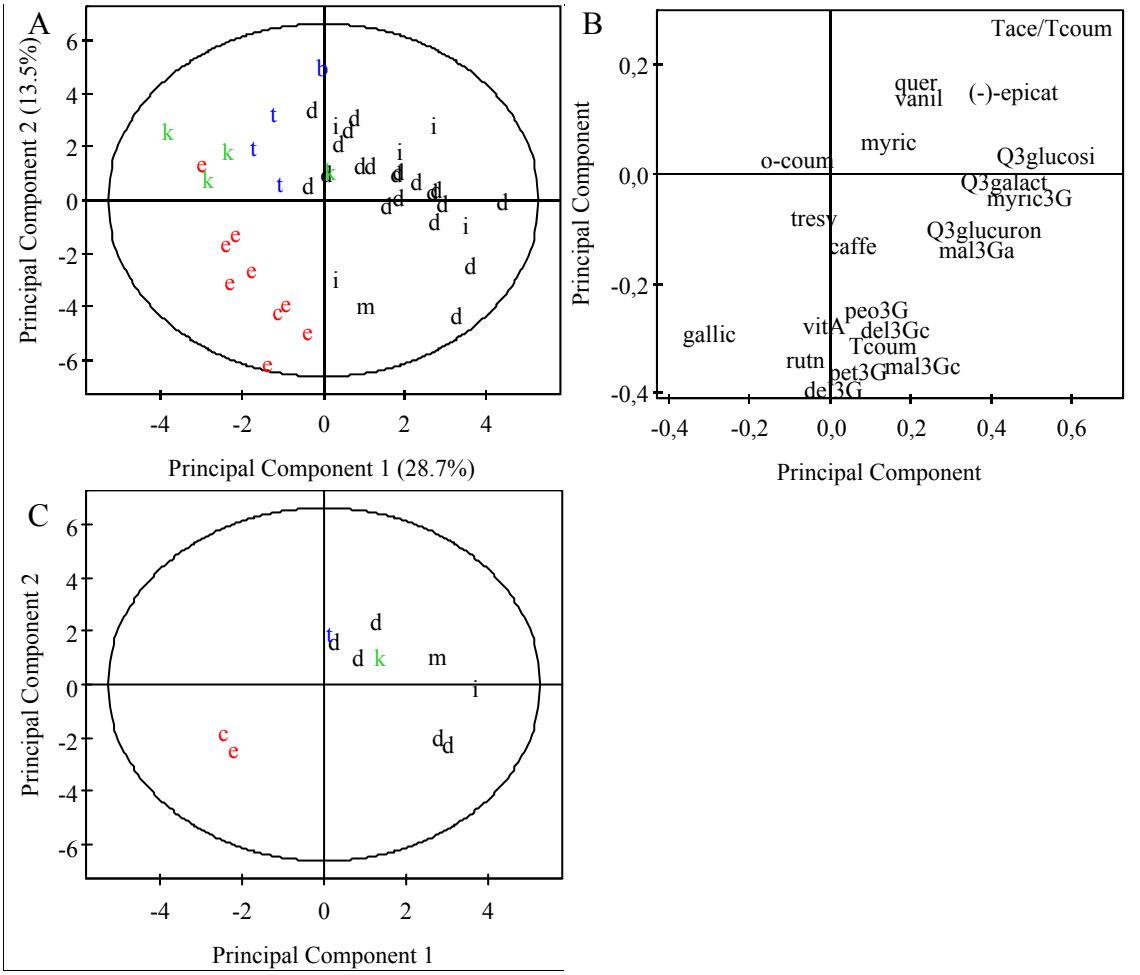

Figure 5.21. PLS-DA score (A), loading (B) and validation (C) plots of red wines based on polyphenol contents discriminated according to geographic region: PC1 vs PC2. Coloring: **Elazığ(e)-Diyarbakır(c)**, **Kapadokya(k)**, **Tekirdağ(t)-Bozcaada(b)**, **Denizli(d)-İzmir(i)-Manisa(m)**. Loadings: peo3G: peonidin-3-glucoside, pet3G: petunidin-3-glucoside, del3G: delphinidin-3-glucoside, mal3Gc: malvidin-3-glucoside coumarate, del3Gc: delphinidin-3-glucoside coumarate vitA: vitisin-A, Tcoum: Total coumarates, Tace/Tcoum: Total acetates/ Total coumarates, rutn: rutin, quer: quercetin, myric: myricetin, Q3glucosi: quercetin-3-glucoside, Q3galact: quercetin-3-galactoside, Q3glucuron: quercetin-3-glucuronide, myric3G: myricetin-3-glucoside, caffe: caffeic acid, tresv: resveratrol, gallic: gallic acid, vanill: vanillic acid, epicat: (-)-epicatechin, o-coum: o-coumaric acid

The regional discrimination of white wines using PLS-DA technique was highly influenced by the harvest years. Of the 8 wines from 2009, 4 of them were scattered to the lower side of the score plot (Figure 5.22A). They were from different geographic regions and grape varieties and were richer in kaempferol, quercetin-3-glucuronide and (+)-catechin than the other vintages. Denizli and İzmir regions could be discriminated from Kapadokya and Tokat regions based on the higher (-)-epicatechin and procyanidin B_1 contents of Kapadokya and Tokat regions. Moreover, Tokat region wines were the richest in (+)-catechin and Kapadokya region wines were the richest in resveratrol, vanillic and o-coumaric acids. In the ANOVA results, Denizli was found significantly low in resveratrol, (+)-catechin and (-)-epicatechin and o-coumaric acid contents ($p<0.05$). However, all Kapadokya and Tokat wines belong to Emir and Narince variety wines, respectively. The wine sample from Manisa within the Kapadokya-Tokat cluster belonged to Narince variety. Moreover, in the cluster of West Anatolia, there was also discrimination between Muscat and Sultaniye wines. All the samples in the validation set were classified correct (membership probability values: 0.27-0.97) (Figure 5.22C).

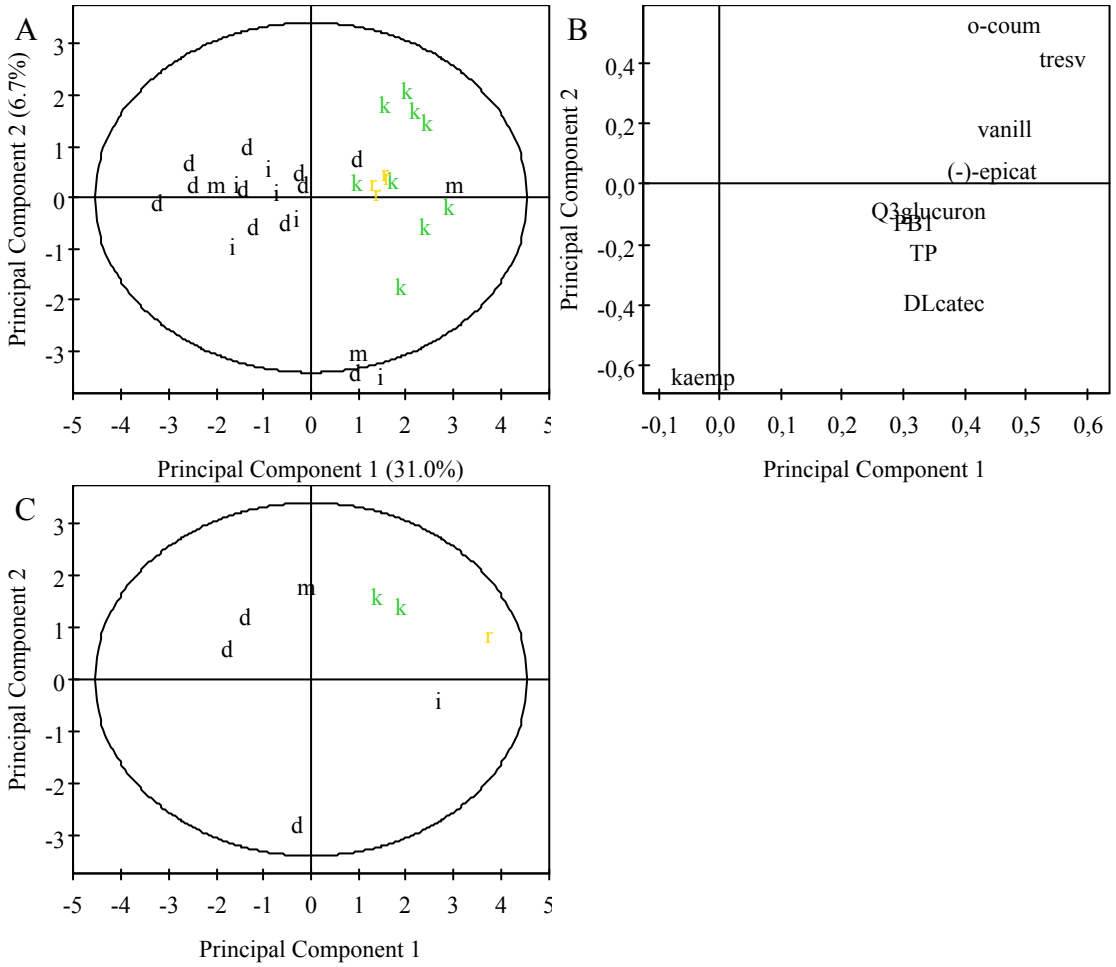

Figure 5.22. PLS-DA score (A), loading (B) and validation (C) plots of white wines based on polyphenol contents discriminated according to geographic region: PC1 vs PC2. Coloring: **Kapadokya(k)**, **Tokat(r)**, **Denizli(d)-İzmir(i)-Manisa(m)**. Loadings: TP: Total phenol, tresv: resveratrol, vanill: vanillic acid, epicat: (-)-epicatechin, DLcatec: (+)-catechin, kaemp: kaempferol, PB1: procyanidin B_1, Q3glucuron: quercetin-3-glucuronide, o-coum: o-coumaric acid

The combination of significant element and polyphenol variables in the PLS-DA models produced similar discrimination with better model parameters (higher R^2_{pred}). The discrimination of red wines from East Anatolia, West Anatolia, Tekirdağ and Kapadokya regions was demonstrated in Figure 5.23A. On the other hand, the discrimination of white wines from Tokat, Kapadokya and West Anatolia was demonstrated in Figure 5.24A. The samples in the validation sets of red and white wines were classified correct (membership probability values: 0.05-0.97 for red wines and 0.19-0.86 for white wines).

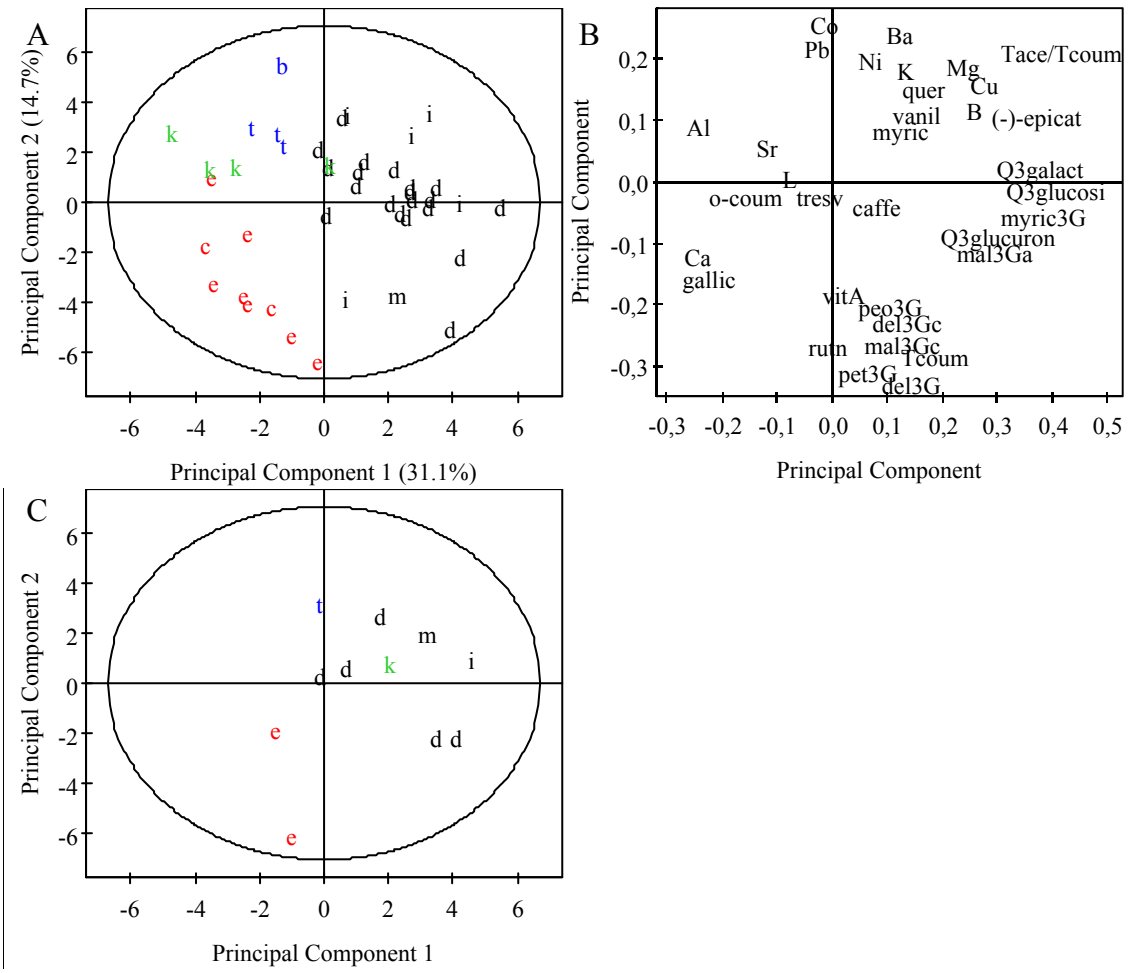

Figure 5.23. PLS-DA score (A), loading (B) and validation (C) plots of red wines based on polyphenol and element contents discriminated according to geographic region: PC1 vs PC2. Coloring: **Elazığ(e)-Diyarbakır(c)**, **Kapadokya(k)**, **Tekirdağ(t)-Bozcaada(b)**, **Denizli(d)-İzmir(i)-Manisa(m)**. Loadings: peo3G: peonidin-3-glucoside, pet3G: petunidin-3-glucoside, del3G: delphinidin-3-glucoside, mal3Gc: malvidin-3-glucoside coumarate, del3Gc: delphinidin-3-glucoside coumarate vitA: vitisin-A, Tcoum: Total coumarates, Tace/Tcoum: Total acetates/ Total coumarates, rutn: rutin, quer: quercetin, myric: myricetin, Q3glucosi: quercetin-3-glucoside, Q3galact: quercetin-3-galactoside, Q3glucuron: quercetin-3-glucuronide, myric3G: myricetin-3-glucoside, caffe: caffeic acid, tresv: resveratrol, gallic: gallic acid, vanill: vanillic acid, epicat: (-)-epicatechin, o-coum: o-coumaric acid

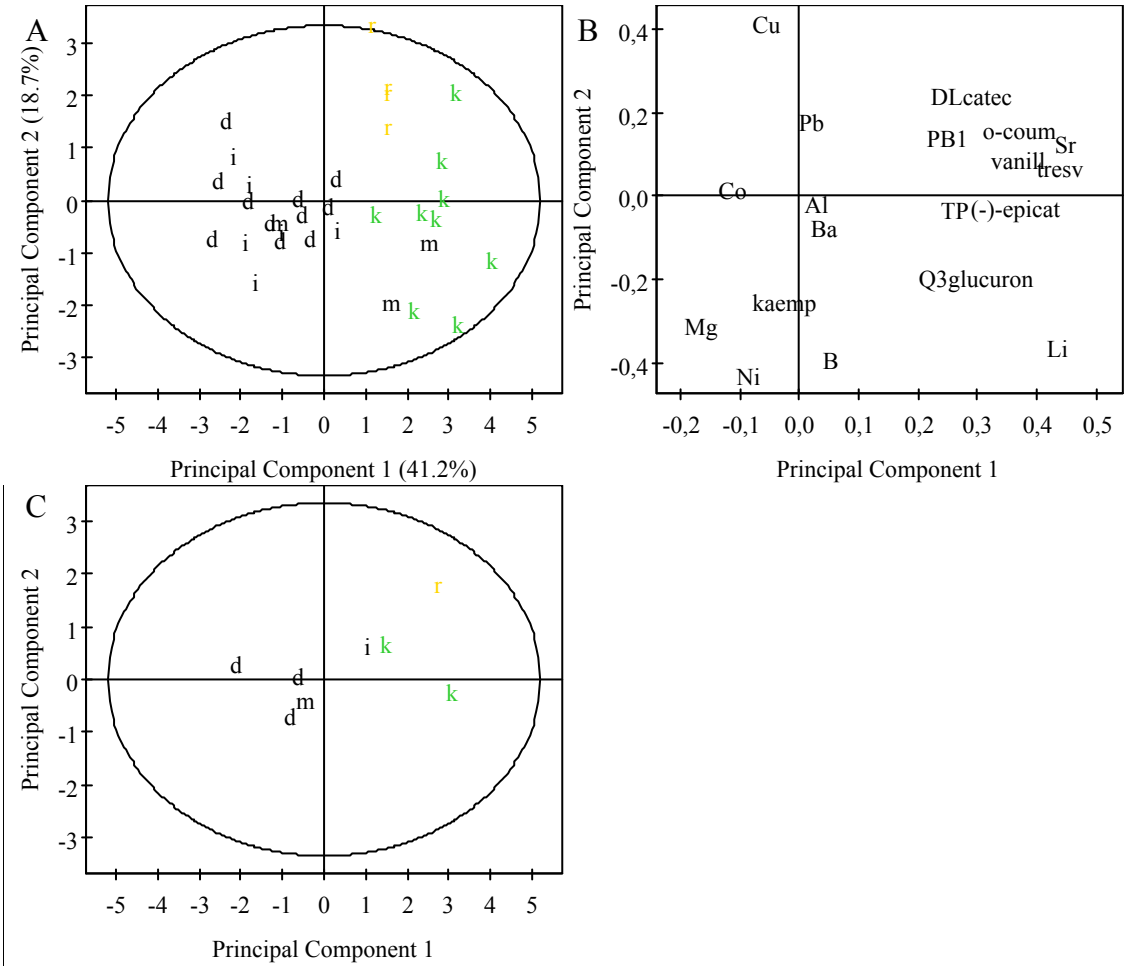

Figure 5.24. PLS-DA score (A), loading (B) and validation (C) plots of white wines based on polyphenol and element contents discriminated according to geographic region: PC1 vs PC2. Coloring: **Kapadokya(k)**, **Tokat(r)**, **Denizli(d)-İzmir(i)-Manisa(m)**. Loadings: TP: Total phenol, tresv: resveratrol, vanill: vanillic acid, epicat: (-)-epicatechin, DLcatec: (+)-catechin, kaemp: kaempferol, PB1: procyanidin B_1, Q3glucuron: quercetin-3-glucuronide, o-coum: o-coumaric acid

5.7.2.1.3. Harvest Year Discrimination

The final application of PLS-DA technique was the harvest year discrimination of wine samples according to the significant variables determined by the VIP feature of Simca-P software. In the PLS-DA models, the four harvest years were set as different classes. The numbers of sample for each harvest year (2006, 2007, 2008 and 2009) were 14, 21, 11 and 8 for red wines whereas they were 8, 10, 8 and 8 for white wines, respectively. The model parameters are listed in Table 5.20.

