

**AUTHENTICATION AND PREDICTION OF
SOME QUALITY PARAMETERS OF ALCOHOLIC
BEVERAGE RAKI WITH INFRARED
SPECTROSCOPY**

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ABSTRACT

AUTHENTICATION AND PREDICTION OF SOME QUALITY PARAMETERS OF ALCOHOLIC BEVERAGE RAKI WITH INFRARED SPECTROSCOPY

Mid-infrared spectra of thirty- two Raki samples of different brands, two types of Ouzo were collected and several chemical parameters were measured with analytical and instrumental methods. Moreover, Raki samples were adulterated with methanol at 0.5-10% (vol/vol) and IR spectra were also obtained. Aims of this study are to classify Raki samples according to raw material of Raki and processing type, to predict some quality parameters of Raki from IR spectra and also to detect adulteration of methanol in pure Raki by using Fourier transform infrared spectroscopy in association with multivariate chemometric techniques and SIMCA.

All samples were used to classify with respect to grape types (fresh or dried), raw material (only suma or suma and ethanol) and production process (double or triple distilled) by chemometric models. No clear classification could be found as parameters investigated because of predominant of alcoholic content in Raki.

In quantification analysis; pH, brix, electrical conductivity, total phenol and sugar content were determined with analytical reference methods for pure samples, then PLS were used to construct models to establish relationships between reference methods and FTIR spectra and to predict these quality parameters from spectral data. After PLS regression, highly good models were developed for brix and sugar.

For adulteration analysis, six different Raki samples were adulterated. First, PCA was used to detect Raki samples adulterated with methanol. Then, PLS multivariate calibration model was constructed to determine the adulterant methanol content in Raki . Consequently, successful results were obtained for detection of methanol in Raki samples.

ÖZET

ALKOLLÜ İÇECEK RAKI'NIN OTANTİSİTESİNİN VE BAZI KALİTE PARAMETERLERİNİN KIZILÖTESİ SPEKROSKOPİ İLE BELİRLENMESİ

Bu çalışma 2 tanesi Ouzo marka olmak üzere 32 çeşit Rakı kullanılarak 3 amaç için yapılmıştır. Birincisi; Rakı da kullanılan üzüm çeşidine(yaş yada kuru üzüm) , farklı üretim koşullarına ve kullanılan hammaddeye (sadece suma yada suma ile birlikte tarımsal kökenli etil alkol) göre sınıflandırılma yapılması, ikincisi kızıl ötesi spektroskopi ve kemometrik yöntemler kullanılarak Rakı daki bazı kalite kriterlerinin tahmin edilmesi için modeller geliştirilmesi, üçüncüsü ise Rakı örneklerine farklı konsantrasyonlarda ilave edilmiş metanolün tespit edilmesidir. 34 adet Rakı tüm orta kızıl ötesi bölgede ($4000-650\text{ cm}^{-1}$) ve seçilen üç spektral bölgede ($965-1565\text{ cm}^{-1}$, $1900-1700\text{ cm}^{-1}$ ve $3040-2800\text{ cm}^{-1}$) taranmıştır.

Rakı örneklerinden elde edilen spektral veriler temel bileşenler analizine göre (PCA) sınıflandırılmıştır. Üzüm çeşidi ve üretim koşullarına göre belirli bir sınıflandırma elde edilememiştir çünkü alkol içeriği Rakı'daki diğer özelliklere göre daha baskın olduğundan alkol seviyelerine göre bir dağılım gözlemlenmiştir.

Nicelik belirlenmesinde; Rakı daki bazı parametrelerin (pH, briks, elektrik iletkenliği, toplam fenol ve şeker miktarının) analitik referans metodlarla ölçülen değerleri ile Rakı spectraları kısmi en küçük kareler (PLS) regresyon analiz yöntemiyle karşılaştırılmış ve aralarındaki ilişkiye göre tahmin modelleri oluşturulmuştur. Regresyon analizi sonucunda ,briks ve şeker miktarının tahmini için çok iyi model geliştirilmiştir.

Rakı örneklerindeki hilenin tespit edilmesinde ise, 6 farklı Rakı'ya % 0.5 ile %10 oranları arasında katılan metanol önce temel bileşenler analizine (PCA) göre farklı sınıflandırma oluşturmasında başarı göstermiştir. Sonra da kısmi en küçük kareler (PLS) regresyon analiz yöntemiyle eklenen metanol oranları için iyi tahmin modeli oluşturulmuştur.

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CHAPTER 1

INTRODUCTION

Rakı is a traditional alcoholic beverage and is the most preferred spirits in Turkey (Yılmaztekin, et al. 2011). It is produced from raw materials including grape, raisins and molasses by twice distillation of only suma or suma mixed with agricultural ethanol and flavored with aniseed (Turkish Food Codex, 2005).

Rakı has a specific taste and flavor. Rakı composition might have differences depending on grape types (fresh or dried grape), the origin of grape, maturity degree of grape, ripening conditions, the characteristic of soil, geographical conditions, production process (fermentation, distillation and dilution process) and additives. These characteristic differences among Rakı could affect consumer preferences (Wetzel, 1998; Kokkinofita, et al. 2005; Arrizon, et al. 2006; Geroyiannaki, et al. 2007; Jurado, et al. 2009; Garza, et al. 2010; Cabaroglu, et al. 2011).

With the increasing demand on consumption of Rakı, and in the meanwhile increasing tax on Rakı products, counterfeiting has become widespread. From 1990 to present more than 300 people died and dozens were hospitalized due to the consumption of fake Rakı (Elmas, et al. 1996; Inancı, et al. 2000; Turla, et al. 2001; Azmak, et al. 2002; Yaycı, et al. 2003; Yaycı, et al. 2005; Eke, et al. 2007). Generally, the adulteration is done with adding methanol because methanol is cheap and has easy availability; therefore, fake Rakı is simply produced with dilution of raw material by adding water, a mixture of ethanol- methanol and flavoring aniseed (Gülmen, et al. 2006). Methanol is a very hazardous substance owing to its metabolites. The consumption of methanol causes some serious effects on ocular and nervous system of humans when its level in body exceeds lethal dose (with consumption of 50-75 g methanol) (Fidan, et al. 1996; Di Maio and Di Maio 2001). After ingestion, it is metabolized into formaldehyde and then converted into formic acid (Aufderheide, et al. 1993; Onder, et al. 1999; Yaycı, et al. 2005; Kapur, et al. 2007; Taheri, et al. 2010). Therefore, methanol adulteration detection and identification of fake Rakı is important for human health.

Besides this, classification according to grape varieties and production process provide insight about which quality parameters affect variation in Rakı composition, and also give an idea about authentication of Rakı (Tzouros, et al. 2001).

Alcoholic beverages have complex compounds such as organic acids, phenolic and volatile components which make differences on the chemical composition and organoleptic properties of samples, so analysis of these compounds is used to classify samples and to determine food authenticity (Arvanitoyannis, 2005; Pontes, et al. 2006; Reid, et al. 2006). Analytical methods are performed to identify composition variation following physical, chemical and sensory tests that could be time consuming and expensive. Therefore, reliable, simple and fast methods with no sample preparation to screen large numbers of samples is required to classify and to determine the adulterant amount added into the sample (Edelman, et al. 2001; Nieuwoudt, et al. 2004; Patz, et al. 2004). Due to complexity of food composition chemometric techniques and strategies are necessary to obtain chemical information from rough measured data using instrumental methods. Thus, models are constructed for identification of and controlling the samples (Brereton, 2003; Brown, et al. 2005).

FTIR spectroscopy combined with chemometric techniques provides a powerful tool for monitoring a variety of processes and for obtaining accurate and simple calibration models for the identification of chemical properties of different Rakı samples. In addition, Rakı samples having similar properties could be classified in representing groups (Almela, et al. 1996; Arozarena, et al. 2000; Aleixandre, et al. 2002; Lachenmeier, et al. 2007; Lee, et al. 2009). Soft Independent Modeling of Class Analogies (SIMCA) based on PCA and PLS by decomposing all spectral data in a few relevant principal components is also used as a useful multivariate method for the classification and discrimination.

