VARIETAL CLASSIFICATION AND PREDICTION OF CHEMICAL PARAMETERS OF TURKISH WINES BY INFRARED SPECTROSCOPY

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ABSTRACT

VARIETAL CLASSIFICATION AND PREDICTION OF CHEMICAL PARAMETERS OF TURKISH WINES BY INFRARED SPECTROSCOPY

This study was performed with the aim of varietal classification of mono-varietal Turkish wines and development of models to predict basic enological parameters from mid-IR spectra with the use of chemometric methods. Mid-infrared (MIR) spectroscopy combined with multivariate data analysis was employed to make a varietal classification of commercial Turkish wines (Boğazkere, Cabarnet Sauvignon, Çalkarası, Kalecik Karası, Merlot, Öküzgözü, Papazkarası, Shiraz, Emir, Misket, Narince, Sultaniye and Chardonnay) from 2006 and 2007 vintages. Wine samples (n=79) including red, rose and white wines were scanned in the mid-IR region (4000-650 cm^{-1}) and three spectral regions (965-1565 cm^{-1}, 1700-1900 cm^{-1} and 2800-3040 cm^{-1}) were used to classify wines on the basis of grape variety. The principal component analysis (PCA) was applied to the spectral data of the wine samples. Although a clear classification could not be achieved according to varieties, almost complete classification of red and white wines was observed.

For the quantification analysis, a total of eleven enological parameters, including total phenol and anthocyanin content, pH, brix, titratable acidity, colour intensity (CI), tint, yellow%, red%, blue% and the proportion of red colour produced by anthocyanins (dA%) were determined with analytical reference methods. Correlation between the results of the reference methods and MIR spectral data was tested with partial least square (PLS) regression analysis and prediction models were developed with the use of these correlations. The calibration and validation sets were established to evaluate the predictive ability of the models. As a result of PLS analysis, the best models were developed for total phenols and CI with excellent predictions (R^2=0.93 and 0.89, respectively and residual predictive deviation RPD=3.68 and 3.83, respectively). The model of pH determination and yellow% gave a good prediction (R^2=0.85 and 0.85, respectively and RPD=2.7 and 2.04, respectively).

ÖZET

KIZIL ÖTESİ SPEKTROSKOPİ İLE TÜRK ŞARAPLARININ ÇEŞİTLERE GÖRE SINIFLANDIRILMASI VE KİMYASAL PARAMETRELERİNİN TESPİTİ

Bu çalışma tek çeşit üzümden oluşan Türk şaraplarının üzüm çeşidine göre sınıflandırılması ve şarabın temel parametrelerini orta bölge kızıl ötesi spektrası ve kemometrik yöntemler kullanarak tahmin eden modeller geliştirmek amacıyla yapılmıştır. Orta bölge kızıl ötesi spektroskopi çok değişkenli veri analizleri ile birlikte 2006 ve 2007 yıllarında üretilen Türk şaraplarının (Boğazkere, Cabarnet Sauvignon, Çalkarası, Kalecik Karası, Merlot, Öküzgözü, Papazkarası, Shiraz, Emir, Misket, Narince, Sultaniye ve Chardonnay) üzüm çeşidine göre sınıflandırılmasını gerçekleştirmek amacıyla kullanılmıştır. Kırmızı, roze ve beyaz şarapları içeren toplam yetmiş dokuz adet şarap örneği orta bölge kızıl ötesi bölgede (4000-650 cm^{-1}) taranmış ve üç spektral bölge (965-1565 cm^{-1}, 1700-1900 cm^{-1} ve 2800-3040 cm^{-1}) şarapları üzüm çeşidine göre sınıflandırmak amacıyla kullanılmıştır. Şarap örneklerinden elde edilen spektral verilere asal bileşenler analizi uygulanmıştır. Üzüm çeşitlerine göre belirgin bir sınıflandırma elde edilemese de; kırmızı ve beyaz şaraplarda yaklaşık bir sınıflandırma gözlenmiştir.

Nicelik belirleme analizi için toplam fenol miktarı, antosiyanin miktarı, pH, briks, titrasyon asitliği, renk yoğunluğu (CI), renk tonu, % sarılık, % kırmızılık, % mavilik ve antosiyaninler tarafından oluşturulan kırmızılık (% dA) olmak üzere toplam on bir şarap parametresi analitik referans metotlarıyla saptanmıştır. Referans metotların sonuçları ve orta bölge kızıl ötesi spektrası arasındaki ilişki kısmi en küçük kareler (PLS) regresyon analizi ile belirlenmiştir ve saptanan bu ilişki kullanılarak tahmin modelleri oluşturulmuştur. Modellerin tahmin yeterliliğini değerlendirmek için ise, kalibrasyon ve validasyon setleri oluşturulmuştur. Regresyon analizi sonucunda nicelik tespitinde en iyi modellerin toplam fenol miktarı ve renk yoğunluğu (korelasyon katsayıları sırasıyla 0.93 ve 0.89; artık tahmin sapması sırasıyla 3.68 ve 3.83) tahmin modelleri olduğu gözlenmiştir. pH ve % sarılık tespit modelleri (korelasyon katsayıları 0.85; artık tahmin sapması sırasıyla 3.68 ve 3.83) iyi tahmin sonuçları vermiştir.

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CHAPTER 1

INTRODUCTION

On account of the favourable climatic conditions and various soil structures, Turkey is one of the most convenient countries in the world for the cultivation of wine grape vineyards. The vineyards are spread all over the country but Aegean, Mediterranean, Central Anatolian and Thrace regions are the main wine production areas. Turkey has a rich variety of grape types which provide high quality wines.

Both wine producers and consumers are interested in wine quality. For instance, in the selection of wines consumers take into consideration of several factors such as pleasant colour and odour, taste and aroma, geographical origin, vintage and nowadays ecological production is also one of the important criteria in the preference. These characteristics form as a result of complex combinations of hundreds of various components (Saurina 2010). Several factors such as variety of the grape used in wine production, ripeness of the grape, geographic origin, vintage, viticultural and vinification techniques have a considerable influence on the compositional variations of the wine. Among these factors the characteristics of a wine are mainly designated by the grape variety.

The grape varieties provide a basis for wine characterisation due to their differentiating chemical compositions and organoleptic properties. Wine characterisation and differentiation is traditionally performed with the data from sensory and wet chemical analysis. However, the complex profile of the wine makes the differentiation hard with limited number of chemical analysis. Furthermore, most of the analytical methods require time consuming, expensive and labouring experimental principles. Therefore, there is a need of simple, rapid and reproducible techniques (Almela, et al. 1996; Arozarena, et al. 2000b; Aleixandre, et al. 2002).

FT-IR spectroscopy in combination with chemometric methods provides rapid, repeatable and non-destructive analysis of wine without any sample preparation and chemical consumption. In addition, information about the complete composition of the wine can be supplied from the FT-IR spectra (Edelman, et al. 2001).

On the other hand, knowledge of chemical composition of wine is extremely important from the point of wine producers. For the production of high quality wine, the changes in the composition are periodically controlled at every stage of the grape and wine since the chemical composition of grape and wine alters from the start of grape ripening to harvest, the maturity, the grape acceptance, in the course of wine production and ageing. Fast and accurate analytical techniques are also desirable for monitoring and screening of the product. FT-IR spectroscopy combined with chemometrics enables a simultaneous determination of a wide range of enological parameters. Thus, this new process control methodology is quite useful for quality assurance purposes as it enables multiparametric determination of wine composition (Patz, et al. 2004; Urbano-Cuadrado, et al. 2005).

Many studies were reported as to the usage of chemometrics in wine production and research areas. The IR spectroscopy in combination with chemometric methods has been applied to distinguish wines in accordance with the different varieties, geographical origin and wine making practices. In the differentiation applications, chemometric techniques provide the explanation of the complex data and point out the variables that best represent the differences between wines from different varieties (Arozarena, et al. 2000b). Additionally, IR spectroscopy combined with chemometrics has been employed for development of the models to predict some quantitative parameters of the wines. This newly developed technique has been applied in the research field, routine laboratory analysis and process control of wine composition.

The objective of this study is to investigate the potential use of FT-MIR spectroscopy in combination with chemometric techniques to classify Turkish wines from two vintages according to variety. Furthermore, the prediction of the certain enological properties such as total phenol content, anthocyanin content, brix, pH, titratable acidity and colour parameters from FT-MIR spectra was studied by means of chemometric techniques.

CHAPTER 2

WINE

2.1. Historical Background of Wine

The history of wine extends over thousands of years and there is an intimate relationship between the wine evolution and the history of agriculture, cuisine and civilisation (McGovern 2003). There is a general thought that first vines originated from the Caucasus area of Russia, between the Baltic and the Caspian Seas. After Stone Age, wild vines botanically known as *Vitis vinifera sylvestris* became domesticated in consequence of the improvement in the settled agricultural implementations in Mesopotamia and Egypt (Clarke, et al. 2004).

Vines and wine making techniques were spread over Greece and Mediterranean from Mesopotamia and Egypt. With the invention of distillation by Arabians, fortified wines like Sherries were developed. The long term storage and spoilage problems were continued till the end of 1800s. The role of yeasts in wine fermentation and the role of some lactic acid bacteria in wine spoilage were revealed by Louis Pasteur in the 1860s and the discovery of microbiological process concerned with wine making served as the basis for the modern wine industry (Clarke, et al. 2004). Till the ends of 18th century wines were mostly known as sweet. Especially the sweetness of the Roman wines were resembled to syrups and it was reported that wines were stored and transported in amphorae, earthenware jugs closed with waxy materials. The fact that corks started to be utilised to seal wine bottles gave rise to maturing the wines in bottles. Romans are also known with their important contribution in classifying grape varieties and colours, observing ripening characteristics, identifying diseases and spreading viticulture through today's major winemaking regions of France, Germany, Italy, Portugal and Spain (Robinson 2006). The domesticated version of the native vine plant was grown in the North and South America where a balanced climate with the right combination of sun and rain exist in the 19th century. In the late 19th century wine production was fully spread over Europe (Clarke, et al. 2004).

2.2. Wine Classification

Wines can be classified by various methods. Classification applications vary in different countries and regions of origins, and many practices have undergone changes over time. The wine producing countries have their own regulations and legislations for determination of the product characteristics aiming at enabling proper production, preparation, process, storage, transportation and marketing of wine. The wines manufactured in member countries of European Union are divided into two quality categories, table wines and quality wines produced in specified regions. The wine production is regulated by the wine notification of Turkish Food Codex covering wines, liquor wines, natural sparkling wines, artificial sparkling wines, natural semi-sparkling wines, artificial semi-sparkling wines and wines registered with geographical origin in Turkey. As far as the notification is concerned, the basic classification of Turkish wines was made by considering vinification methods and geographical origin (Turkish Food Codex 2005).

The classification of wines is basically made by considering the place of origin or appellation, vinification techniques, sugar contents, vintage, and variety of the grapes used to produce wine.

2.2.1. Classification According to Vinification Techniques

Wines may be classified by vinification methods including red, rose or white wine, sparkling, fortified and dessert wines.

Red and white wines differ at the stage of processing after crushing; the skins and seeds are not removed and fermentation is carried out in the presence of the grape juice, skins and seeds together for red wine production. In addition, the red wines are generally fermented at higher temperatures in the range of 25-30^{o}C, whereas fermentation temperature for white wines is ranging between 18-24^{o}C (Boulton, et al. 1996). Rose wines are produced with similar method as red wines; the only difference is the temperature and length of the fermentation period of grape juice with seeds and skins. The fortified wines are strengthened through the addition of spirit, brandy or ethyl alcohol (70-75%) solution to increase the alcohol level to 15-20% and Port wine, Sherry and Madeira are the well known examples of fortified wines (Clarke, et al. 2004).

Dessert wines have alcoholic content between 14-24% by volume derived from the added grape brandy or alcohol. On the other hand, sparkling wines are produced by traditional fermentation in sealed vats and fermentation in the bottle (natural sparkling wines) or they can be produced by carbonation (aerated sparkling wines) (Food Standards Agency 2010).

2.2.2. Classification According to Sugar Content

Wines are divided into four groups with respect to sugar content in the final product (Food Standards Agency 2010):

Dry wines: This group contains maximum of 4 g/L sugar. Dry wines can also contain maximum 9 g/L if the total acidity content is not more than 2 g/L below the residual sugar content.

Medium Dry Wines: The residual sugar content exceeds the maximum for dry wines but must not exceed 12 g/L, or maximum 18 g/L where the total acidity content is not more than 10 g/L below the residual sugar content.

Medium Sweet Wines: The residual sugar content must exceed the maximum for medium dry but not exceed 45 g/L.

Sweet Wines: The sweet wines contain at least 45 g/L sugar.

2.2.3. Classification by Appellation

Wines are regulated with the laws controlling the naming of wine by geographic origin, which is referred to as *appellation controlée* (original name derived from France). Appellation Control laws establish the regions where particular grape varieties are cultivated, since the soil type and climatic conditions give regional wines their unique characteristics (Jackson 2000). Appellation system and relevant control laws regulate not only where the grapes in a wine were grown but also the grape cultivars and vinification technique employed in wine production (Jackson 2000). The appellation system was firstly established in France in 1938. The French wine appellation system consists of four appellation categories; Vins de Table (VCC), Vins de Pays, Vins De´limités de Qualité Supérieure (VDQS), and Appellation d'Origine Contrôlée (AOC) (Zhao 2005). Among them AOC is the main and the most important

qualification category of the whole appellation system. The qualifications within AOC are classified as regional (e.g.Bordeaux, Burgundy), communal or village (i.e. Pauillac in Bordeaux) (Coates 2000). On the other hand, appellation system in the USA consists of 'the United States', a state (for instance California), two or no more than three States which are all neighbour, a county (i.e. Sonoma County), two or no more than three counties in the same States and an American Viticultural Area, AVA (e.g. Napa Valley) (Zhao 2005). According to German appellation system, the largest appellations are designated as *bestimmte Anbaugebiete*. These regions are divided into one or more areas stated as Bereich. The succeeding divisions are predicated either on group vineyards (Grosslage) or on individual vineyards (Einzellage). The further divisions are related with the name of the nearest village or suburb (Ortsteil) (Jackson 2000).

2.2.4. Classification by Grape Variety

The wine variety defines the names of the dominant grapes used in the wine production. The wine may not be entirely composed of one grape variety; two or more varieties are blended for the purpose of obtaining a balanced taste and texture. The varietal labelling laws differ in different countries. In the EU countries the wines are labelled as single variety if at least 85% of the products is made from that variety (EC 607 2009). However, the US varietal designations require at least 75% of the grapes used to make the wine of a single variety (Alcohol and Tobacco Tax and Trade Bureau 2010). On the other hand, in accordance with the International Wine and Viniculture Organization (OIV) labelling standard the wines are labelled as mono-varietal if they are produced from at least 75% of the grapes of defined variety which determines the specific character of the wine (OIV 2006).

2.2.5. Classification by Vintage

The vintage of the wine is defined as the wine produced from grapes which are all or majorly harvested in a particular year. Quality variations can be seen from year to year resulting from differences in chemical and textural profile of the wines. Most of the wine producing regions have their own vintage labelling laws. The wines must be made with grapes coming 100% from the year indicated, if they are from a recognised

geographic indication or appellation of origin (OIV 2006). According to EU and the US wine labelling regulations, the wines are labelled with vintage provided that at least 85% of the grapes used to produce the wines have been harvested in the year at issue (EC 607 2009, European Union Wine Regulations 2010).

2.3. World Wine Production

With the fluctuations in wine production three countries from EU; Italy, France and Spain are the world's leader of wine production (Table 2.1). France is the leader wine producer till 2007, but Italy has taken its row owing to a decrease in the wine production in France in 2008. The US follows these three countries in wine production. With its 530,000 hectares vineyard, Turkey is the fourth largest grape grower country in the world. Nearly 40% of the grapes is consumed as raisin, 35% is consumed freshly, 2% is used in the wine production and the rest is used for production of traditional foods like grape molasses, dried fruit pulp, etc (Gümüş and Gümüş 2008). Almost 1200 grape varieties are grown in Turkey but only 34 types are used for wine production. The wine production in Turkey is mainly concentrated in provinces Tekirdağ, Edirne, Kırklareli, Çanakkale, Bilecik, Yozgat, Çorum, Amasya, Tokat, Nevşehir, Mersin, İzmir, Manisa, Denizli, Burdur, Isparta, Elazığ, Malatya, Gaziantep and Diyarbakır (Appendix A).

Even though Turkey, with its large vineyards and numerous of grape cultivars, is located in the most suitable climatic zone for vine growing, it is situated in the last ranks in the world wine production (Table 2.1). Special Consumption Tax and the government's behaviour towards the wine sector are reported as the most important problems in Turkey (Gümüş and Gümüş 2009).