Table 5.20. PLS-DA model parameters of harvest year discrimination of red and white wines

		Variables	# of PC	R^2_X	R^2_Y	R^2_{pred}	Calib. set	Valid. set	Observations in the validation set
Red Wines	Polyphenols	25: mal3G, peo3G, pet3G, del3G, del3Ga, pet3Ga, peo3Ga, mal3Ga, del3Gc, mal3Gc, vitA, pinA, Tace, Tcoum, rutn, myric, kaemp, Q3glucosi, Q3galact, myric3G, p-coum, ferul, tresv, gallic, o-coum	5	0.801	0.608	0.352	54	11	B8k8, C7i11, K6a4, K8d11, M7d7, M8d10, O6k8, O9e4, S6d6, S7d7
	Color	8: a, b., C, H, T, Da, R, Y	2	0.974	0.221	0.164	54	11	B8k8, C7i11, K6a4, K7d11a, K8d6, M7d7, O6e6, O8e4, O9e4, S6d6, S7d7
	Polyphenol-Color	33 variables	3	0.655	0.528	0.411	54	11	B8k8, C7i11, K6a4, K8d11, M7d7, M8d10, O6k8, O9e4, S6d6, S7d7
White Wines	Polyphenols	13: TP, quer, myric, kaemp, Q3galact, Q3glucuron, myric3G, ferul, gallic, DLcatec, vanill, (-)-epicat, o-coum	3	0.585	0.543	0.260	34	9	E7k4, E8k8, H8i11, H9i11, N7r5, T6i6s, T8i6s, U6d5, U7d5

The quality of harvest varies from year to year and the effect on grape composition can be varying sugar, acidity, nitrogen and phenolic compound balance (Pereira et al., 2006). The environmental factors such as rainfall, sunshine-exposure or average temperature of each harvest year might have affected the polyphenol content and color parameters of wine samples as reported elsewhere (Ferrandino & Lovisolo, 2014; Jaitz et al., 2010; Lee et al., 2009; Lorrain et al., 2011). The effect of vintage was clearly observed in the PCA discriminations of red and white wines using the polyphenol and color variables.

The polyphenol variables produced a PLS-DA model and the first PC discriminated 2009 harvest year wines from the other harvest years due to higher flavonol (myricetin, kaempferol), pinotin-A, vitisin-A and anthocyanin contents of 2009 harvest year wines (Figure 5.25A). It was the only vintage with vitA/pinA ratio lower than 1.0. It was explained in the PCA section that the high anthocyanin concentration of 2009 vintage wines might be based on the increased biosynthesis of flavonoids due to water deficiency during veraison period (August). The strongest water deficit during the veraison period was observed in 2009 though it has the highest precipitation during blooming. Lorenzo et al. (2012) have positively correlated the precipitation during bloom with wine quality while precipitation during bud break and veraison were negatively correlated. Ojeda et al. (2002) have reported that the anthocyanin content of Syrah grapes increased 5 days after the beginning of veraison period and strong water deficiency during veraison increased the biosynthesis of anthocyanins. Moreover, the intense early water deficit can limit the biosynthesis of anthocyanins.

Sofo et al. (2012) reported that the non-irrigated soils produced berries with significantly higher anthocyanin contents than the irrigated soils and the irrigated berries had significantly lower Tace/Tcoum ratios. Similar results were reported by Bucchetti et al. (2011) for Merlot grapes. Lee et al. (2009) have also discriminated 2006 and 2007 vintage Meoru red wines of Korea due to the higher content of polyphenols in 2006 vintage wines. 2006 vintage veraison period (August) had lower rainfall and higher sunshine-exposure time than 2007 which might be responsible for the high polyphenol contents of red wines. On the other hand, unlike our findings, Lorrain et al. (2011) have found lower anthocyanin and proanthocyanin concentrations in 2009 vintage grapes of Cabernet Sauvignon and Merlot than 2006, 2007 and 2008 harvest years. They attributed the low phenolic concentrations of grapes on the high rainfall amounts before flowering in 2009 and the high sunshine and temperature values

between June and September. The high sunshine and temperature values could have damaged the anthocyanins and proanthocyanins in grape skins and reduced their amount. In Turkey, the mean temperature values during veraison were the lowest in 2009 compared to 2006, 2007 and 2008. However, the veraison period of 2006 had the highest sunshine-exposure and temperature values and wines of this vintage were the poorest in anthocyanin and flavonol content.

The discrimination between 2006, 2007 and 2008 harvest years was clear in the score plot of PC2 and PC3 (Figure 5.25C). 2008 vintage wines were discriminated with their low rutin levels. On the other hand, 2006 and 2007 harvest year wines had significantly high resveratrol and low kaempferol ($p<0.05$). 2007 harvest year wines were also discriminated with their significantly high ferulic acid and low gallic acid contents ($p<0.05$) and they had high flavonol-glycosides (myricetin-3-glucoside and quercetin-3-glucoside), malvidin-3-glucoside and its acetylated derivative. According to the Pearson coefficients, these 2 flavonol-glycosides were positively and significantly correlated to the average temperature value of May (both 0.62 with $p<0.05$). This indicated that the higher the temperatures in May, the higher the quercetin- and myricetin-3-glucoside concentrations in wine. Among all vintages, 2007 had the highest temperature values in May for all regions. van Leeuwen et al. (2004) have stated that quercetin-3-glucoside level of berries under direct solar radiation were higher than the shaded berries. All the samples in the prediction set were classified correct with membership probability values between 0.05-0.98 (Figure 5.25D).

The flavan-3-ols and procyanidin B_1 had no impact on the harvest year discrimination of red wines. The tannin concentration in the berry skin increased early during the development (before veraison) and reached a maximum close to veraison. The accumulation of tannins is less sensitive to water deficiency than anthocyanins and the mechanisms causing rise were different. Water deficiency during ripening increased anthocyanin concentrations but not tannins. On the other hand, early water deficits affected the tannin content of berries. The high tannin content was related to the higher skin/ berry weight rather than the increased biosynthesis of tannins (Bucchetti et al., 2011). However, in the literature flavan-3-ols [(+)-catechin and (-)-epicatechin], flavonols (quercetin, myricetin, kaempferol) and some of the phenolic acids (gallic, ferulic, p-coumaric and caffeic acids) were found to be useful in the discrimination of Austrian red wines according to the five vintages (2003-2007) (Jaitz et al., 2010).

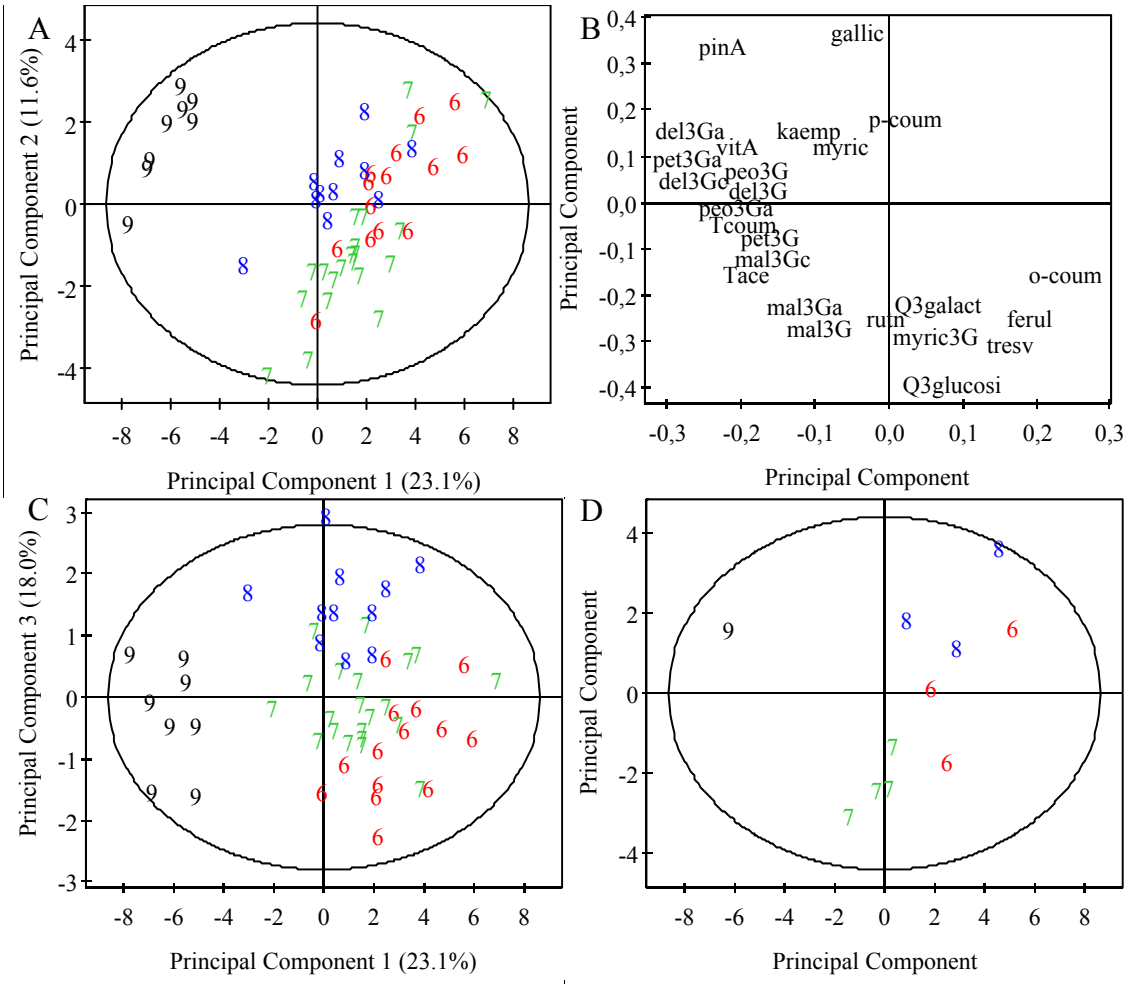

Figure 5.25. PLS-DA scores (A, C), loading (B) and validation (D) plots of red wines based on polyphenol contents discriminated according to harvest year: (A) PC1 vs PC2, (C) PC1 vs PC3. Coloring: **2006**, **2007**, **2008**, **2009.** Loadings: mal3G: malvidin-3-glucoside, peo3G: peonidin-3-glucoside, pet3G: petunidin-3-glucoside, del3G: delphinidin-3-glucoside, mal3Ga: malvidin-3-glucoside acetate, peo3Ga: peonidin-3-glucoside acetate, pet3Ga: petunidin-3-glucoside acetate, del3Ga: delphinidin-3-glucoside acetate, vitA: vitisin-A, pinA: pinotin-A, Tcoum: Total coumarates, Tace: Total acetates, rutn: rutin, myric: myricetin, kaemp: kaempferol, Q3glucosi: quercetin-3-glucoside, Q3galact: quercetin-3-galactoside, myric3G: myricetin-3-glucoside, ferul: ferulic acid, p-coum: p-coumaric acid, tresv: resveratrol, gallic: gallic acid, o-coum: o-coumaric acid

The harvest year discrimination of white wines was achieved with the flavonol and their glycoside derivatives as well as flavan-3-ols and some of the phenolic acids. 2006-2007 and 2008-2009 harvest years could be discriminated from each other according to the first PC (Figure 5.26A). The first two harvest year wines were poorer in quercetin-3-galactoside, (+)-catechin, total phenol content than the latter two. Moreover, 2009 harvest year wines were significantly rich in quercetin, kaempferol,

quercetin-3-galactoside and quercetin-3-glucuronide levels and poor in o-coumaric acid, ferulic acid and gallic acid levels among the other harvest years ($p<0.05$). The second PC was responsible for the discrimination of 2008 and 2009 vintage wines due to the higher contents of o-coumaric, vanillic and ferulic acids in 2008 vintage wines. All the samples in the validation set were classified correct with membership probability values between 0.09-0.97 (Figure 5.26C).

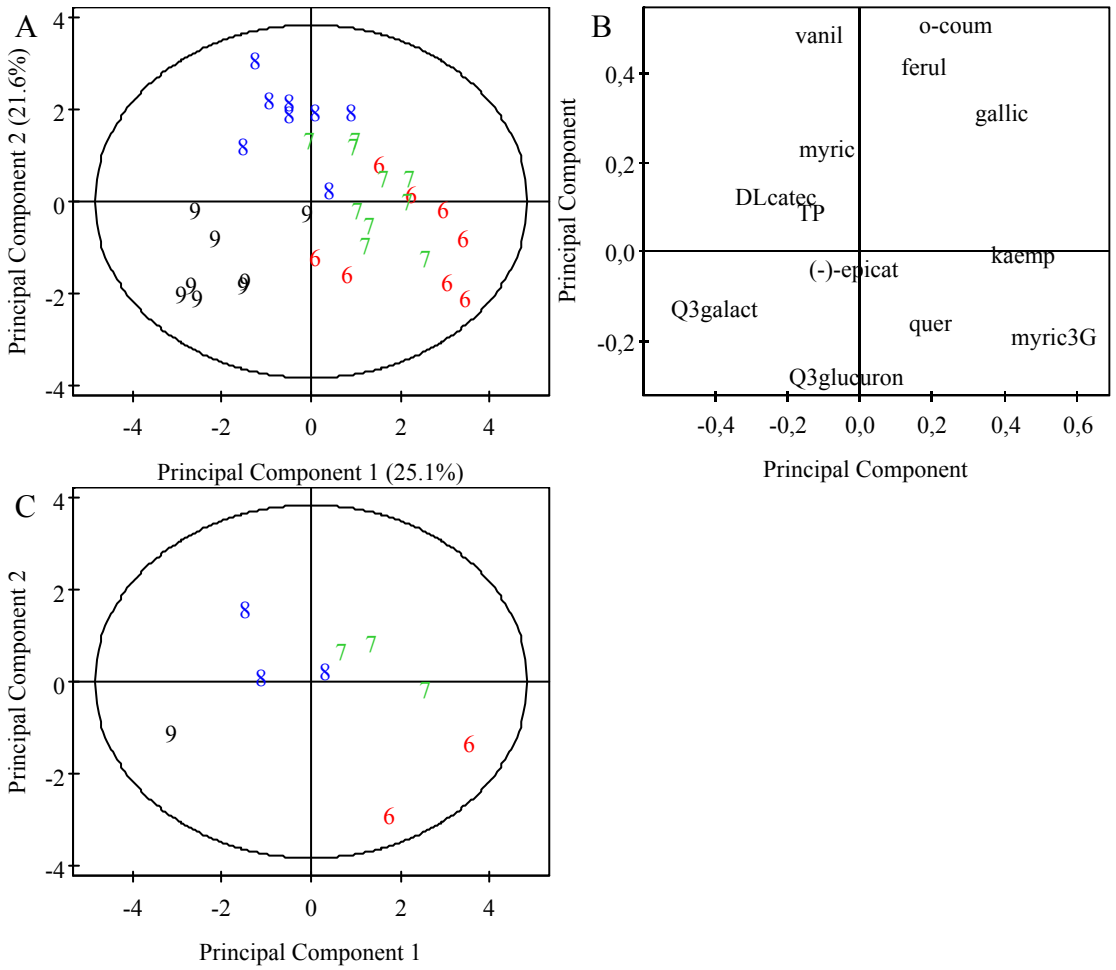

Figure 5.26. PLS-DA score (A), loading (B) and validation (C) plots of white wines based on polyphenol contents discriminated according to harvest year: PC1 vs PC2. Coloring: **2006**, **2007**, **2008**, **2009**. Loadings: TP: Total phenol content, quer: quercetin, myric: myricetin, kaemp: kaempferol, Q3glucuron: quercetin-3-glucuronide, Q3galact: quercetin-3-galactoside, myric3G: myricetin-3-glucoside, ferul: ferulic acid, vanill: vanillic acid, gallic: gallic acid, o-coum: o-coumaric acid, DLcatec: (+)-catechin, (-)-epicat: (-)-epicatechin

In the varietal discrimination of red wines using color parameters by PCA, the influence of harvest year was emphasized. Therefore the PLS-DA technique was employed on the color parameters to discriminate red wines by their harvest years. 2006-2007 and 2008-2009 harvest year wines were discriminated from each other due to lower red/green chromaticity, yellow/blue chromaticity, chroma, tint and yellow% values and higher proportion of red coloration and red% values of 2008-2009 harvest year wines (Figure 5.27A). All the samples in the prediction set were classified correct with membership probability values between 0.15-0.99 (Figure 5.27C).

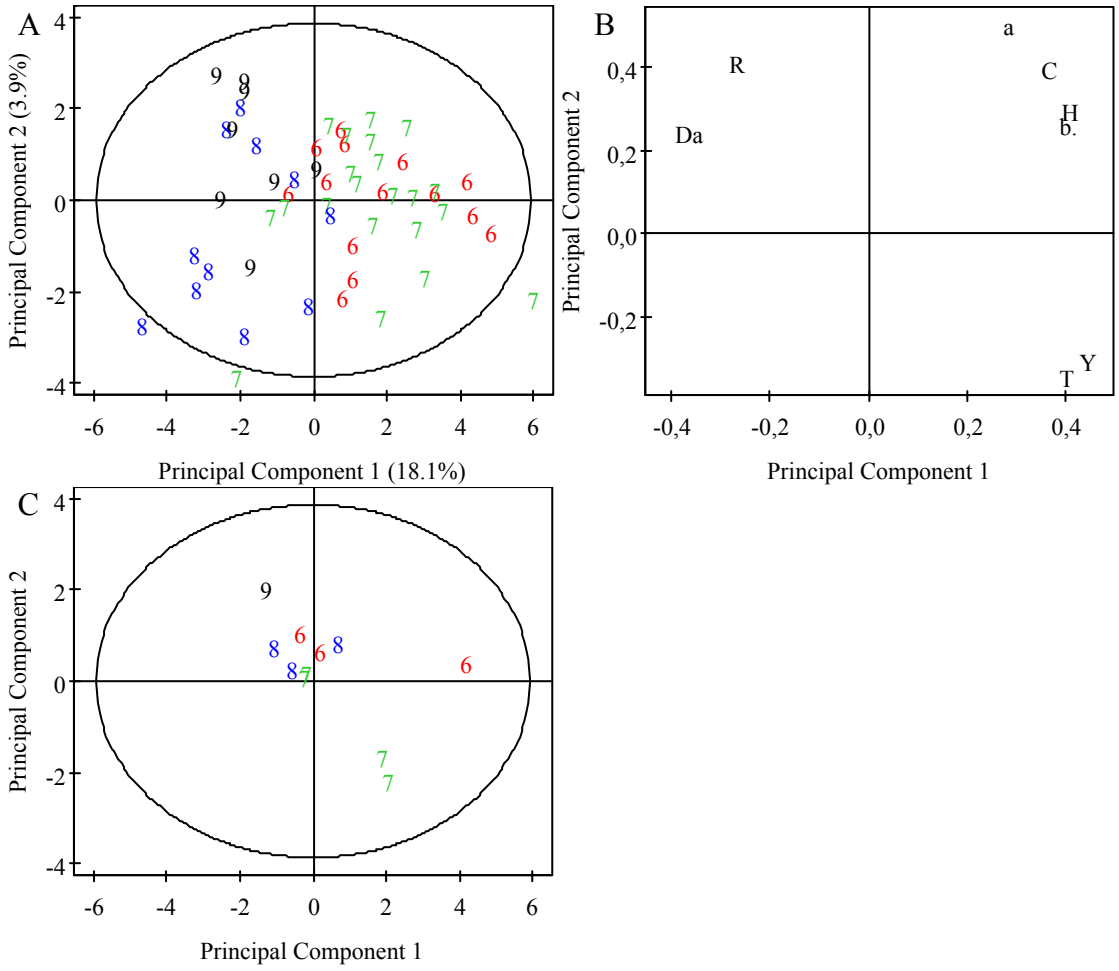

Figure 5.27. PLS-DA score (A), loading (B) and validation (C) plots of red wines based on color parameters discriminated according to harvest year: PC1 vs PC2. Coloring: **2006**, **2007**, **2008**, **2009**. Loadings: a: red/green chromaticity, b.: yellow/blue chromaticity, C: chroma, H: hue, T: tint, Da: proportion of red coloration, R: red%, Y: yellow%

The best harvest year discrimination of red wines was achieved with the combination of significant polyphenol variables and color parameters (Figure 5.28A). The model with R^2_{pred} of 0.411 discriminated the four harvest years and predicted all the samples in the validation set correct with membership probability values between 0.26-0.99 (Figure 5.28D).