In this study three objectives were investigated. One of the objectives was to develop a technique for detecting methanol adulteration using mid-IR spectroscopy in combination with chemometric methods. The other one is to classify Rakı samples with respect to raw materials and production processes again using IR spectroscopic data. Finally, it was also aimed to predict some chemical parameters including pH, brix, electrical conductivity, total phenol and sugar contents from FTIR spectral data with chemometric techniques.

CHAPTER 2

RAKI

2.1. Aniseed Flavored Spirits

Alcoholic beverages can be produced by fermentation and/or distillation and distilled alcoholic spirit categories include two groups in itself: namely distilled spirits and liquors. Fermented drinks are prepared by the action of yeasts that metabolize sugars or other carbohydrates in grapes, other fruits, vegetables and grains by producing alcohol as a waste product whereas distilled spirits are produced by the distillation of fermented raw materials with the separation of alcohol from fermented liquid by increasing the alcohol content. Wine, beer, whisky, rum, vodka, brandy, gin and liquor are the most consumed alcoholic beverages all around the world (Son, et al. 2011; Arrizon, et al. 2006).

Aniseed flavored spirit drinks are distilled spirits which are produced with the separation of ethyl alcohol from fermented saccharate raw materials and flavored with aniseed-type plants, mostly star anise (*Illicium verum* Hook f.), anise (*Pimpinella anisum* L.) and fennel (*Foeniculum vulgare* Mill.), or any other plant containing the same principal aromatic constituent. Anise is the most preferred flavoring constituent in alcoholic beverages because of its predominant taste (Official Journal of the European Union, 2008). Anise has been used in alcoholic beverages as a popular aromatic plant and spice as well as in folk medicine as a relaxant, a carminative, a diuretic, an appetizer and a sedative agent and as an agent to increase milk secretion (Bisset, 1994). Anise (*Pimpinella anisum* L.) is a member of Umbelliferae family which is a grassy herb with white flowers and green to yellow seeds and grows particularly in Asian countries and many warm regions including Egypt, Greece, Turkey, Russia, South Africa and Latin America (Tripelli, et al. 2007). In Turkey, aniseed is a significant agricultural crop. Aniseed of Turkey is usually harvested from Çeşme, but there are other important production regions such as Tefenne, Acıpayam and Elmalı. Annually, 10 000 000 kg of aniseed is cultivated in Turkey and 7 000 000 kg of total aniseed are used in the production of Turkish alcoholic spirit “Rakı” and the rest of all are exported

(Fidan, et al. 1993; Tabanca, et al. 2006). Aniseed is used in alcoholic beverages as a flavoring agent in many countries such as Rakı in Turkey, ouzo in Greece, pastis in France, sambuca in Italy, allasch in Russia, aguadiente in South America, anis in Spain and arak in Syria. However, each kind of aniseed spirit is different because of their special production processes and their traditional uses (Jurado, et al. 2007).

2.2. Rakı

Rakı is a traditional Turkish aniseed spirit. In the Turkish Food Codex (2005), Rakı is described as a spirit produced by twice distillation of only suma or suma mixed with agricultural-based ethanol in traditional maximum 5000 L copper alambics and flavored with aniseed (*Pimpinella anisum*), and it must be manufactured only in Turkey (Turkish Food Codex, 2005). Grape, raisins and molasses are the main raw materials for suma production. Suma is a distillate and it is distilled to maximum 94.5% ethanol for the purpose of preserving the taste and odor of grape or other raw materials (Anlı, et al. 2010). Several criteria are defined in Turkish Food Codex (2005) for the Rakı production:

- ◆ suma constitutes a minimum of 65% of total alcohol of final product,
- ◆ white refined sugar must be used in the preparation phase and,
- ◆ Rakı contains less than 10 grams of sugar per liter and 4-6 g/L of sucrose is added during ageing process,
- ◆ volatile compounds must be a minimum of 100 g/hL in 100% (v/v) alcohol,
- ◆ the methanol content must be a maximum of 150 g/hL in 100% (v/v) alcoholic final product,
- ◆ the amount of anethol of volatile oil that comes from aniseed herb must be a minimum of 800 mg/L of product,
- ◆ Rakı has minimum 40% alcoholic content,
- ◆ finally, Rakı is rested at least one month before filling.

The main raw materials and the use of post distillation processes like ageing, coloring and flavoring define the characteristics of Rakı. Sultania, Dimrit, Yapınacak and Muscat Blanc are the grape varieties used in Rakı production (Fidan, et al. 1993).

2.3. Historical Background of Rakı

Rakı is a traditional alcoholic spirit and its history goes back to 300 years. It is not known exactly where or when Rakı was produced first. The drink was known by different names such as Araka, Araki, Ariki, Iraqi and Razaki that came from the same origin in the Near and Middle East countries. Some claimed that it was called Iraqi because it was first produced in Iraq then spread to other regions. Others said it got its name from Razaki grapes which were used as the raw material. Another theory is that the distillation process was started in the Arab world then with addition of aniseed, Rakı took on its own characteristic. In 1630, Evliya Çelebi, a famous Turkish traveler, mentioned Rakı in his book about his voyages. According to his account people produced their own Rakı in small towns. In old times, when alcoholic beverages were prohibited, Rakı was also produced by underhand producers. The illegal production of Rakı caused blindness in some cases. As the Rakı industry developed, it was produced according to technical rules with appropriate equipment by monopoly (Zat, 2011; Fidan, 1993).

Turkish Rakı is the most preferred beverage among distilled spirits in Turkey and it has protected its own characteristic of being a traditional beverage since 1930. Rakı was awarded a geographical indication statue (Menşe İşareti) to protect its identity and to distinguish it from similar alcoholic beverages in 1999. In Turkey, Rakı was the first product owning a geographical indication which is obtained by the European Union in alcoholic drinks industry (Yılmaztekin, et al. 2011; Fidan et al. 2002; Fidan, et al. 2000).

Before 2003, Rakı produced by only a state monopoly (Tekel) under 4 brands (“Altınbas”, “Kulüp”, “Tekirdag” and “Yeni Rakı”). However, in recent years, several companies have started producing Rakı (TAPDK, 2011). Therefore, number of Rakı varieties has increased. For example, Rakı samples have been started to be produced from various grape varieties such as Misket and Bogazkere other than Sultaniye (Ozkandan, 2009).

Rakı which is considered as the national alcoholic drink of Turkey is also an important product from the economic point of view for Turkey. Rakı production consists of 80% of total alcoholic beverages and Rakı export constitutes 95% of total alcoholic beverages. According to Tobacco, Tobacco Products and Alcoholic Beverages

Market Regulatory Authority (TAPDK) of Turkey; In 2003, 60 million liters of Rakı were produced. After the approval of high tax regulation Rakı consumption fell down to approximately 45 million liters in 2008. Recent increase in taxation of Rakı further led to falling down of Rakı production and consumption of about 40 million liters per years (DPT, 2000).

2.4. Rakı Production

Rakı is produced mainly in two steps, namely “suma production” and “Rakı production”. According to Turkish Food Codex (2005), suma is a colorless and clear grain alcohol and it owns a special odor and flavor. Suma is obtained by the distillation of fermented raw materials and it contains minimum 65% of Rakı (Turkish Food Codex, 2005). For the production of suma, the main raw materials such as grape, raisin and molasses are crushed so that sugar dissolves in water easily during the fermentation process. After the yeast is added into the fermentation medium alembic distillation continues up to the minimum of 85% ethanol ratio. Consequently, suma is obtained (Anlı, et al. 2010; Cabaroglu, et al. 2011; Gözen, 2005; Fidan, et al. 1999).