Table 2.1. World wine production by country between 2004-2008 in 1000 Hectolitres and % change 2008/2004 (the red coloured terms express decrease in production) (Source: Wine Institute 2010)

COUNTRY	2004	2005	2006	2007	2008	%CHANGE 2008/2004
WORLD TOTAL	291,987	301,363	285,035	284,700	283,898	2.80%
ITALY	44,086	53,135	50,566	49,631	51,500	16.80%
FRANCE	57,386	52,105	53,400	52,127	45,692	20.40%
SPAIN	41,843	43,168	36,158	38,29	36,781	12.10%
UNITED STATES	24,11	27,859	24,298	25,125	24,274	0.70%
ARGENTINA	15,464	15,222	15,396	15,046	15,013	2.90%
AUSTRALIA	15,048	14,669	14,628	9,620	14,750	2.00%
CHINA	11,700	12,000	13,000	14,000	14,500	23.90%
GERMANY	10,107	9,150	9,256	9,000	10,363	2.50%
SOUTH AFRICA	9,279	9,052	10,130	10,200	10,300	11%
CHILE	6,550	8,046	8,450	8,280	8,690	32.70%
PORTUGAL	7,340	7,481	7,267	7,542	6,049	17.60%
ROMANIA	5,555	6,166	2,602	5,015	5,288	4.80%
RUSSIA	5,120	5,035	5,000	5,000	5,000	2.30%
MOLDOVA	3,488	3,509	3,597	3,600	3,650	4.60%
GREECE	3,815	4,295	3,997	3,874	3,337	12.50%
HUNGARY	3,88	5,271	3,103	3,144	3,222	17.00%
BRAZIL	3925	3,199	2,372	3,000	3,000	23.60%
UKRAINE	2,400	2,400	2,460	2,400	2,400	0.00%
AUSTRIA	2,735	2,264	2,256	2,300	2,300	15.90%
BULGARIA	2,327	1,961	1,708	1,757	1,800	22.60%
CROATIA	1,800	1,571	1,592	1,600	1,600	11.10%
NEW ZEALAND	1,192	1,020	1,195	1,250	1,300	9.10%
GEORGIA	950	950	1,100	1,100	1,100	15.80%
SWITZERLAND	1,159	1,001	1,108	1,100	1,100	5.10%
MEXICO	1,100	1,028	1,028	1,050	1,060	3.60%
JAPAN	862	900	960	960	960	11.40%
MACEDONIA	900	940	760	900	900	0.00%
URUGUAY	1,126	892	900	900	900	20.10%
SLOVENIA	731	944	846	738	857	17.20%
ALGERIA	770	770	770	770	770	0.00%
CANADA	522	417	504	520	540	3.40%
PERU	130	435	400	453	480	269.20%
UZBEKISTAN	450	450	450	450	450	0.00%
CZECH	560	580	438	434	434	22.50%
CYPRUS	404	197	340	400	400	1.00%
MOROCCO	350	375	345	350	350	0.00%
SLOVAKIA	515	409	302	328	328	36.30%
TUNISIA	375	331	300	300	300	20.00%
KAZAKHSTAN	250	210	270	270	270	8.00%
TURKEY	250	287	250	255	260	4.00%
TURKMENISTAN	240	240	240	240	240	0.00%

2.4. Important Wine Quality Parameters

The persistence and diversity of flavours of a wine, its ability to age, its appearance in the glass, and the taste of it are the most significant attributes appreciated by both wine makers and consumers. All of these characteristics are highly related to

each other and basically depend on the chemical profile of the wine which arises from complicated reactions beginning with grape maturation and continuing until the consumption.

Wine is a complex mixture of chemical compounds existing at different concentrations. The basic chemical compounds of the wines are listed in Table 2.2.

Table 2.2. Typical range of basic chemical compounds in the wine
(Source: Clarke, et al. 2004)

Component		**Typical range found (g/L)**		
Sugars	Glucose	0.2-0.8 (dry)*, up to 30 (sweet)		
	Fructose	1-2 (dry), up to 60 (sweet)		
	Arabinose	0.30-1.0		
Alcohols (volatile)	Ethyl alcohol	72-120 (9.1-1.5 % v/v)		
	Glycerol	5.0-15.0		
(nonvolatile)	Butan,2,3,-diol	0.3-1.5		
	Inositol	0.2-0.7		
	Sorbitol	0.1		
Acids	Total	3.5-15.0 (some present as salts with metallic cations)		
(nonvolatile)				
(volatile)	Acetic acid	0.5-1.0 (can be higher in spoiled wines)		
Metal cations	Potassium	0.5-1.5		
	Sodium	0.03-0.05		
Metal cations	Magnesium	0.05-0.15		
	Calcium	0.05-0.15		
Tannins	Total	**Content in mg/L**		
(Folin Ciocalteau test)		**Wine type**	**Range**	**Average**
		White	40-1300	360
		Red	190-3800	2000
		White-dessert	100-1100	350
		Red-dessert	400-3300	900
Other volatile substances	Total	0.8-1.2		
		(esters, terpens, phenols, hydrocarbones and other volatiles)		

* EU definition of ‘dry’, < 9g/L sugar

Water and ethanol are the major compounds of the wine. The minor components such as sugars, organic acids, phenolic compounds, salts, glycerol, aliphatic and aromatic alcohols act on the basic flavour and colour formation. Their concentration in the wine is of great importance in terms of both quality assurance of wine making industries and the consumer preferences (Tarantilis, et al. 2008).

The chemical compounds affect sensory perception by interacting in complicated ways. The taste and mouth-feel sensations of a wine are primarily related to the certain compounds including water, alcohols (basically ethanol), fixed acids (mainly tartaric, malic and lactic acids), phenolic compounds, sugars (glucose and fructose) and glycerol (Jackson 2008). Sweetness of the wine, important in classification according to taste, is majorly correlated with residual amount of sugars (saccharides), glucose and fructose. The ethyl alcohol and glycerol have also small contribution to sweet taste of the final wine. Acidity of the wine is important owing to its role in control of fermentation and stability of the wine. The acid taste is majorly due to tartaric, malic and citric acids. Lactic acid is especially important in malolactic fermentation, in addition acetic acid is highly significant as a potential spoilage agent. Astringency, described as dry and puckery sensation, is primarily due to high molecular weight phenolic compounds and also small wine flavanoids, (+)-catechin and (-)-epicatechin (Clarke, et al. 2004). Furthermore, tannins have significant role in the final quality of red wines, especially their astringency. In addition, they provide important health benefits due to their antioxidant activity (Fernández and Agosin 2007). Mouth-feel, a term often corresponds with the term ‘body’, is correlated with viscosity and hydrophilic proline-rich protein-phenol complex plays a significant role on mouth-feel perception (Clarke, et al. 2004).

Colour, another quality parameter, is a part of the overall perceived organoleptic property. The colour of the red wines derives from the condensation reactions between anthocyanins, natural phenolic glycosides, and other phenolic compounds naturally occurring in wines and tannins also play important role in the long-term colour stability. Anthocyanins are found in the skin of the black and red grapes and are extracted to the wine during vinification and particularly maceration step and the colour of anthocyanins is affected by the pH differences until the consumption (Janik, et al. 2007). On the other hand, colour of white wines is related to the non-flavanoid chemicals such as caffeoyl tartaric and its derivatives (Clarke, et al. 2004, Fernández and Agosin 2007). It is also believed that the yellow colour of young white wine is resulted from the limited

extraction and oxidation of flavonols such as quercetin and kaempferol and yellow gold colour of older white wines is derived from the oxidation of phenols or galacturonic acid (Jackson 2008).

Odour and flavour of the wine is one of the important parameters influencing the consumer preference. Certain primary aromas characterize a wine like grapey aroma which is mainly related to the terpenes. There are numerous volatile components acting on the flavour of the wine and basically esters, terpens, alcohols, volatile acids, aldehydes and ketons are known to act on the flavour of the wine to a large extent (Clarke, et. al. 2004).

2.5. Factors Affecting Wine Composition

The composition of wines is influenced by many factors like the variety of the grapes used in winemaking, terroir, ripening conditions of the grapes, viticultural techniques (fertilisation, irrigation techniques), the characteristics of the soil, geographical condition, the climatic conditions, the vintage and different wine making methods (temperature and duration of maceration, catalysts and enzymes). Variability among different wines is arising from the interaction of all of these factors with each other (Arozarena, et al. 2000; Tarantilis, et al. 2008).

Climate, one of the most important factors, is related to the vine and grape composition which influence the wine chemistry and microbiology, and sensory properties. Among the most important climate-related effects are the late harvest and high temperatures during the grape ripening period. These conditions cause high grape sugar that induces high alcohol levels, lower acidities and differences in the varietal aroma compounds. In addition, anthocyanin synthesis and accumulation are affected by the length of sun exposure because of excessive or low temperatures (Mira de Orduña 2010). Sun exposure also influences the formation of monoterpenes which are responsible for fruity, floral and spicy aromas and it was reported that white wine aromas comprise more conveniently in cool climates (Duchene and Schneider 2005). The variations between different vintage wines basically stem from the climatic differences between the years of harvest.

The grape variety is another differentiating factor on the chemical composition of the wine. Every grape plant has individual phenolic composition (anthocyanins, flavonoids, procyanidins, hydroxycinnamic acids, and their derivatives) which is

characteristic for each grape variety (Tarantilis , et al. 2008). In another words, phenolic compounds acts as a fingerprint in differentiation of the wines from different varieties and this distinguishing property of the wine was previously studied for the purpose of differentiation of the wines on the basis of grape variety for many times (Arozarena, et al. 2000; Tzouros and Arvanitoyannis 2001; Aleixandre, et al. 2002; Bevin, et al. 2008).

Wine composition is also diverging with the influence of differences in the wine making step. Differentiations in the maceration and fermentation stages, usage of different fining agents, and addition of yeasts and enzymes are some of the examples from the different enological treatments which have been studied (Castillo-Sánchez, et al. 2006; Castillo-Sánchez, et al. 2008). In a previous study, the effect of skin contact treatment on the content of aroma compounds of Narince wines was investigated by comparing the untreated wines of two vintages. The study revealed that skin contact treatment increased the total concentration of wine volatiles (Selli, et al. 2006). In another study, the effects of different wine making processes (conventional maceration and fermentation after initial carbonic maceration) with and without the use of four different fining agents on anthocyanin content and the colour stability were evaluated. On the basis of the results, carbonic maceration led lower anthocyanin content but higher stability during the storage and usage of fining agents resulted in variation on colour intensity and stability (Castillo-Sánchez, et al. 2006). The influence of maceration temperature, clarification method, storage temperature and length of storage time on the phenolic compounds and colour of young red wines was investigated by Gomez-Plaza, et al. (2000). Multivariate analysis indicated significant differences among all of the variables according to vinification techniques and length of storage time (Gomez-Plaza, et al. 2000).

Soil properties also cause differences in the composition of the wines. Among the soil properties water retention and drainage capacity are important as far as the physical and chemical characteristics are concerned. Since the vine plant doesn't like water logging, extremely clayed soils are undesirable. On the other hand, clay has the advantage of attracting and bearing humus, containing nitrogen and other nutrients. Depending on the composition and amounts of chemicals naturally found in the soil, the chemical composition of the wines shows variations (Clarke et al. 2004). Soil characteristics and climate together cause variations in the wine composition and these properties are used in the geographical classification (or appellation of origin in some countries) (Liu, et al. 2006).

2.6. Wine Characterisation

Characteristics of a wine show differences with the effect of a wide range of factors like the type of the grape (variety), soil characteristics, cultural and enological conditions (differentiation in the production steps), climatic and geographical conditions. Grape variety, geographic origin and vinification techniques are also important from the point of consumer expectations.

Varietal classification of the wines is based on the chemical differentiation between mono-varietal wines. Even though the mono-varietal wine can be composed of two or more varieties, it is defined with the dominant grape variety accounting for 75-85% of the wine and the dominant variety is decisive on varietal differentiation. The chemical and corresponding sensory profile of a mono-varietal wine are distinguishing, on account of the fact that each grape variety has individual chemical composition. Table 2.3. shows the variations in the chemical composition of four mono-varietal wines from Valencian community, a region determined with 'Appellation d'Origine' in Spain. All the grapes used in the study given in Table 2.3. were collected at the optimum stage of grape harvest for manufacturing young wines with equal maceration times.

Table 2.3. Chemical composition of the Cabarnet Sauvignon, Tempranillo, Monastrell and Bobal wines of Valencia region from 1994 and 1995 vintages (Source: Aleixandre, et al. 2002)

	Variety			
	Cabarnet Sauvignon	Tempranillo	Monastrell	Bobal
volatile acidity[a]	0.67	0.64	0.61	0.58
total acidity[b]	6.32	4.82	5.07	6.14
pH	3.46	3.82	3.76	3.51
Density	0.993	0.993	0.994	0.995
ethanol [c]	12.71	12.57	12.77	11.3
Sugar [d]	1.99	1.9	2.02	2.67
SO2 total [e]	58.2	60.24	65.63	36.13
SO2 free [e]	18.36	26.98	21.41	20.11
acetaldehyde [e]	36.04	26.5	31.55	19.57
methanol [e]	161.98	142.39	213.92	147.05
1-propanol [e]	21.09	27.25	22.5	24.09
isobutyric alcohol [e]	50.49	44.58	53.49	57.11
isoamylic alcohol [e]	337.4	225.62	305.84	246.16
glycerol [e]	12012.25	10145.69	10200.32	8932.38
2.3-butanediol [e]	591.15	684.46	569.75	525.74
1-butanol [e]	3.32	2.28	3.93	1.49
1-pentanol [e]	0.06	0.05	0.09	0.05
cis-3-hexenol [e]	0.08	0.24	0.04	0.09
2-phenylethanol [e]	65.88	44.93	76.55	34.78
methyl acetate [e]	11.99	11.27	13	11.71
ethyl acetate [e]	55.15	57.39	54.64	50.95
ethyl propionate [e]	0.15	0.18	0.18	0.16
ethyl butyrate [e]	0.83	0.98	1.34	0.81
isoamyl acetate [e]	0.67	1.19	0.79	0.48
isobutyl acetate [e]	0.05	0.06	0.05	0.04
hexyl acetate [e]	0.04	0.05	0.03	0.05
ethyl lactate [e]	78.94	69.72	70.37	71.13
ethyl octanoate [e]	0.56	0.59	0.5	0.58
ethyl decanoate [e]	0.43	0.65	0.3	0.34
γ-butyrolactone [e]	9.24	6.41	10.7	6.53
diethyl succinate [e]	5.2	6.53	5.99	6.17
diethyl glutarate [e]	0.16	0.07	0.11	0.07
ethyl laurate [e]	0.03	0.04	0.03	0.03

[a] Grams per liter of acetic acid. [b] Grams per liter of tartaric acid. [c] Percent v/v.
[d] Grams per liter. [e] Milligrams per liter.

Wine characterisation is generally performed with sensory and chemical analysis in traditional way. However, with the improvement of the up-to-date methods especially spectroscopic techniques in combination with multivariate data analysis have been started to be used for the classification. Such being the case, a large number of chemical compounds can be simultaneously handled to differentiate wines from different varieties. Characterizing or classifying the wines according to variety, geographical origin and different wine making techniques has been studied for many times previously. Wine characterisation was also performed to detect the adulteration and authenticity. Almela et al. (1996) investigated the discrimination of young red wines from six different varieties produced in Spain by using colour parameters. PCA and discriminant analysis (DA) were employed to determine the differences among the wine samples and a level of classification of 80.7% was achieved by using three colour variables (L, a and C values). When the number of variables were increased to 10 including colour intensity, pH, ionised anthocyanins, total phenols, percentage of yellow pigments, total anthocyanins, hue and L value 95% correct discrimination was succeeded by discriminant analysis (Almela , et al. 1996). In another study wines made from Cabarnet Sauvignon, Tempranillo, Monastrell and Bobal varieties from 1994 and 1995 vintages were differentiated according to variety by using conventional chemical parameters such as, alcohols, polyols and esters. Discriminant analysis composed of 11 variables was able to differentiate 100% of the 1994 vintage and 97% of the 1995 vintage (Aleixandre, et al. 2002). Tarantilis, et al. (2008) achieved to differentiate Greek red wines from Agiorgitiko, Xinomavro and Merlot varieties by mid-infrared spectroscopy in combination with chemometrics. The phenolic extracts obtained with solid phase extraction using C-18 column were examined by (FTIR) spectroscopy. By comparison of the unknown wine extract with the libraries of spectra constituted from each wine samples, the match values were measured and a successful varietal differentiation was achieved with this method (Tarantilis, et al. 2008). A similar study was performed by using phenolic wine extracts for discrimination of seven different red wine cultivars (Edelman, et al. 2001). Multivariate data analysis was applied by using mid-infrared spectroscopy and UV-vis spectroscopy to differentiate Austrian red wines from Cabarnet Sauvignon, Merlot, Pinot Noir, Blaufränkisch (Lemberger), St. Laurent and Zweigelt. Phenolic extracts taken by solid phase extraction with C-18 column were analysed by both mid-infrared spectroscopy and UV-vis spectroscopy and the results were tested with hierarchial cluster analysis. The use of mid-infrared spectra was given

more satisfactory results for cultivar discrimination, since the cultivar differentiation was observed to be limited to the authentication of Pinot Noir wines (Edelmann, et al. 2001).

Data from sensory analysis were also used to classify the wines. Kallithraka, et al. (2001) made a classification by using both instrumental and sensory analysis in combination with statistical analysis. Thirty three red wines from different geographical origins of Greece were analysed in terms of phenolic contents, non-coloured phenolics, anthocyanins, minerals and sensory profile including astringency, sweetness, acidity, body, flavour, after taste and overall acceptability. PCA method was employed to differentiate wines and PCA analysis of anthocyanin and sensory properties ended up with satisfactory classification for differentiating wines into two groups as south and north regions. Differentiation with anthocyanins resulted in identification of three groups, two belonging to North Greece and one to South Greece (Kallithraka, et al. 2001).

2.7. Application of Fourier Transform Infrared Spectroscopy in Wine Analysis

Knowledge of the change in the chemical composition is crucial from the beginning of grape ripening, for deciding on the optimum the maturity level, for controlling fermentation, for storage and aging stages and for monitoring and screening the vine and wine at all the stages to obtain a high quality wine. It is well known that wine is composed of numerous chemical compounds (Patz, et al. 2004). Wet chemical methods for determination of high number of chemicals, whether qualitative or quantitative, require time consuming, environment polluting, laborious and expensive procedures (Bevin, et al. 2006). That's why there is a need of simple and rapid methods that can be easily applied in routine laboratory analysis. With the advantages of its time saving and high resolution, Fourier transform infrared (FTIR) spectroscopy has been applied for both quantification and classification of wines (Moreira and Santos 2004).