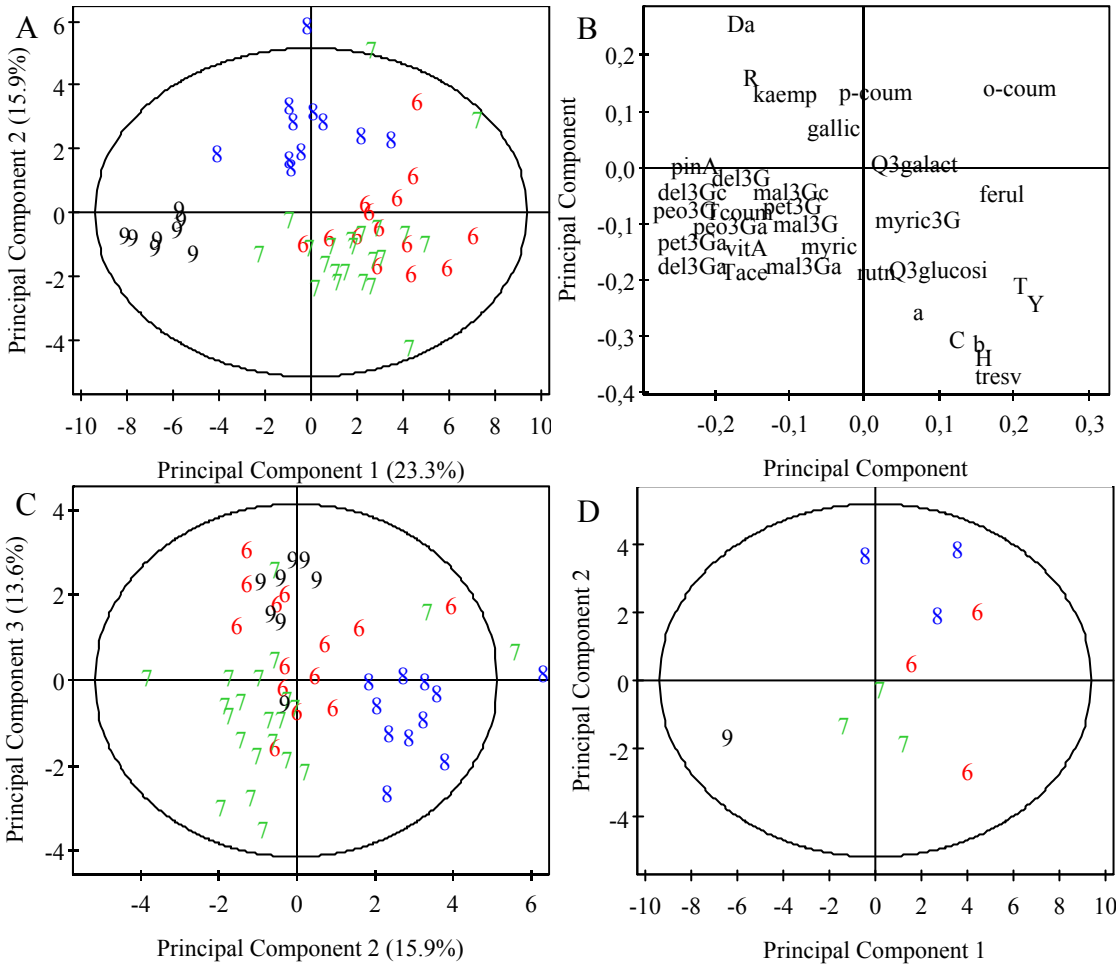

Figure 5.28. PLS-DA scores (A, C), loading (B) and validation (D) plots of red wines based on polyphenol variables and color parameters discriminated according to harvest year: (A) PC1 vs PC2, (C) PC2 vs PC3. Coloring: **2006**, **2007**, **2008**, **2009**. Loadings: mal3G: malvidin-3-glucoside, peo3G: peonidin-3-glucoside, pet3G: petunidin-3-glucoside, del3G: delphinidin-3-glucoside, mal3Ga: malvidin-3-glucoside acetate, peo3Ga: peonidin-3-glucoside acetate, pet3Ga: petunidin-3-glucoside acetate, del3Ga: delphinidin-3-glucoside acetate, vitA: vitisin-A, pinA: pinotin-A, Tcoum: Total coumarates, Tace: Total acetates, rutn: rutin, myric: myricetin, kaemp: kaempferol, Q3glucosi: quercetin-3-glucoside, Q3galact: quercetin-3-galactoside, myric3G: myricetin-3-glucoside, ferul: ferulic acid, p-coum: p-coumaric acid, tresv: resveratrol, gallic: gallic acid, o-coum: o-coumaric acid, a: red/green chromaticity, b.: yellow/blue chromaticity, C: chroma, H: hue, T: tint, Da: proportion of red coloration, R: red%, Y: yellow%

The combination of various chemical parameters in the harvest year discrimination of wines has been reported in the literature. For instance, Giaccio & Del Signore (2004) discriminated Montepulciano d'Abruzzo wines from a small wine growing region in Italy using several chemical parameters (reducing sugar, total alcohol, volatile and total acidity, Mg, Ca, tartaric, malic and lactic acids, anthocyanins, resveratrol, and aroma compounds). They concluded that the single variety wine originating from a small geographic region was at most affected by vintage rather than winemaking processes (yeasts, pressing, fermentation, clarification, maceration). Another study by Pereira et al. (2006) discriminated 2002-2003-2004 harvest year grapes of Merlot noir, Cabernet franc and Cabernet Sauvignon using ^{1}H NMR instrument. They associated the discrimination to the significant variables: sugar and phenolic contents. They found that cultivar was not a discriminating factor. Moreover, the higher phenolic and organic acid contents were observed in the grapes from the coldest vintage with the highest precipitation.

5.7.2.2. Soft Independent Modeling of Class Analogy (SIMCA)

In this section, another supervised statistical technique SIMCA was employed for the classification of wine samples. The element content, organic acid, sugar and chemical parameters were not useful to discriminate wine samples according to grape variety, geographic region and harvest year using this technique ($R^2_{pred} \leq 0.0$). The polyphenols and color parameters were able to produce models for each class (Table 5.21). The PCA class models were established with the significant variables that were previously determined according to the VIP feature of Simca-P software. For the varietal discrimination of wine samples, the PCA classes were established for each grape variety: Boğazkere and Öküzgözü as one class (BO), Cabernet Sauvignon (C), Kalecik Karası (K), Merlot (M), Syrah (S), Emir (E), Chardonnay (H), Narince (N), Muscat (T), Sultaniye (U). Papazkarası and Çalkarası red wines were not given a class as the number of observations was too less (n:1).

Table 5.21. SIMCA model parameters of varietal discrimination of wines

		Classes	# of PC	R^2_X	R^2_{pred}	Calib. set	Observations in the validation set
Red Wines	Polyphenols	BO	2	0.691	0.364	15	B7r3, B9w4, C7i11, K7a4, K7d7, K8d6, M7d7, M9h6, O6k8, O9e4, S6d6, S7d7, S8m5
		C	2	0.694	0.029	7	
		K	2	0.633	0.281	12	
		M	3	0.850	0.119	7	
		S	4	0.905	0.194	9	
	Color Parameters	BO	3	0.999	0.968	15	B8c6, B8k8, C6t1, C7r3, K7d10, K7d6, K8d6, M7d7, O6k8, O8e6, S7d4, S8m5
		C	2	0.987	0.928	6	
		K	1	0.678	0.586	12	
		M	3	0.992	0.882	8	
		S	3	0.999	0.985	10	
White Wines	Color Parameters	E	3	0.999	0.938	7	E7k5, E8k8, H6d10, H9i11, N7r3, T6i6s, T8m5, U6d5
		H	3	0.995	0.975	8	
		N	3	0.999	0.980	7	
		T	3	0.995	0.973	7	
		U	2	0.916	0.639	5	

Using the polyphenol variables, the discriminations of Boğazkere-Öküzgözü wines from the other varieties were demonstrated in Figure 5.29. The best discrimination was observed between Boğazkere-Öküzgözü and Cabernet Sauvignon wines (Figure 5.29A). Among the five samples in the validation set, one Cabernet Sauvignon (C7i) wine was predicted as outlier. On the other hand, the discrimination of Boğazkere-Öküzgözü wines from Merlot was the worst (Figure 5.29C). The Cooman's plots of Boğazkere-Öküzgözü and Kalecik Karası demonstrated a good discrimination with one Kalecik Karası (K8d) sample predicted as outlier (Figure 5.29B). The discrimination between Boğazkere-Öküzgözü and Syrah was also satisfactory (Figure 5.29D).

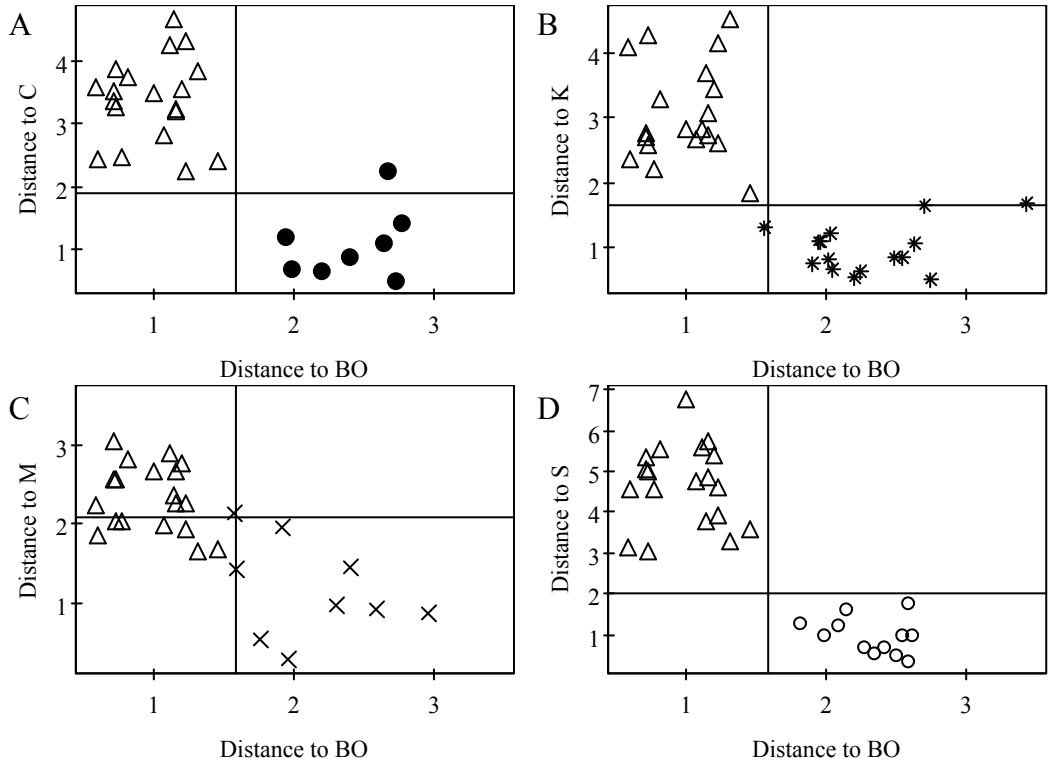

Figure 5.29. Cooman's plots for the discrimination of Boğazkere-Öküzgözü-BO (Δ), Cabernet Sauvignon-C (●), Kalecik Karası-K (*), Merlot-M (×), Syrah-S (○) wines based on polyphenol contents of red wines

The color parameters were less effective than the polyphenol variables in the discrimination of red wines. Boğazkere-Öküzgözü class could be discriminated only from Cabernet Sauvignon wines. Of the six samples in the prediction set, one Cabernet Sauvignon wine (C7r) was predicted in the common region and one Öküzgözü sample was predicted as outlier (Figure 5.30).

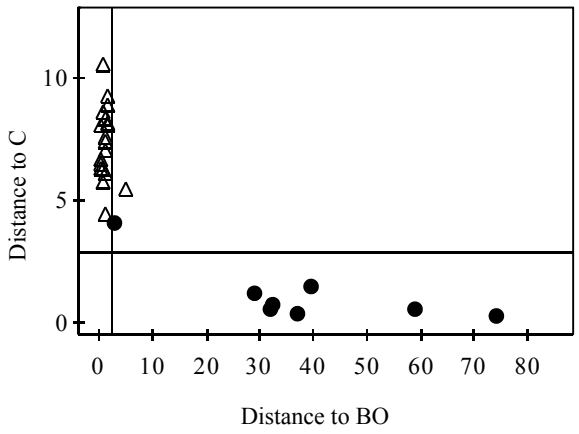

Figure 5.30. Cooman's plot for the discrimination of Boğazkere-Öküzgözü-BO (Δ) and Cabernet Sauvignon-C (●) wines based on color parameters of red wines

The color parameters of white wines were able to discriminate Muscat wines from Chardonnay wines (Figure 5.31). Of the four samples in the validation set, one Muscat (T8m) sample was predicted as outlier. Discrimination of other varieties was poor using SIMCA technique since the majority of the samples were located in the common region. The application of SIMCA technique using the CIELab color parameters (L*, a*, b*, C*, H*, S*) as variables has been studied to discriminate the rose, claret and blended wines from each other (Meléndez et al., 2001).

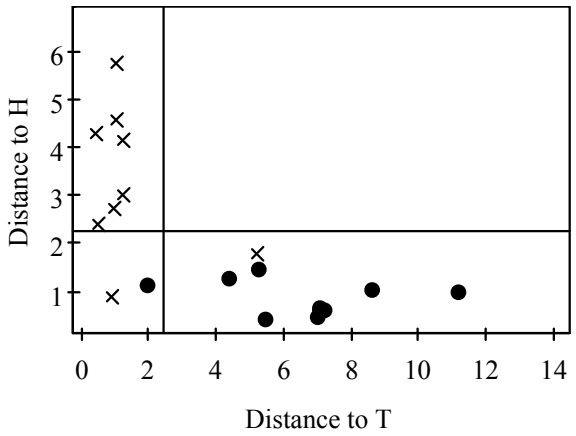

Figure 5.31. Cooman's plot for the discrimination of Muscat-T (×), Chardonnay-H (●) wines based on color parameters of white wines

In the geographic discrimination of red wine samples, the PCA classes were built for Denizli-İzmir-Manisa (DIM), Kapadokya (K), Elazığ-Diyarbakır (EC) and Tekirdağ-Bozcaada (TB) (Table 5.22). Ankara, Tokat, Denizli-Urla-Trakya, Denizli-Ankara and Denizli-Diyarbakır regions were not given any class since the number of wine samples was insufficient (n< 3). Among the element, polyphenol, color parameters and organic acid and sugar parameters, only polyphenol variables produced PCA class models for DIM and EC classes. However for white wines, neither of the variables produced PCA class models. In the Cooman's plot of red wines, the discrimination between the east and west was clear with the exception of two samples (O8e, M7d) in the validation set predicted as outliers (Figure 5.32).

Table 5.22. SIMCA model parameters of geographic discrimination of red wines

	Classes	# of PC	R^2_Y	R^2_{pred}	Calib. set	Observations in the validation set
Polyphenols	DIM	5	0.876	0.480	26	B8c6, C6t1, C7i11, C7k8, K7d6, K8d11, M7d7, O8e4, S6d6, S8d4, S8m5
	EC	4	0.904	0.355	9	

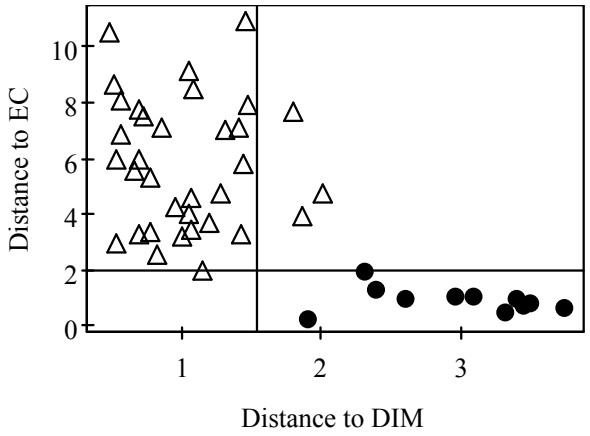

Figure 5.32. Cooman's plot for the discrimination of Denizli-İzmir-Manisa-DIM (Δ) and Elazığ-Diyarbakır-EC (●) region red wines based on polyphenol variables

5.7.3. Discrimination of Wine Samples Using Visible Spectra

Spectral techniques enable rapid and non-destructive analysis of wines in industry (Martelo-Vidal & Vazquez, 2014). The visible transmittance scans were recorded for the calculation of CIELab color parameter (Figure 5.33). These transmittance data were transformed into absorbance values using UVPC color analysis software feature (ver. 2.7) and their discrimination power was investigated with the supervised and unsupervised statistical techniques.

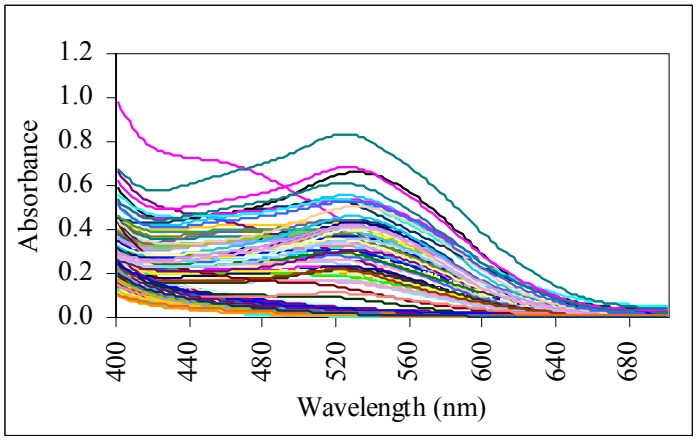

Figure 5.33. The visible absorbance spectra of wine samples

Prior to modeling, the visible absorbance spectra was standardized by subtracting the averages and dividing them to the standard deviations and then pre-processed by Wavelet Compression Spectra filtering technique (a Simca-P software

feature). The data was effective to discriminate solely red wines by grape variety using PCA and PLS-DA techniques. On the other hand, no discrimination was observed for white wines. The PLS-DA model of red wines included five classes: Boğazkere-Öküzgözü, Cabernet Sauvignon, Kalecik Karası, Merlot and Syrah (Table 5.23).

Table 5.23. The model parameters of varietal discrimination of red wines by visible absorbance spectra

	# of PC	R^2_X	R^2_Y	R^2_{pred}	Calib. set	Valid. set	Observations in the validation set
PCA	4	0.994	-	0.975	65	-	B8c6, B9w4, C7b17, C7k8, K6d6, K7d10, K7d7, M7d7,
PLS-DA	4	0.989	0.453	0.325	52	13	M9h6, O7e4, O9e4, S7d10a, S8d4

The discrimination of red wines by the visible spectra was similar to that observed with the color parameters. The PCA score plot indicated the clusters of native varieties, Kalecik Karası and Boğazkere-Öküzgözü, below the horizontal axis, and the non-native varieties, Cabernet Sauvignon, Merlot and Syrah were located above the horizontal axis (Figure 5.34A). According to the loadings plot, the variables were less significant at wavelengths greater than 620 nm. On the other hand, the turns at around 520 nm and 560 nm indicate the anthocyanins compounds showing absorbance peak at 520 nm. Syrah wines were affected from the wavelengths between 520-560 nm. The turn at 420 nm were related to the yellow colored pigments which affected the discrimination of Cabernet Sauvignon wines (Figure 5.34B). The PLS-DA technique provided a similar discrimination as the PCA technique and all the samples in the validation set were classified correct (membership probability values: 0.05-0.94) (Figure 5.35). According to Saavedra et al. (2011), the UV-Vis data appeared to be a good choice for the characterization of wine samples; however, it was known to cause auto-correlation of data and overfitting of models. Therefore, they combined the UV-Vis data with the polyphenol and element variables in the multivariate model for the geographic characterization of Chilean wines. Martelo-Vidal, Domínguez-Agis, & Vázquez (2013) have also discriminated the Albarino white wines according to their geographical subzones in Spain using UV, Visible and NIR transmittance spectra and their combinations.