For Rakı production, suma, aniseed and sucrose are used. Distillation produces three groups of product; initial, middle and end products (Fidan, et al. 1996). In Turkish Rakı process, only *Pinpinella anisum L.* can be used for aromatization, especially aniseed manufactured in Çeşme and Tefenni are the most preferred and used at the ratio of 6-10% (Karaali, et al. 1995). As essential oil in the aniseed is variable to its type, climatic condition and freshness, the amount of aniseed used in Rakı production is determined according to its fat content. First suma and aniseed are added in the alembic (5000L), and the amount of total alcohol is lowered to about 45% v/v with deionized water. Distillation process continues about 40-45 hours. During distillation process initial, middle and end products are separated. Middle product constitutes Rakı. Initial product with a strong odor comprises aldehyde and ester compounds which can be vaporized at the low temperature. A little fusel oil and volatile organic acids are also in the initial product. The quantity of initial product is 10% of total volume and the alcohol level is about 82.5%. After separation of initial product, middle product is taken from the alembic. Middle product, which is used for Rakı production, is 63-70% of suma and its alcoholic level changes from 75 to 84%. It is clearer and contains less waste product

of fermentation than initial and end product. When the color and odor of distillate change, end product is extracted. End product contains compounds like fusel oil and furfural and these compounds correspond to approximately 25% of total volume and alcohol level is between 30 to 40%. Later, middle product is taken to the settling barrels. Rakı is diluted to desired alcoholic level of 45 to 50% with deionized water and 4-6 gL⁻¹ sugar is added. After dilution process Rakı is filtered and aged for at least one year before bottling (Turkish Food Codex, 2005).

Freshness of anise, quality of suma and good separation of initial, middle and end products as well as volatile compounds are important quality parameters in Rakı production (Anlı, et al. 2007; Fidan, 1993).

2.5. Adulteration of Rakı

Rakı is the most consumed alcoholic spirit drink in Turkey. Therefore, it is prone to adulteration (Anlı, et al. 2010; Cabaroglu, et al. 2011; Fidan, et al. 1996). Owing to the fact that Rakı is a distilled alcoholic beverage, some volatile compounds such as methanol, ethanol and essential oils which originate from anise and other volatile compounds are associated with the quality of Rakı. The amount and the ratio of volatile compounds determine the characteristic odor and taste of Rakı and also affect the quality (Wechgama, et al. 2008). Methanol, aldehydes (acetaldehydes, acetate), esters (ethyl acetate, methyl acetate) and higher alcohols (2-butanol, n-propanol, isobutanol, n-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol) are by products formed during fermentation process. These compounds have toxic effects on human health if they are consumed at higher levels than a specific amount. Among these, methanol is the most hazardous (Apostolopoulou, et al. 2005; Gonza'lez, et al. 2010; Kinoshita, et al. 1998).

In Turkey, Rakı is produced commercially in large scales by corporations equipped with modern systems. However, in recent years, Rakı has been also produced through illegal channels, or traditionally at home. One example is known by the name of 'Boğma Rakı' which is a home-made Rakı. If wooden material and reed pipes or not qualified raw materials are used during distillation methanol could also be produced accidentally by the equipment (Anlı, et al. 2007; Gülmen, et al. 2006). Most of clandestine Rakı is prepared by simple dilution of raw materials by adding water, ethanol or by a mixture of ethanol-methanol and flavored with aniseed. Methanol is

added to Rakı instead of ethanol because of its cheapness and easy availability. In addition, since home-made Rakı is not produced under controlled conditions there might be a problem about its methanol level (Gülmen, et al. 2006).

Besides adding directly methanol into Rakı, all alcoholic beverages have a little content of methanol because it forms as a result of hydrolization of methoxyl group from pectic substances present in crushed grapes by pectolytic enzymes during fermentation. Methanol levels in distilled spirits depend on raw material (grape variety, grape health, ripeness of grape, robustness of grape) because the presence of overripe, soft and spoiled grapes increases methanol levels. Methanol level is also affected by processing technique, extraction time, fermentation temperature and pectolytic enzyme the treatments (Apostolopoulou, et al. 2005; Cabaroglu, 2005).

Methanol is a clear and colorless volatile liquid that smells like ethyl alcohol. It is a very toxic substance which is commonly used as an industrial solvent and in antifreeze production for auto industry (Kinoshita, et al. 1998). It is obtained by distillation of wood. Its consumption results in some serious effects on ocular and neurologic systems. Although methanol itself is harmless, its metabolites are toxic and this cause methanol poisoning in both human and animals. Methanol spreads rapidly into the body via all routes. As shown in Figure 2.1 after dispersion in body compartments; methanol is firstly digested into formaldehyde by alcohol dehydrogenize enzyme. Formaldehyde is more toxic than methanol (thirty-three times), and it is speedily converted into formic acid by aldehyde dehydrogenize enzyme. Finally formic acid is metabolized into carbon dioxide and water then is moved away from body (Aufderheide, et al. 1993; Kapur, et al. 2007; Onder, et al. 1999; Yaycı, et al. 2005).

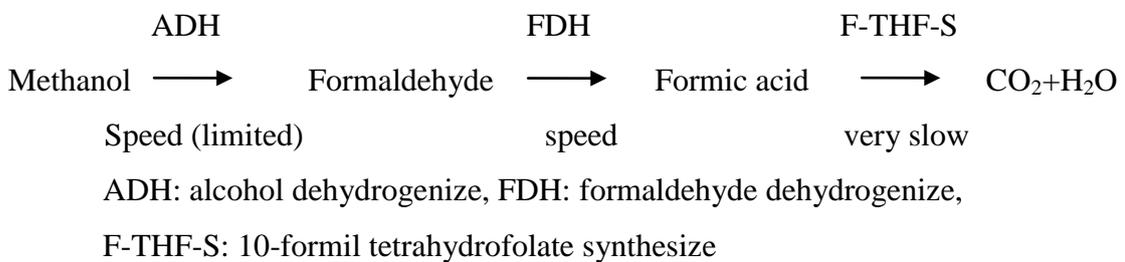


Figure 2.1. The metabolism of methanol
 (Source: Yaycı, eta l. 2005)

Methanol intoxication leads to severe metabolic disturbances; starting with headache, fatigue, nausea and goes on with blindness, permanent neurologic dysfunction and occasionally death. After methanol consumption, it reaches maximum level in the blood, and then symptoms are observed like sickness, vomiting, nausea, headache, blood pressure drop and tachypnea (Taheri, et al. 2010).

According to literature, from 1990 to present the total number of deaths due to methanol poisoning which was arisen from consumption of adulterated Rakı, has been more than 300 (Azmak, et al. 2002; Eke, et al. 2007; Elmas, et al. 1996; Inanıcı, et al.2000; Turla, et al. 2001; Yaycı, et al. 2003; Yaycı, et al. 2005). Lethal dose of methanol in humans is related to age, sex, metabolic rate, methanol blood level and the amount of consumed Rakı. It was estimated that consumption of 50-75 g methanol causes deaths due to methanol intoxication however, there are also reports showing that toxication level could be as low as 11.5 g (Di Maio and Di Maio 2001; Fidan, et al. 1996).

2.6. Rakı Classification

There are many factors that cause differences between Rakı varieties. Commercially, Rakı may be classified according to grape variety as “fresh grape Rakı”, “dried grape Rakı” and their combination. In addition, Rakı could be produced only from suma or suma and ethanol from agricultural resources. The basis of the differentiation among Rakı samples could also depend on; grape types used in Rakı production, the methods of production, its additives, volatile compounds which come into existence during fermentation and affect both quality assurance of Rakı and consumer preferences (Anlı, et al. 2010; Cabaroglu, et al. 2011; Fidan, et al. 1996; Turkish Food Codex, 2005; Yılmaztekin, et al. 2011).

2.6.1. Classification with respect to Grape Varieties

Dried, fresh grapes or their mixtures are used in the production of Rakı. If fresh grape are used in Rakı production, mash is obtained with crushing fresh grapes then this mash is directly processed in fermentation. On the other hand if the raw material is dried grapes, a paste is obtained from dried grapes by maceration then fermentation

takes place. Apart from that, the other steps are similar for using both dried and fresh grapes to obtain suma (Anlı, et al. 2010). Rakı could be produced from only suma or suma mixed with agricultural ethyl alcohol. This also leads to some differences in especially volatile compounds and sugar values. Total volatiles, methanol, trans-anethole, estragole and sugar content are higher in the Rakı samples produced from only suma (Cabaroglu, et al. 2011).

Composition of grapes may differ depending on their varieties, origin (geological region and climatic conditions), and harvest time and affect quality of grapes (Arozarena, et al. 2000; Tarantilis, et al. 2008).