The working principle of IR spectrometers is related to the various chemical bonds and functional groups that exist in the molecules (Table 2.4). A beam of infrared light (wavelength ~0.7-500 μm) is focused on the sample thanks to reflective devices. With the absorption of electromagnetic radiation by the molecules constituting the sample, gradation between the rotational and vibrational energy levels of the lowest

electronic energy state is stimulated and the excitement of vibrational (stretching and bending) positions of the molecules in the sample indicated in Table 2.4. create the peaks in the spectrum within IR region (Ismail, et al. 1997).

Table 2.4. Functional group absorption in mid-IR region
(Source: Ismail, et al. 1997)

Functional group	Frequency (cm^{-1})	Remarks
-OH	3600-3200	O-H (H-bonded) stretching vibration
-NH and NH_2 (H-bonded)	3300-3000	-N-H stretching vibration
$-CH_3$	2962 (±10), 2872 (±10)	C-H stretching doublet
$-CH_2-$	2926 (±10), 2853 (±10)	asymmetric and symmetric vibration
=C-H	3082-3000	
-C≡N	2260-2200	stretching
-C≡C	2250-2040	stretching
-C=O		
Acids	1770-1750	monomeric stretching, C=O
Acid salts	1610-1550	asymmetric stretching of CO_2^- group (strong)
Esters	1745-1725	C=O stretching
Aldehydes	1735-1715	C=O stretching
Ketones	1720-1710	C=O stretching
Amides	1700-1600	C=O stretching (Amide I band)
-C=N	1670-1618	C=N stretching
-C=C- (*trans*)	1678-1665	C=C stretching
-C=C- (*cis*)	1662-1648	C=C stretching
-N-H	1590-1500	-N-H bending
-N-D	1490-1400	-N-D bending
-C-N	1280-1030	C-N stretching

The IR spectroscopy is based on the study of the bands in the near infrared region (12800-4000 cm^{-1}) of the spectrum and in the mid- infrared region (5000-400 cm^{-1}) of the spectrum (Wilson 1994).

Internal reflectance, also referred to as attenuated total reflectance (ATR), is the most adaptable type of the sample representation which consists of a prism of infrared transmitting material of high refractive index (Figure 2.1). Infrared radiation enters the sample making good optical contact with the crystal after it is focused on the surface of the crystal and reducing of the reflected light eventuates when the sample medium absorbs infrared beam (Wilson 1994).

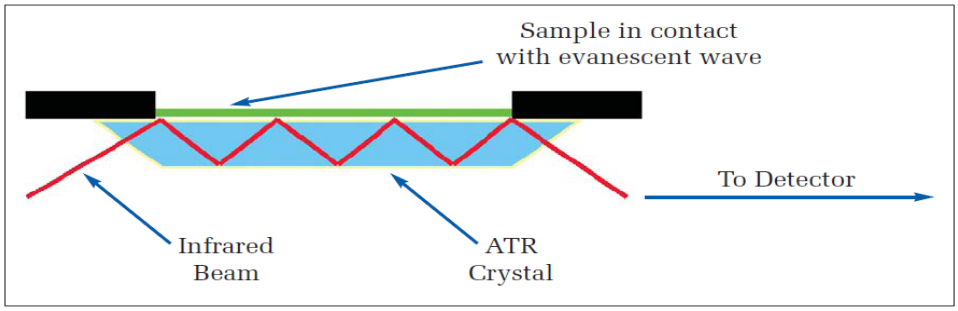

Figure 2.1. The typical scheme representing Attenuated Total Reflectance (ATR) system (Source: Perkin-Elmer 2010)

FT-IR spectroscopy can be used in the differentiation analysis since the IR spectrum of a compound is characteristic property which can be accepted as 'fingerprint' (Ismail, et al. 1997). Therefore, FT-IR spectroscopy is widely applied in the grape and wine for classification. For instance, FT-IR spectroscopy technique was utilised in differentiation of the 'Fino' sherry wines according to six different aging levels by Palma and Barroso (2002). PCA analysis was performed to analyse the spectral data for differentiation among the wines of six different ageing scales. FT-IR spectra have provided a successful differentiation according to ageing levels (Palma and Barroso 2002). Quantitative analysis can be also applied with the use of FT-IR spectroscopy technique on account of the fact that the amount of the IR energy absorbed by the individual component is directly proportional to the amount of each compound present (Beer's Law) (Ismail, et al. 1997). Nieuwoudt, et al. (2006) performed a FT-IR spectroscopy application for the quantification of volatile acidity, glycerol, ethanol, reducing sugar and glucose concentrations in fermented Chenin Blanc and synthetic musts. FT-IR spectra were collected for screening of the fermentation profile of wine yeast and calibration set was established to control accuracy of the prediction model. At the stage of evaluating the models, standard error of laboratory (SEL) and standard error of prediction (SEP) values were considered. Excellent predictive accuracy was achieved for the regression model for prediction of volatile acidity and regression of ethanol, reducing sugar and glucose ended up with satisfactory prediction models (Nieuwoudt, et al. 2006). In another study, FT-MIR spectroscopy combined with chemometric techniques was applied for quantitative analysis of red wine tannins (Fernández and Agosin 2007). With the use PLS regression and spectral interval selection procedures (iPLS and CSMWPLS), prediction models for tannin and mean degree of

polymerization of the tannins (mDP) were developed. The investigation resulted in accurate predictions for tannin concentrations and mDP of the tannins in accordance with the logical root mean square of prediction RMSEP, root mean square of calibration RMSEC and regression coefficient R^2 values calculated for calibration and prediction sets established.

2.8. Implementation of Multivariate Statistical Techniques in Wine Analysis

With the development of modern analytical instrumentation, nowadays food analysis depends more on full-spectrum measurements of both chemical and physical characteristics of a sample including multivariate responses (Bauer , et al. 2008). The results obtained from spectroscopic and chromatographic methods can include complex data with a large number of variables. Therefore, multivariate data analysis, also referred to as chemometrics, is required to evaluate the data by extracting the relevant information from these complex data. Chemometric methods perform the data analysis with comprehensive interpretation of the overall data matrix by taking into account the interactions among the numerous constituents present in the sample (Downey 1998; Cozzolino, et al. 2005; Bauer, et al.2008).

Chemometric methods are mainly employed for basically three purposes. Chemometric techniques are most commonly used for characterizing the data visually by appropriate graphic plots including useful summaries of simple means, standard deviations and correlations. Discrimination or classification is another field of usage to separate the large data into small groups. The wines show variability according to grape varieties, different wine-making practises, vintage and geographical origins. Chemometric methods for classifying wines in combination with spectral or chromatographic data have been widely applied for many years (Moret, et al. 1994). Regression and prediction of the chemical or sensory properties from the spectral or chromatographic data are among one of the most used application fields of multivariate data analysis. Regression analysis relates two sets of variables (X and Y) to each other. X data matrix, generally spectral or chromatographic data, and one or more Y variables obtained from analytical methods are correlated to each other to attain a prediction of y variables calculated from the regression model created. The purpose of regression analysis is to develop a model also known as calibration model to predict a property of

interest, for example, the concentration of a particular chemical in the sample (Esbensen 2002; Cozzolino, et al. 2009). Regression analysis combined with modern analytical methods provides simultaneous determination of large numbers of enological parameters, therefore, regression analysis have been applied in wine industry from the start of grape ripening until bottling of the finished wine for many years (Bauer, et al. 2008).

The most commonly used chemometric techniques applied to grape and wine analysis are principal component analysis (PCA) and partial least squares (PLS) regression.

2.8.1. Principal Component Analysis (PCA)

Principal component analysis is a multivariate projection method designed for screening, extracting and compressing multivariate data matrix X with N rows and K columns representing observations and variables, respectively (Eriksson, et al. 2001; Cozzolino 2009).

PCA is developed to reduce the number of variables to a smaller number of indices, referred to as principal component, by projecting the original multivariate data to a low-dimensional space (Figure 2.2). Linear combinations of variables, represented by lines, planes and hyperplanes, are produced by PCA on the basis of least square sense, which minimizes the residual variance and maximizes the variance of the scores (Eriksson, et al. 2001; Cozzolino 2009).

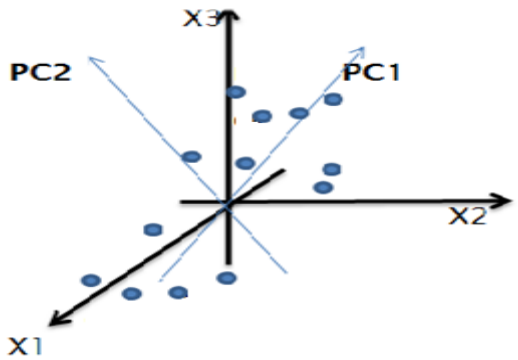

Figure 2.2. Projection of the multivariate data matrix to low dimensional space as a ‘swarm of points’ (Source: Eriksson, et al. 2001)

Prior to PCA, the data are firstly pre-processed by subtracting the mean from each of the data dimensions (mean centering) and by dividing each column of X by its standard deviation (UV scaling). There are several scaling techniques and the most commonly used one is unit variance (UV) scaling (Brereton 2003).

PCA method utilises a mathematical transformation procedure of the original data matrix X, which can be stated as:

$$X= T*P+E \qquad (2.1)$$

where the scores having as many rows as the original data matrix are symbolised by T; the loadings having as may columns as the original data are symbolised by P matrix and E is the error matrix (Brereton 2003). Scores matrix constituted from column vectors, and loadings matrix composed of row vectors together model the structure of principal components as the matrix product T*P (Figure 2.3). Several vectors construct the scores matrices T and loading matrices P and the first scores and loading vector are named as eigenvectors of the first PC. Each loading constitute a bridge between variable space and PC space while scores are described as the distance of the projected observation to PC-axis which are represented as t_i for the observation i (Figure 2.2.) (Brereton 2003; Esbensen 2002).

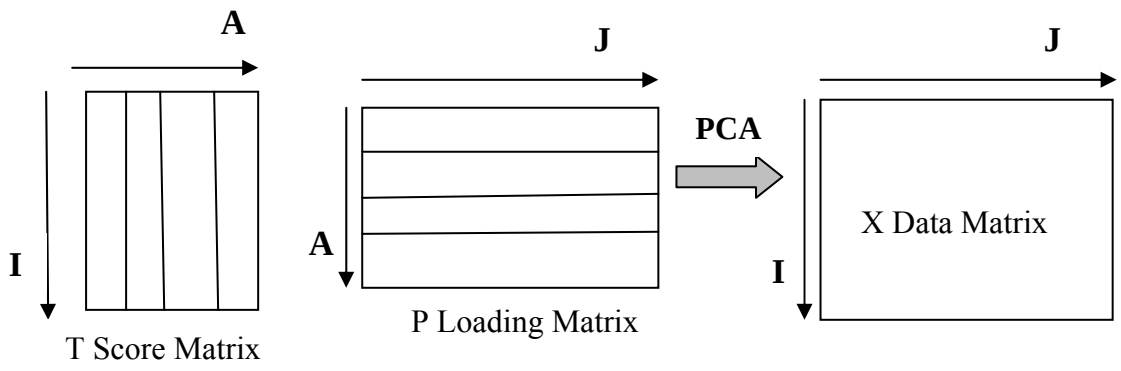

Figure 2.3. A matrix representation of how a data matrix is modelled by PCA (Source: Brereton 2003)

PCA method is basically a transformation of the data by using the PC lines that mostly describe the relationships between the data. Therefore, a classification of the

data point with the contributions from each of the PC lines is achieved which can be used for discrimination and classification purposes (Esbensen 2002).

Principal component analysis is used in the classification method referred to as Soft Independent Modelling of Class Analogies (SIMCA). SIMCA method is based on development of models to be used for prediction of class memberships by PCA for each class independently. In SIMCA method class boundaries are defined by two parameters as Euclidean distance and leverage. Euclidean distance (sample to model distance) defines the distance from class centre to the sample which forms a basis for Coomans' plot in PCA technique. Leverage is described as the measure of similarity of a sample to the whole samples of the class at issue (Legin, et al. 2003; Brereton 2003).

2.8.2. Partial Least Square (PLS)

Partial least square (PLS), a regression extension of PCA, is a method for construction of predictive models by relating two data matrices, X matrix of factors/predictors (particularly, spectral or chromatographic data) and Y matrix of responses (data taken from analytical methods) by linear multivariate model (Eriksson et al. 2001).

Like PCA method pre-processing of the data is required prior to performing PLS regression analysis. Same pre-processing techniques (mean centering and UVscaling) are employed before PLS analysis.

PLS regression analysis is summarised with the following set of models:

$$X = T*P + E \tag{2.2}$$

$$c = T*q + f \tag{2.3}$$

where q, which is normalised, has similarity to a loading vector, the product of T and P approaches to spectral data (predicted) concentration and the product of T and q approaches to actual concentrations, E is the error matrix of X matrix and f is the error matrix of c matrix (Brereton 2003). Principle of PLS is illustrated in Figure 2.4.

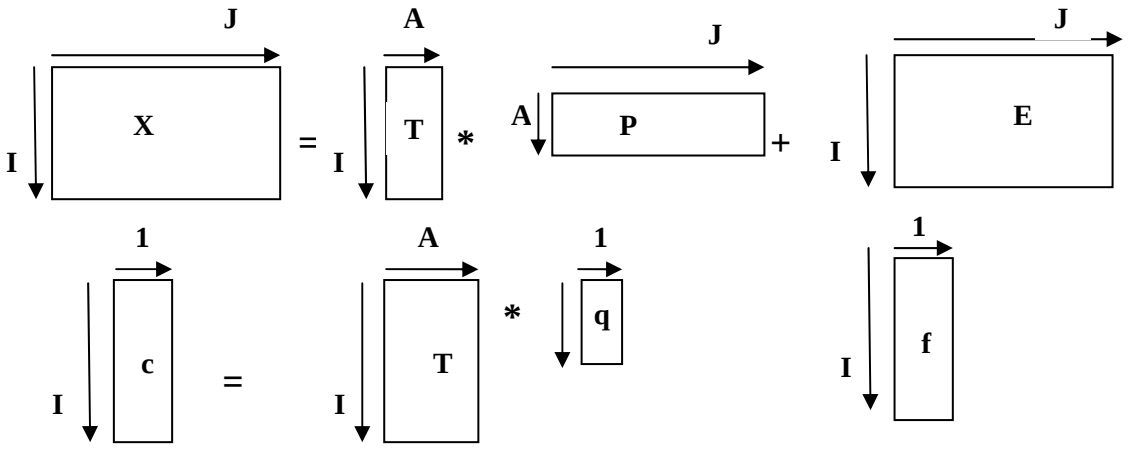

Figure 2.4. Matrix relationships in PLS regression analysis
(Source: Brereton 2003)

PLS components are the results of the projection of the information taken from the original x variables onto a small number of latent factors or variables. These latent variables are considered while the variable correlations are modelled. PLS regression analysis does not assume that the errors appear only for y. Thus, regression model requires to be validated before it is used. With the help of newly constructed independent and representative validation set, the predictive ability of the model can be evaluated (Wold, et al. 2001).

One or more responses can be modelled and analysed by PLS regression. When the Y-variables are strongly correlated to each other, they can be analysed together. However, the variables should be analysed separately to avoid high number of PLS components which creates difficulty in interpretation if the Y variables have no correlation (Eriksson, et al. 2001).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Wine Samples

A total of 79 samples from mono-varietal Turkish wines were used in this study. To cover a wide range of variety, dry and semi-sweet wines produced from the grapes of all winery regions in Turkey were selected. 41 samples from 2006 and 38 samples from 2007 vintage, were bought from the local markets in Turkey (Table 3.1 and 3.2). The wines were stored in the Schott bottles of 100 mL at 4° C till usage. To avoid any chemical changes all the bottles were covered with aluminium foil and the headspace of the samples was flushed with nitrogen gas.

Table 3.1. The wine samples from 2006 vintage

Sample Number	Producer	Label	Grape Type	Code
1	Yazgan	Boğazkere	Boğazkere	1BK6
2	Kavaklıdere	Lal	Çalkarası	2ÇK6
3	Doluca	DLC	Kalecik karası	3KK6
4	Doluca	DLC	Öküzgözü	4OG6
5	Doluca	Nevşah	Emir	5E6
6	Doluca	Safir	Misket	6M6
7	Pamukkale	Anfora	Narince	7N6
8	Kavaklıdere	Angora	Sultaniye	8S6
9	Pamukkale	Anfora	Chardonnay	9CH6
10	Kocabağ	Boğazkere	Boğazkere	10BK6
11	Sevilen	Cabarnet Savignon	Cabarnet Savignon	11CS6
12	Kayra	Cumartesi	Çalkarası	12CK6
13	Kayra	Terra	Kalecik karası	13KK6
14	Pamukkale	Anfora	Merlot	14ME6
15	Melen	Papazkarası	Papazkarası	15PK6
17	Melen	Muscat Reine DeVin	Misket	17M6
18	Turasan	Narince	Narince	18N6
19	Yazgan	Kulüp	Sultaniye	19S6
20	Umurbey	Chardonnay	Chardonnay	20CH6
21	Büyülübağ	Cabarnet Savignon	Cabarnet Savignon	21CS6

(Cont. on the next page)

Table 3.1. (Cont.)