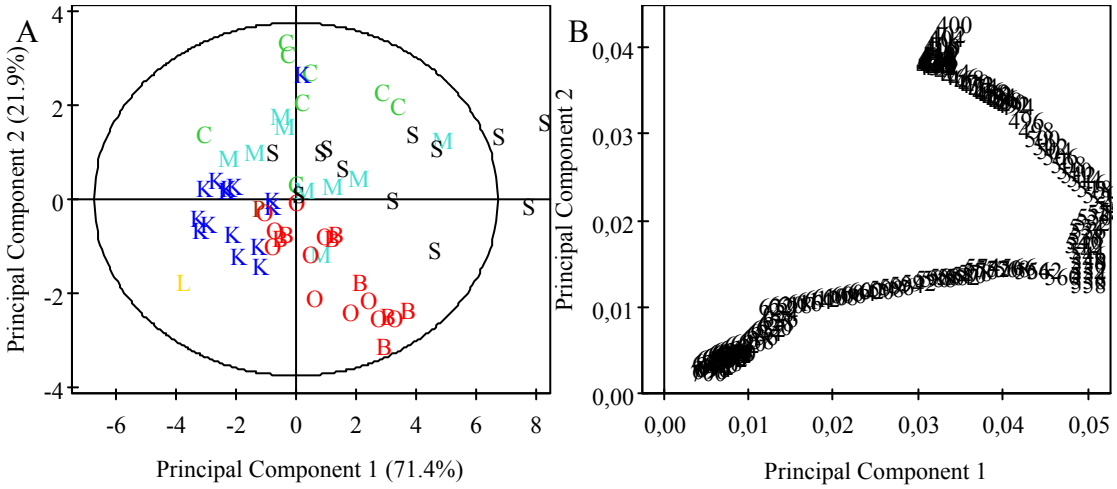

Figure 5.34. PCA score (A) and loading (B) plots of red wines based on based on visible absorbance spectra discriminated according to grape variety. Coloring: **Boğazkere**, **Öküzgözü**, **Çalkarası**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Papazkarası**, **Syrah**

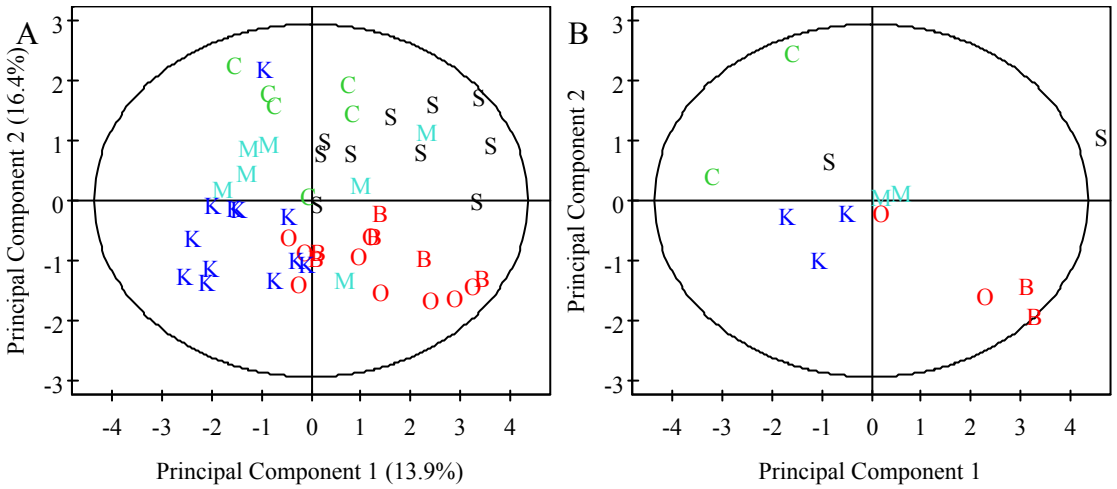

Figure 5.35. PLS-DA score (A) and validation (B) plots of red wines based on visible absorbance spectra discriminated according to grape variety. Coloring: **Boğazkere**, **Öküzgözü**, **Cabernet Sauvignon**, **Kalecik Karası**, **Merlot**, **Syrah**

5.7.4. Prediction of Polyphenol Composition of Wine Samples Using Visible Spectra

The polyphenol composition of wine samples that was determined by the HPLC method was related to the visible spectral data using the partial least squares regression technique (PLS). Prior to PLS modeling, the visible absorbance spectra were standardized by subtracting the averages and dividing them to the standard deviations. Standardization was followed by data pre-processing using second order derivation which produced the best results. The PLS models for quantification purposes have been established both for individual phenols and group of phenolic compounds for red and white wines. The prediction of phenolic composition of white wine samples via visible absorbance spectra was not successful. The calibration model of red wine samples was established with 65 samples. The validation set of red wine samples included 15 samples (B7w, B8w, C7k, C8k, K6d, K7a, K7d, K8d, M6i, M7t, O6f, O8f, O9f, S6d, S8i). Figure 5.36 and Table 5.24 summarized the results obtained with the proposed equations of red wine samples. According to the results, the visible spectra were useful in the quantification of quercetin-3-glucoside (Q3glucoside) and myricetin-3-glucoside (myricetin3G) in red wine samples. The quantification of flavonols rather than the anthocyanin compounds was not surprising since the wine color not only depends on the concentration of anthocyanins and pH but on the concentration of other phenolic compounds and their copigmentation cofactor and the level of other polymeric pigments. The color of young wines may be dominated by the anthocyanins but these compounds are not stable and their impact varies with pH (Jensen et al., 2008; Montealegre et al., 2006). According to Table 5.24, the acetylated delphinidin- and petunidin-glycosides had high R^2_{val} and RPD values indicating good PLS models. However, the samples in the validation set were not distributed homogeneously. The majority was accumulated at the origin. For this reason, the PLS plots were not demonstrated. In literature, combination of visible data with UV and near-infrared (NIR) data was employed in the prediction of polyphenol composition of red wines. For instance, Martelo-Vidal & Vazquez (2014) have predicted the polyphenol concentrations of red wines from different subzones of Spain using UV-visible-NIR spectral data. They concluded that different geographic regions highly influence the

number of phenolic compounds to be predicted. Regardless of geographic regions, the malvidin-3-glucoside and catechin contents of all red wines were predicted using PLS.

Table 5.24. Results of proposed PLS models of red wines

Parameter	PC	R^2cal	R^2_{pred}	Calibration Equation	RMSEC	R^2 val	RMSEP	RPD
Mal3G	2	0.595	0.435	y = 0.9682x + 2.339	11.43	0.297	12.68	1.13
Peo3G	2	0.772	0.525	y = 0.9971x + 0.2459	1.18	0.553	1.55	1.21
Pet3G	2	0.747	0.631	y = 0.9125x + 0.5297	1.75	0.406	2.41	1.22
Del3G	2	0.764	0.644	y = 0.9440x + 0.3342	1.38	0.510	1.75	1.41
Del3Ga	3	0.875	0.647	y = 0.8602x + 0.1923	0.75	0.941	0.34	4.08
Pet3Ga	2	0.849	0.582	y = 0.7363x + 0.3168	0.91	0.915	0.39	3.45
Peo3Ga	2	0.821	0.530	y = 1.08x + 0.0829	0.82	0.515	0.94	1.41
Mal3Ga	2	0.616	0.403	y = 1.013x + 0.6356	3.87	0.125	4.92	0.97
Del3Gc	2	0.782	0.529	y = 1.132x + 0.0978	0.82	0.538	0.87	1.52
Mal3Gc	2	0.676	0.501	y = 0.8957x + 0.6624	1.92	0.281	3.07	1.12
Q3glucoside	3	0.804	0.513	y = 0.978x + 1.252	5.25	0.711	5.43	1.77
Myricetin3G	3	0.822	0.611	y = 0.933x + 1.49	4.51	0.700	4.66	1.63
vitA	3	0.796	0.663	y = 0.7407x + 0.415	0.73	0.732	0.55	1.93

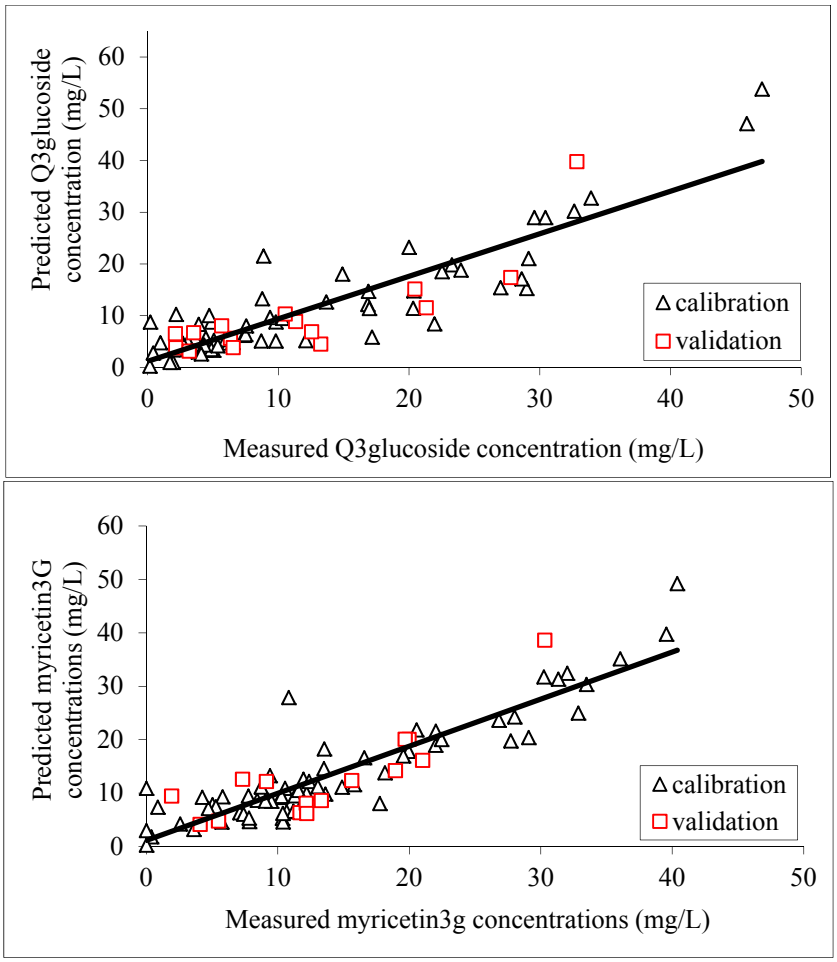

Figure 5.36. Regression plots of quercetin-3-glucoside and myricetin-3-glucoside

5.8. Final Remarks

In this section important points of the results were highlighted:

-All the statistical techniques were able to cluster the native red wines of grape varieties Boğazkere and Öküzgözü and discriminate them from the other wines. The significant variables were the high Ca, gallic acid, o-coumaric acid, delphinidin-3-glucoside and coumaroylated malvidin derivatives, red%, proportion of red coloration, tartaric acid and low B, Cu, yellow%, tint, Tace/Tcoum, quercetin-3-glucoside, quercetin-3-galactoside, (-)-epicatechin, pH, sugar, glycerol, original malic acid and lactic acid contents. The similarities in their chemical composition lead to cluster of these two varieties, but there were differences as well. For instance, Öküzgözü wines had higher anthocyanin and lower total phenol contents than Boğazkere wines. These two varieties were mainly from Elazığ and Diyarbakır with a few wines from Tokat, Kapadokya and Diyarbakır-Denizli.

-The majority of Syrah and Kalecik Karası wines were from the same region (Denizli); however, a distinct discrimination of these two varieties was achieved based on the lower vitisin-A, vitA/pinA, quercetin, myricetin, anthocyanin (peonidin-3-glucoside, petunidin-3-glucoside, delphinidin-3-glucoside) contents, color density, color intensity, proportion of red coloration, logarithmic color density, red% and higher CieLab parameters (L*, b*, C*, H*), tint and yellow% of Kalecik Karası wines.

-Among the foreign varieties, Merlot samples were the most scattered wines and they generally overlapped with Syrah, Cabernet Sauvignon and Kalecik Karası clusters. Besides having higher acetylated anthocyanin derivatives following Syrah wines they didn't have any distinct chemical composition than the other wines.

-Syrah wines gathered in distinct clusters due to their polyphenol, organic acid, sugar contents and color parameters. They were rich in anthocyanins, flavonols, lactic acid, original malic acid contents, logarithmic color density, color density, color intensity. Cabernet Sauvignon wines resembled Syrah wines with their high logarithmic color density, color density, color intensity, lactic acid, and original malic acid contents. However, they differed with their lower anthocyanin and flavonol contents and higher tint and yellow%.

-Among the white wines, the discrimination between Muscat, Emir and Sultaniye wines was influenced by most of the variables. Emir wines of Kapadokya

region were rich in Sr, Li, resveratrol and poor in Cu content while Narince wines had high (+)-catechin, procyanidin B_1 and vanillic acid contents. Muscat wines were recognized with their high Pb, Co, Mn, hydroxycinnamic acid contents, yellow%, tint, hue, total acidity, tartaric and acetic acid contents and low yellow/blue chromaticity, chroma, color intensity, logarithmic color density and pH. Chardonnay wines had higher organic acids then the Sultaniye wines. The Sultaniye wines were also the poorest in terms of polyphenol, malic, lactic, original malic acid contents and had the highest yellow/blue chromaticity and chroma values.

-The geographic discrimination of wine samples was possible with the element contents and to a lesser extent with the polyphenol contents. The red wines originating from Western Anatolia had higher Pb, Cu, B and lower Ca levels than the wines from East and that may be due to the growing industrial development of western Turkey. The polyphenol content of eastern region red wines (Boğazkere and Öküzgözü) were rich in gallic acid and coumaroylated malvidin derivatives and poor in (-)-epicatechin. Moreover, Tekirdağ region wines were the poorest in terms of flavonol-glycosides which might be affected from the low sunshine-exposure of grapes in this region. For white wines, Emir wines of Kapadokya region had higher Li, Sr and resveratrol levels. On the other hand, the western Anatolia wines were rich in Pb. Limited number of wine samples made it difficult to be certain about the effect of variety and vineyard location on the chemical composition.

-The influence of harvest year was observed in the PCA and PLS-DA models established with polyphenol contents and color parameters. Moreover, the element contents were able to discriminate 2009 harvest year wines using HCA. The higher anthocyanin and flavonol content of 2009 harvest year might be based on the increased biosynthesis of flavonoids due to water deficiency during veraison period (August). The strongest water deficit during the veraison period was observed in 2009. Moreover, 2006 vintage wines were the poorest in anthocyanin and flavonol content which might be related to the damage of flavonoids by high sunshine-exposure and temperature during 2006 veraison.

-The visible spectra were found to be useful in the varietal discrimination of red wines. The score plot was similar to that of color parameters. Moreover, it was employed in the prediction of polyphenol composition of wines. According to the results, quercetin-3-glucoside and myricetin-3-glucoside contents of red wine samples were quantified.

CHAPTER 6

CONCLUSION

136 monovarietal commercial wines from four vintages between 2006 and 2009 were characterized in terms of their element, polyphenol, organic acid, sugar contents and color parameters. For classification purpose, several multivariate statistical techniques such as PCA, HCA, PLS-DA and SIMCA were employed. The unsupervised statistical techniques (PCA and HCA) showed the general distribution of observations and variables. With the selected significant variables, varietal, regional and harvest year classifications were performed by using supervised techniques (PLS-DA and SIMCA).

This study revealed the similarity of native Boğazkere and Öküzgözü wines, originating mainly from Diyarbakır and Elazığ and their differences from the other red variety wines. In the same way, Emir wines of Kapadokya and Narince wines of Tokat showed their clusters using the polyphenol contents. The so-called natural element contents (Sr, Li, Ba, B, Ni, Pb, Ca and Al) were effective in the classification of wine samples according to their geographic origins. It can be concluded that the high Pb content of wines from the western vineyards might be related to the high industrialization of these regions. The polyphenol and color parameters were mainly effective in the varietal classification of wine samples, particularly for red wines. The anthocyanins and flavonol-glycosides were discriminative tools for Syrah wines. High coumaroylated anthocyanin derivatives and red color properties were distinctive for Boğazkere and Öküzgözü wines. Color parameters were also effective to classify Cabernet Sauvignon wines. Kalecik Karası red wines and Sultaniye white wines were characterized with their low total phenol contents, while high hydroxycinnamic acid content was characteristic of Muscat wines. 2009 vintage wines were shown to have the highest anthocyanin and flavonol contents.

For our study, the commercial wine samples originate from different geographic regions, harvest years or were produced under different process conditions. The expected variability in their chemical composition due to the different vineyards, harvest year or grape varieties might also be affected by the different production

experiences. Despite of these various sources of variations, the wines of some varieties and some geographical origins separated themselves from others.

This study served a preliminary step for the determination of authenticity of monovarietal wines of grapes cultivated in Turkey using their chemical compositions. The distinctive classification of wine samples in this study was related to the influence of at least one of the factors: grape variety, geographic region and harvest year. Their difference or similarity in their chemical composition can be used in geographic origin labeling. The territorial Turkish wines produced from specific grape varieties cultivated within a specific geographic region can protect the desired characteristics of wine and give a monetary value to the product and provide consumer interest.

REFERENCES

Aceto, M., Abollinoz, O., Bruzzonitiz, M. C., Mentastiz, E., Sarzaniniz, C., & Malandrinoz, M. (2002). Determination of metals in wine with atomic spectroscopy (Fame-AAS, GF-AAS and ICP-AES); a review. *Food Additives and Contaminants, 19*(2), 126-133. doi: 10.1080/0265203011007133

Aktan, N., & Kalkan, H. (2000). *Şarap Teknolojisi*. Ankara: Kavaklıdere Eğitim Yayınları.

Alkış, İ. M., Öz, S., Atakol, A., Yılmaz, N., Anlı, R. E., & Atakol, O. (2014). Investigation of heavy metal concentrations in some Turkish wines. *Journal of Food Composition and Analysis, 33*(1), 105-110. doi: 10.1016/j.jfca.2013.11.006

Almeida, C. M. R., & Vasconcelos, M. T. S. D. (2003). Lead Contamination in Portuguese Red Wines from the Douro Region: from the Vineyard to the Final Product. *J Agric Food Chem, 51*, 3012-3023.

Álvarez, M., Moreno, I. M., Jos, Á. M., Cameán, A. M., & González, A. G. (2007). Study of mineral profile of Montilla-Moriles "fino" wines using inductively coupled plasma atomic emission spectrometry methods. *Journal of Food Composition and Analysis, 20*(5), 391-395. doi: 10.1016/j.jfca.2006.07.010

Angus, N. S., O'Keeffe, T. J., Stuart, K. R., & Miskelly, G. M. (2006). Regional classification of New Zealand red wines using inductively-coupled plasma-mass spectrometry (ICP-MS). *Australian Journal of Grape and Wine Research, 12*, 170-176.

Anli, E., Vural, N., Demiray, S., & Özkan, M. (2006). Trans-resveratrol and Other Phenolic Compounds in Turkish Red Wines with HPLC. *Journal of Wine Research, 17*(2), 117-125. doi: 10.1080/09571260601004328

Anli, R. E., & Vural, N. (2009). Antioxidant phenolic substances of Turkish red wines from different wine regions. *Molecules, 14*(1), 289-297. doi: 10.3390/molecules14010289

Arnous, A., Makris, D. P., & Kefalas, P. (2001). Effect of Principal Polyphenolic Components in Relation to Antioxidant Characteristics of Aged Red Wines. *Journal of Agricultural Food Chemistry, 49*, 5736-5742.

Bernal, J. L., Del Nozal, M. J., Toribio, L., & Del Alamo, M. (1996). HPLC Analysis of Carbohydrates in Wines and Instant Coffees Using Anion Exchange

Chromatography Coupled to Pulsed Amperometric Detection. *J Agric Food Chem, 44*, 507-511.

Bouloumpasi, E., Soufleros, E. H., Tsarchopoulos, C., & Biliaderis, C. G. (2002). Primary amino acid composition and its use in discrimination of Greek red wines with regard to variety and cultivation region. *Vitis, 41*(4), 195-202.

Boulton, R. B., Singleton, V. L., Bisson, L. F., & Kunkee, R. E. (1996). *Principles and Practices of Winemaking*. New York: Kluwer Academic Publishers.

Bozan, B., Tosun, G., & Ozcan, D. (2008). Study of polyphenol content in the seeds of red grape (Vitis vinifera L.) varieties cultivated in Turkey and their antiradical activity. *Food Chemistry, 109*(2), 426-430. doi: 10.1016/j.foodchem.2007.12.056

Brereton, R. G. (2003). *Chemometrics Data Analysis for the Laboratory and Chemical Plant*. UK: John Wiley & Sons Ltd.

Bucchetti, B., Matthews, M. A., Falginella, L., Peterlunger, E., & Castellarin, S. D. (2011). Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. *Scientia Horticulturae, 128*(3), 297-305. doi: 10.1016/j.scienta.2011.02.003

Cabaroglu, T., Canbas, A., Baumes, R., Bayonove, C., Lepoutre, J. P., & Günata, Z. (1997). Aroma Composition of a White Wine of Vitis vinifera L. cv. Emir as Affected by Skin Contact. *Journal of Food Science, 62*, 680-683.