The first important factor affecting the grape composition is the grape variety. Grapes vary from species to species in respect to all chemical properties. They have different physicochemical parameters according to geographic origin, such as total sugar content, phenolic compounds, macro- and trace elements, physical and chemical characteristic, amino acids and biogenic amines and volatile compounds. Especially phenolic compounds act as a fingerprint for differentiation of alcoholic beverages produced from different varieties (Alexandre, et al. 2002; Andreu-Navarro, et al. 2011; Arozarena, et al. 2000; Bevin, et al. 2008; Castellari, et al. 2000; Garza, et al. 2010; Huai-Feng, et al. 2006; Liu, et al. 2006; Tzouros, et al. 2001). Although sucrose is added in the production of Rakı, total sugar content present in each grape are also different. Glucose and fructose are the predominant sugars of most cultivars. Sugar determines alcohol content and sweetness of Rakı because the microbial fermentation of carbohydrates results in the production of alcohol (Huai-Feng, et al. 2006; Castellari, et al. 2000; Turkish Food Codex, 2005).

Geological region is another factor which has influence on composition of grape varieties since growing regions contribute to characteristic taste of grapes. The chemical composition of grapes are affected from soil structure and ripening conditions. Water retention, drainage capacity, bearing humus, nitrogen content and having other nutrients are important for soil (Clarke, et al. 2004; Huai-Feng, et al. 2006; Jackson 2000; Liu, et al. 2006).

Besides these, climatic conditions also affect maturity degree, grape chemistry, microbiology and sensory properties. Temperature, rain, sun exposure and winds are important for growing of grape (Mira de Orduña 2010). When average temperature in a day is 10⁰C, grapes start to ripen until the temperature go down below this point. While

the raining in winters and spring has good effect on ripening conditions, wind affects badly (Bagcılık, 2011).

Finally, harvest time shows differences from grape to grape. All grapes should be collected at the optimum stage of harvest. Moreover, grapes harvested at different years can cause a variation between Rakı samples. The studies of Csomós, et al. (2002) about wine classification with PCA showed the effect of the harvest year.

2.6.2. Classification with respect to Production Methods

Rakı types could differ from each other according to process step in Rakı making. Production processes consist of three main parts; fermentation, distillation and dilution. Also after dilution; ageing, filtration and bottling are the steps that need to be taken into consideration for quality of the end products (Turkish Food Codex, 2005).

In fermentation process; time, temperature, quality of material and equipments used in process are important for producing good quality end product. The organoleptic properties of distilled spirits depend on their content of volatile compounds, organic and inorganic components which form during fermentation. These compounds can vary from high to low levels as fermentation process continues (Anlı, et al. 2010; Turkish Food Codex, 2005). Some examples of differences in the maceration and fermentation process with using different fining agents, yeast and enzymes on wines were studied by Castillo-Sánchez, et al. According to used three techniques (hourly pumping-over, rotation of rotary at every 12 h and carbonic maceration) organoleptic quality of wine, anthocyanin contents and color densities were shown different from each other (Castillo-Sánchez, et al. 2006)

During Rakı processing, some Rakı types are distilled twice, others are distilled thrice. Distillation can influence all compounds in Rakı content, thus classification might change according to alcohol levels (Turkish Food Codex, 2005).

In dilution process deionized water is used and alcohol level is set to desired scale. Depending on dilution in Rakı, the level of alcohol changes, and this affects the composition of Rakı; thus the classification of Rakı could also change (Lachenmeier, et al. 2008; Turkish Food Codex, 2005).

2.6.3. Classification with respect to Additives

Distilled spirits include some additives such as flavorful seeds, aniseed, sugar, mastic and other sweetening products. In addition, the raw material shows variation because of the characteristics of different fruits. On the other hand, Rakı is quite different from other distilled spirits with respect to the raw materials and additives. The additives affect sensory perception, therefore, might contribute to classification (Cabaroğlu, et al. 2011; Jurado, et al. 2009; Wetzel, et al. 1998).

2.7. Rakı Characterization

The characterization of Rakı samples could be performed using chemical composition as input data. The distinctness among Rakı samples arises from grape types, geological origin and ripening conditions of grapes and differences in the production steps as mentioned above. Considering that all Rakı samples have individual characteristic properties as a result of these factors; classification and characterization of Rakı samples with spectroscopic techniques in combination with multivariate data analysis could contribute to the authenticity studies and also to the detection of adulteration (Geroyiannaki, et al. 2007; Jurado, et al. 2007; Petrakis, et al. 2005).

There are several studies about classification techniques of alcoholic beverages. Various statistical methods are used in the characterization and classification of spirits and other foodstuffs according to origin, volatile compounds, sugar content, phenolic compounds, amino acids, and other features. These methods are pattern recognition methods (PRT), principal component analysis (PCA), linear discriminant analysis (LDA), stepwise linear discriminant analysis (SLDA), canonical discriminant analysis (CDA), cluster analysis (CA), hierarchical cluster analysis (HCA), regularized discriminant analysis (RDA) and regression trees. The review by Saurina (2010) summarized classification of wine according to compositional profiles using CA, PCA and related methods. In Andreu-Navarro, et al. (2011) Italian and Spanish wines were classified with supervised (LDA) and un-supervised (FA) pattern recognition methods according to phenolic compound content. Chinese wines with regard to their volatile compounds were classified by SLDA and PCA models in the study about Zhang, et al. (2010). Classification of 33 Greek wines with PCA on the basis of their geographical

origin was studied by Kallithraka, et al. (2001). In another study Cyriot spirit “Zivania” was differentiated by using HCA, PCA, CA, RDA and regression trees with respect to alcoholic grade, methanol, furfural, 2- and 3-methyl-butanol, 2-methyl-propanol and the chemical shift of $-CH_3$ (Kokkinofita, et al. 2005). 98 grape cultivars were studied according to their organic acids composition and sugar content with PCA for classification (Liu, et al. 2006).

2.8. Fourier Transform Infrared Spectroscopy (FTIR)

Distilled spirits constitute a complex mixture of numerous volatile and flavor compounds. The changes in the chemical composition of products, which contribute to their unique sensory properties, are related to raw materials, production process, geological and climatic conditions and additives as mentioned above (Jurado, et al. 2007; Lachenmeir, 2007; Namara, et al. 2005). Differentiating between different groups of distilled spirits gives an idea about quality of products and authenticity assessment as well as providing a control in all stages in production process. The detection and quantification of adulterants are very important for the protection of consumers' health. Some analytical methods have been developed for food authentication studies of alcoholic beverages such as using FTIR, GC, HPLC, VIS, NIR, NMR, ICP and AAS. Among these, the application of FTIR spectroscopy for analysis of distilled spirits is one of the most useful methods because it is a simple and rapid method with no sample preparation and is environmental friendly. In addition, it has high resolution, good precision and accuracy, and comparability to the reference methods (Bauer, et al. 2008; Fernandez, et al. 2007; Lachenmeier, 2007; Patz, et al. 2004; Pontes, et al. 2006; Saurina, 2010; Soriano, et al. 2007).

Chemometric techniques in combination with FTIR spectroscopy was successfully applied to detect adulteration of olive oil (Rohman, et al. 2010), honey (Sivakesava, et al. 2001), lard adulteration in chocolate products (Che Man, et al. 2005), dietary supplements (Champagne, et al. 2011), instant coffee (Briandet, et al. 1996) and pomegranate juice concentrate (Vardin, et al. 2008)

Table 2.1. Functional group assignments 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 asymmetric and symmetric vibration
CH ₂	2926 (±10), 2853(±10)	
=C-H	3082-3000	Stretching
-C≡N	2260-2200	
-C≡C		
-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
Ketons	1720-1710	C=O stretching
Amides	1700-1600	C=O stretching(Amide 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

FTIR spectroscopy is related to various chemical bonds and functional groups which present in the molecules (Table 2.1). The importance of infrared spectrum is that a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibration between the bonds of the atoms making up the material could be obtained. Infrared spectroscopy can provide a positive identification because each material is a unique combination of atoms and no two components or samples represent the same infrared spectrum in terms of amount and intensity of peaks. Measuring principle of IR spectroscopy is based on the reflection of light. With passing IR light through the samples at different wavelengths, the absorbance and transmittance of light beam constitute a vibration which occurred by electronic energy. These stretching and

bending positions of the molecules in the sample create the peaks in the spectrum (Fernandez, et al. 2007; Ismail, et al. 1997; Lachenmeier, et al. 2007; Lee, et al. 2009). Attenuated total reflectance (ATR) crystal is a commonly used FTIR sampling technique. ATR crystal has high refractive index with a prism of infrared transmitting material because of very thin sampling path length and depth of penetration of IR beam into the sample. An infrared beam is directed onto optically dense crystal then an evanescent wave which extends beyond the surface of crystal into samples is created by this internal reflectance (Figure 2.2) (Kemsley, et al. 1996).