Sample Number	Producer	Label	Grape Type	Code
22	Pamukkale	Anfora	Kalecik karası	22KK6
23	Umurbey	Merlot	Merlot	23ME6
24	Yazgan	Dolce Vita	Öküzgözü	24OG6
25	Yazgan	Papazkarası	Papazkarası	25PK6
26	Doluca	DLC	Shiraz	26SH6
27	Kocabağ	Öküzgözü	Öküzgözü	27OG6
28	Kayra	Tılsım	Misket	28M6
30	Sevilen	Chardonnay	Chardonnay	30CH6
31	Çankara	Cabarnet Savignon	Cabarnet Savignon	31CS6
32	Yazgan	Kalecik Karası	Kalecik karası	32KK6
33	Çankara	Alev	Merlot	33ME6
34	Yazgan	Emir	Emir	34E6
35	Cankara	Misket	Misket-muscat-bornova misketi	35M6
36	Büyülübağ	Sultaniye	Sultaniye	36S6
37	Melen	Melencik	Kalecik karası	37KK6
38	Sevilen	Merlot	Merlot	38ME60
40	Pamukkale	Senfoni	Sultaniye	40S6
41	Sevilen	Kalecik Karası	Kalecik karası	41KK6
42	Sevilen	Kara Salkım	Papazkarası	42PK6
43	Kayra	Terra	Shiraz	43SH6
44	Pamukkale	Anfora	Shiraz	44SH6

Table 3.2. The wine samples from 2007 vintage

Sample Number	Producer	Label	Grape Type	Code
1	Doluca	DLC	Boğazkere	1BK7
2	Sevilen	Cabarnet Savignon	Cabarnet Savignon	2CS7
3	Kavaklıdere	Rosato	Çalkarası	3CK7
4	Sevilen	Syrah	Shiraz	4SH7
5	Sevilen	Kalecik Karası	Kalecik karası	5KK7
6	Doluca	Safir	Misket	6M7
7	Kipa	Vaha	Merlot	7ME7
8	Doluca	Nevşah	Emir	8E7
9	Kavaklıdere	Ancyra	Narince	9N7
10	Pamukkale	Beyaz Harman	Sultaniye	10S7
11	Kipa	Vaha	Chardonnay	11CH7
12	Doluca	DLC	Öküzgözü	12OG7
13	Diren	Boğazkere	85%Boğazkere+7.5%Kalecik Karası +7.5%CabarnetSavignon	13CS7
14	Diren	Cabarnet Savignon	85%CabarnetSavignon+7.5%Syrah +7.5%Boğazkere	14CS7
15	Doluca	DLC	Kalecik karası	15KK7
16	Kavaklıdere	Angora	Sultaniye	16S7
17	Kipa	Vaha	Kalecik karası	17KK7

(Cont. on the next page)

Table 3.2. (Cont.)

Sample Number	Producer	Label	Grape Type	Code
18	Yazgan	Dolce Vita	Öküzgözü	18OG7
19	Kocabağ	Velvet	Emir	19E7
20	Doluca	Moscado	Misket	20M7
21	Diren	Dimes	Narince	21N7
22	Pamukkale	Anfora	Shiraz	22SH7
23	Doluca	DLC	Shiraz	23SH7
24	Melen	Merlot	Merlot	24ME7
25	Kocabağ	Cabarnet Savignon	Cabarnet Savignon	25CS7
26	Pamukkale	Anfora	Kalecik karası	26KK7
27	Kayra	Terra	Kalecik karası	27KK7
28	Kocabağ	Kalecik Karası	Kalecik karası	28KK7
29	Kavaklıdere	Ancyra	Merlot	29ME7
30	Kavaklıdere	Ancyra	Öküzgözü	30OG7
31	Diren	Öküzgözü	Öküzgözü	31OG7
32	Pamukkale	Anfora	Shiraz	32SH7
33	Kipa	Vaha	Shiraz	33SH7
34	Turasan	Emir	Emir	34E7
35	Turasan	Narince	Narince	35N7
36	Yazgan	Kulüp	Sultaniye	36S7
37	Sevilen	Merlot	Merlot	37ME7
38	Kavaklıdere	Sade	Emir	38E7

3.1.2. Chemical Reagents

The chemicals used in the experiments are all analytical grade and indicated in Table 3.3.

Table 3.3. Chemical reagents used in the analysis

Chemical Name	Manufacturer	Code
Ethanol absolut puriss	Sigma-Aldrich	32221
Folin Ciocalteu's Phenol Reagent	Sigma	F9252
Gallic Acid	Sigma	SIG7384-100G
HCl (37%)	Sigma Aldrich	7102
Methyl Red Indicator	Sigma Aldrich	250198
NaCO3	Merck	1.06392
NaOH	Merck	1.06462.1000
pH 4 Buffer	Sigma Aldrich	82598
pH 7 Buffer	Merck	1.09477.0500
pH 10 Buffer	Merck	1.09438.1000
Potassium Chloride	Riedel-de Haen	12636
Sodium Acetate	Merck	1.06268.1000

3.2. Methods

3.2.1. Total Phenol Determination

Total phenol of the wine samples were determined with the use of Folin-Ciocalteau micro method, a method derived from total phenol analysis (Slinkard and Singleton 1977). Due to the fact that minimum volume of sample and reagent is needed for micro method, reduced waste and disposal volume makes this modified method preferable.

The procedure is based on the fact that phenols ionize completely under alkaline conditions, and can be readily oxidized by the Folin-Ciocalteau reagent. As initial step gallic acid stock solution and sodium carbonate solution were prepared.

- Preparation of Gallic Acid Stock Solution: 0.500 g of dry gallic acid was weighed and dissolved in 10 mL of ethanol. The volume was then completed to 100 mL with distilled water. The solution was kept in the closed flask covered with aluminium foil and saved in the refrigerator for two weeks.

- Preparation of Sodium Carbonate Solution: 200 g of anhydrous sodium carbonate ($NaCO_3$) was weighed and dissolved in 800 mL of distilled water. The solution was boiled and cooled. After cooling a small amount of sodium carbonate was added. It was waited for 24 hours than filtered and the volume was completed to 1 L.

- Preparation of Calibration Curve: 0, 1, 2, 3, 5, 10, 13, 15 and 17 mL of gallic acid stock solution was taken into 100 mL volumetric flasks, and diluted to volume with distilled water. 40 µL from each calibration solution was added into different tubes, and 3.16 mL of distilled water was added to each. 200 µL of Folin- Ciocalteau reagent was added and immediately mixed via vibratory mixer (Yellow Line TTS 2, Ireland). After waiting for 4 minutes 600 µL of sodium carbonate solution was added and mixed. The solutions were kept for 2 hours in a dark place at room temperature, then the absorbance of each solution was read against the blank at 765 nm with a spectrophotometer

(Schimadzu UV-2450, Japan). The absorbance results were plotted against concentrations.

- Phenol Determination: From each white wine 40 µL was added into different tubes, and 3.16 mL of distilled water was added. 200 µL of Folin- Ciocalteau reagent was added and immediately mixed. After waiting for 4 minutes, 600 µL of sodium carbonate solution was added and mixed. The solutions were kept for 2 hours in a dark place at room temperature then the absorbance of each solution was read against the blank at 765 nm. The red and rose wines were firstly diluted by 10 to see the colour change clearly, and then the same procedure was applied. Through the use of calibration curve total phenol concentration of wines were calculated as mg gallic acid per L (Figure 3.1).

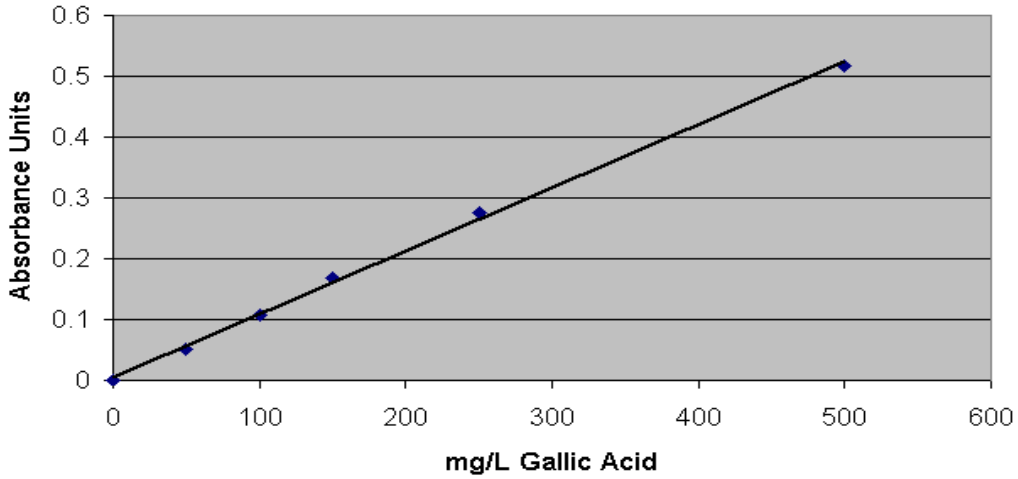

Figure 3.1. Calibration curve of gallic acid for total phenol calculation

(Source: Slinkard and Singleton 1977)

3.2.2. Anthocyanin Determination

The total anthocyanin content of wines was detected using pH differential method (AOAC Official Method 2005). The principle of this method is measuring the colour change resulted in the anthocyanin pigment (the coloured oxonium at pH 1.0, and the colourless hemiketal form at pH 4.5) at different wavelengths.

- Preparation of pH 1.0 Buffer: 0.93 g of potassium chloride (KCl) into a flask and dissolved in 490 mL of distilled water. The pH of the solution was adjusted to pH 1.0 (±0.05) via HCl (37%). The solution was transferred to a 500 mL volumetric flask and the volume was completed with distilled water.

- Preparation of pH 4.5 Buffer: 27.22 g of sodium acetate was weighed into a flask and dissolved in 480 mL of distilled water. The pH of the solution was adjusted to pH 4.5 (±0.05) with HCl (37%). The solution was interchanged to a 500 mL volume flask and the volume was completed with distilled water.

- Adjustment of the Dilution Factor: The proper dilution factor was determined by diluting the sample with pH 1.0 buffer till observing the absorbance at 520 nm within the range of 0.2 and 1.4.

- Determination of Anthocyanins: The anthocyanin content of the wines was determined by measuring the absorbances of the samples diluted with both pH 1.0 and pH 4.5 solutions at 520 and 700 nm with the help of UV visible spectrophotometer (Schimadzu UV-2450, Japan). The anthocyanin concentration, expressed as cyanidin-3-glucoside equivalent, was calculated with the following formula:

$$Anthocyaninpigment(cyd-3-glu.eq.,mg/L)=\frac{A*MW*DF*10^{3}}{\varepsilon*1} \qquad (3.1)$$

$$A=(A_{520nm}-A_{700nm})\text{pH 1.0}-(A_{520nm}-A_{700nm})\text{pH 4.5} \qquad (3.2)$$

MW (molecular weight) = 449.2 g/mol for cyaniding-3-glucoside

DF= proper dilution factor

1= pathlength of spectro cuvet in cm

ε= 26900 molar extinction coefficient in $moL*mol^{-1}*cm^{-1}$ for cyd-3-glu

10^3= factor for conversion from g to mg.

3.2.3. Titratable Acidity Measurement

The titratable acidity of the wine samples was measured with the modified AOAC method. This procedure depends on titrating the sample with standard sodium hydroxide solution to pH 8.2. The stages of the experiment can be listed as follows:

- Preparation of the 0.1 N Standard Sodium Hydroxide Solution: For a 0.1 N solution 4.00 g of sodium hydroxide (NaOH) was weight and dissolved in one liter of water. Since NaOH is considerably hygroscopic, the solution was firstly standardized before usage. The standardization was performed by titrating the NaOH solution with 0.1 N HCl solution. 0.1 N HCl solution was prepared by dilution of the 1 mL of HCl of 37% to 100 mL. For standardization 10 mL of HCl was taken into a flask. 50 mL of distilled water and three drops of methyl red indicator was added to HCl solution. A 25 mL buret was filled with 0.1 N NaOH solution and hydrochloric acid solution was titrated to a stable lemon yellow colour appearance.

$$\text{Normality of NaOH} = \frac{\text{Volume of HCl} * \text{Normality of HCl}}{\text{Volume of NaOH consumed}} \tag{3.3}$$

- Titration of wine samples: The pH meter (WTW PH 720, Inolab, Germany) was fist calibrated before usage consistent with the operator's manual. 100 mL of deionized water was taken to a beaker and 5 mL of wine sample was added. A magnetic stirrer was used to provide a homogeneous mixing. The pH electrode was immersed into the dilution away from the stir bar and the diluted sample was quickly titrated with standard NaOH solution to pH 8.2. The consumption of NaOH was recorded and the trials were repeated twice.

- Calculation: Titratable acidity of wines is expressed in equivalent of tartaric acid content (g/ L).

$$\text{Titratable acidity(g tartaric acid/L)} = \frac{(mL_{NaOH} * (N_{NaOH}) * (75) * (1000)}{L_{sample}} \tag{3.4}$$

3.2.4. pH Measurement

pH of the wine samples were measured with the use of pH meter (WTW Series, Inolab, Germany) with AOAC Official Methods(960.19, 17th Ed 2010). Before the measurements pH meter was calibrated according to the user's manual of the pH meter with buffer solutions (pH 4, pH 7, and pH 10). The electrode of the pH meter was cleaned with distilled water after every measurement.

3.2.5. Brix Measurement

The refractive index, in other words total soluble solids, of the wine samples was directly measured with a refractometer (Re50, Mettler Toledo, USA) in accordance with the analytical methods. The refractometer was firstly calibrated with air and distilled water according to the user's manual and cleaned with distilled water after each measurement.

3.2.6. Colour Measurement

A spectrophotometric method (Kelebek, et al. 2007) was utilised to measure the colour of the wine samples. As a pre-treatment all the wine samples were centrifugated at 2500 RCF and 4°C for 4 minutes (2-16, Sigma, UK) for the separation of wine sediment. After centrifugation the absorbance of the wines were directly read at 420, 520, and 620 nm using a spectrophotometer. Calculations were made with the help of the following formulas:

$$\text{Colour Intensity(CI): } Abs_{420} + Abs_{520} + Abs_{620} \quad (3.5)$$

$$\text{Tint: } Abs_{420} / Abs_{520} \quad (3.6)$$

$$\text{Proportion of yellow colour - Ye(\%)= } Abs_{420} / CI \quad (3.7)$$

$$\text{Proportion of red colour - Red(\%)= } Abs_{520} / CI \quad (3.8)$$

Proportion of blue colour - $Bl(\%) = Abs_{620} / CI$ (3.9)

Proportion of red colour produced by the flavylium cations of free and bound anthocyanins - $dA(\%) = (1-(Abs_{420}+Abs_{620})/2*Abs_{520})*100$ (3.10)

3.2.7. FTIR- Spectroscopy Analysis

All wine samples were scanned through an IR spectrometer (Perkin Elmer Spectrum 100 FT-IR spectrometer, Wellesley, MA) within the range of 4000-650 cm^{-1} wave number. This equipment has a horizontal attenuated total reflectance (HATR) accessory with ZnSe crystal (45 deg. Trough Plate) and deuterated tri-glycine sulphate (DTGS) detector.

The scanning was carried out at 4 cm^{-1} resolution, and 0,50 cm/s scan speed. The number of scans for each spectrum was adjusted to 64. The sampling crystal was cleaned with, ethanol, toothpaste and distilled water after each measurement and dried under nitrogen flow. The measurements were repeated at least three times.

3.3. Statistical Analysis

The data from the FT-MIR spectrometer was analysed by using multivariate statistical techniques with SIMCA software (SIMCA P-10.5 Umetrics Inc. Sweden). Certain ranges of spectra were used to avoid interferences resulted from the large bands. Three spectral regions (965-1565 cm^{-1}, 1700-1900 cm^{-1}, and 2800-3040 cm^{-1}) were selected to be used in the statistical analysis (Versari, et al. 2010).

3.3.1. Pre-treatment of the Data

Pre-treatment procedure is necessary prior to multivariate data analysis to standardize the data complex by subtracting their averages and dividing by their standard deviations (Kettaneh, et al. 2005). To that aim, the data was firstly transformed into a more suitable form by the well known pre-treatment techniques such as scaling and mean centering.

Measured frequencies can often be pre-processed thanks to a group of mathematical procedures including various forms of derivatives, and signal corrections to yield better results. Certain spectral filtering techniques including first and second order derivation, wavelet compression of spectra (WCS) were utilised in data analysis for better classification before PCA. The working principle of WCS technique depends on retaining significant coefficients from the representation of the data in the new ordinate system in order to compress and de-noise the complicated signals. The technique includes several functions like Beylkin, Coiflet, Daubechies and SymmLet (Eriksson, et al. 2000). Daubechies-4 function was chosen and WCS method was applied in 99.95% confidence interval. Spectral filtering techniques like wavelet compression of spectra (WCS), wavelet in combination with orthogonal signal correction (WOSC) and orthogonal signal correction in combination with wavelet (OSCW) were all employed before the PLS regression analysis to MIR spectra. The best results were obtained from the OSCW for the quantification of chemical parameters and OSCW technique was applied prior to all PLS regression analysis.

3.3.2. Classification

Classification was performed using FT-MIR spectra for separation of the groups of spectral data. Principal component analysis (PCA) is a commonly employed method to reduce a spectral data set into a small number of new orthogonal variables (Karouri, et al. 2010). The data matrix composed of wine samples (observations) and spectral data (variables) were used to classify the wine samples into disjoint data classes based on the similarities among members of the same data class (Bauer, et al. 2008). PCA reduces the number of variables to a small number of principal components (PC) which are linear combinations of original variables. The number of components to be used in the PCA models is of great importance in the beginning of the classification analysis. Determination of the sufficient number of PCs depends on the goodness of fit, which is represented by the parameter R^2, and predictive ability of the model (Eriksson, et al. 2000).