Cabaroglu, T., Canbas, A., Lepoutre, J. R., & Gunata, Z. (2002). Free and bound volatile composition of red wines of Vitis vinifera L. cv. Okuzgozu and Bogazkere grown in Turkey. *American Journal of Enology and Viticulture, 53*(1), 64-68.

Calull, M., Mar, R. M., & Borrull, F. (1992). Determination of carboxylic acids, sugars, glycerol and ethanol in wine and grape must by ion-exchange high performance liquid chromatography with refractive index detection. *Journal of Chromatography, 590*, 215-222.

Capron, X., Smeyersverbeke, J., & Massart, D. (2007). Multivariate determination of the geographical origin of wines from four different countries. *Food Chemistry, 101*(4), 1585-1597. doi: 10.1016/j.foodchem.2006.04.019

Cassidy, A., Hanley, B., & Lamuela-Raventos, R. M. (2000). Isoflavones, lignans and stilbenes - origins, metabolism and potential importance to human health. *Journal of the Science of Food and Agriculture, 80*(7), 1044-1062. doi: Doi 10.1002/(Sici)1097-0010(20000515)80:7<1044::Aid-Jsfa586>3.0.Co;2-N

Castellari, M., Versari, A., Spinabelli, U., Galassi, S., & Amati, A. (2000). An Improved Hplc Method for the Analysis of Organic Acids, Carbohydrates, and Alcohols in Grape Musts and Wines. *Journal of Liquid Chromatography & Related Technologies, 23*(13), 2047-2056. doi: 10.1081/jlc-100100472

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2010). Flavonol profiles of Vitis vinifera white grape cultivars. *Journal of Food Composition and Analysis, 23*(7), 699-705. doi: 10.1016/j.jfca.2010.03.017

Castillo-Sánchez, J. X., García-Falcón, M. S., Garrido, J., Martínez-Carballo, E., Martins-Dias, L. R., & Mejuto, X. C. (2008). Phenolic compounds and colour stability of Vinhão wines: Influence of wine-making protocol and fining agents. *Food Chemistry, 106*(1), 18-26. doi: 10.1016/j.foodchem.2007.04.069

Castro, I., Martín, J. P., Ortiz, J. M., & Pinto-Carnide, O. (2011). Varietal discrimination and genetic relationships of Vitis vinifera L. cultivars from two major Controlled Appellation (DOC) regions in Portugal. *Scientia Horticulturae, 127*(4), 507-514. doi: 10.1016/j.scienta.2010.11.018

Charters, S. (2003). *Perceptions of Wine Quality*. (Philosophy of Doctorate), Edith Cowan University, Australia.

Clifford, M. N. (2000). Review Anthocyanins – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture, 80*, 1063-1072.

Coetzee, P. P., Steffens, F. E., Eiselen, R. J., Augustyn, O. P., Balcaen, L., & Vanhaecke, F. (2005). Multi-element Analysis of South African Wines by ICP−MS and Their Classification According to Geographical Origin. *J. Agric. Food Chem., 53*, 5060-5066.

Cozzolino, D., Cynkar, W. U., Shah, N., Dambergs, R. G., & Smith, P. A. (2009). A brief introduction to multivariate methods in grape and wine analysis. *International Journal of Wine Research, 1*, 123-130.

Cozzolino, D., Cynkar, W. U., Shah, N., & Smith, P. A. (2011). Can spectroscopy geographically classify Sauvignon Blanc wines from Australia and New Zealand? *Food Chemistry, 126*(2), 673-678. doi: 10.1016/j.foodchem.2010.11.005

Cugnetto, A., Santagostini, L., Rolle, L., Guidoni, S., Gerbi, V., & Novello, V. (2014). Tracing the "terroirs" via the elemental composition of leaves, grapes and derived wines in cv Nebbiolo (Vitis vinifera L.). *Scientia Horticulturae, 172*, 101-108. doi: 10.1016/j.scienta.2014.03.055

Davis, C. R., Wibowo, D., Eschenbruch, R., Lee, T. H., & Fleet, G. H. (1985). Practical Implications of Malolactic Fermentation - a Review. *American Journal of Enology and Viticulture, 36*(4), 290-301.

de Andrade, R. H. S., do Nascimento, L. S., Pereira, G. E., Hallwass, F., & Paim, A. P. S. (2013). Anthocyanic composition of Brazilian red wines and use of HPLC-UV–Vis associated to chemometrics to distinguish wines from different regions. *Microchemical Journal, 110*, 256-262. doi: 10.1016/j.microc.2013.04.003

De Maesschalck, R., Candolfi, A., Massart, D. L., & Heuerding, S. (1999). Decision criteria for soft independent modelling of class analogy applied to near infrared data. *Chemometrics and Intelligent Laboratory Systems, 47*, 65-77.

Demyttenaere, J. C. R., Dagher, C., Sandra, P., Kallithraka, S., Verhe, R., & De Kimpe, N. (2003). Flavour analysis of Greek white wine by solid-phase microextraction–capillary gas chromatography–mass spectrometry. *Journal of Chromatography A, 985*, 233-246.

Ding, M.-Y., Koizumi, H., & Suzuki, Y. (1995). Comparison of Three Chromatographic Systems for Determination of Organic Acids in Wine. *Analytical Sciences, 11*, 239-243.

Doyle, M., Beuchat, L. R., & Montville, T. J. (2001). *Food Microbiology: Fundamentals and Frontiers* (Second ed.). Washington DC: ASM Press.

Eriksson, L., Johanson, E., Wold, N. K., & Wold, S. (2001). *Multi- and megavariate data analysis: Principles and applications*. Sweden: Umetrics AB.

Etievant, P., Schlich, P., Bouvier, J. C., Symonds, P., & Bertrand, A. (1988). Varietal and Geographic Classification of French Red Wines in Terms of Elements, Amino-Acids and Aromatic Alcohols. *Journal of the Science of Food and Agriculture, 45*(1), 25-41. doi: DOI 10.1002/jsfa.2740450105

Fabani, M. P., Arrúa, R. C., Vázquez, F., Diaz, M. P., Baroni, M. V., & Wunderlin, D. A. (2010). Evaluation of elemental profile coupled to chemometrics to assess the geographical origin of Argentinean wines. *Food Chemistry, 119*(1), 372-379. doi: 10.1016/j.foodchem.2009.05.085

Fanzone, M., Pena-Neira, A., Gil, M., Jofre, V., Assof, M., & Zamora, F. (2012). Impact of phenolic and polysaccharidic composition on commercial value of Argentinean Malbec and Cabernet Sauvignon wines. *Food Research International, 45*(1), 402-414.

Fernández-Mar, M. I., Mateos, R., García-Parrilla, M. C., Puertas, B., & Cantos-Villar, E. (2012). Bioactive compounds in wine: Resveratrol, hydroxytyrosol and

melatonin: A review. *Food Chemistry, 130*(4), 797-813. doi: 10.1016/j.foodchem.2011.08.023

Ferrandino, A., & Lovisolo, C. (2014). Abiotic stress effects on grapevine (Vitis vinifera L.): Focus on abscisic acid-mediated consequences on secondary metabolism and berry quality. *Environmental and Experimental Botany, 103*, 138-147. doi: 10.1016/j.envexpbot.2013.10.012

Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., & Santos, J. A. (2014). Climate factors driving wine production in the Portuguese Minho region. *Agricultural and Forest Meteorology, 185*, 26-36. doi: 10.1016/j.agrformet.2013.11.003

Garcia Falcon, M., Perezlamela, C., Martinezcarballo, E., & Simalgandara, J. (2007). Determination of phenolic compounds in wines: Influence of bottle storage of young red wines on their evolution. *Food Chemistry, 105*(1), 248-259. doi: 10.1016/j.foodchem.2006.11.006

Geana, I., Iordache, A., Ionete, R., Marinescu, A., Ranca, A., & Culea, M. (2013). Geographical origin identification of Romanian wines by ICP-MS elemental analysis. *Food Chemistry, 138*(2-3), 1125-1134. doi: 10.1016/j.foodchem.2012.11.104

Giaccio, M., & Del Signore, A. (2004). Multivariate classification of Montepulciano d'Abruzzo wine samples according to vintage year. *Journal of the Science of Food and Agriculture, 84*(2), 164-172. doi: 10.1002/jsfa.1624

Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. *Journal of Food Composition and Analysis, 20*(7), 618-626. doi: 10.1016/j.jfca.2007.03.002

Gomez, M. D. M. C., Feldmann, I., Jakubowski, N., & Andersson, J. T. (2004). Classification of German White Wines with Certified Brand of Origin by Multielement Quantitation and Pattern Recognition Techniques. *J Agric Food Chem, 52*, 2962-2974.

González-Fernández, A. B., Marcelo, V., Valenciano, J. B., & Rodríguez-Pérez, J. R. (2012). Relationship between physical and chemical parameters for four commercial grape varieties from the Bierzo region (Spain). *Scientia Horticulturae, 147*, 111-117. doi: 10.1016/j.scienta.2012.09.009

Gonzálvez, A., Llorens, A., Cervera, M. L., Armenta, S., & de la Guardia, M. (2009). Elemental fingerprint of wines from the protected designation of origin Valencia. *Food Chemistry, 112*(1), 26-34. doi: 10.1016/j.foodchem.2008.05.043

Greenough, J. D., Longerich, H. P., & Jackson, S. E. (1997). Element fingerprinting of Okanagan Valley wines using ICP–MS: Relationships between wine composition, vineyard and wine colour. *Australian Journal of Grape and Wine Research, 3*, 75-83.

Grindlay, G., Mora, J., Maestre, S., & Gras, L. (2008). Application of a microwave-based desolvation system for multi-elemental analysis of wine by inductively coupled plasma based techniques. *Anal Chim Acta, 629*(1-2), 24-37. doi: 10.1016/j.aca.2008.09.023

Gumus, S. G., & Gumus, A. H. (2008). The Wine Sector in Turkey: Survey on 42 Wineries. *Bulgarian Journal of Agricultural Science, 14*(6), 549-556.

Gurbuz, O., Gocmen, D., Dagdelen, F., Gursoy, M., Aydin, S., Sahin, I., . . . Usta, M. (2007). Determination of flavan-3-ols and trans-resveratrol in grapes and wine using HPLC with fluorescence detection. *Food Chemistry, 100*(2), 518-525. doi: DOI 10.1016/j.foodchem.2005.10.008

Hernandez-Orte, P., Cacho, J. F., & Ferreira, V. (2002). Relationship between Varietal Amino Acid Profile of Grapes and Wine Aromatic Composition. Experiments with Model Solutions and Chemometric Study. *J Agric Food Chem, 50*, 2891-2899.

Hışıl, Y. (1994). *Enstrümental Gıda Analizleri-1*. İzmir: Ege Üniversitesi Basımevi.

http://ec.europa.eu/. European Comission Agriculture and Rural Development/Agricultural Markets/Wine. Retrieved May, 2014, from http://ec.europa.eu/agriculture/markets/wine/index_en.htm

http://karya.mu.edu.tr/. Şarabın Hikayesi. Retrieved May, 2014, from http://karya.mu.edu.tr/sarap/uzum2.html

http://www.food.gov.uk/. Food Standards Agency: Labelling of European Community Still Wines. Retrieved May, 2014, from http://www.food.gov.uk/multimedia/pdfs/eustillwine.pdf

http://www.ibp.gov.tr/. Turkish Contact Point Alkollü ve Alkolsüz İçecekler. Retrieved May, 2014, from http://www.ibp.gov.tr/pg/sektorpdf/tarim/alkollu_alkolsuz_icecekler.pdf

http://www.oiv.int/oiv/info/enlisteindication. OIV List of Geographical Indications and Appellations of Origin. Retrieved May, 2014, from http://www.oiv.int/oiv/info/enlisteindication

http://www.oiv.int/oiv/info/en_press_conference_may_2014. OIV The Wine market: Evolution and Trends. Retrieved May, 2014, from http://www.oiv.int/oiv/info/en_press_conference_may_2014

http://www.oiv.int/oiv/info/enstatistiquessecteurvitivinicole#secteur. OIV Vine and Wine Outlook. Retrieved May, 2014, from http://www.oiv.int/oiv/info/enstatistiquessecteurvitivinicole#secteur

http://www.tcp.gov.tr/. Turkish Contact Point Food and Agriculture: Wine. Retrieved May, 2014, from http://www.tcp.gov.tr/english/sectors/sectoringpdf/agric/wine.pdf

http://www.tpe.gov.tr/. Turkish Patent Institute Registered Geographic Indications. Retrieved May, 2014, from http://www.tpe.gov.tr/portal/default2.jsp?sayfa=431

http://www.ttb.gov/. U.S. Alcohol and Tobacco Tax and Trade Bureau. Retrieved May, 2014

Ilbery, B., & Kneafsey, M. (2000). Registering regional speciality food and drink products in the United Kingdom: the case of PDOs and PGIs. *Area, 32*(3), 317-325.

Illuminati, S., Annibaldi, A., Truzzi, C., & Scarponi, G. (2014). Recent temporal variations of trace metal content in an Italian white wine. *Food Chemistry, 159*, 493-497. doi: 10.1016/j.foodchem.2014.03.058

Ivanova, V., Stefova, M., Vojnoski, B., Dörnyei, Á., Márk, L., Dimovska, V., . . . Kilár, F. (2011). Identification of polyphenolic compounds in red and white grape varieties grown in R. Macedonia and changes of their content during ripening. *Food Research International, 44*(9), 2851-2860. doi: 10.1016/j.foodres.2011.06.046

Jackson, R. S. (2000). *Wine Science Principles, Practice, Perception* (Second ed.). San Diego: Academic Press.

Jackson, R. S. (2002). *Wine Tasting: A Professional Handbook*. San Diego: Academic Press.

Jaitz, L., Siegl, K., Eder, R., Rak, G., Abranko, L., Koellensperger, G., & Hann, S. (2010). LC–MS/MS analysis of phenols for classification of red wine according to geographic origin, grape variety and vintage. *Food Chemistry, 122*(1), 366-372. doi: 10.1016/j.foodchem.2010.02.053

Jarisch, R., & Wantke, F. (1996). Wine and headache. *International Archives of Allergy and Immunology, 110*(1), 7-12.

Jensen, J. S., Demiray, S., Egebo, M., & Meyer, A. S. (2008). Prediction of Wine Color Attributes from the Phenolic Profiles of Red Grapes (Vitis vinifera). *J. Agric. Food Chem., 56*, 1105-1115.

Johnson, R. A., & Wichern, D. W. (2007). *Applied Multivariate Statistical Analysis* (Sixth ed.). NJ: Pearson Prentice Hall.

Kallithraka, S., Arvanitoyannis, I. S., Kefalas, P., El-Zajouli, A., Soufleros, E., & Psarra, E. (2001). Instrumental and sensory analysis of Greek wines; implementation of principal component analysis (PCA) for classification according to geographical origin. *Food Chemistry, 73*(4), 501-514.

Kamsu-Foguem, B., & Flammang, A. (2014). Knowledge description for the suitability requirements of different geographical regions for growing wine. *Land Use Policy, 38*, 719-731. doi: 10.1016/j.landusepol.2014.01.018

Kelebek, H., Canbas, A., Jourdes, M., & Teissedre, P.-L. (2010). Characterization of colored and colorless phenolic compounds in Öküzgözü wines from Denizli and Elazig regions using HPLC-DAD–MS. *Industrial Crops and Products, 31*(3), 499-508. doi: 10.1016/j.indcrop.2010.01.012

Kızılgöz, İ., Sakin, E., & Gürsöz, S. (2011). Ovacık Köyü'nde (Şanlıurfa) Yetiştirilen Asma (Vitis vinifera L.) Çeşitlerinin Mineral BeslenmeDurumunun Değerlendirilmesi *Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 25*(1), 1-10.

Kment, P., Mihaljevič, M., Ettler, V., Šebek, O., Strnad, L., & Rohlová, L. (2005). Differentiation of Czech wines using multielement composition – A comparison with vineyard soil. *Food Chemistry, 91*(1), 157-165. doi: 10.1016/j.foodchem.2004.06.010

Koundouras, S., Hatzidimitriou, E., Karamolegkou, M., Dimopoulou, E., Kallithraka, S., Tsialtas, J. T., . . . Kotseridis, Y. (2009). Irrigation and rootstock effects on the phenolic concentration and aroma potential of Vitis vinifera L. cv. cabernet sauvignon grapes. *J Agric Food Chem, 57*(17), 7805-7813. doi: 10.1021/jf901063a

Lago-Vanzela, E. S., Rebello, L. P. G., Ramos, A. M., Stringheta, P. C., Da-Silva, R., García-Romero, E., . . . Hermosín-Gutiérrez, I. (2013). Chromatic characteristics and color-related phenolic composition of Brazilian young red wines made from the hybrid grape cultivar BRS Violeta ("BRS Rúbea"×"IAC 1398-21"). *Food Research International, 54*(1), 33-43. doi: 10.1016/j.foodres.2013.06.008

Landrault, N., Poucheret, P., Ravel, P., Gasc, F., Cros, G., & Teissedre, P.-L. (2001). Antioxidant Capacities and Phenolics Levels of French Wines from Different Varieties and Vintages. *J Agric Food Chem, 49*, 3341-3348.

Lee, J. E., Hwang, G. S., Van Den Berg, F., Lee, C. H., & Hong, Y. S. (2009). Evidence of vintage effects on grape wines using 1H NMR-based metabolomic study. *Anal Chim Acta, 648*(1), 71-76. doi: 10.1016/j.aca.2009.06.039

Li, Z., Pan, Q., Jin, Z., Mu, L., & Duan, C. (2011). Comparison on phenolic compounds in Vitis vinifera cv. Cabernet Sauvignon wines from five wine-growing regions in China. *Food Chemistry, 125*(1), 77-83. doi: 10.1016/j.foodchem.2010.08.039

López-Tamames, E., Puig-Deu, M. A., Teixeira, E., & Buxaderas, S. (1996). Organic Acids, Sugars, and Glycerol Content in White Winemaking Products Determined by HPLC: Relationship to Climate and Varietal Factors *Am. J. Enol. Vitic., 47*(2), 193-198.

Lorenzo, C., Pardo, F., Zalacain, A., Alonso, G. L., & Salinas, M. R. (2005). Effect of Red Grapes Co-winemaking in Polyphenols and Color of Wines. *J Agric Food Chem, 53*(19), 7609-7616.

Lorenzo, M. N., Taboada, J. J., Lorenzo, J. F., & Ramos, A. M. (2012). Influence of climate on grape production and wine quality in the Rías Baixas, north-western Spain. *Regional Environmental Change, 13*(4), 887-896. doi: 10.1007/s10113-012-0387-1

Lorrain, B., Chira, K., & Teissedre, P.-L. (2011). Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: Comparison to 2006, 2007 and 2008 vintages. *Food Chemistry, 126*(4), 1991-1999. doi: 10.1016/j.foodchem.2010.12.062

Lorrain, B., Ky, I., Pechamat, L., & Teissedre, P. L. (2013). Evolution of analysis of polyhenols from grapes, wines, and extracts. *Molecules, 18*(1), 1076-1100. doi: 10.3390/molecules18011076

Makris, D. P., Kallithraka, S., & Mamalos, A. (2006). Differentiation of young red wines based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents. *Talanta, 70*(5), 1143-1152. doi: 10.1016/j.talanta.2006.03.024

Marengo, E., & Aceto, M. (2003). Statistical investigation of the differences in the distribution of metals in Nebbiolo-based wines. *Food Chemistry, 81*(4), 621-630. doi: 10.1016/s0308-8146(02)00564-2

Marquez, A., Serratosa, M. P., & Merida, J. (2014). Influence of bottle storage time on colour, phenolic composition and sensory properties of sweet red wines. *Food Chemistry, 146*, 507-514. doi: 10.1016/j.foodchem.2013.09.103

Martelo-Vidal, M. J., Domínguez-Agis, F., & Vázquez, M. (2013). Ultraviolet/visible/near-infrared spectral analysis and chemometric tools for the discrimination of wines between subzones inside a controlled designation of origin: a case study of Rías Baixas. *Australian Journal of Grape and Wine Research, 19*(1), 62-67. doi: 10.1111/ajgw.12003

Martelo-Vidal, M. J., & Vazquez, M. (2014). Determination of polyphenolic compounds of red wines by UV-VIS-NIR spectroscopy and chemometrics tools. *Food Chemistry, 158*, 28-34. doi: 10.1016/j.foodchem.2014.02.080

Martin, A. E., Watling, R. J., & Lee, G. S. (2012). The multi-element determination and regional discrimination of Australian wines. *Food Chemistry, 133*(3), 1081-1089. doi: 10.1016/j.foodchem.2012.02.013

Martinez-carrasco, L., Brugarolas, M., & Martinez-poveda, A. (2005). Quality wines and wines protected by a designation of origin: Identifying their consumption determinants. *Journal of Wine Research, 16*(3), 213-232. doi: 10.1080/09571260600556690

Mato, I., Suárez-Luque, S., & Huidobro, J. F. (2005). A review of the analytical methods to determine organic acids in grape juices and wines. *Food Research International, 38*(10), 1175-1188. doi: 10.1016/j.foodres.2005.04.007

Meija, J., Mounicou, S., & Caruso, J. A. (2004). Plasma Spectrometry for Elemental Speciation and Characterization in Beverages. *Journal of AOAC International, 87*(1), 205-224.