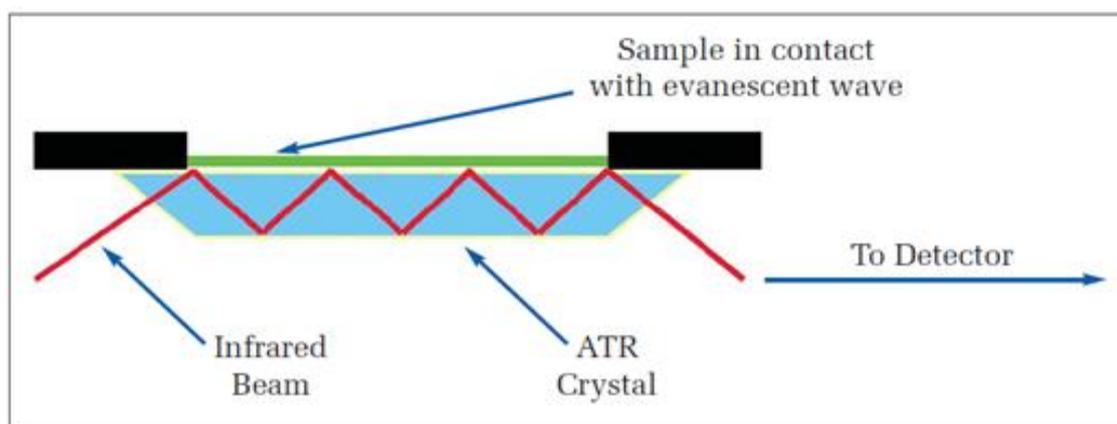


Figure 2.2. The typical scheme representing attenuated total reflectance (ATR) system (Source: Perkin-Elmer 2011)

FTIR with attenuated total reflectance (ATR) crystal could be an excellent tool for qualitative and quantitative analysis in a variety of samples with small sample size. Qualitative analysis is performed by identifying the existence of functional groups or by estimating the identity of a compound by comparing with a standard spectrum. Quantitative analysis is done with measuring the absorbance or transmittance of the bands which are proportional to the composition of the tested compound (Kemsley, et al. 1996; Lachenmeier, 2007; Lee, et al. 2009; Patz, et al. 2004; Nieuwoudt, et al. 2004).

Combined with multivariate calibration of PLS and PCA, FTIR spectroscopy in the mid infrared region ($4000\text{-}650\text{ cm}^{-1}$) is used for quantitative analysis and classification of complex mixture in this study. Moreover, this technique was employed to identify Rakı samples adulterated with methanol.

2.9. Multivariate Statistical Techniques

There has been growing interest in food science to analyze both chemical and physical characteristic of samples by analytical instruments which depend on spectrum measurement (Andreu, et al. 2011; Reid, et al. 2006). Chemometrics is an essential and important impact on the spectroscopic field because of the results obtained from spectroscopic methods including complex data with a large number of variables also some having redundant information. Therefore, chemometric techniques which is a part of multivariate data analysis is applied to the data to obtain meaningful information about objects and variables from these complex data by taking into account the interactions among numerous components that exist in the samples (Bauer, et al. 2008; Cozzolino, et al. 2009; Eriksson, et al. 2001; Geladi, et al. 2004; Geleadi, 2003; Wold, et al. 2002).

Chemometric methods with the employment of Soft Independent Modeling of Class Analogy (SIMCA) are commonly used for discrimination, characterization and classification of data. In addition, chemometric methods have been increasingly needed for detection of adulteration because spectra contain particularly characteristic features between original and adulterated products (Edelmann, et al. 2001; Lee, et al. 2009; Nieuwoudt, et al. 2004; Pontes, et al. 2006). The characterization of the samples is expressed by graphic plots including useful summaries of simple means, standard deviation and correlations. Discrimination or classification techniques help to explain the differences between groups of samples on the basis of their chemical characteristics by reducing the large data into small groups and classification could be visualized with score and loading plots (Andreu-Navarro, et al. 2011; Cozzolino, et al. 2009).

Using multivariate methods for regression and prediction analysis provides richness of information for summarizing spectral data by using two set of variables (X and Y). Generally X data matrix is related to spectral or chromatographic data; on the other hand, Y variable is obtained from analytical methods. As a result of the regression analysis, Y variables are predicted by creating the calibration model (Esbensen 2002; Cozzolino, et al. 2009; Geladi, 2003; Wold, et al. 2002).

The application of multivariate data analysis techniques such as principal component analysis (PCA) and partial least square (PLS) regression opens up the possibility to determine and interpret the chemical properties of the sample and allows

classification, characterization and authentication without the use of chemical information (Saurine, et al. 2010; Versari, et al. 2010).

2.9.1. Principal Component Analysis (PCA)

Principal component analysis is one of the multivariate projection methods used for screening, extracting and compressing multivariate data matrix X with N rows (observation) and K columns (variables) (Eriksson, et al. 2001; Geladi, et al. 2003; Liu, et al. 2006).

The purpose of PCA is to reduce the dimensionality of a data set consisting of a large number of variables to a much smaller number of principal components. This is achieved by transforming to a new set of variables regardless of the nature of the original variables. Each variable having an equal weight in the analysis is ready for subjecting to PCA. Because of this, standardization is required. Standardization of variables is done by subtracting the mean from each of the data and then dividing by the standard deviation of each original variable in each column (centering and scaling) (Brereton, 2003 ; Eriksson, et al. 2001; Liu, et al. 2006; Wold, et al. 2001).

Principal components describe the variance in the independent variables with mathematical transformation, which can be formulized as

$$\mathbf{X} = \mathbf{T} * \mathbf{P} + \mathbf{E} \quad (2.1)$$

PCA is to approximate the original data matrix X by a product of two small matrices; score (T) and loading matrices (P) and error matrix which are symbolized by E . While score matrix constitute of column vectors, loading vectors are row vectors. Score and loading plot which is obtained from PCA is used to show the relationship between samples and which phenomena cause this relationship. Score plot is used to detect and exclude outliers among the sample spectra; loading plot is used to detect outliers or noisy region in the spectra (Figure 2.3) (Brereton, 2003; Geladi, et al. 2004; Geladi, 2003).

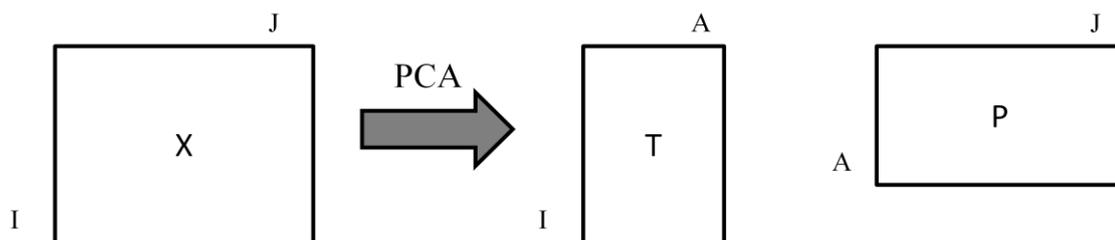


Figure 2.3. A matrix modeling in PCA
(Source: Brereton, 2003)

In Figure 2.3, I is the number of objects, J is the number of variables, A is the number of the new latent factors (PCs), T is the score matrix, P represents loading matrix and E represents error matrix containing unexplained part of X.

Soft Independent Modeling of Class Analogies (SIMCA) is used as a class modeling technique which is helpful in detecting outliers, clustering of objects and gradients between clusters in classification and discrimination analysis. Classification and detection of adulteration based on PCA can be done in supervised and unsupervised way. Objects are specified to the belonging classes in supervised case; on the other hand, in unsupervised case data matrix is constructed without knowledge of the objects belonging to different classes (Esbensen, 2002; Geladi, 2003; Pontes, et al. 2006).