The results of classification were visualized by scores and Coomans' plots. The score plots show the locations of the samples along each model component and are used to detect sample groupings, similarities or differences between the samples. The score

plots are simply plotted for the first two principle components on a two dimensional windows. The Coomans' plot were also used to demonstrate the grouping more clearly by using two models created for the basic sample groups as white and red wine. The Coomans' plot compares the distance to the model results against the distance from the model centre for unknown samples of selected models (Esbensen, et al. 2002). The resulting plot constitutes from four regions, two regions indicate discrimination of two classes (red and white) described by the models with which plot is constructed, one region indicates overlap of the two classes, and remaining region indicates the observations far from two classes. The Coomans' plot demonstrates differentiation of the selected groups and separation of dissimilar observations like semi-sweet wines clearly. FT-MIR spectral data was used to classify the wine samples according to variety.

3.3.3. Quantification

Multivariate regression analysis is used to develop models to predict a property of interest. There are many examples of the use of modelling and prediction with regression analysis such as PLS method. PLS regression analysis can be used for relating two data matrices, X (FT-MIR spectral data) and Y (analytical results of chemical parameters) by linear multivariate model (Eriksson, et al. 2000). The main purpose of PLS regression in this study was to construct linear calibration models that enable prediction of chemical parameters like total phenol content, anthocyanin content, brix, titratable acidity, pH and certain colour parameters like colour intensity, tint, yellow%, red%, blue% , anthocyanin proportionality to red colour dA% of wine samples using FT-MIR spectral data.

The observation set was divided into calibration (2/3 of samples) and validation (1/3 of samples) sets. The calibration and validation sets were created with randomly selected observations and the results of the regression analysis were also visualized with prediction plots showing the regression correlation coefficient (R^2) of the created models. The regression coefficient expresses the connection between predictions and the actual results of the chemical parameters and gives an idea about the predictive efficiency of the model (Bauer, et al. 2008). The evaluation of the calibration models was performed by computing the standard error of prediction (SEP) from validation set,

root mean square error of calibration and prediction (RMSEC, RMSEP), residual predictive deviation (RPD). The SEP value indicates the average prediction error and RMSEP is a measurement of the average difference between the predicted and reference actual values at the validation step. Similarly RMSEC describes predictive ability of calibration model with reference to the actual data. The RPD value is a significant criterion to be utilised for evaluation of the predictive ability of regression models (Esbensen, et al. 2002). The calculations were performed by using the following formulas:

$$\text{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n}(\widehat{y_i} - y_i)^2}{n-2}} \tag{3.11}$$

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(\widehat{y_i} - y_i)^2}{n-1}} \tag{3.12}$$

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}(\widehat{y_i} - y_i - bias)^2}{n-1}} \tag{3.13}$$

$$Bias = \frac{\sum_{i=1}^{n}(\widehat{y_i} - y_i)}{n} \tag{3.14}$$

$$RPD = \frac{SD}{RMSEP} \tag{3.15}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i - \overline{y}\right)^2}{n-1}} \tag{3.16}$$

where y_i is the actual value obtained from analytical methods for the i-th sample; $\widehat{y_i}$ is the predicted value by mid-IR spectra for the same sample; $\overline{y}$ is the mean of each set; n is the number samples used in each set; SD is standard deviation in each set (Saeys, et al. 2005; Zornoza, et al. 2008).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Varietal Classification of Wines

4.1.1. Results of Chemical Analysis

The wine samples experimented in this study were selected from the wines of the well known grape varieties of Turkey and also foreign varieties grown in Turkey. In consequence of varietal, regional and vintage distinctions among the grapes, the wines have different chemical properties. The results of chemical analysis of wines from 2006 and 2007 vintages are listed with their minimum and maximum values in Tables 4.1-4.4.

As far as the chemical results are concerned, significant variability in the phenol and anthocyanin contents among the same variety wines were observed. For example, Boğazkere, Cabarnet Sauvignon and Merlot from 2006 vintage have wide ranges in terms of their phenol content. These significant differences may be resulted from the variation in regional origins of the grapes of the same variety. Boğazkere wines come from Diyarbakır, Cappadocia and Tokat region and Merlot wines come from Aegean region and also Thrace region. When the chemical parameters of the same variety wines produced in different years were compared, variation between different vintage wines could be observed. The mean anthocyanin content was measured as 43 mg/L for Boğazkere wines from 2006 vintage, while the same wine has the mean anthocyanin content of 103 mg/L in 2007 vintage (Tables 4.1 and 4.3). The climatic changes between the vintage years account for the source the variation.

The results of titratable acidity, pH and brix were found within the critical limits of Turkish Food Codex (2008).

Table 4.1. The chemical parameters of the wine samples from 2006 vintage (ranges are shown in parentheses)

Wine Variety	Number of samples	Total Phenol*	Anthocyanin**	TA***	Brix	p H
Boğazkere	2	2659.46±1146.65 (1848.66 - 3470.27)	42.62±34.34 (18.34 - 66.91)	4.59±0.004 (4.58 - 4.59)	7.39±1.01 (6.68 - 8.10)	3.43±0.03 (3.41 - 3.44)
Cabarnet Sauvignon	3	2847.21±808.15 (2273.95 - 3771.52)	37.28±17.05 (23.55 - 56.36)	4.14±0.16 (4.03 - 4.31)	8.08±0.54 (7.68 - 8.69)	3.70±0.06 (3.64 - 3.76)
Çalkarası	3	1163.87±174.04 (1054-1364)	3.63±2.21 (2.83-6.12)	4.42±0.40 (3.97-4.72)	7.48±0.75 (7.02-8.35)	3.29±0.091 (3.21-3.39)
Kalecik Karası	6	1485.18±295.81 (1153.846 - 1703.07	36.89±20.68 (10.66 - 70.97)	4.48±0.99 (3.79 - 6.36)	7.29±0.38 (6.79 - 7.70)	3.57±0.13 (3.37 - 3.74)
Merlot	4	2577.41±1305.75 (1439.39 - 4080.59)	33.29±13.40 (18.45 - 48.01)	4.13±0.47 (3.70 - 4.56)	8.03±0.59 (7.18 - 8.56)	3.53±0.18 (3.31 - 3.71)
Öküzgözü	3	1328.28±245.48 (1045.45 - 1486.01)	36.18±35.01 (0.42 - 70.39)	4.24±0.41 (3.85 - 4.67)	6.97±0.66 (6.22 - 7.48)	3.37±0.04 (3.34 - 3.41)
Papazkarası	3	1788.30±264.50 (1483.68 - 1959.77)	35.39±15.16 (18.70 - 48.32)	5.35±0.57 (5.02 - 6.01)	7.30±0.33 (7.05 - 7.67)	3.45±0.06 (3.39 - 3.51)
Shiraz	3	1969.99±184.12 (1791.19 - 2159)	73.48±13.79 (60.03 - 87.59)	4.36±0.76 (3.76 - 5.21)	7.90±0.44 (7.63 - 8.41)	3.47±0.06 (3.44 - 3.54)
Emir	2	242.91±7.86 (237.36 - 248.47)		4.43±0.22 (4.28 - 4.59)	6.18±0.62 (5.74 - 6.62)	3.19±0.21 (3.04 - 3.33)
Misket	4	411.82±151.12 (242.34 - 583.33)		4.44±0.72 (3.38 - 4.90)	7.70±1.26 (6.60 - 9.18)	3.19±0.22 (3.03 - 3.50)
Narince	2	312.39±113.16 (232.38 - 392.41)		3.95±0.40 (3.67 - 4.23)	6.54±0.24 (6.38 - 6.71)	3.47±0.01 (3.46 - 3.48)
Sultaniye	4	230.15±55.76 (175.67 - 296.17)		3.86±0.35 (3.77 - 4.40)	6.53±0.21 (6.34 - 6.83)	3.29±0.15 (3.16 - 3.44)
Chardonnay	3	246.66±51.32 (187.93 - 282.86)		3.94±1.04 (3.03 - 5.07)	6.77±0.31 (6.54 - 7.12)	3.49±0.28 (3.26 - 3.79)

(*Total Phenol expressed in mg gallic acid/ L. **Anthocyanin in cyd-3-glu eq.mg/L and ***Titratable Acidity in g tartaric acid/L)

Table 4.2. The colour parameters of the wine samples from 2006 vintage (ranges are shown in parentheses)

Wine Variety	Number of samples	CI	Tint	Yellow%	Red%	Blue%	dA% *
Boğazkere	2	7.38±2.10 (5.90 - 8.86)	0.99±0.35 (0.75 - 1.24)	0.44±0.09 (0.38 - 0.51)	0.46±0.07 (0.41 - 0.51)	0.096±0.02 (0.085 - 0.108)	-600.32±396.60 (-880.76 - -319.88)
Cabarnet Sauvignon	3	9.37±1.45 (7.91 - 10.89)	0.93±0.06 (0.87 - 0.98)	0.42±0.01 (0.42 - 0.43)	0.45±0.01 (0.44 - 0.46)	0.13±0.003 (0.12 - 0.13)	-1004.00±340.81 (-1350.25 - -668.91)
Çalkarası	3	0.88±0.20 (0.67-1.08)	1.84±0.08 (1.75-1.89)	0.61±0.007 (0.60-0.61)	0.33±0.01 (0.32-0.34)	0.058±0.005 (0.053-0.063)	91.06±3.97 (87-94)
Kalecik Karası	6	5.58±0.85 (4.45 - 6.61)	0.93±0.10 (0.81 - 1.08)	0.42±0.01 (0.40 - 0.44)	0.45±0.03 (0.41 - 0.50)	0.13±0.02 (0.099 - 0.15)	-291.37±117.14 (-439.16 - -139.01)
Merlot	4	7.48±1.77 (5.66 - 9.64)	0.93±0.12 (0.85 - 1.11)	0.42±0.03 (0.40 - 0.46)	0.46±0.03 (0.41 - 0.49)	0.12±0.01 (0.097 - 0.13)	-620.21±336.20 (-1056 - -299.53)
Öküzgözü	3	4.56±3.71 (0.40 - 7.54)	1.00±0.29 (0.73 - 1.31)	0.45±0.08 (0.37 - 0.52)	0.46±0.05 (0.40 - 0.51)	0.098±0.02 (0.078 -0.13)	-273.97±355.37 (-609.86 - 98.11)
Papazkarası	3	6.65±0.82 (6.08 - 7.58)	0.91±0.02 (0.89 - 0.93)	0.42±0.0004 (0.41 - 0.42)	0.46±0.01 (0.45 - 0.47)	0.13±0.01 (0.12 - 0.14)	-453.64±140.56 (-615.19 - -359.39)
Shiraz	3	9.73±0.74 (8.88 - 10.19)	0.77±0.03 (0.73 - 0.79)	0.38±0.01 (0.37 - 0.39)	0.50±0.01 (0.49 - 0.51)	0.12±0.001 (0.119 - 1.121)	-1088.28±175.37 (-1196.75 - -885.96)
Emir	2	0.21±0.083 (0.15 - 0.27)	5.85±2.54 (4.05 - 7.65)	0.80±0.082 (0.75 - 0.86)	0.15±0.05 (0.11 - 0.18)	0.048±0.032 (0.026 - 0.070)	
Misket	4	0.19±0.11 (0.085 - 0.31)	5.57±1.67 (4.02 - 7.42)	0.81±0.06 (0.76 - 0.87)	0.15±0.04 (0.12 - 0.19)	0.032±0.02 (0.01 - 0.05)	
Narince	2	0.17±0.01 (0.17 - 0.18)	5.29±1.98 (3.89 - 6.69)	0.79±0.08 (0.74 - 0.85)	0.16±0.04 (0.13 - 0.19)	0.05±0.04 (0.022 - 0.072)	
Sultaniye	4	0.19±0.03 (0.15 - 0.22)	4.49±1.30 (3.28 - 5.65)	0.77±0.06 (0.71 - 0.82)	0.18±0.04 (0.14 - 0.22)	0.053±0.02 (0.032 - 0.083)	
Chardonnay	3	0.20±0.03 (0.18 - 0.23)	4.42±0.98 (3.32 - 5.20)	0.77±0.07 (0.69 - 0.82)	0.18±0.03 (0.16 - 0.21)	0.056±0.04 (0.022 - 0.10)	

([a] dA%: The proportion of red colour produced by flavylium cations of free and bound anthocyanins)

Table 4.3. The chemical parameters of the wine samples from 2007 vintage (ranges are shown in parentheses)

Wine Variety	Number of Wines	Total Phenol*	Anthocyanin**	T A***	Brix	pH
Boğazkere	2	3467.41± 148.77 (3362.21 - 3572.61)	107.79± 29.80 (86.72 - 128.86)	4.37± 0.97 (3.68 - 5.05)	7.70± 0.21 (7.55 - 7.84)	3.38± 0.16 (3.27 - 3.49)
Cabarnet Sauvignon	3	3336.08± 1232.54 (2368 - 4723.60)	71.38± 28.82 (45.98 - 102.70)	4.47± 0.31 (4.20 - 4.83)	8.62± 0.55 (8.10 - 9.19)	3.82± 0.18 (3.68 - 4.03)
Kalecik Karası	6	2061.61± 362.71 (1585.81 - 2698.02)	46.51± 21.09 (31.78 - 85.83)	4.20± 0.32 (3.91 - 4.73)	7.75± 0.28 (7.28 - 7.99)	3.71± 0.18 (3.45 - 3.91)
Merlot	4	2808.58± 604.65 (2318.48 - 3634.49)	66.10± 24.12	4.47± 0.24 (4.14 - 4.73)	7.64± 1.16 (5.94 - 8.56)	3.56± 0.13 (3.41 - 3.71)
Öküzgözü	4	1876.42± 776.64 (735.04 - 2437.29)	58.74± 42.02 (1.73 - 102.98)	4.74± 0.24 (4.53 - 5.02)	6.75± 0.88 (5.84 - 7.51)	3.51± 0.20 (3.34 - 3.80)
Shiraz	5	2929.37± 514.00 (2476.80 - 3593.23)	87.00± 41.85 (65.90 - 161.81)	4.72± 0.50 (4.21 - 5.54)	7.37± 1.44 (5.85 - 8.99)	3.58± 0.13 (3.39 - 3.74)
Emir	4	325.92± 32.93 (294.60 - 368.40)		4.13± 0.70 (3.44 - 5.05)	5.70± 0.73 (4.67 - 6.35)	3.27± 0.33 (2.97 - 3.74)
Misket	2	321.16± 48.72 (286.72 - 355.61)		5.33± 0.30 (5.12 - 5.54)	8.07± 1.33 (7.13 - 9.01)	3.02± 0.08 (2.96 - 3.07)
Narince	3	369.50± 63.44 (329.62 - 442.66)		4.39± 0.75 (3.88 - 5.25)	5.81± 0.89 (4.78 - 6.35)	3.31± 0.18 (3.11 - 3.46)
Sultaniye	3	246.53±19.59 (232.84 - 268.98)		4.38±1.61 (2.67 - 5.87)	5.93±0.87 (4.94 - 6.54)	3.62±0.44 (3.16 - 4.03)
Chardonnay	1	333.75		3.72	6.55	3.56

(*Total Phenol expressed in mg gallic acid/ L. **Anthocyanin in cyd-3-glu eq.mg/L and ***Titratable Acidity in g tartaric acid/L)

Table 4.4. The colour parameters of the wine samples from 2007 vintage (ranges are shown in parenthesis)

Wine Variety	Number of Wines	CI	Tint	Yellow%	Red%	Blue%	dA% *
Boğazkere	2	8.89± 0.36 (8.64 - 9.15)	0.69± 0 (0.69)	0.36± 0.01 (0.35 - 0.36)	0.51± 0.01 (0.51 - 0.52)	0.13± 0.02 (0.12 - 0.15)	-888.75±78.67 (-944.38 - -833.13)
Cabarnet Sauvignon	3	9.83± 1.57 (8.50 - 11.56)	0.96± 0.16 (0.79 - 1.10)	0.42± 0.04 (0.38 - 0.45)	0.44± 0.04 (0.40 - 0.48)	0.14± 0.01 (0.13 - 0.15)	-1106.58± 393.92 (-1539.13 - -768.44)
Kalecik Karası	6	7.11± 1.50 (5.49 - 7.88)	0.89± 0.15 (0.69 - 0.97)	0.41± 0.04 (0.36 - 0.44)	0.47± 0.04 (0.45 -0.52)	0.12± 0.01 (0.10 - 0.14)	-547.36± 268.40 (-673.14 - -272.12)
Merlot	4	10.20± 1.20 (8.49 - 11.08)	0.93± 0.06 (0.88 - 1.02)	0.41± 0.03 (0.38 - 0.45)	0.44± 0.01 (0.44 - 0.45)	0.15± 0.03 (0.12 - 0.18)	-1197.16± 289.40 (-1419.75 - -786.32)
Öküzgözü	4	6.21± 3.94 (0.52 - 9.44)	0.82± 0.22 (0.69 - 1.16)	0.40± 0.06 (0.36 - 0.49)	0.49± 0.05 (0.42 - 0.53)	0.11± 0.02 (0.09 - 0.13)	-527.10± 466.77 (-500.56 - 96.76)
Shiraz	5	11.54± 0.37 (11.06 - 12.03)	1.01± 0.02 (0.99 - 1.05)	0.43± 0.01 (0.41 - 0.44)	0.42± 0.01 (0.42 - 0.43)	0.15± 0.02 (0.14 - 0.18)	-1528.58± 99.72 (-1657.14 - -1399.72)
Emir	4	0.17± 0.05 (0.13 - 0.25)	3.78± 1.04 (2.31 - 4.58)	0.73± 0.04 (0.67 - 0.77)	0.20± 0.06 (0.17 - 0.29)	0.07± 0.02 (0.04 - 0.09)	
Misket	2	0.15± 0.02 (0.14 - 0.17)	2.52± 0.79 (1.96 - 3.08)	0.67± 0.04 (0.65 - 0.70)	0.28± 0.07 (0.23 - 0.33)	0.05± 0.04 (0.024 - 0.076)	
Narince	3	0.17± 0.05 (0.13 - 0.23)	4.96± 1.86 (3.13 - 6.84)	0.77± 0.07 (0.70 - 0.85)	0.17± 0.05 (0.12 - 0.22)	0.06± 0.02 (0.031 - 0.075)	
Sultaniye	3	0.29±0.14 (0.14 - 0.40)	3.50±1.32 (2.43 - 4.98)	0.69±0.09 (0.59 - 0.77)	0.21±0.05 (0.15 - 0.24)	0.10±0.06 (0.051 - 0.17)	
Chardonnay	1	0.19	4.51	0.79	0.17	0.04	

(*dA%: The proportion of red colour produced by flavylium cations of free and bound anthocyanins)

4.1.2. FT-MIR Spectral Data

Although the whole spectral range (4000 – 650 cm^{-1}) was collected and stored for each sample, only useful intervals were taken into consideration to avoid interference: 965-1565 cm^{-1} , 1700-1900 cm^{-1} , and 2800-3040 cm^{-1}. The intermediate zones were not employed in this study on account of the unrepeatability due to high absorbance values (Urbano-Cuadrado, et al. 2005). Figure 4.1 represents typical spectra of the wine samples obtained in this study with the used regions.