Meléndez, M. E., Sánchez, M. S., Íñiguez, M., Sarabia, L. A., & Ortiz, M. C. (2001). Psychophysical parameters of colour and the chemometric characterisation of wines of the certified denomination of origin 'Rioja'. *Anal Chim Acta, 446*, 159-169.

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International, 43*(7), 1844-1855. doi: 10.1016/j.foodres.2010.05.001

Mooi, E., & Sarstedt, A. (2011). *A Concise Guide to Market Research: The Process, Data, and Methods Using IBM SPSS Statistics*. Berlin Heidelberg: Springer Verlag.

Moran, C., & Saliba, A. (2012). Reasons for drinking wine and other beverages - comparison across motives in older adults. *International Journal of Wine Research, 4*, 25-32. doi: 10.2147/ijwr.s33323

Morata, A., Calderón, F., González, M. C., Gómez-Cordovés, M. C., & Suárez, J. A. (2007). Formation of the highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde. *Food Chemistry, 100*(3), 1144-1152. doi: 10.1016/j.foodchem.2005.11.024

Moreno, I. M., Gonzalez-Weller, D., Gutierrez, V., Marino, M., Camean, A. M., Gonzalez, A. G., & Hardisson, A. (2007). Differentiation of two Canary DO red wines according to their metal content from inductively coupled plasma optical emission spectrometry and graphite furnace atomic absorption spectrometry by using Probabilistic Neural Networks. *Talanta, 72*(1), 263-268. doi: 10.1016/j.talanta.2006.10.029

Moro, E., Majocchi, R., Restani, P., Ballabio, C., & Molfino, S. (2007). A Rapid and Simple Method for Simultaneous Determination of Glycerol, Fructose, and Glucose in Wine. *Am. J. Enol. Vitic., 58*(2), 279-282.

Mortimer, R., & Polsinelli, M. (1999). On the origins of wine yeast. *Res. Microbiol., 150*, 199-204.

Nicholas, K. A., Matthews, M. A., Lobell, D. B., Willits, N. H., & Field, C. B. (2011). Effect of vineyard-scale climate variability on Pinot noir phenolic composition. *Agricultural and Forest Meteorology, 151*(12), 1556-1567. doi: 10.1016/j.agrformet.2011.06.010

Odabaş, F. (1980). Doğu Anadolu'nun Bağcılık Durumu ve İklim Faktörlerinin Bölge Bağcılarına Etkileri. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 11*(3-4), 143-156.

OIV. (2011). Compendium of International Methods of Analysis: Maximum Acceptable Limits of Various Substances Contained in Wine. Paris France: International Organisation of Vine and Wine.

OIV. (2012a). International Standard for the Labelling of Wines. Paris France: International Organisation of Vine and Wine.

OIV. (2012b). Statistical Report on World Vitiviniculture.

OIV. (2013). Compendium of International Methods of Wine and Must Analysis Volume 1: International Organisation of Vine and Wine.

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., & Deloire, A. (2002). Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of Vitis vinifera cv. Shiraz. *Am. J. Enol. Vitic., 53*(4), 261-267.

Oliveira, C. M., Ferreira, A. C. S., De Freitas, V., & Silva, A. M. S. (2011). Oxidation mechanisms occurring in wines. *Food Research International, 44*(5), 1115-1126. doi: 10.1016/j.foodres.2011.03.050

Özkan, G., & Baydar, N. G. (2006). A Direct RP-HPLC Determination of Phenolic Compounds in Turkish Red Wines. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 19*(2), 229-234.

Pereira, G. E., Gaudillere, J.-P., Leeuwen, C. v., Hilbert, G., Maucourt, M., Deborde, C., . . . Rolin, D. (2006). 1H NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. *Anal Chim Acta, 563*(1-2), 346-352. doi: 10.1016/j.aca.2005.11.007

Pérez Trujillo, J. P., Conde, J. E., Pérez Pont, M. L., Câmara, J., & Marques, J. C. (2011). Content in metallic ions of wines from the Madeira and Azores archipelagos. *Food Chemistry, 124*(2), 533-537. doi: 10.1016/j.foodchem.2010.06.065

Pinho, C., Couto, A. I., Valentão, P., Andrade, P., & Ferreira, I. M. P. L. V. O. (2012). Assessing the anthocyanic composition of Port wines and musts and their free radical scavenging capacity. *Food Chemistry, 131*(3), 885-892. doi: 10.1016/j.foodchem.2011.09.072

Porgalı, E., & Büyüktuncel, E. (2012). Determination of phenolic composition and antioxidant capacity of native red wines by high performance liquid chromatography and spectrophotometric methods. *Food Research International, 45*(1), 145-154. doi: 10.1016/j.foodres.2011.10.025

Rapp, A. (1998). Volatile flavour of wine: Correlation between instrumental analysis and sensory perception. *Nahrung, 42*(6), 351-363.

Rastija, V., Srečnik, G., & Marica Medić, Š. (2009). Polyphenolic composition of Croatian wines with different geographical origins. *Food Chemistry, 115*(1), 54-60. doi: 10.1016/j.foodchem.2008.11.071

Rentzsch, M., Schwarz, M., Winterhalter, P., Blanco-Vega, D., & Hermosín-Gutiérrez, I. (2010). Survey on the content of vitisin A and hydroxyphenyl-pyranoanthocyanins in Tempranillo wines. *Food Chemistry, 119*(4), 1426-1434. doi: 10.1016/j.foodchem.2009.09.023

Rodríguez Montealegre, R., Romero Peces, R., Chacón Vozmediano, J. L., Martínez Gascueña, J., & García Romero, E. (2006). Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. *Journal of Food Composition and Analysis, 19*(6-7), 687-693. doi: 10.1016/j.jfca.2005.05.003

Rovio, S., Sirén, K., & Sirén, H. (2011). Application of capillary electrophoresis to determine metal cations, anions, organic acids, and carbohydrates in some Pinot Noir red wines. *Food Chemistry, 124*(3), 1194-1200. doi: 10.1016/j.foodchem.2010.07.044

Römisch, U., Jäger, H., Capron, X., Lanteri, S., Forina, M., & Smeyers-Verbeke, J. (2009). Characterization and determination of the geographical origin of wines. Part III: multivariate discrimination and classification methods. *European Food Research and Technology, 230*(1), 31-45. doi: 10.1007/s00217-009-1141-x

Rupasinghe, H. P. V., & Clegg, S. (2007). Total antioxidant capacity, total phenolic content, mineral elements, and histamine concentrations in wines of different fruit sources. *Journal of Food Composition and Analysis, 20*(2), 133-137. doi: 10.1016/j.jfca.2006.06.008

Saavedra, J., Fuentealba, C., Yáñez, L., Bravo, M., Quiroz, W., Lukacsy, G., & Carot, J. M. (2011). Chemometric approaches for the zoning of Pinot Noir wines from the Casablanca valley, Chile. *Food Chemistry, 127*(4), 1842-1847. doi: 10.1016/j.foodchem.2011.01.132

Saeys, W., Mouazen, A. M., & Ramon, H. (2005). Potential for Onsite and Online Analysis of Pig Manure using Visible and Near Infrared Reflectance Spectroscopy. *Biosystems Engineering, 91*(4), 393-402. doi: 10.1016/j.biosystemseng.2005.05.001

Salinas, M. R., Garijo, J., Pardo, F., Zalacain, A., & Alonso, G. L. (2005). Influence of prefermentative maceration temperature on the colour and the phenolic and volatile composition of rosé wines. *Journal of the Science of Food and Agriculture, 85*(9), 1527-1536. doi: 10.1002/jsfa.2133

Schlesier, K., Fauhl-Hassek, C., Forina, M., Cotea, V., Kocsi, E., Schoula, R., . . . Wittkowski, R. (2009). Characterisation and determination of the geographical origin of wines. Part I: overview. *European Food Research and Technology, 230*(1), 1-13. doi: 10.1007/s00217-009-1140-y

Schwarz, M., Hofmann, G., & Winterhalter, P. (2004). Investigations on anthocyanins in wines from Vitis vinifera cv. pinotage: factors influencing the formation of pinotin A and its correlation with wine age. *J Agric Food Chem, 52*(3), 498-504. doi: 10.1021/jf035034f

Sehovic, D., Petrovic, V., & Maric, V. (2004). Glycerol and Wine Industry Glycerol Determination in Grape Must and Wine. *Kem. Ind., 53*(11), 505-516.

Selih, V. S., Sala, M., & Drgan, V. (2014). Multi-element analysis of wines by ICP-MS and ICP-OES and their classification according to geographical origin in Slovenia. *Food Chemistry, 153*, 414-423. doi: 10.1016/j.foodchem.2013.12.081

Selli, S., Canbas, A., Cabaroglu, T., Erten, H., Lepoutre, J.-P., & Gunata, Z. (2006a). Effect of skin contact on the free and bound aroma compounds of the white wine of Vitis vinifera L. cv Narince. *Food Control, 17*(1), 75-82. doi: 10.1016/j.foodcont.2004.09.005

Selli, S., Canbas, A., Cabaroglu, T., Erten, H., & Günata, Z. (2006b). Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment. *Food Chemistry, 94*(3), 319-326. doi: 10.1016/j.foodchem.2004.11.019

Serapinas, P., Venskutonis, P. R., Aninkevičius, V., Ežerinskis, Ž., Galdikas, A., & Juzikienė, V. (2008). Step by step approach to multi-element data analysis in testing the provenance of wines. *Food Chemistry, 107*(4), 1652-1660. doi: 10.1016/j.foodchem.2007.09.003

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic., 16*(3), 144-158.

Skoog, D. A., Holler, F. J., & Nieman, T. A. (1998). *Principles of Instrumental Analysis* (Fifth ed.). Florida: Harcourt Brace College Publisher.

Smeyers-Verbeke, J., Jäger, H., Lanteri, S., Brereton, P., Jamin, E., Fauhl-Hassek, C., . . . Römisch, U. (2009). Characterization and determination of the geographical origin of wines. Part II: descriptive and inductive univariate statistics. *European Food Research and Technology, 230*(1), 15-29. doi: 10.1007/s00217-009-1142-9

Sofo, A., Nuzzo, V., Tataranni, G., Manfra, M., De Nisco, M., & Scopa, A. (2012). Berry morphology and composition in irrigated and non-irrigated grapevine (Vitis vinifera L.). *J Plant Physiol, 169*(11), 1023-1031. doi: 10.1016/j.jplph.2012.03.007

Soufleros, E. H., Bouloumpasi, E., Tsarchopoulos, C., & Biliaderis, C. G. (2003). Primary amino acid profiles of Greek white wines and their use in classification according to variety, origin and vintage. *Food Chemistry, 80*, 261-273.

Sperkova, J., & Suchanek, M. (2005). Multivariate classification of wines from different Bohemian regions (Czech Republic). *Food Chemistry, 93*(4), 659-663. doi: 10.1016/j.foodchem.2004.10.044

Stafilov, T., & Karadjova, I. (2009). Atomic Absorption Spectrometry in Wine Analysis-A Review-. *Macedonian Journal of Chemistry and Chemical Engineering, 28*(1), 17-31.

Şimşek, O., Şenol, G. S., & Velioğlu, S. D. (2008, 21-23 Mayıs). *Trakya Bölgesinde Üretilen Şarapların Ağır Metal İçeriklerinin Belirlenmesi Üzerine Bir Araştırma.* Paper presented at the Türkiye 10. Gıda Kongresi, Erzurum.

Tarara, J. M., Lee, J., Spayd, S. E., & Scagel, C. F. (2008). Berry Temperature and Solar Radiation Alter Acylation, Proportion, and Concentration of Anthocyanin in Merlot Grapes. *Am. J. Enol. Vitic., 59*(3), 235-247.

Tekelioğlu, Y., & Demirer, R. (2008). *Küreselleşme Sürecinde, Yöresel Ürünler ve Coğrafi İşaretlerin Geleceği.* Paper presented at the Küreşelleşme, Demokratikleşme ve Türkiye, Akdeniz Üniversitesi İ.İ.B.F.

Thiel, G., Geisler, G., Blechschmidt, I., & Danzer, K. (2004). Determination of trace elements in wines and classification according to their provenance. *Anal Bioanal Chem, 378*(6), 1630-1636. doi: 10.1007/s00216-003-2444-6

Thomas, R. (2004). *Practical Guide to ICP-MS*. New York: Marcel Dekker, Inc.

Thomas, R. (2013). *Practical Guide to ICP-MS: A Tutorial for Beginners* (Third ed.). NW: CRC Press.

TSE. (1981). Food staffs-Determination of metals and other elements- Atomic absorption Spectrophotometric method (Vol. 3606).

Turkish Food Codex. (2008a). Communication of Wine (Vol. 2008/67).

Turkish Food Codex. (2008b). Communique on Maximum Residue Limits of Contaminants in Foods (Vol. 26).

Uzun, İ. (2004). *Bağcılık*. Antalya: Hasad Yayıncılık.

Ünsal, T. (2007). *Kalecik Karası, Gamay ve Cabernet Sauvignon Şaraplarında Bazı Fenolik Bileşenlerin Karşılaştırılması.* (Yüksek Lisans Tezi), Ankara Üniversitesi, Ankara.

van Leeuwen, C., Friant, P., Tregoat, O., Koundouras, S., Choné, X., & Dubourdieu, D. (2004). Influence of Climate, Soil, and Cultivar on Terroir. *Am. J. Enol. Vitic., 55*(3), 207-214.

van Leeuwen, C., & Seguin, G. (2006). The concept of terroir in viticulture. *Journal of Wine Research, 17*(1), 1-10. doi: 10.1080/09571260600633135

Versari, A., Laurie, V. F., Ricci, A., Laghi, L., & Parpinello, G. P. (2014). Progress in authentication, typification and traceability of grapes and wines by chemometric

approaches. *Food Research International, 60*, 2-18. doi: 10.1016/j.foodres.2014.02.007

Versari, A., Parpinello, G. P., & Laghi, L. (2012). Application of Infrared Spectroscopy for the Prediction of Color Components of Red Wines. *Spectroscopy, 27*(2), 36-47.

Ververidis, F., Trantas, E., Douglas, C., Vollmer, G., Kretzschmar, G., & Panopoulos, N. (2007). Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnol J, 2*(10), 1214-1234. doi: 10.1002/biot.200700084

Villagra, E., Santos, L. S., Vaz, B. G., Eberlin, M. N., & Felipe Laurie, V. (2012). Varietal discrimination of Chilean wines by direct injection mass spectrometry analysis combined with multivariate statistics. *Food Chemistry, 131*(2), 692-697. doi: 10.1016/j.foodchem.2011.08.078

Vitaglione, P., Sforza, S., Galaverna, G., Ghidini, C., Caporaso, N., Vescovi, P. P., . . . Marchelli, R. (2005). Bioavailability of trans-resveratrol from red wine in humans. *Mol Nutr Food Res, 49*(5), 495-504. doi: 10.1002/mnfr.200500002

Volpe, M. G., La Cara, F., Volpe, F., De Mattia, A., Serino, V., Petitto, F., . . . Di Stasio, M. (2009). Heavy metal uptake in the enological food chain. *Food Chemistry, 117*(3), 553-560. doi: 10.1016/j.foodchem.2009.04.033

Vystavna, Y., Rushenko, L., Diadin, D., Klymenko, O., & Klymenko, M. (2014). Trace metals in wine and vineyard environment in southern Ukraine. *Food Chemistry, 146*, 339-344. doi: 10.1016/j.foodchem.2013.09.091

weppi.gtk.fi. Strontium. Retrieved May, 2014, from http://weppi.gtk.fi/publ/foregsatlas/text/Sr.pdf

Wold, S. (1987). Principal Component Analysis. *Chemometrics and Intelligent Laboratory Systems, 2*, 37-52.

www.dmi.gov.tr. Turkish State Meteorological Service. Retrieved January, 2012

www.ecfr.gov. U.S. Electronic Code of Federal Regulations. Retrieved May, 2014, from http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&sid=f32f67bf76f21056fc9377d73deaca60&rgn=div8&view=text&node=27:1.0.1.1.2.3.25.4&idno=27

www.gap.gov.tr. Asma Yetiştiriciliği. Retrieved May, 2014, from http://www.gap.gov.tr/proje-ve-faaliyetler/ekonomik-kalkinma-ve-girisimcilik-genel-koordinatorlugu/tarim-orman-ve-kirsal-kalkinma/mevcut-durum/gap-

bitkisel-uretim/gap-bolgesi-bitki-yetistirme-teknikleri/guneydogu-anadolu-bolgesi-meyve-yetistirme-teknikleri/asma-yetistiriciligi

www.kavaklidere.com. Bağcılık. Retrieved May, 2014, from http://www.kavaklidere.com/Bagcilik.aspx

Xiang, L., Xiao, L., Wang, Y., Li, H., Huang, Z., & He, X. (2014). Health benefits of wine: don't expect resveratrol too much. *Food Chemistry, 156*, 258-263. doi: 10.1016/j.foodchem.2014.01.006

Yang, Y. M., Duan, C. Q., Du, H. J., Tian, J. J., & Pan, Q. H. (2010). Trace Element and Rare Earth Element Profiles in Berry Tissues of Three Grape Cultivars. *American Journal of Enology and Viticulture, 61*(3), 401-407.