Consequently, the data matrix is reduced to these terms for easy understanding, thus it is more stable and the residuals constitute noise and less useful information in the data. The visualization of the data can be explained in PC lines, so plots and figures help in understanding the spectral information for discrimination and classification purposes (Geladi, 2003; Wold, et al. 2001)

2.9.2. Partial Least Square (PLS) Analysis

Partial least square (PLS) analysis is one of the most commonly used multivariate calibration method. This method is based on a linear multivariate model and is used to determine a linear relationship that exists between desired chemical, biological or physical properties of samples and results in numerous cases a satisfactory solution (Fernández, et al. 2007). This method contributes to get information about the parameter that is under the interest for the spectral interval by constructing a prediction model. The model is related to X data matrix of predictors (spectral or chromatographic

data) and Y matrix of responses (the results of analytical methods) (Eriksson et al. 2001) Besides PLS analysis can be used to establish a regression model for the prediction of adulterant concentration (variable matrix Y) based on the spectra (variable matrix X) (Pontes, et al. 2006). Pre- processing of the data could be required for applying PLS like PCA.

PLS regression is explained with the following set of models:

$$\mathbf{X} = \mathbf{T} * \mathbf{P} + \mathbf{E} \quad (2.2)$$

$$\mathbf{c} = \mathbf{T} * \mathbf{q} + \mathbf{f} \quad (2.3)$$

Where T is score vector, P and q represent loading vector, E and f are the error of the data matrix X and c, respectively.

PLS method relates X and Y data matrix which corresponds to the process variable X and responses, respectively. The model reduces dimensionality by splitting predictor variable X into a small number of latent factors or variables Y. Thus distance between the covariance matrix X and Y are maximized by PLS. Matrix X and Y are separated into score, loading vector and residual error. In PLS model variable correlation are modeled by latent variables, also new and independent validation set are constructed then the model are used to predict parameter which is under interest (Figure 2.4) (Brereton, 2003; Geladi, 2003; Wold, et al. 2001).

Using PLS regression one or more responses can be modeled together if it is strongly correlated to each other using a few number of PLS component, (Eriksson, et al. 2001; Wold, et al. 2001).

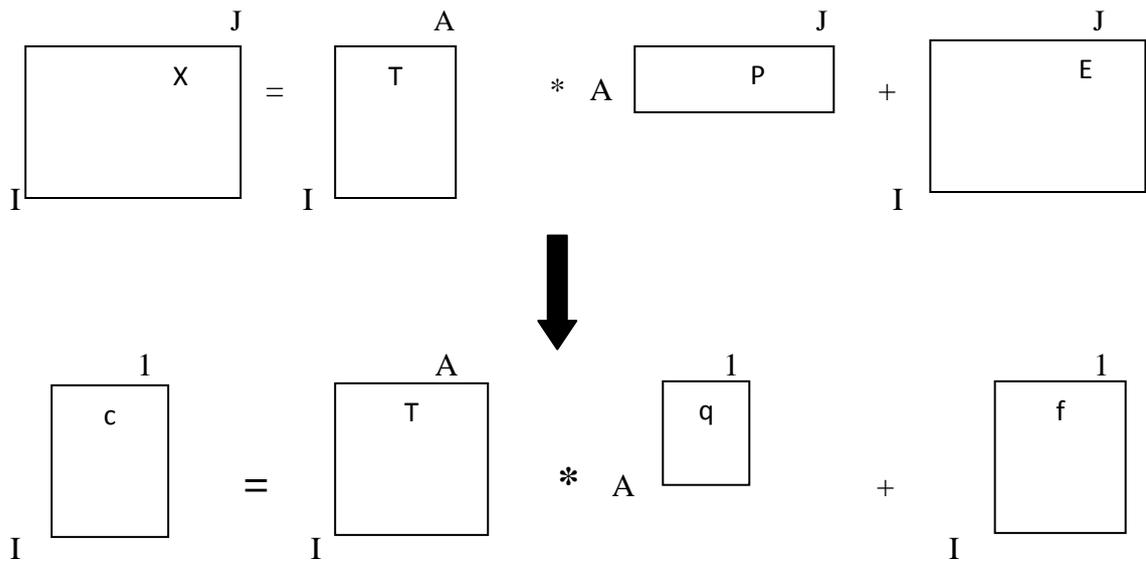


Figure 2.4. Matrix relationships in PLS regression analysis
(Source: Breton 2003)

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Rakı Samples

Twenty-six different Turkish Rakı samples and two Greek Ouzos were used in this study. All Rakı samples were purchased from local markets and cover most of the brand names and their sub-brands produced in Turkey. More than one sample from some brands was included into the sample set. Rakı samples differed depending on their production and the grape type used as the raw material. The list of the samples and their characteristics as defined on the label are presented in Table 3.1. The Rakı samples were stored in their original bottles at 4°C. The lids of the bottles were further covered with parafilm in order to avoid alcohol evaporation.

Table 3.1. List of Rakı samples

Sample Number	Rakı Types	Production Process	Grape Type	Alcohol content %	Code
1	Yeni Rakı-2	distillated twice	Fresh-dried	45	YNR
2	Yeni Rakı Yeniseri	distillated twice	Fresh-dried	45	YNY Y
3	Anadolu 2000	distillated twice	N.D	40	AN, ANYE
4	Anadolu	distillated twice	Fresh	45	ANY
5	Anadolu	distillated twice	Dried	45	ANM
6	Anadolu Sohbet	distillated twice	N.D	43	ANS
7	Ata	N.D	N.D	45	AT
8	Burgaz	N.D	Fresh-dried	45	BRG
9	Altınbaş	N.D	N.D	50	AB, ABY

(Cont. on the next page)

Table 3.1. (Cont.)

Sample Number	Rakı Types	Production Process	Grape Type	Alcohol	
				content %	Code
10	Tekirdağ	distillated twice	N.D	45	TKR
11	Tekirdağ Gold	distillated twice	N.D	45	TKRG
12	Mercan	distillated twice	N.D	45	MRC
13	Yekta	distillated twice	Fresh-dried	43	YKT
14	Kulup	N.D	Fresh	50	KLP
15	Kara Efe2007	distillated triple	N.D	47	EFK, EFKKK
16	Efe 2007	distillated triple	Dried	45	EFM, EFMV
17	Efe 2009	distillated twice	Fresh	47	EFY, EFYS
18	Sarı Zeybek	distillated twice	N.D	45	SRZ
19	İzmir Rakı	N.D	N.D	N.D	IZM
20	Beylerbeyi	distillated thrice	Dried	45	BEM
21	Beylerbeyi	distillated thrice	Fresh	45	BEY
22	Çilingir	distillated twice	N.D	43	CLN
23	Beyoğlu	distillated twice	N.D	43	BYG
24	Mest Misket	distillated twice	N.D	45	MSM
25	Mest Sultaniye	distillated twice	N.D	45	MSR
26	İzmir sakızlı	distillated twice	N.D	45	IZMS
27	Ouzo Metaxa	Traditional	N.D	38	OZM
28	Ouzo Pakistico	Traditional	N.D	38	OZP

*N.D: not defined

*[AB- ABY], [AN, ANYE], [AB- ABY], [EFK, EFKK], [EFM, EFMV], [EFY, EFYS] are the same types of Rakı with different batch numbers

3.1.2. Chemical Reagents

The chemicals used in the experiments are all analytical grades and shown in Table 3.2.

Table 3.2. Chemical reagents used in the analysis

Chemical Name	Manufacturer	Code
Ethanol absolut puriss	Sigma-Aldrich	32221
Methanol	Sigma-Aldrich	34885
Folin Ciocalteu's Phenol Reagent	Sigma	F9252
NaCO ₃	Riedel-de Haen	13418
Gallic Acid	Sigma	SIG7384-100G
pH 4 Buffer	Sigma Aldrich	82598
pH 7 Buffer	Merck	1.094.770.500
pH 10 Buffer	Merck	1.094.381.000
Electrical conductivity calibration solution	Hanna Instrument	HI7031

3.1.3. Preparation of Adulterated Raki

For adulteration study, six Raki samples were chosen to represent different production processes and grape types. EFY, EFMV, EFKK, YNR, MSM and TKR were the samples which were adulterated with methanol. Samples were adulterated by addition of methanol in the levels of 0.5%, 1%, 2.5%, 5%, 7.5% and 10% (v/v) to the pure samples. Adulterated samples were coded by including adulteration rate into pure sample code.