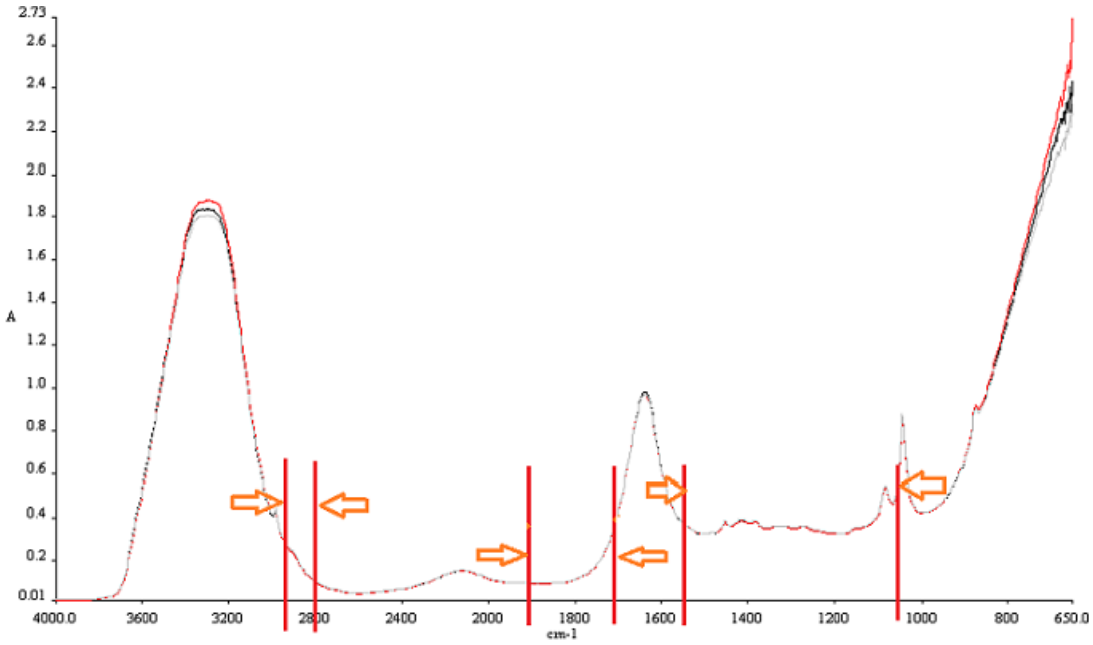

Figure 4.1. Typical FT-MIR spectra of a wine sample

4.1.3. Classification Using FT-IR Data

First part of this study investigates the varietal classification of red, rose and white wines produced in Turkey. The mono-varietal wines were selected to avoid any confusion that can result from the chemical differences between different varieties in each wine samples. Although the study grounds on the classification of each wine in accordance with variety, mono-varietal term comprises the wines blended in different wine proportions. The ratio of wine varieties differs from country to country and the limits are established by the legislations. In most of the countries, the blending proportionality determined as minimum

85% to 75% of wine from basic grape variety with other wines derived from other wines from different varieties (Food Standards Agency 2010).

The selected regions of FTIR spectra were transferred into SIMCA software (version P-10.5. Umetrics Inc., Sweden) for chemometric analysis. PCA method was employed for classification. The PCA plot obtained from the raw data allows an approximate separation of the white and red wines (Figure 4.2). Although the white and red wines were separated on top and bottom halves of score plot, respectively, a clear subdivision of the wine samples in accordance with variety could not be obtained.

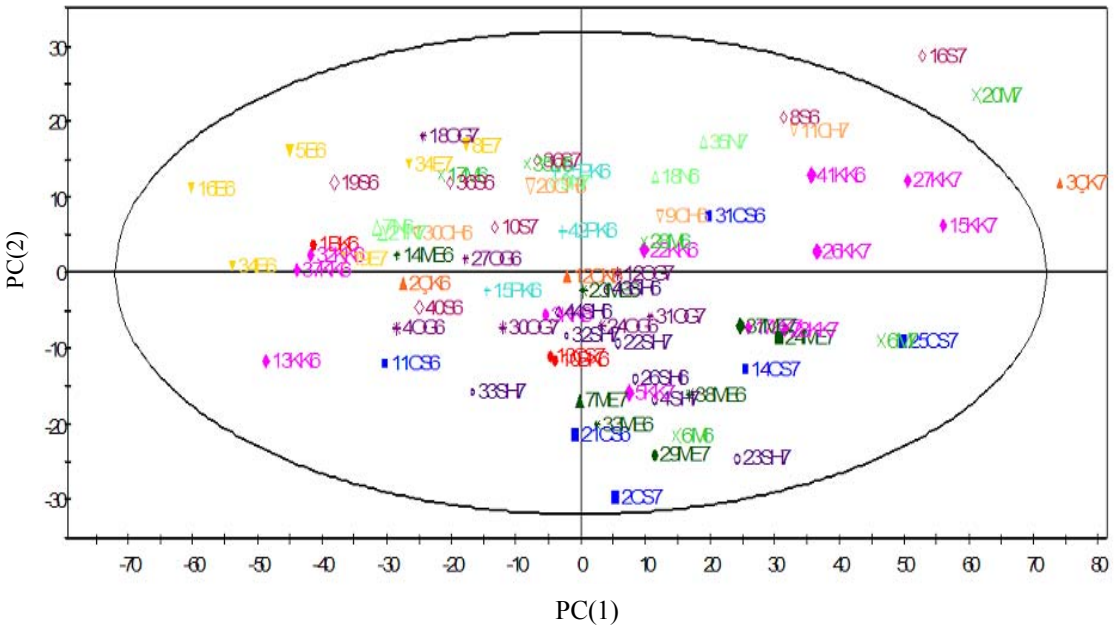

Figure 4.2. Score plot based on FT-IR spectra of wine samples grouped according to variety (Different colours belong to different varieties)

The result obtained from the PCA analysis of the raw data exhibited separation of a group of semi-sweet wines from Misket (20M7) and Çalkarası (3ÇK7) grapes. The rose wines were spread over both sides of the plot (Figure 4.2). Some of the wines from the same variety were also spread over a large area on PCA plots. The wines from Kalecik Karası, Cabarnet Sauvignon and Boğazkere grapes can be seen on every region of plot. One of the reasons for the large clusters of the same variety can be their differentiating vineyard area. It is reported that differences among the same variety grapes from different region is originating from the heat fluctuation during their growing stage. The length of exposure to sunlight, the temperature difference between the day and night, and temperature of the soil result in the

difference among the grapes (Duchêne and Schneider 2005). The soil type is another factor to create this difference. In addition, the wines were bought from different wineries, and that may result in variation in the same variety due to differences in the processing techniques. Different maceration temperatures, clarification treatments and length of storage time were reported to be the basic factors influencing the chemical composition (Gomez-Plaza, et al. 2000). Although rather in small amounts, the mono-varietal wines may include wines from different varieties, which can cause problems in varietal classification.

In a previous study, red, rose and white wines from different origin and grape varieties were investigated in combination of the chemical parameters with FT-MIR and NIR spectroscopy techniques (Urbano-Cuadrado, et al. 2005). Grouping of only white, rose and red wines was achieved clearly on different quarters of PCA score plot of NIR spectra.

In order to visualise the differentiation of the wine samples according to varieties evidently, Coomans'plot was also constructed (Figure 4.3). The discrimination of the red and white wines can be also seen on this plot. The semi- sweet wines from Çalkarası and Misket varieties were located apart from the other wines. Rose wines from Çalkarası and Öküzgözü varieties are among both red and white wine regions. The other wine samples were completely spread over the plot and did not show any grouping.

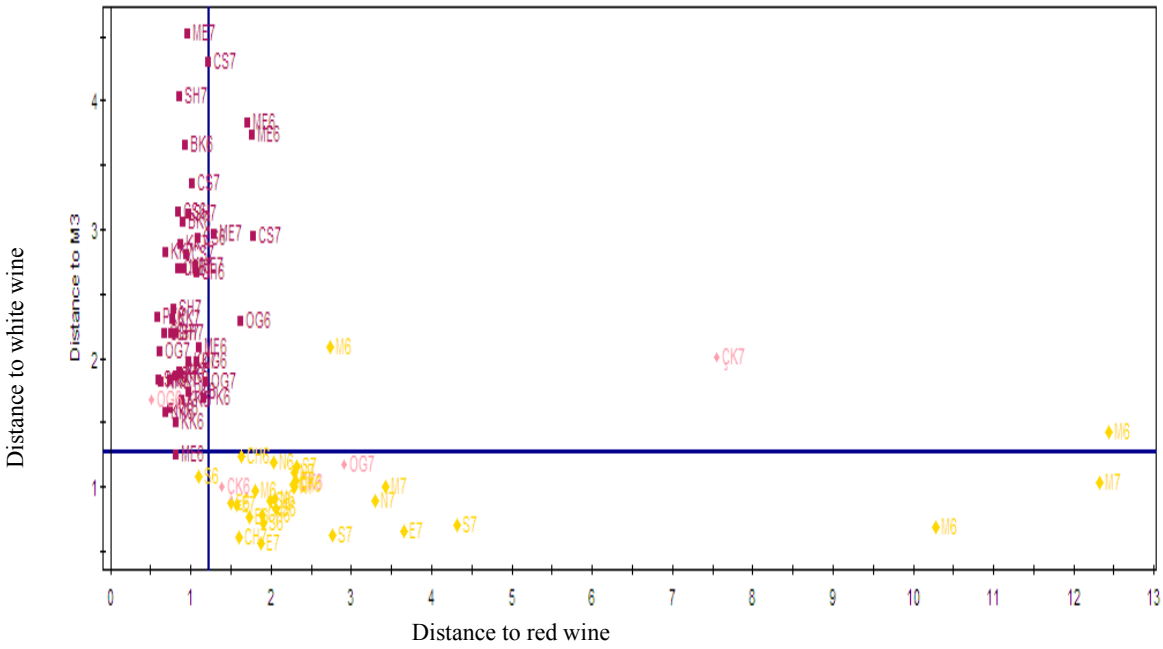

Figure 4.3. Coomans'plot for the classification of the wine samples according to varieties using spectral FT-MIR data (red, rose and white wines are represented with purple, pink and yellow colour, respectively)

Although the data was pre-processed with the spectral filtering techniques (first and second derivation, WCS) as a pre-processing step to remove the spectral noise and improve the varietal classification, no improvement in classification was observed (Figure 4.4).

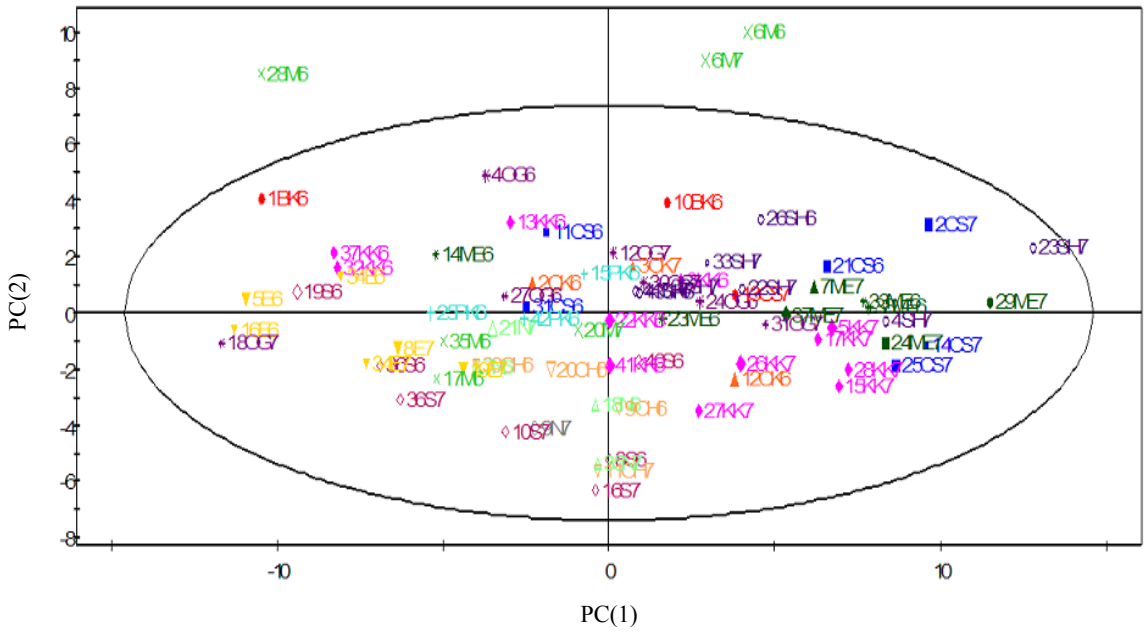

Figure 4.4. The score plot based on WCS scaled FT-MIR spectra of wine samples grouped according to variety (Different colours belong to different varieties)

As a further attempt to achieve a better classification, the data of red and white wines were handled separately. The PCA result of the red wines from the raw FT-MIR data was shown in Figure 4.5.

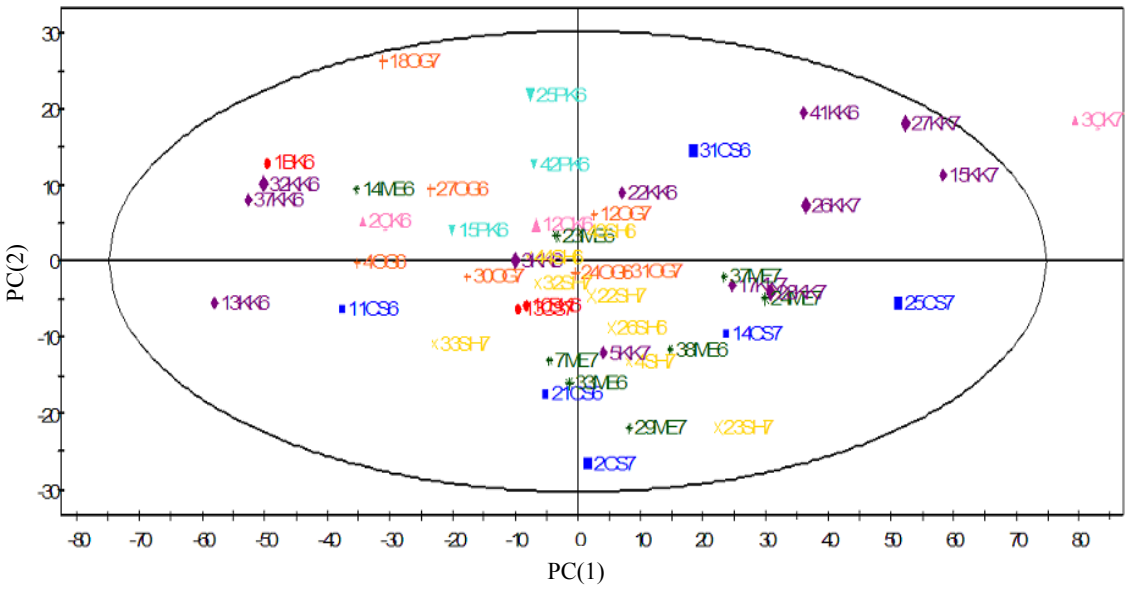

Figure 4.5. The score plot based on FT-MIR spectra of red wine samples grouped according to variety (Different colours belong to different varieties)

The semi- sweet rose wine from Çalkarası variety (3ÇK7) was separated from the other wines on the outer region of the plot. The remainder wines were spread on the inner region and the clusters of all the varieties overlapped with each other. In another study, four mono-varietal red wines from Montilla-Moriles reagion were searched to analyse the enological variables and differentiate wines via PCA analysis (Viviani, et al. 2007). Volatile chemicals were used as variables and a clear varietal differentiation was obtained with PCA analysis. Wine samples of that study were produced with the grapes from a narrow vineyard region and with the use of similar winemaking techniques, which are advantageous for a good variety differentiation. A similar study was performed with 54 Greek red wine samples of 3 different varieties from vintages of 1998 to 2005 (Tarantilis, et al. 2008). Extracts of phenolic components were obtained with C-18 columns and investigated by FT-MIR spectroscopy. The MIR spectra were recorded and compared with the library created by software (OMNIC). According to calculated match values, the differentiation of 3 varieties was performed and the varietal discrimination was achieved using mid-infrared spectra (Tarantilis, et al. 2008).