Yıldırım, H. K., Elmacı, Y., Ova, G., Altuğ, T., & Yücel, U. (2007). Descriptive Analysis of Red Wines From Different Grape Cultivars in Turkey. *International Journal of Food Properties, 10*(1), 93-102. doi: 10.1080/10942910600755128

Yildirim, K. H. (2006). Evaluation of colour parameters and antioxidant activities of fruit wines. *Int J Food Sci Nutr, 57*(1-2), 47-63. doi: 10.1080/09637480600655993

Zhao, W. (2005). Understanding classifications: Empirical evidence from the American and French wine industries. *Poetics, 33*(3-4), 179-200. doi: 10.1016/j.poetic.2005.09.010

Zou, J. F., Peng, Z. X., Du, H. J., Duan, C. Q., Reeves, M. J., & Pan, Q. H. (2012). Elemental Patterns of Wines, Grapes, and Vineyard Soils from Chinese Wine-Producing Regions and Their Association. *American Journal of Enology and Viticulture, 63*(2), 232-240. doi: DOI 10.5344/ajev.2012.11087

APPENDIX A

THE GEOGRAPHIC ORIGINS OF WINE SAMPLES

Figure A.1. The geographic origins of wine samples

APPENDIX B

THE CORRELATION COEFFICIENTS AND ANALYTICAL CONDITIONS OF CALIBRATION MODELS OF INSTRUMENTS

Table B.1. ICP-OES instrument parameters

Analysis Date	Element	Ca	Fe	K	Mg	Na
31.05.11	r^2	1.000	1.000	1.000	0.997	0.999
10.01.11	r^2	1.000	1.000	0.999	0.997	0.999
11.01.11	r^2	1.000	1.000	0.999	1.000	0.999
25.10.11	r^2	0.999	1.000	0.999	0.996	0.999

Table B.2. ICP-MS instrument parameters

Analysis Date	Element	Li	Be	B	Al	Cr	Mn	Co	Ni	Cu	Zn	Ga	Sr	Cd	Ba	Tl	Pb
10.06.10	r^2	1.0000	1.0000	0.9999	1.0000	0.9999	0.9999	1.0000	0.9999	1.0000	0.9999	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000
	a	0.0234	0.1224	0.0073	0.0291	0.0341	0.2388	0.4057	0.1059	0.2699	0.0569	0.3312	0.3391	0.1921	0.6226	0.9611	1.4560
	b	0.0001	0.0002	0.0151	0.0260	0.0018	1.1330	0.0063	0.0010	-0.0028	0.0349	0.0003	0.0002	-0.0001	-0.0713	0.0057	0.0865
17.06.10	r^2	1.0000	0.9999	0.9998	1.0000	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	1.0000	1.0000
	a	0.0306	0.0158	0.0104	0.0430	0.0410	0.3028	0.4862	0.1210	0.3086	0.0588	0.3534	0.3302	0.1744	0.5146	0.8086	1.1850
	b	0.00438	-0.00005	0.07588	0.02534	0.00011	0.67810	0.00043	0.02018	0.04283	0.04571	0.00253	0.03101	0.00075	0.02676	0.00170	0.05264
24.06.10	r^2	1.0000	1.0000	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000
	a	0.0292	0.1525	0.0098	0.0432	0.0406	0.3094	0.4727	0.1166	0.2988	0.0604	0.3845	0.3583	0.1824	0.5608	0.8384	1.2400
	b	0.0020	-0.0001	0.0445	-0.0310	0.0000	-0.1554	-0.0004	0.0060	-0.0033	0.0165	0.0003	-0.1217	0.0004	-0.0281	0.0018	0.0582
17.09.10	r^2	1.0000	1.0000	0.9999	0.9999	0.9999	0.9998	1.0000	0.9999	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	a	0.0237	0.1096	0.0070	0.0358	0.0424	0.2869	0.4966	0.1261	0.3283	0.0611	0.3757	0.3364	0.1802	0.5402	0.8020	1.3290
	b	0.00194	0.00004	0.16180	0.04532	0.00128	0.26890	0.00066	0.01498	0.01536	0.06752	0.00198	-0.04035	0.00032	-0.01613	-0.00021	0.05206
05.01.11	r^2	1.0000	1.0000	0.9999	0.9999	0.9999	1.0000	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999
	a	0.0285	0.2291	0.0102	0.0251	0.0420	0.2627	0.5446	0.1529	0.4016	0.0603	0.4284	0.2307	0.1462	0.3651	0.5412	0.7257
	b	0.0004	0.0006	0.0148	0.0394	0.0028	-0.2702	0.0030	-0.0080	0.0008	0.2222	0.0001	0.0074	0.0010	-0.0218	-0.0007	0.0701
25.10.11	r^2	0.9997	0.9999	0.9997	1.0000	0.9997	0.9999	0.9999	1.0000	0.9997	0.9997	0.9995	0.9999	1.0000	0.9998	0.9994	0.9996
	a	0.0226	0.1992	0.0064	0.0212	0.0384	0.2312	0.4502	0.1158	0.2997	0.0565	0.4054	0.2625	0.1778	0.5098	0.6257	1.1580
	b	0.0068	-0.0001	-0.0162	0.0282	0.0019	-0.4961	0.0061	0.0123	0.0920	0.1245	0.0003	0.0067	0.0049	0.0908	0.0194	0.1093

Table B.3. HPLC instrument parameters of polyphenol analysis for 2006 and 2007

Compounds	Max ג	Rt* 2006	$R^2$2006	Calibration Curve2006	Rt2007	$R^2$2007	Calibration Curve2007
gallic acid	266	8.595	0.9986	Y= 45.4725X-241.3436	8.933	0.9994	Y=38.4836X-137.6082
(-) epicatechin	278	27.666	1.0000	Y=12.4042X-4.8088	28.532	0.9999	Y=11.7389X-1.8781
DL catechin	278	22.67	0.9997	Y=10.5335X+1.6929	23.284	0.9997	Y=9.8526X+4.7919
o-coumaric acid	278	46.04	0.9996	Y=105.9454X+4.8173	46.783	0.9999	Y=99.9832X+9.5091
vanillic acid	262	23.664	1.0000	Y=35.9653X-34.0781	24.212	1.0000	Y=34.2719X-25.8974
caffeic acid	324	25.166	0.9999	Y=77.1651X+1649.0820	25.877	1.0000	Y=75.0148X+1523.6584
ferulic acid	322	36.373	0.9994	Y=93.4526X+0.6938	36.907	1.0000	Y=89.8010X+7.8981
p-coumaric acid	320	32.978	1.0000	Y=127.2245X-95.6174	33.662	1.0000	Y=120.5936X-107.0239
rutin	354	37.485	0.9998	Y=29.7546X-81.8929	38.605	0.9997	Y=28.2345X-47.4610
myricetin	378	49.582	0.9996	Y=56.7600X-232.1307	50.679	0.9992	Y=58.3227X-236.4601
quercetin	370	61.545	0.9993	Y=37.3825X-125.7724	62.219	0.9991	Y=30.1314X-54.0269
kaempferol	366	69.492	0.9994	Y=25.0866X-12.1214	70.099	0.9991	Y=39.3628X-17.8263
t-resveratrol	306/318	-	-	-	54.652	1.0000	Y=156.6163X-5.785064
malvidin	526	30.315	0.9994	Y=59.3872X-3.6848	30.43	0.9993	Y=51.4282X+53.6318
Quercetin-3-glucoside	354/258	40.025	0.9997	Y=38.0060756X	40.025	0.9997	Y=38.0060756X
Quercetin-3-galactoside	354/258	39.187	0.9995	Y=15.7036697	39.187	0.9995	Y=15.7036697
PB1	280	19.466	0.9998	Y=10.5863632X	19.466	0.9998	Y=10.5863632X

*Rt: Retention time

Table B.4. HPLC instrument parameters of polyphenol analysis for 2008 and 2009

Compounds	Max ג	Rt*2008	$R^2$2008	Calibration Curve2008	Rt2009	$R^2$2009	Calibration Curve2009
gallic acid	266	8.881	0.9993	Y=35.8723X-134.6274	8.862	0.9991	Y=25.8028X+80.9423
(-) epicatechin	278	28.198	0.9995	Y=13.5727X-1.4159	27.915	0.9997	Y=13.9627X-10.9680
DL catechin	278	23.181	0.9992	Y=10.5443X-14.4070	22.922	0.9993	Y=11.0774X-22.9298
o-coumaric acid	278	46.708	0.9994	Y=114.2893X-1.2922	46.566	0.9998	Y=219.5921X-1.4802
vanillic acid	262	24.133	0.9990	Y=37.0179X-30.7797	24.062	1.0000	Y=36.2166X-1.3172
caffeic acid	324	25.784	0.9991	Y=111.4179X-123.3616	25.695	0.9995	Y=95.7198X-42.863
ferulic acid	322	36.859	0.9996	Y=105.4916X-7.6058	36.709	0.9988	Y=124.7188X-8.2650
p-coumaric acid	320	33.627	0.9993	Y=132.8416X-112.3391	33.507	1.0000	Y=90.9531X+2.5095
rutin	354	38.119	0.9999	Y=29.0539X+2.6030	37.964	0.9952	Y=28.7880X+5.9529
myricetin	378	50.125	0.9997	Y=65.3947X-35.8348	50.034	0.9969	Y=58.8359X-146.9229
quercetin	370	62.174	0.9998	Y=65.0524X-39.5902	62.069	0.9981	Y=68.8017X-148.5596
kaempferol	366	70.026	0.9998	Y=70.5400X-21.9996	69.913	0.9984	Y=87.5475X-79.3510
t-resveratrol	306/318	54.51	0.9992	Y=1462.2679X-145.7002	54.057	0.9995	Y=151.0510X+2.0372
malvidin	526	30.854	0.9998	Y=51.4057X+69.9234	30.547	0.9992	Y=64.5116X-284.002
Quercetin-3-glucoside	354/258	40.025	0.9997	Y=37.6578X+25.1722	39.669	0.9993	Y=37.8341X-45.2316
Quercetin-3-galactoside	354/258	39.187	0.9992	Y=15.9876X-21.3938	38.821	0.9999	Y=68.6387X-4.3956
PB1	280	19.466	0.9998	Y=10.5863632X	19.466	0.9996	Y=10.5372X+5.535

*Rt: Retention time

Table B.5. HPLC instrument parameters of organic acid, sugar and alcohol analyses

Compound	Rt* 2006-2007	R^2 2006-2007	Calibration Curve 2006-2007	Rt 2008	R^2 2008	Calibration Curve 2008	Rt 2009	R^2 2009	Calibration Curve 2009
glucose	10.373	0.9990	Y=74718.4028+643.0435X	11.031	0.9998	Y=88783.2153+641.2432X	10.979	0.9999	Y=13991.3643+629.8979X
fructose	11.286	0.9987	Y=75283.8591+631.5845X	12.077	0.9998	Y=71408.4662+648.2692X	12.015	0.9999	Y=37774.9195+617.8409X
glycerol	15.358	0.9999	Y=-11960.3011+531.9206X	16.126	0.9996	Y=56056.8343+531.0106X	16.23	0.9990	Y=102713.5526+499.2549X
ethanol	24.119	0.9962	Y=-470480.5631+1679904.1219X	24.978	0.9995	Y=-121037.1459+1659006.6579X	25.309	0.9999	Y=-43741.6606+1682617.3620X
citric acid	8.721	0.9990	Y=3069.3110+2401.8979X	9.277	0.9992	Y=-1383.1203+2962.5836X	9.338	0.9984	Y=-3713.4209+2683.9062X
tartaric acid	9.498	0.9983	Y=-46953.9106+3174.0932X	10.098	0.9998	Y=-504.0915+2932.1592X	10.176	0.9954	Y=-16243.8536+3277.1480X
malic acid	10.331	0.9983	Y=-22548.8636+1688.6313X	10.996	0.9995	Y=-2270.5405+1445.5009X	11.063	0.9984	Y=-36.6439+1542.3938X
pyruvic acid	11.555	0.9990	Y=10167.7932+8636.1233X	12.115	0.9992	Y=-9556.6257+9155.8335X	12.254	0.9998	Y=4265.3166+10221.1384X
succinic acid	12.121	0.9989	Y=88.3511+1637.2361X	12.941	0.9991	Y=-345.4287+1148.2372X	12.976	0.9995	Y=1460.5999+1793.3785X
lactic acid	13.73	0.9980	Y=-25797.1862+1560.6426X	14.55	0.9997	Y=-2629.3993+1422.5436X	14.664	0.9979	Y=3340.9069+1411.2429X
acetic acid	16.19	0.9997	Y=1107.391614X	17.071	0.9998	Y=-962.2347+1114.9962X	17.146	0.9997	Y=-883.5802+1172.3535X

*Rt: Retention time

APPENDIX C

THE HPLC CHROMATOGRAMS OF WINE SAMPLES

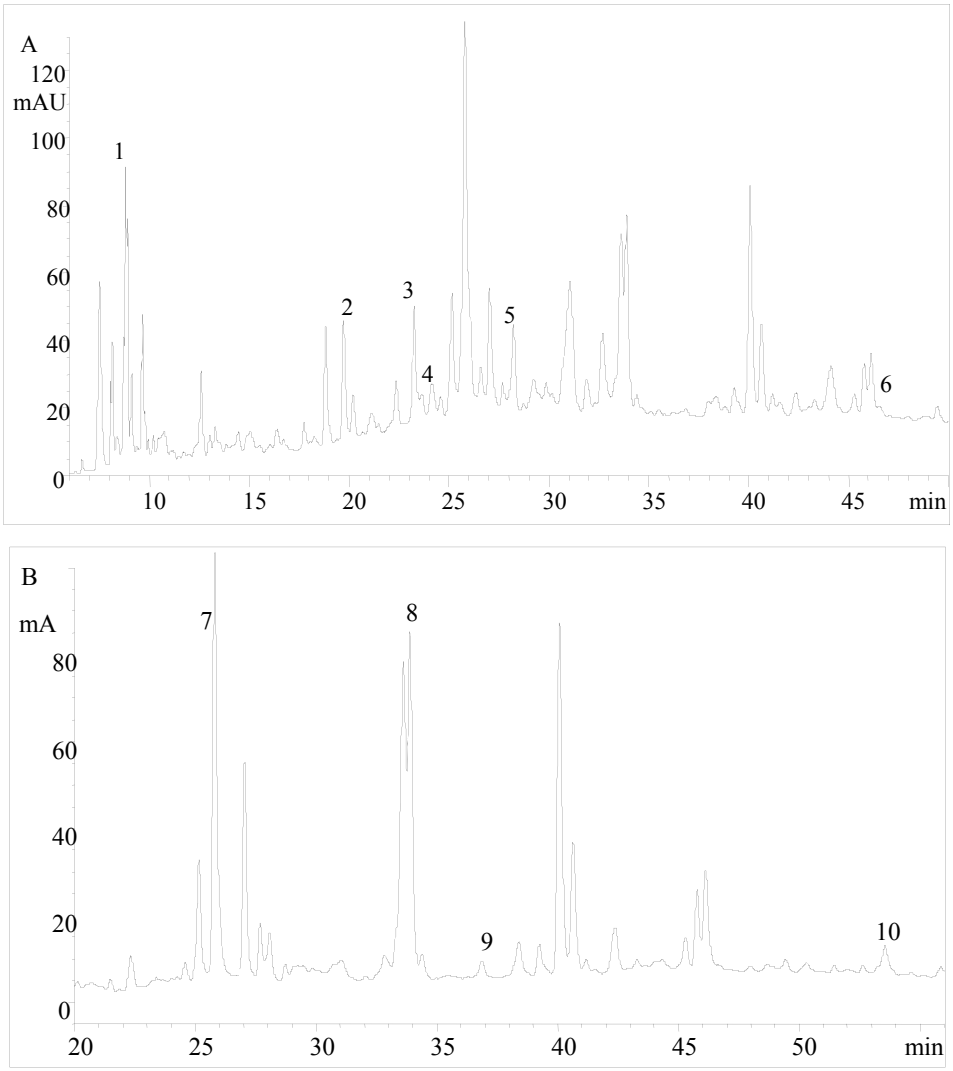

Figure C.1. HPLC polyphenol chromatograms of Kalecik Karası red wine at 280 nm (A), 320 nm (B), 360 nm (C) and 520 nm (D). Peak Assignment: 1 gallic acid, 2 Procyanidin B_1, 3 (+)-catechin, 4 vanillic acid, 5 (-)-epicatechin, 6 o-coumaric acid, 7 caffeic acid, 8 p-coumaric acid, 9 ferulic acid, 10 resveratrol, 11 myricetin-3-glucoside, 12 rutin, 13 Quercetin-3-galactoside, 14 Quercetin-3-glucoside, 15 Quercetin-3-glucuronide, 16 myricetin, 17 quercetin, 18 kaempferol, 19 delphinidin-3-glucoside, 20 petunidin-3-glucoside, 21 peonidin-3-glucoside, 22 malvidin-3-glucoside, 23 vitisin-A, 24 delphinidin-3-glucoside acetate, 25 petunidin-3-glucoside acetate, 26 peonidin-3-glucoside acetate, 27 malvidin-3-glucoside acetate, 28 delphinidin-3-glucoside acetate, 29 pinotin-A, 30 malvidin-3-glucoside coumarate **(cont. on next page)**

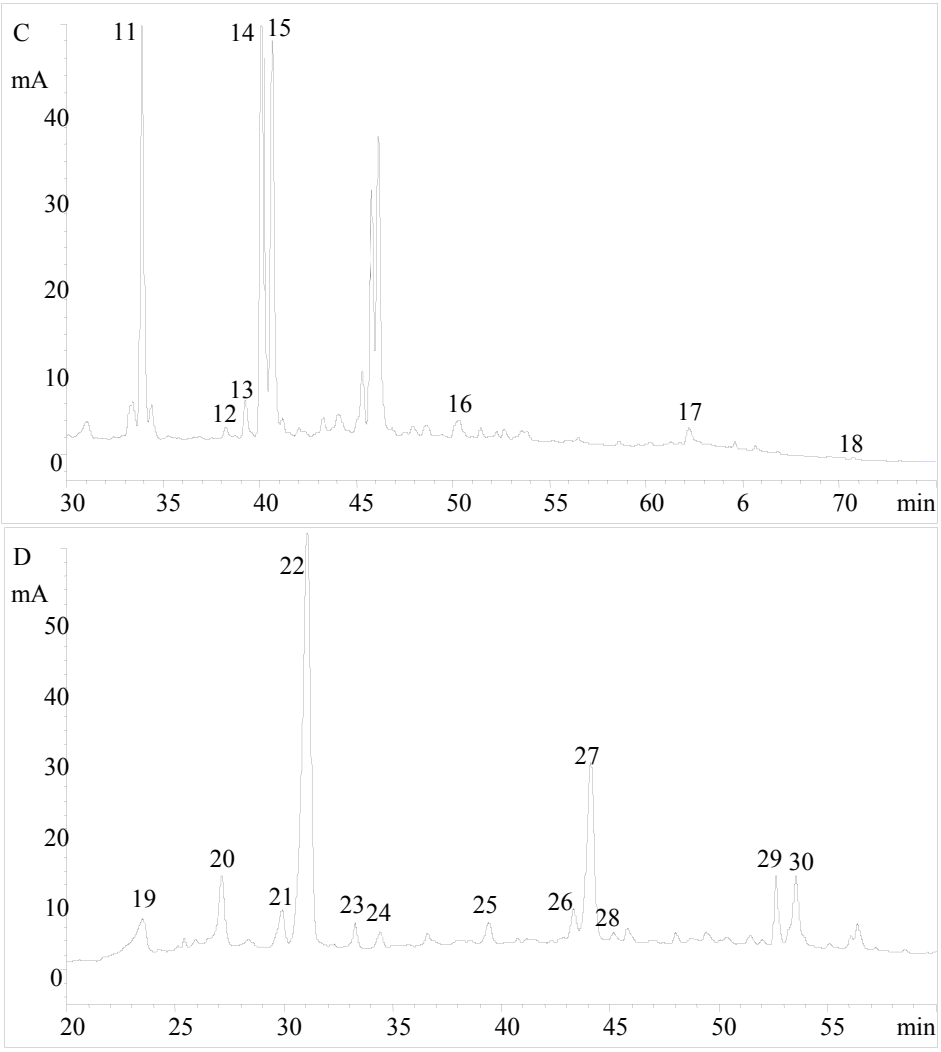

Figure C.1. (Cont.)