3.2. Methods

3.2.1. pH Measurement

pH of the Raki samples was measured with a pH meter (HI221 Microprocessor pH meter, Italy) with AOAC Official Method (960.19, 17th Ed 2010). Primarily pH meter was calibrated with buffer solutions (pH 4, pH 7, and pH 10) according to the user's manual of the pH meter. The electrode of the pH meter was cleaned with distilled water after every measurement.

3.2.2. Brix Measurement

Total soluble solid content of Rakı samples was measured with a refractometer (Re50, Mettler Toledo, USA) according to the analytical methods. Before each measurement the refractometer was calibrated with air and distilled water according to the user's manual, and it was cleaned with distilled water after each measurement.

3.2.3. Electrical Conductivity Measurement

Electrical conductivity or specific conductance of Rakı was measured with a Bench Conductivity Meter (EC-215R Hanna Instrument, ITALY). Before measurements conductivity meter was calibrated using its own calibration solution (HI7031) by adjusting the temperature coefficient from 0 to 2.5%/ °C (199.9 μ s ranges). The probe was immersed in the solution submerging the holes of the sleeve and tapped lightly on the bottom of the erlenmeyer to remove any air bubbles trapped inside the sleeve, then cleaned with distilled water after each measurement.

3.2.4. Total Phenol Content Measurement

Folin-Ciocalteu micro method was used to determine the total phenol content of Rakı samples (Slinkard and Singleton 1977). The method, reported in Slinkard et al (1977), was adapted to use the minimum volume of reagents and samples, so that waste of reagents is eliminated and disposal volume is reduced.

First, gallic acid stock solution and sodium carbonate solution were prepared as following;

Gallic Acid Stock Solution: 0.500 g of dry gallic acid was dissolved in 10 mL of ethanol, and then solution was completed to 100 mL with distilled water. The solution was covered with aluminum foil was stored in the refrigerator up to two weeks.

Sodium Carbonate Solution: 200 g of anhydrous sodium carbonate (NaCO_3) was dissolved in 800 mL of distilled water. The solution was boiled then waited to cool down. After cooling, a few crystals of sodium carbonate were added. Twenty-four hours later it was filtered and completed to 1 L.

Calibration Curve: To prepare a calibration curve in the range of 0-150 mg/L gallic acid, 0, 0.5, 1, 1.5, 2, 2.5 and 3 mL of gallic acid stock solution was added into 100 mL volumetric flasks, and diluted to volume with distilled water. 40 μ L from each calibration solution was added into different tubes, and 3.16 mL of distilled water was added to each. 200 μ L of Folin- Ciocalteau reagent was added and immediately mixed with a test tube shaker (Yellow Line TTS 2, IKA, Germany). After 4 minutes 600 μ L of sodium carbonate solution was added and mixed. Before reading the absorbance, each solution was kept for 2 hours in a dark place at 20 $^{\circ}$ C. The absorbance was determined at 765 nm against a blank with a UV-visible spectrophotometer (Schimadzu UV-2450, Japan). The absorbance results were plotted against concentration (Figure 3.1).

Phenol Determination: From each Rakı sample 40 μ L was put into different tubes, and 3.16 mL of distilled water was also added into the tubes. 200 μ L of Folin- Ciocalteau reagent was added and shaken. After 4 minutes, 600 μ L of sodium carbonate solution was added and mixed. The solutions were kept for 2 hours in a dark place at 20 $^{\circ}$ C, and then the absorbance of each solution was measured at 765 nm against the blank. Total phenol concentration of Rakı samples was calculated as mg gallic acid per L by using calibration curve in Figure 3.1.

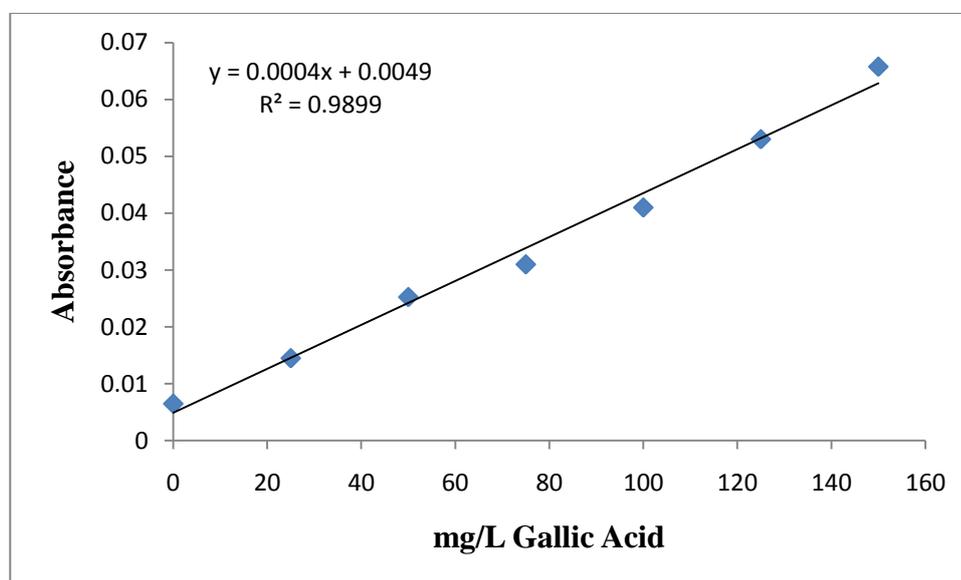


Figure 3.1. Calibration curve of gallic acid for total phenol calculation

3.2.5. Sugar Determination

Sugar content of Rakı was measured using an HPLC (Perkin Elmer Series 200, USA) with a refractive index detector. Twenty microliters of each Rakı sample were filtered through 0.45- μm cellulose-acetate membrane filter (Sartorius, Germany) then injected directly into the HPLC. 0.045*N* sulphuric acid containing 6% acetonitrile was used as mobile phase, with a flow rate of 0.5 mL/min. The column was maintained at 45 $^{\circ}\text{C}$ (Biorad hpx 87 h, column size= 300x7.8 mm, 9 μm). The identification of sugars was confirmed by using integrated peak areas according to external standard solution calibration. This method was adapted from Castellari, et al. (2000).

3.2.6. FTIR- Spectroscopy Analysis

Rakı samples were scanned through an FT-IR spectrometer (Perkin Elmer Spectrum 100 FT-IR spectrometer, Wellesley, MA) equipped with a deuterated triglycine sulphate (DTGS) detector. Samples were placed on horizontal attenuated total reflectance (HATR) accessory with zinc selenide (ZnSe) crystal (45 deg. Trough Plate) and covered with a lid to prevent evaporation of alcohol. 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 64. All spectra were collected within the range of 4000-650 cm^{-1} wave number. The sampling crystal was first cleaned with a toothpaste then using ethanol and distilled water and finally dried under nitrogen flow. The measurements were repeated at least three times.

3.3. Statistical Analysis

Spectral data obtained with an FT-IR spectrometer were analyzed by multivariate statistical techniques with SIMCA software (SIMCA P-10.5 Umetrics Inc. Sweden). Whole spectrum and also three useful spectral intervals selected to avoid interferences and noises were used in the data analysis. These selected spectral regions were 965-1565 cm^{-1} , 1700-1900 cm^{-1} and 2800-3040 cm^{-1} (Lachenmeir, 2007; Versari, et al. 2010).

3.3.1. Pre-Treatment of the Data

Pre-processing of Rakı spectra obtained from FTIR can be very complicated to use in multivariate data analysis. Therefore, complex data is generally standardized by subtracting their averages and dividing by their standard deviation. Spectral data set was firstly transformed into suitable form by pre-processing techniques of scaling and centering, Rakı samples constitute the columns and the variables (spectral data) are located in the rows (1043 selected wave numbers) (Kettaneh, et al. 2005). Pre-processing can be improved by using mathematical procedures including signal corrections, some various derivative forms and using some filtering techniques such as wavelet compression of spectra (WCS), wavelet in combination with orthogonal signal correction (WOSC) and orthogonal signal correction in combination with wavelet (OSWC) (Casela, et al. 2010). For obtaining better classification with PCA, wavelet compression of spectra (WCS) was preferred as spectral filtering technique in this study. Daubechies-10 function wavelet was chosen and confidence interval was set to 99.95%. De-noise and sophisticated signals are compressed with WCS technique which depends on retaining the significant coefficients from the representation of the data in the new ordinate system. For PLS, OSWC technique was applied (Eriksson, et al. 2000; Patz, et al. 2004).