Similar result was obtained in PCA plot of white wines and the only differentiated wines were semi sweet wines from Misket variety which are located in the upper left quarter of the plot (Figure 4.6).

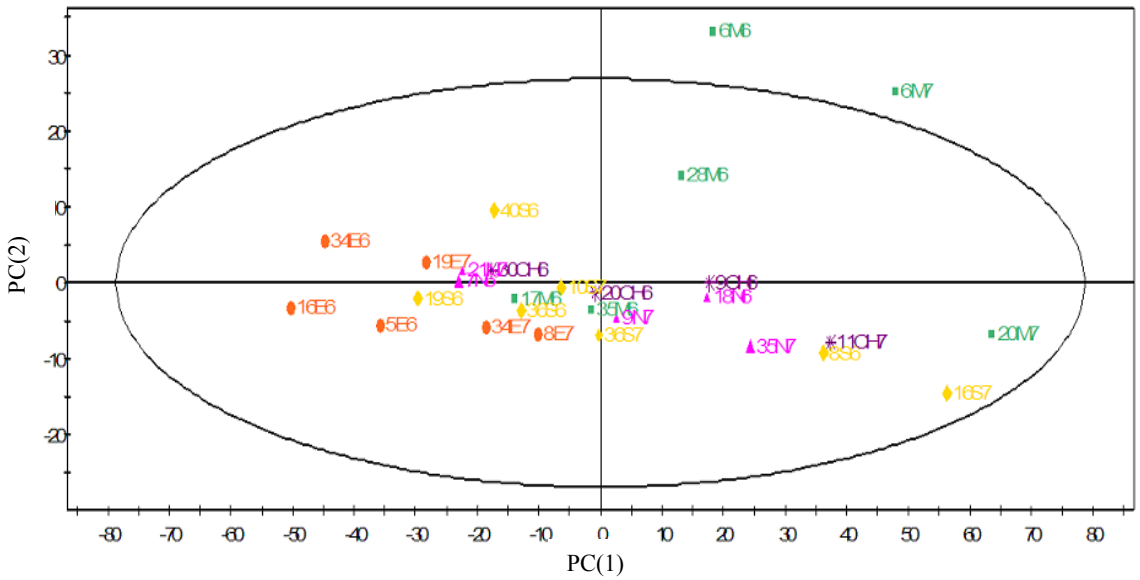

Figure 4.6. The score plot based on FT-MIR spectra of white wine grouped according to variety (Different colours belong to different varieties)

The results show that application of PCA to FT-MIR spectral data can not provide a distinguishing varietal classification of Turkish wines and this could be related to the variability of processing techniques, grape origins and compositional blending of the wines in the course of production stage.

4.1.4. Quantitative Analysis Using FT-MIR Data and Chemical Results

It is well known that wines have complex chemical composition. Determination of chemical parameters of wine in the course of production stage ensures both total control of production process and optimal product characteristics (Soriano, et al. 2007). There is an increasing interest in fast and accurate techniques to determine the quality parameters in product screening and process control. Instead of time consuming analytical methods mid-IR spectroscopy measuring a significant number of important wine parameters within a short time have been investigated in this study. FTIR was designated as an alternative analytical method providing precise and accurate results with high resolution (Moreira, et al. 2002). PLS, one of the most commonly used regression technique in chemometrics, was employed to show the relation between FT-MIR data and the results of chemical analysis and to develop equations for predicting some enological parameters of wine samples. FT-MIR spectral data

and the results of chemical analysis obtained from analytical techniques were implicated with the PLS analysis.

The chemical results including total phenol content, anthocyanin content, brix value, titratable acidity and various colour values were analysed separately in combination with FT-MIR spectral data. The results of only red and rose wines were handled in anthocyanin regression analysis, since anthocyanin pigment only exists in red grapes. As the acidity terms are highly related to each other, the titratable acidity and pH results were analysed together. Similarly colour results were included to the regression analysis as one group. Wine samples in each group were divided into a calibration set (2/3 of the samples) and a validation set (1/3 of the samples). As a pre-treatment step various spectral filtering techniques such as first derivation, second derivation and wavelet compression were applied to the raw data and the best results were obtained with orthogonal signal correction in combination with wavelet (OSCW) method.

Root mean square error of calibration and prediction (RMSEC, RMSEP), standard error of prediction (SEP), residual predictive deviation (RPD) and regression correlation coefficient (R^2) for all the PLS analysis were calculated and summarised in Table 4.5.

Table 4.5. Summary of statistical results for PLS analysis of wine samples

Parameter	Number of PCs	R²(cal)	RMSEC	RMSEP	SEP	RPD
Total phenol (mg/L)	2	0.93	315.16	310.15	294.46	3.61
Anthocyanin (mg/L)	2	0.89	11.53	14.13	14.13	1.84
Brix (%)	3	0.81	0.43	0.41	0.39	2.16
Titratable acidity (g/L)	5	0.75	0.26	0.36	0.36	2.06
pH (pH unit)	5	0.85	0.083	0.10	0.092	2.65
Colour intensity (CI)	6	0.89	1.35	1.22	1.15	3.75
Tint	6	0.72	1.06	1.17	1.11	1.21
Yellow%	6	0.85	0.067	0.079	0.077	2.00
Red%	6	0.78	0.43	0.067	0.067	1.86
Blue%	6	0.73	0.02	0.024	0.023	1.68
dA%	6	0.53	235.77	307.42	281.50	1.38

Accuracy of the prediction models were evaluated by using the R², RPD and slope of the equations determined for the calibration sets (Table 4.6).

Table 4.6. The criteria used for evaluation of the prediction models
(Source: Zornoza, et al. 2008; Saeys, et al. 2005)

R^2	between 0.66-0.80 approximate predidictions between 0.81-0.90 good predictions >0.90 excellent predictions
RPD	<2.0 insufficient between 2.0-2.5 approximate predictions between 2.5-3.0 good predictions >3.0 excellent prediction
Slope	<0.8 or >1.2 less reliable around 0.8-1.2 reliable between 0.9-1.1 very reliable

PLS analysis for prediction of total phenol concentration was performed by relating FT-MIR spectral data as X variables and total phenol content as Y variables obtained with analytical methods. 52 and 26 observations were randomly selected for calibration and validation sets, respectively. The model contains 2 significant components (PCs) explaining 93.1% of the total variation of total phenol content (Y). The regression coefficient of the model determined with calibration set was found as 0.93 (Figure 4.7).

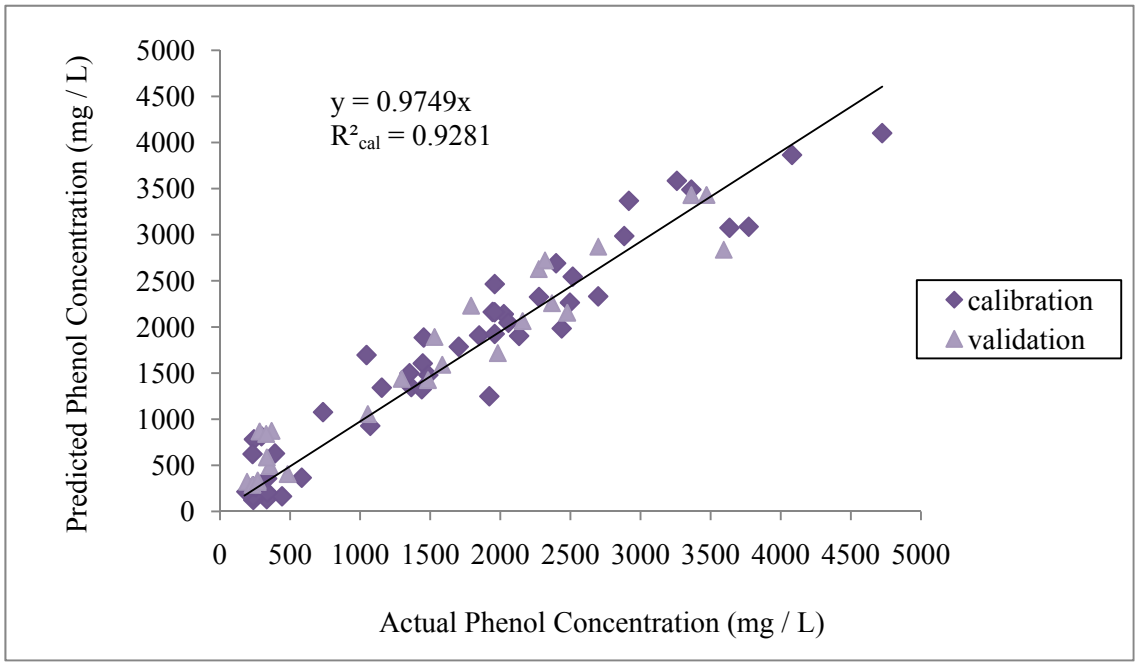

Figure 4.7. PLS regression of actual vs. predicted phenol content of calibration and validation sets

RMSEP value was also calculated with validation set and found as 315.16. By comparing with the criteria set in Table 4.6 the determination of total phenol content by FT-MIR yielded excellent prediction with slope of 0.97, which accounts for high reliability, regression coefficient (0.93) greater than 0.9 and RPD value (3.61) greater than 3.0 (Table 4.5). In another study, 495 red wines were analysed to determine the concentration of phenolic compounds during wine fermentation from the NIR spectral data in combination with PLS regression analysis (Cozzolino, et al. 2004). Cabarnet Sauvignon and Shiraz wines from 2001 and 2002 vintages were analysed via HPLC as a reference method. PLS regression analysis with internal cross validation (one group reserved for validation, three groups used for calibration). R², standard error of calibration and cross validations (SEC and SECV) and residual predictive deviation (RPD) were calculated. The models provided good predictions of phenol concentrations using NIR spectroscopy.

Prediction of anthocyanin content from FT-MIR spectral data with the help of PLS analysis was carried out with calibration and validation sets consist of 32 and 16 observations, respectively. The regression analysis resulted in high correlation of R^2 0.89 for calibration set (Figure 4.8). The model includes 2 PCs explaining 89.4% of total variation (Y) with a predictive ability of 81.7%. Notwithstanding the fact that the slope and R² value of the anthocyanin prediction model were 0.96 and 0.89, respectively which can be regarded as good prediction, the RPD value was calculated as 1.84, resulting in insufficient prediction (Table 4.5 and 4.6).

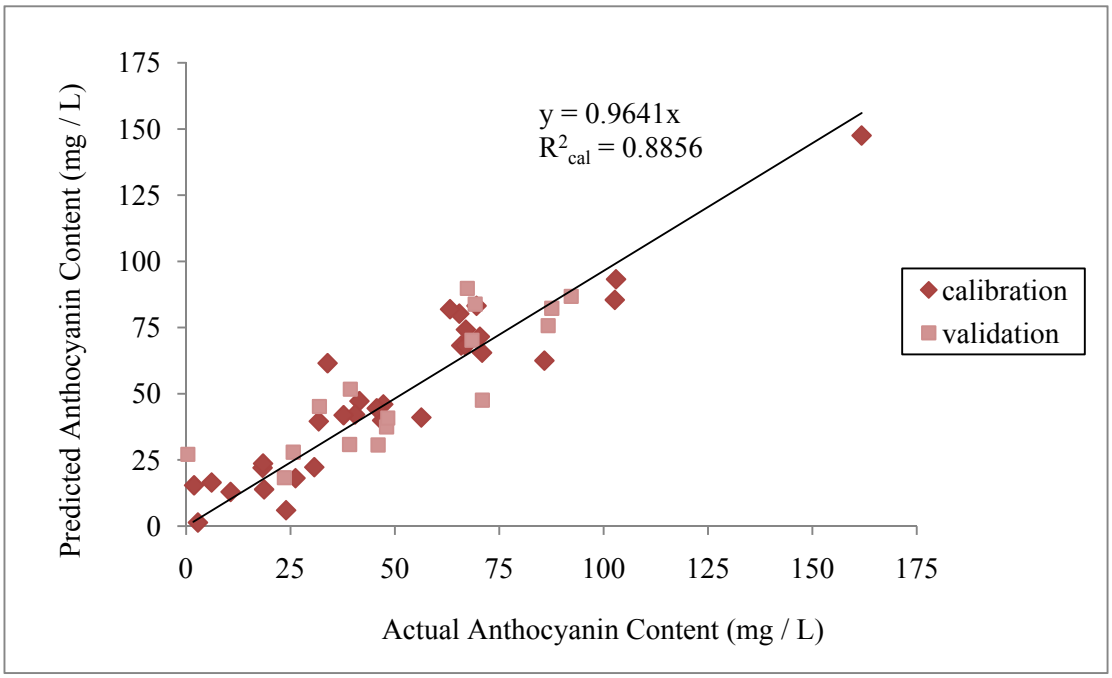

Figure 4.8. PLS regression of actual vs. predicted anthocyanin content of calibration and validation sets

Similar results were obtained in a previous study which was performed with 390 young red wines (vintage of 2004) from Spain (Soriano, et al. 2007). As the reference method HPLC was utilised to determine anthocyanins in different forms. Calibration and validation set were analysed independently and cross validation was performed for detection of the accuracy of the calibration. R^2 values and relative standard deviation (RSD) were calculated for evaluation of the models and it was recorded that RSD values below 6.6% indicated good repeatability. Except cyanidin-3-glucoside, prediction of anthocyanins from FTIR spectra and PLS analysis was successful (Soriano, et al. 2007).

The model created for brix prediction was composed of 3 PCs explaining 84.25% of total variation (Y). The predictive ability of the model constructed for brix values with FT-MIR spectral data was not as good as phenol model (R^2=0.81) (Figure 4.9). Even though the slope of the calibration model was 0.997 which is within the range of 0.9-1.1, the model for prediction of brix gave approximate prediction with regression coefficient 0.81 (around 0.85) and RPD value of 2.16 (between 2.0-2.5) (Table 4.5 and 4.6).

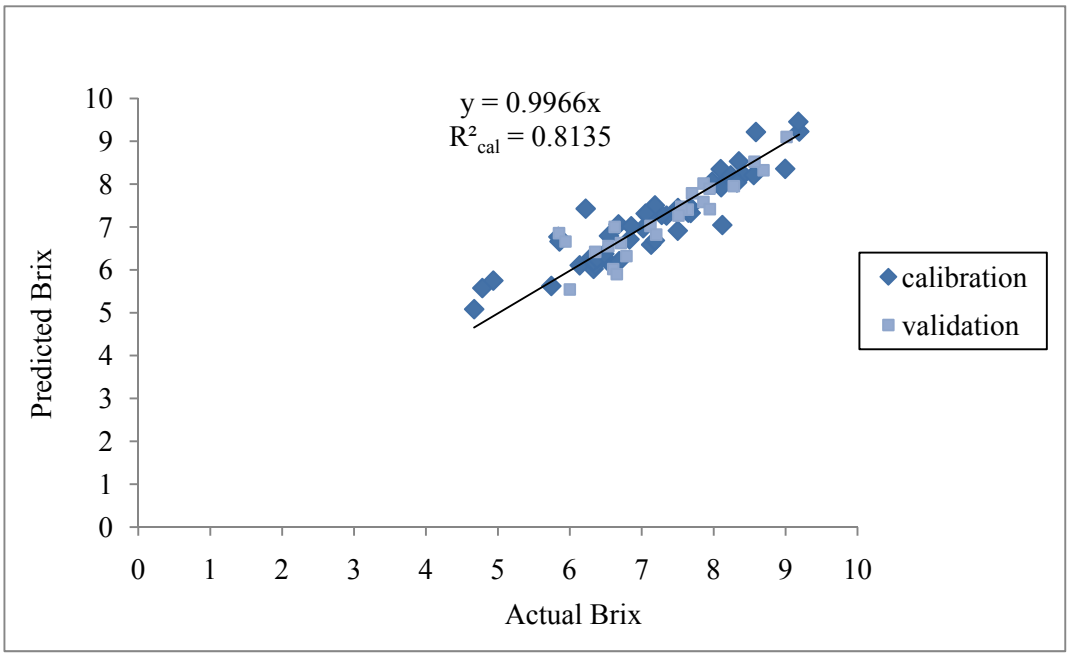

Figure 4.9. PLS regression of actual vs. predicted brix value of calibration and validation sets

Titratable acidity and pH analytical results were also tried to be predicted from mid-IR spectra. As a result of PLS analysis, slope of titratable acidity model was 0.997 (Figure 4.10). Considering the correlation coefficient of 0.75 and RPD value of 2.06, determination of titratable acidity by the model end up with approximate prediction (Table 4.5 and 4.6). However, the analysis resulted in a good correlation for pH prediction with the slope of 0.99 and R^2 value of 0.85 (Figure 4.11). The model contains 5 PCs and showed good predictive ability with high RPD value (2.65) for pH values (Table 4.5).