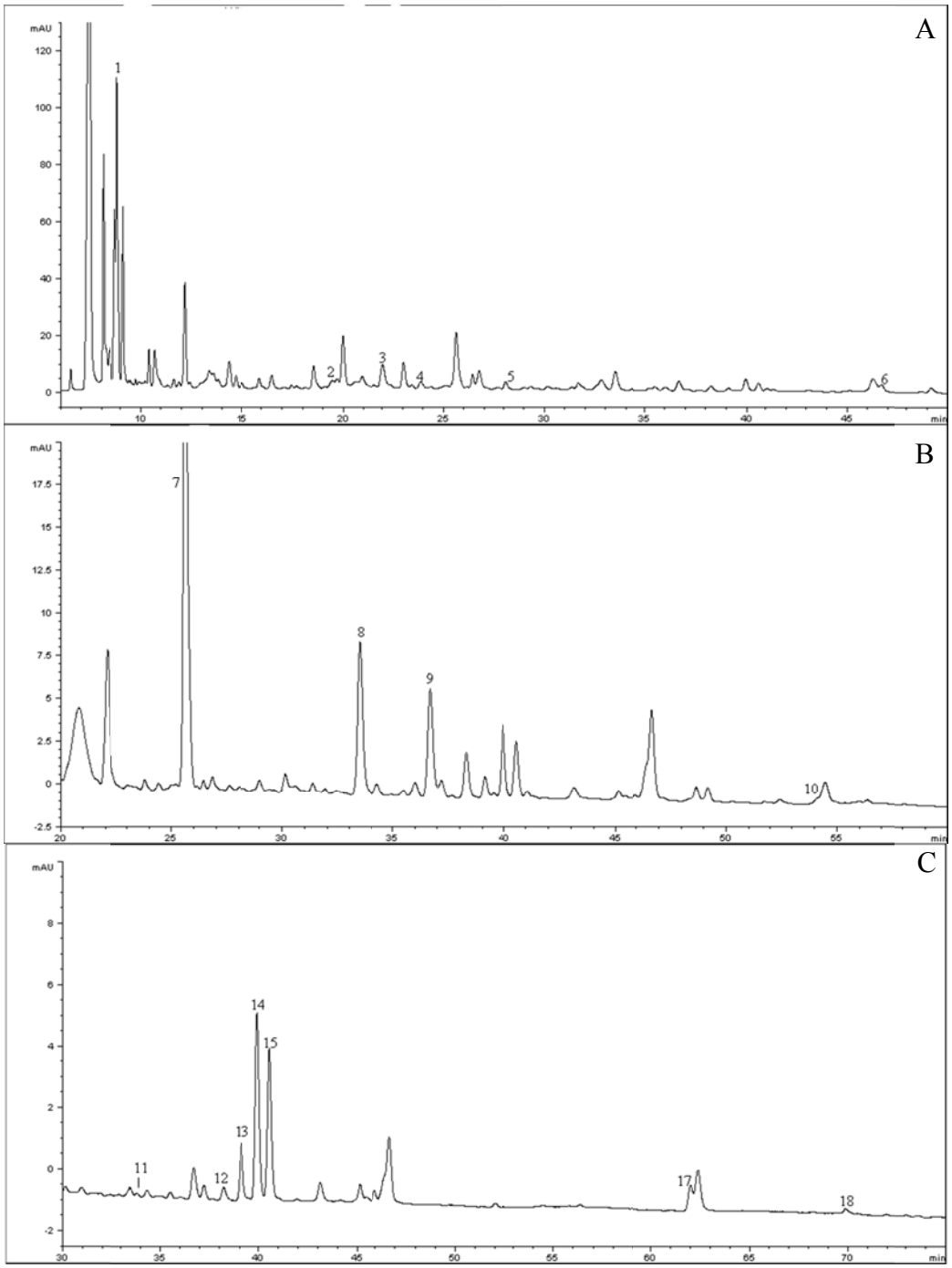

Figure C.2. HPLC polyphenol chromatograms of Narince white wine at 280 nm (A), 320 nm (B) and 360 nm (C). Peak Assignment: 1 gallic acid, 2 Procyanidin B_1, 3 (+)-catechin, 4 vanillic acid, 5 (-)-epicatechin, 6 o-coumaric acid, 7 caffeic acid, 8 p-coumaric acid,9 ferulic acid, 10 resveratrol, 11 myricetin-3-glucoside, 12 rutin, 13 Quercetin-3-galactoside, 14 Quercetin-3-glucoside, 15 Quercetin-3-glucuronide, 16 myricetin, 17 quercetin, 18 kaempferol

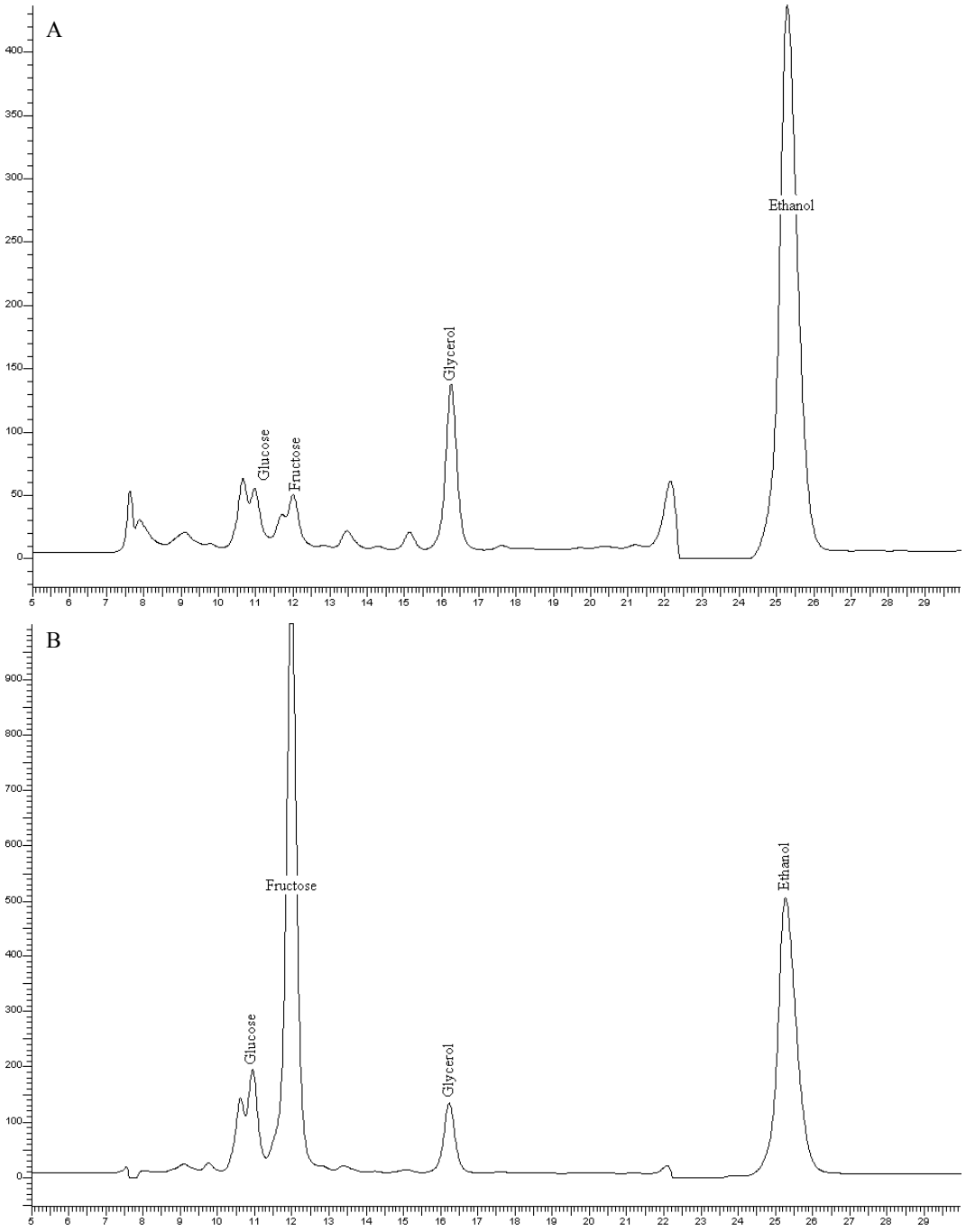

Figure C.3. HPLC sugar chromatograms of red (A) and white (B) wine samples

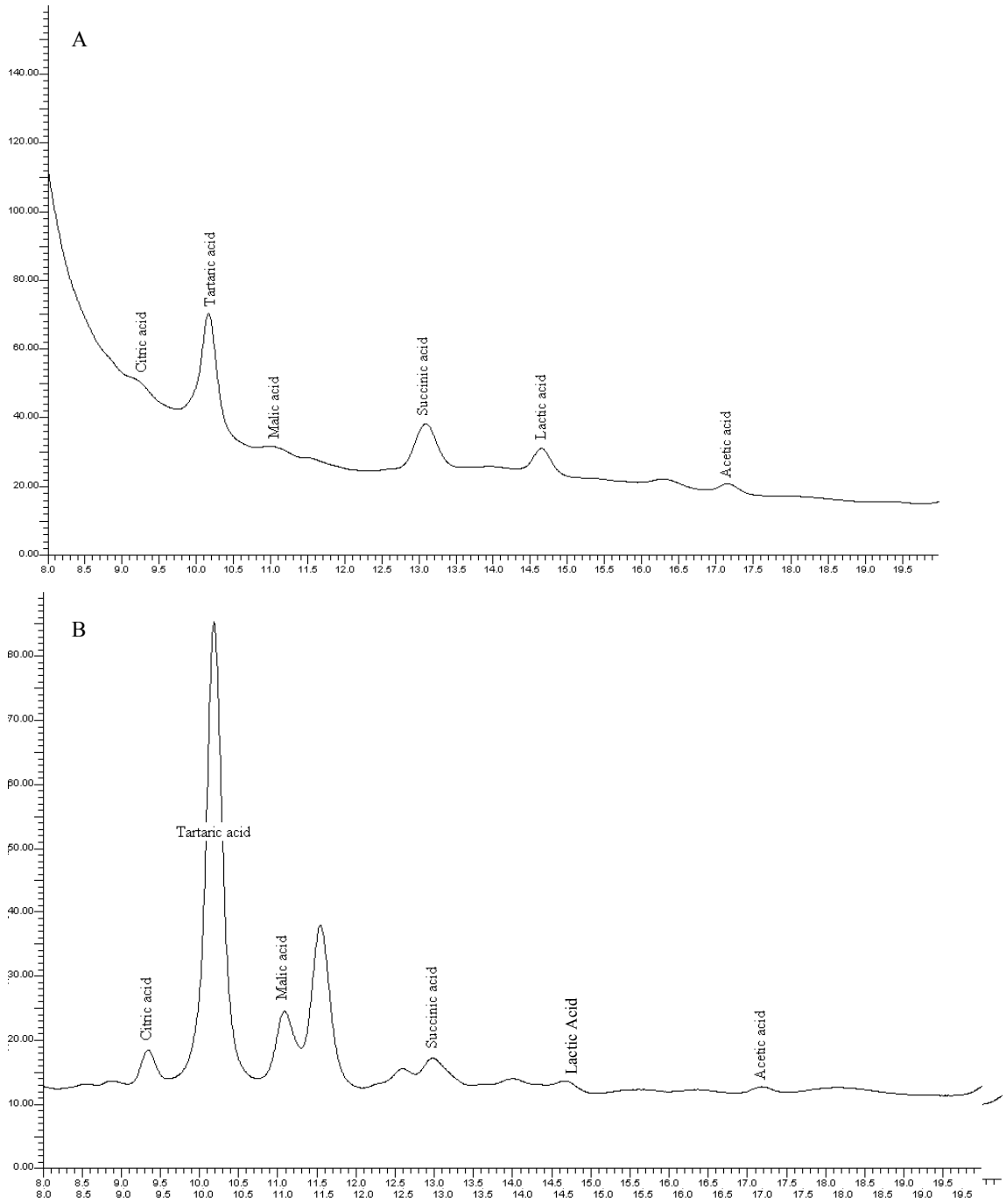

Figure C.4. HPLC organic acid chromatograms of red (A) and white (B) wine samples

APPENDIX D

TYPICAL TRANSMITTANCE SPECTRA OF RED, ROSE AND WHITE WINE SAMPLES

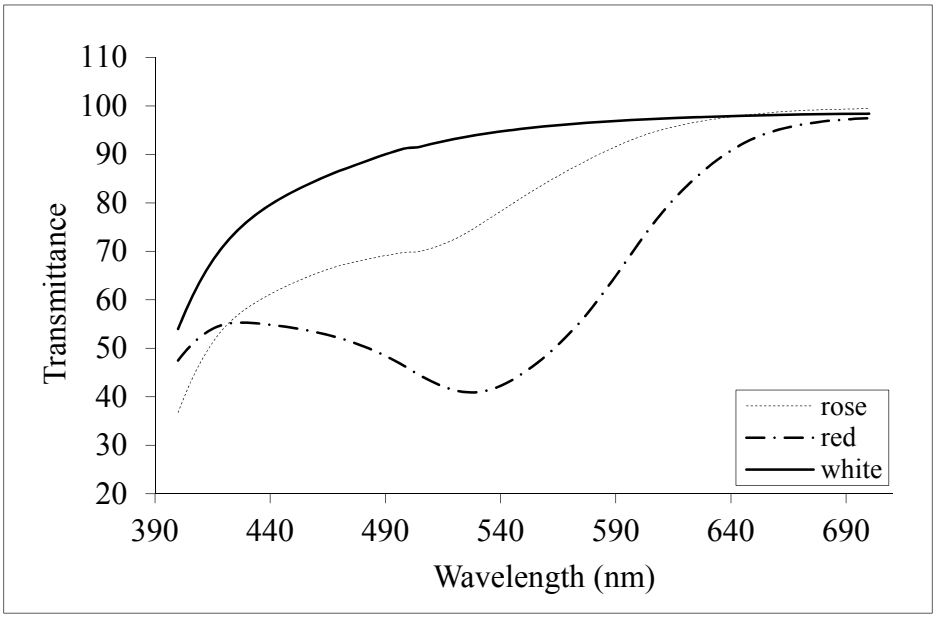

Figure D.1. The transmittance spectra of wine samples

APPENDIX E

THE CALIBRATION CURVES OF TOTAL PHENOL CONTENT ANALYSIS

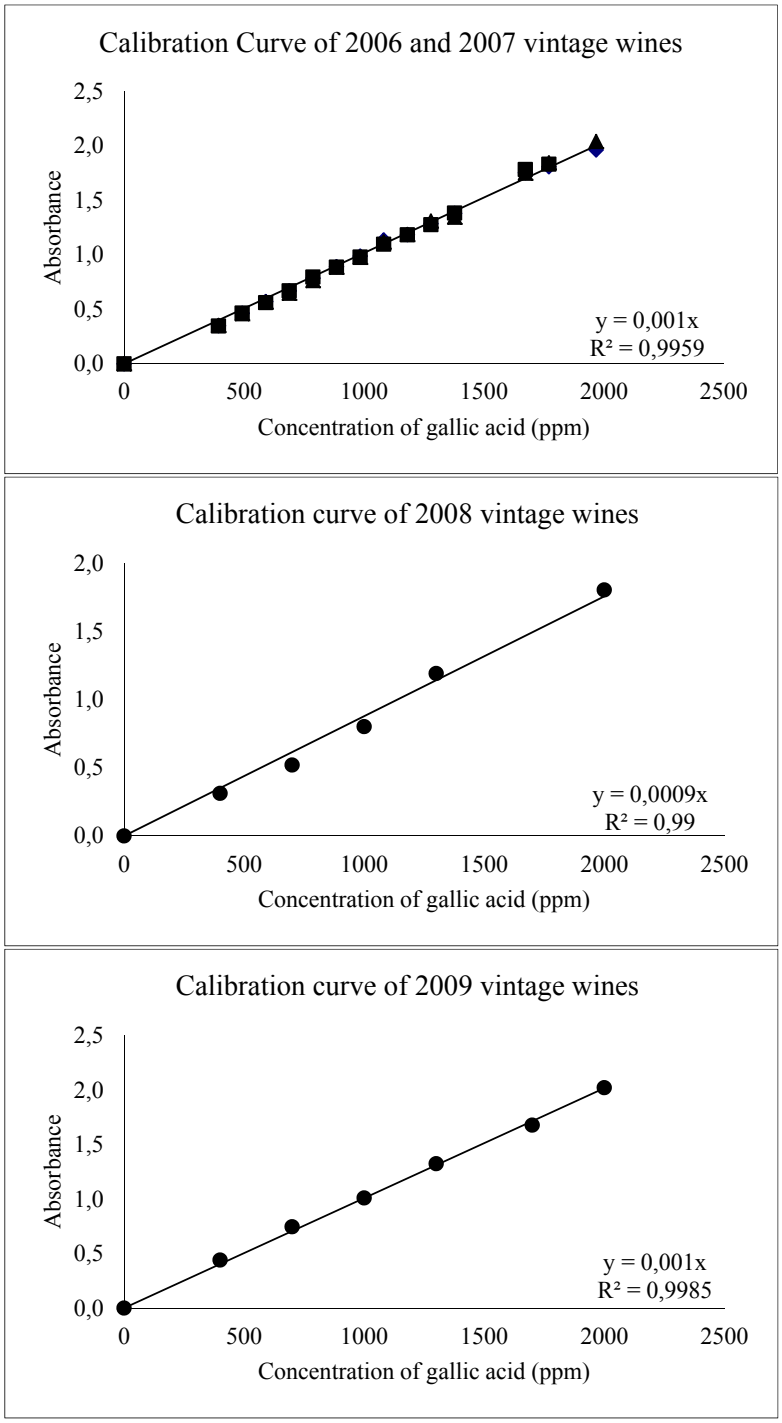

Figure E.1. The calibration curves of total phenol content analysis

APPENDIX F

THE PEARSON CORRELATION COEFFICIENTS

Table F.1. The Pearson correlation coefficients of red wine samples

	Al	Co	Ba	Li	Ni	T	R	Y	mal3G	mal3Ga	PB1	T5
Fe	0.58	0.62										
Cr	0.62											
Co	0.61											
Sr			0.61	0.73								
pet3G						-0.59	0.60	-0.61				
del3G						-0.62	0.63	-0.64				
vitA								-0.58				
del3Gc		-0.64										
rutn									0.60			
myric					0.68							
Q3glucosi										0.58		0.62
myric3G												0.62
Q3glucuron											0.59	
vanill											0.60	

Table F.2. The Pearson correlation coefficients of white wine samples

	Co	Li	Sr	Al	Ba	Ni	b	C	KK	RI	tresv	DLcat
Co				0.69	0.67							
Li			0.63									
Cr				0.59								
Mn										0.62		
caffe									-0.61			
Q3galact						0.68						
p-coum							-0.59	-0.58	-0.62	0.72		
PB1												
CTRC	0.63											
MLC												0.63
OMLC												0.58
T4		-0.59	-0.65								-0.58	
T8		-0.66	-0.58								-0.64	
T9		-0.71	-0.62								-0.64	
R9	0.66			0.63								

b: yellow/blue chromaticity, C: chroma, T: tint, KK: logarithmic color density, R: red%, Y: yellow%, mal3G: malvidin-3-glucoside, pet3G: petunidin-3-glucoside, del3G: delphinidin-3-glucoside, vitA: vitisin-A, del3Gc: delphinidin-3-glucoside coumarate, mal3Ga: malvidin-3-glucoside acetate, rutn: rutin, myric: myricetin, Q3glucosi: quercetin-3-glucoside, Q3galact: quercetin-3-galactoside, Q3glucuron: quercetin-3-glucuronide, myric3G: myricetin-3-glucoside, caffe: caffeic acid, p-coum: p-coumaric acid, tresv: resveratrol, DLcatec: (+)-catechin, vanill: vanillic acid, PB1: procyanidin B_1, CTRC: citric acid, MLC: malic acid, OMLC: original malic acid, T4: average of temperature in April, T5: average of temperature in May, T8: average of temperature in August, T9: average of temperature in September, R9: total rainfall in September.
*All data are significant at 99.5% confidence interval.

VITA

Date and Place of Birth: 09.06.1978, Uşak, Turkey

EDUCATION

2008-2014 Philosophy of Doctorate (PhD), Izmir Institute of Technology, Department of Food Engineering (Characterization and Classification of Wines from Grape Varieties Grown in Turkey)

2000-2003 Master of Science (MSc), Izmir Institute of Technology, Department of Food Engineering (Spectroscopic Determination of Major Nutrients (N, P, K) of Soil)

1995-1999 Bachelor of Science (BS), Ege University, Department of Food Engineering (Flavoring Methods of Edremit Olives)

PROFESSIONAL EXPERIENCE

2007-Cont., Research Staff, Specialist

Izmir Institute of Technology Department of Food Engineering, İzmir

2006-2007, Food Additives and Residue Analysis Lab. Supervisor

Ege Analiz Food and Industrial Analysis Lab. Corp., İzmir

2005-2006, Milk Based Dessert and Ice Cream Production Engineer

Özsütden Food Inc., İzmir

2004-2005, Process Quality Assurance Engineer

Fora Olives, AntFood Inc., Edremit

2000-2003, Research Assistant

Izmir Institute of Technology Department of Food Engineering, İzmir

PUBLICATIONS

Sen, I., Tokatli, F. 2014. Characterization and Classification of Turkish Wines based on the Elemental Composition. *Am. J. Enol. Vitic*. 65(1):134-142.

Sen, I., Tokatli, F. 2014. Authenticity of wines made with economically important grape varieties grown in Anatolia by their phenolic profiles. *Food Control* 46:446-454.

Aktas, A.B., Ozen, B., Tokatli, F., Sen, I. 2014. Phenolics Profile of a Naturally Debittering Olive in Comparison to Regular Olive Varieties. *J Sci Food Agric* 94:691-698.

Aktas, A.B., Ozen, B., Tokatli, F., Sen, I. 2014. Comparison of some chemical parameters of a naturally debittered olive (Olea europaea L.) type with regular olive varieties. *Food Chemistry* 161:104-111.