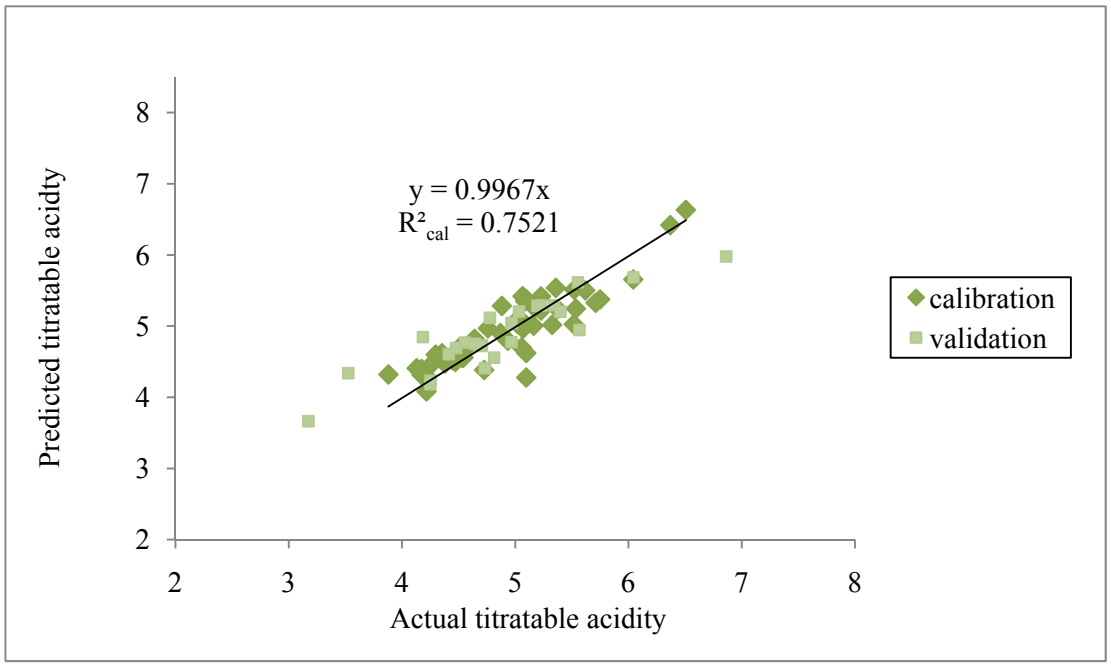

Figure 4.10. PLS regression of actual vs. predicted titratable acidity of calibration and validation sets

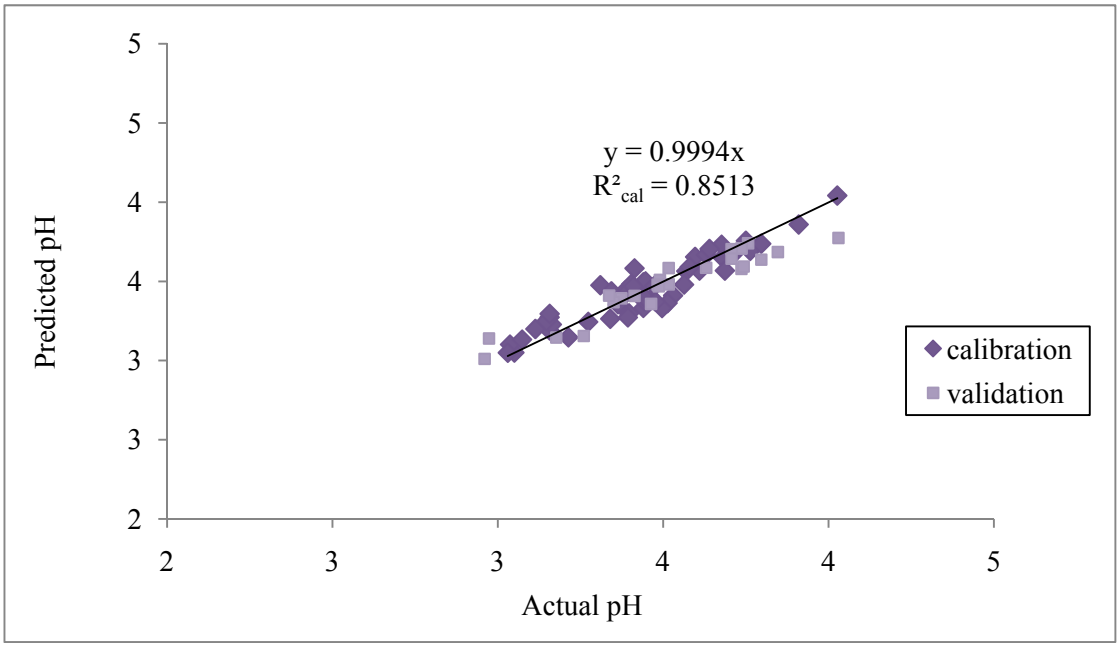

Figure 4.11. PLS regression of actual vs. predicted pH of calibration and validation sets

The colour parameters were also analysed with PLS regression analysis and the best results were obtained with colour intensity and yellow% prediction which were shown in Figure 4.12 and 4.13. The model of colour intensity was tested with calibration and validation

sets, slope of 0.95 and R^2 value of 0.89, indicating good prediction were obtained for calibration set. Furthermore the RPD value of 3.75 has verified the excellent predictability of the model (Table 4.5 and 4.6). The model created for colour parameters prediction contains 6 PCs explaining 82.6% of total variation (Y) and the correlation coefficients for other colour parameters varied between 0.53- 0.85 range (Table 4.5).

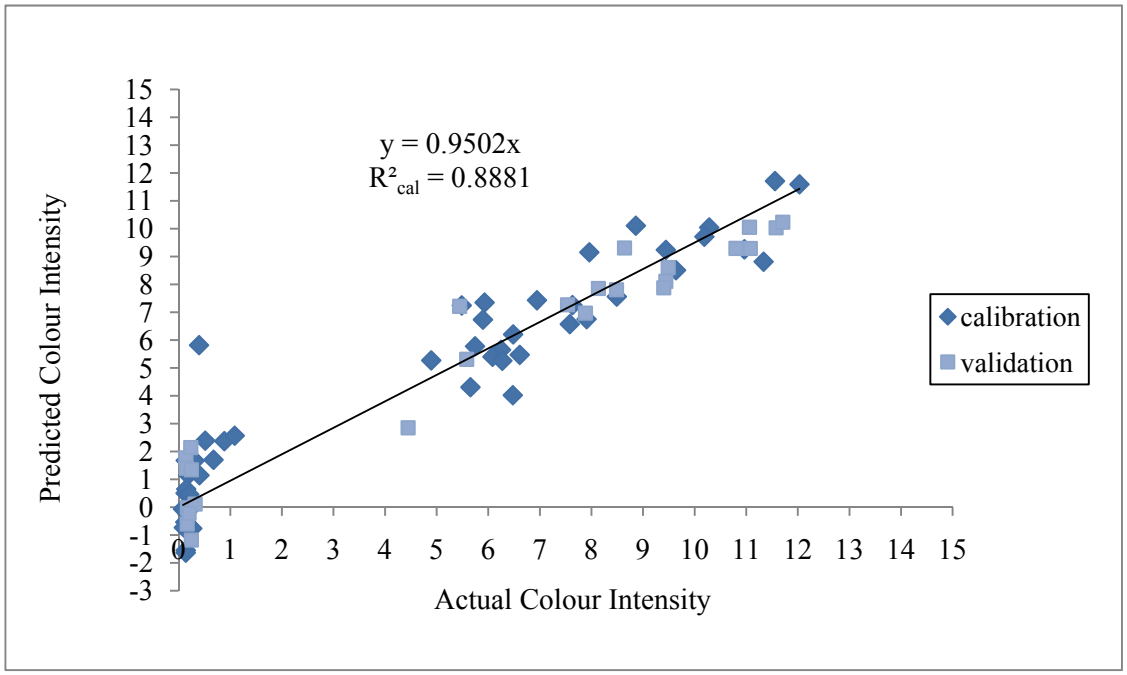

Figure 4.12.PLS regression of actual vs. predicted colour intensity of calibration and validation sets

The model created for determination of yellow% provided approximate prediction with regression coefficient of 0.85 (Figure 4.13-a) and RPD value of 2.0 (Table 4.5 and 4.6). The predictive ability of the models for prediction of tint, red%, blue % and dA% were insufficient with RPD values below 2.0 and R^2 values lower than 0.8 (Table 4.6).

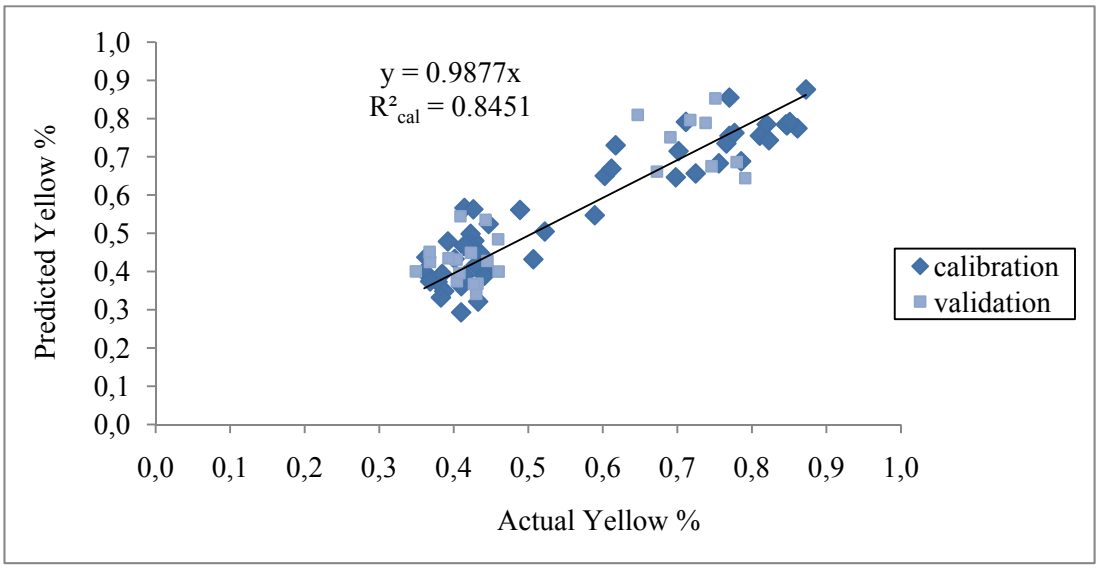

Figure 4.13. PLS regression of actual vs. predicted yellow% of calibration and validation sets

In a previous study, which was performed as an application of FT-MIR spectrometry in wine analysis, 327 German wines from vintages of 1989 to 2001 were analysed with reference methods and also scanned with FT-MIR to obtain spectral data (Patz, et al. 2004). The wine samples were separated into two independent groups as calibration and validation and two groups were analysed separately. The prediction models resulted in high regression correlations and appropriate RMSEP values. A large group of wine parameters like total phenol, relative density, glycerol, total acidity, pH, alcohol%, conductivity, sugars (fructose, glucose) and antioxidant activity were successfully predicted from FT-MIR spectral data in combination with multivariate data analysis (Patz, et al. 2004). In another study, NIR spectroscopy and multivariate analysis was studied for determination of 15 enological parameters (Urbano-Cuadrado, et al. 2004). A total of 180 red, rose and white wines were analysed with analytical methods as reference methods and NIR spectrometry was used to create models for determination of the parameters in combination with PLS regression analysis. Cross validation procedure was also employed for calibration equations and R^2 and standard error of cross validation (SECV) values were used for evaluation of their models. Accurate predictions were observed for determination of ethanol, volumic mass, total acidity, pH, glycerol, colour, tonality (tint) and total polyphenol index by the created equations with high R^2 values and SECV values close to the reference methods (Urbano-Cuadrado, et al. 2004).

As a result of the FT-MIR application combined with multivariate data analysis for prediction of the basic enological parameters, a high performance was achieved for some of the selected chemical parameters of Turkish wines. With this rapid method, a group of wine quality parameters were determined within a short time simultaneously. Thus, this method can be an alternative to determine important enological parameters.

CHAPTER 5

CONCLUSION

In the current study, performance of varietal classification of Turkish wine samples with the use of FT-MIR spectral data combined with multivariate data analysis has been investigated. A total of 79 wine samples including 47 red, 5 rose, 28 white wines from 2006 and 2007 vintages were analysed. Principal component analysis (PCA) was applied to classify wines according to variety. Classification of red and white wines was almost achieved but a distinct grouping of the wine samples with respect to variety could not been attained. The regional differences among the same variety might be a factor giving rise to deficiency on varietal classification due to the effects of climatic and soil diversity. Furthermore, the fact that mono-varietal wines may be composed of wines mainly from one variety but other varieties may be also added in small percentages has significant influence on varietal classification.

Applicability of determining certain wine characteristics (total phenol and anthocyanin content, titratable acidity, pH, brix and colour parameters) from mid-infrared spectra in combination with chemometric methods was also discussed. The experimental results were correlated with MIR spectra by using partial least square (PLS) analysis method. The prediction of total phenols and colour intensity was achieved with high correlation coefficient (R^2 of 0.93 and 0.89, respectively) and high residual predictive deviation (RPD of 3.61 and 3.75, respectively) indicating excellent predictions. With RPD value of 2.65 and R^2 of 0.85 the model of pH determination has good predictive ability. Similarly the correlation of the yellow% resulted in approximate prediction with R^2 of 0.85 and RPD value of 2.0. Although some of the colour parameters resulted in inadequate prediction, a considerable part of the chemical parameters of Turkish wines could be predicted from FT-MIR spectral data.

The study carried out indicates that FT-MIR spectrum has information on chemical structures of the wine sample and this information could be used to predict the basic enological parameters via mid-infrared spectra without the use of large amounts of samples and chemicals or time consuming sample preparation. The combination of FT-MIR spectroscopy with chemometric analysis provided valuable information related to quality of the wines and can be an alternative method in the industrial and research applications in the near future.

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APPENDIX A

TURKEY WINE PRODUCTION

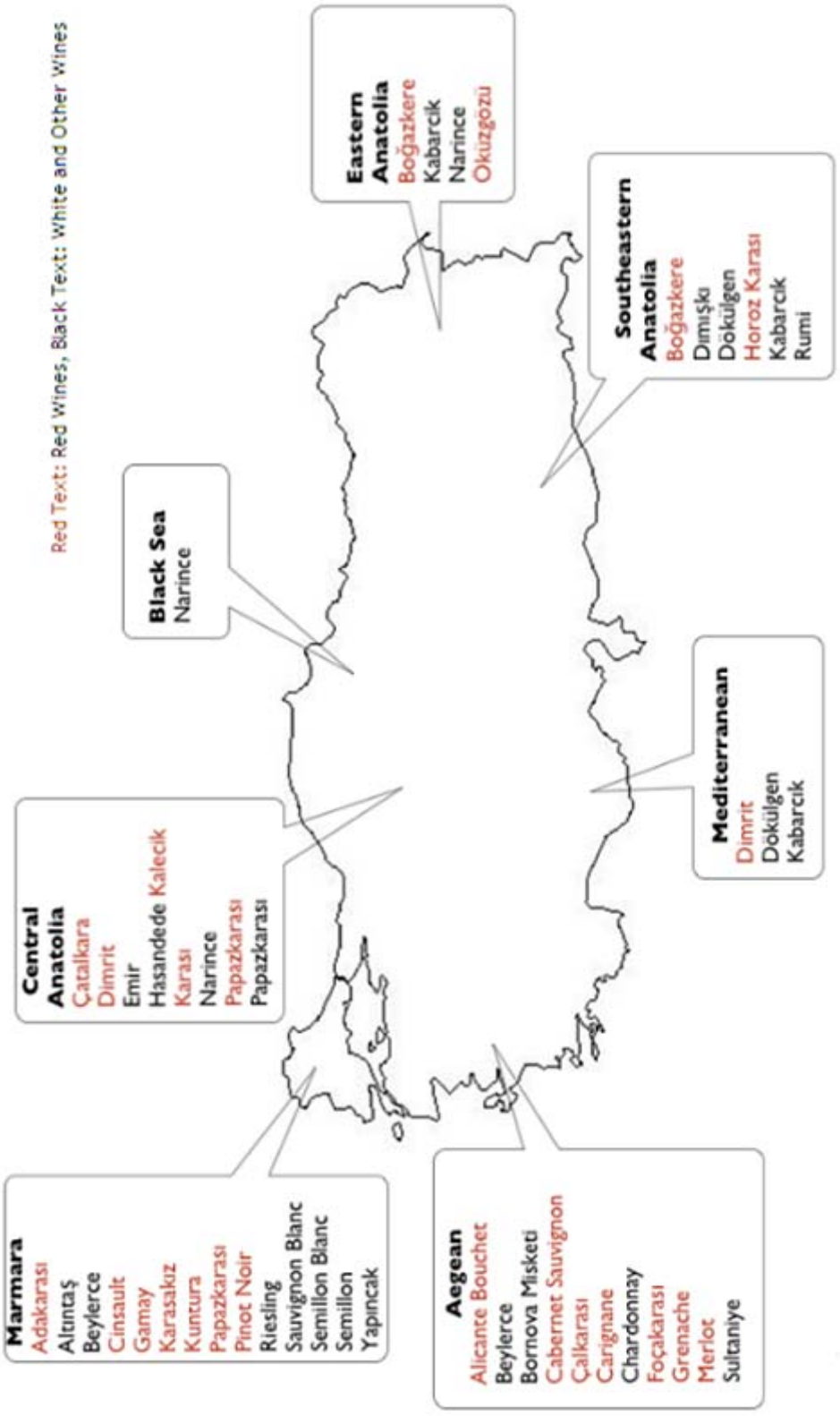

Figure A. Turkey Wine Production Map