3.3.2. Classification of Pure Rakı

PCA of FT-IR spectral data was used to see if classification of pure Rakı samples is possible according to processing (double or triple distillation) and/or raw material (fresh or dried grapes) differences. Spectral data of pure and methanol adulterated Rakı samples were also analyzed by PCA for classification. The data matrix consists of observations represented by Rakı samples and variables represented by FT-IR spectral data. The classification method is used to determine the group of Rakı samples based on the similarities among member of the same data class. (Pontes,et al. 2006). Principal component analysis (PCA) is one of the most preferred methods to reduce the dimensionality of the original data matrix and provide a new set of variable obtained as a linear combination of the original features by determining the sufficient number of principle components (PCs) which explain the highest variance with a

minimum loss of information (Brown et al. 2009; Karouri, et al. 2010). Assignment 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).

Score and Coomans' plots were created as part of classification analysis. If a grouping was observed in score plot then Coomans' plot was also used. The Coomans' plot compares the distance to the model results against the distance from the model center for unknown samples of selected models (Esbensen, et al. 2002). Coomans' plot comprises of four regions, two regions specify pure and adulterated classes defined by the models, common region indicates overlap of the two classes, and the other region indicates the observations far from two classes.

3.3.3. Quantification

PLS regression was applied to pure Rakı samples to quantitate some of their chemical parameters using whole spectral range. This method was also employed to determine adulteration rate of methanol adulterated Rakı using both whole and selected spectral ranges. PLS analysis was performed to establish a regression model for prediction of analytical results of chemical parameters (variable matrix Y) based on FTIR spectral data (variable matrix X) and the second regression model was performed for prediction of adulterant concentration (variable matrix Y) using FTIR spectra (variable matrix X) (Brown, et al. 2005; Eriksson, et al. 2000; Versari, et al. 2010).

PLS is based on a linear model and used as a satisfactory solution in numerous cases where a linear relationship exists between the spectra and chemical parameters of Rakı samples (pH, brix, total phenol content, electrical conductivity and sugar content) as well as enabling the prediction of authenticity of Rakı samples.

Obtained data sets were randomly separated into two groups as calibration (2/3 of samples) and validation (1/3 of samples) set. The predictive ability of the models were expressed by some parameters and visualized with prediction plots of created models. These parameters are root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), residual predictive deviation (RPD) and the regression correlation coefficient (R^2) both for calibration and validation models. The regression coefficients R^2 expresses how close the relationship between prediction (FTIR predicted value) and the response variation (referenced results of the chemical

parameters). The closer and higher R^2 values for both calibration and validation model, the better the relationship between referenced and predicted values. RMSEC and RMSEP values are used to evaluate performance in the prediction process. RMSEP is a measurement of the average differences between the predicted and reference referenced values at the validation step. Similarly RMSEC refers to the calibration uncertainty that can be expected for predictions. A good model would have small value of RMSEC and RMSEP. Assessing the accuracy of model can be also expressed using RPD value. Generally evaluating all these parameters gives an idea about the predictive efficiency of the model (Bauer, et al. 2008; Brereton, 2003; Esbensen, et al. 2002; Tamaki, et al. 2011).

R^2 , RMSEC, RMSEP, and RPD are defined with following equations:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n-2}} \quad (3.1)$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n-1}} \quad (3.2)$$

$$RPD = \frac{SD}{RMSEP} \quad (3.3)$$

$$SD = \sqrt{\frac{\sum_{i=1}^n (y_i - \bar{y})^2}{n-1}} \quad (3.4)$$

Where n is the number of samples used in each set; SD is standard deviation in each set, y_i is the referenced value obtained from analytical methods for the i -th sample, \hat{y}_i is the predicted value determined by FTIR for the same sample and \bar{y} is the mean of each set (Tamaki, et al. 2011; Pizarro, et al. 2011; Saeys, et al. 2005; Zornoza, et al. 2008).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. FT-IR Spectral Data

Figure 4.1 shows the typical spectra of the Raki samples obtained in this study. All pure samples have similar spectra; however, there are changes in the intensity of some peaks depending on the sample. Owing to differences between peaks, Raki classification was performed with a chemometric technique. FTIR spectra show absorption bands at different frequencies and these bands are attributed to the various functional groups. The two distinct absorption bands around 3600-3200 and 1700-1565 cm^{-1} are associated with O-H stretching vibrations. The peak around 1636 cm^{-1} corresponds to C=C stretch. The peak at 3040-2800 cm^{-1} is due to the stretching vibration of C-H bond. The peaks between 1565 and 965 cm^{-1} are in the fingerprint area where many different IR bands, including those corresponding to the vibrations of the C-O, C-C, CH₂, CH₃, C-OH, C-H and C≡N bonds occur. The region from 4000-3600 cm^{-1} and the spectral range from 965 to 650 contain very little useful information (Fernandez, et al. 2007; Ismail, et al. 1997; Lachenmeier, et al. 2007). IR spectral peaks are related to the bonds in the compounds, therefore, could be correlated with the composition of a specific food sample such as its phenol, alcohol, aldehyde, higher alcohol, polyol, acid, sugar, volatile acid and amino acid content (Lee, et al. 2009). In addition to whole spectral range, a selected range (1565-965 cm^{-1} , 1900-1700 cm^{-1} , and 3040-2800 cm^{-1}) was also used in statistical analysis to reduce noises by eliminating areas with no peak and excluding –OH peaks which were very dominant.

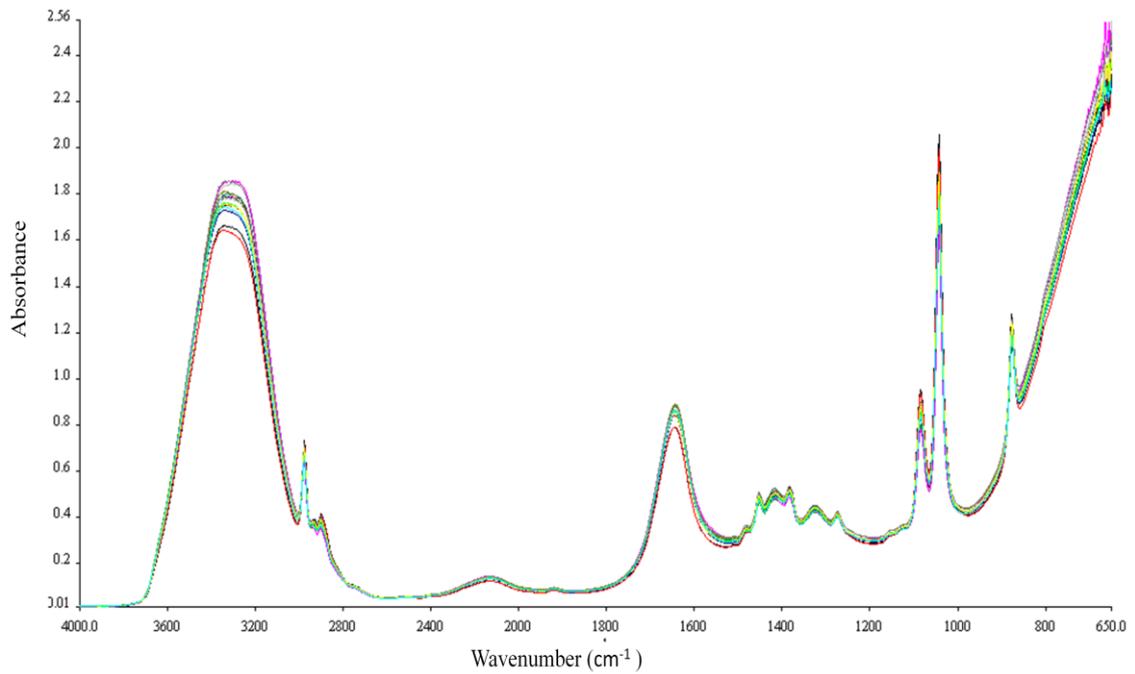
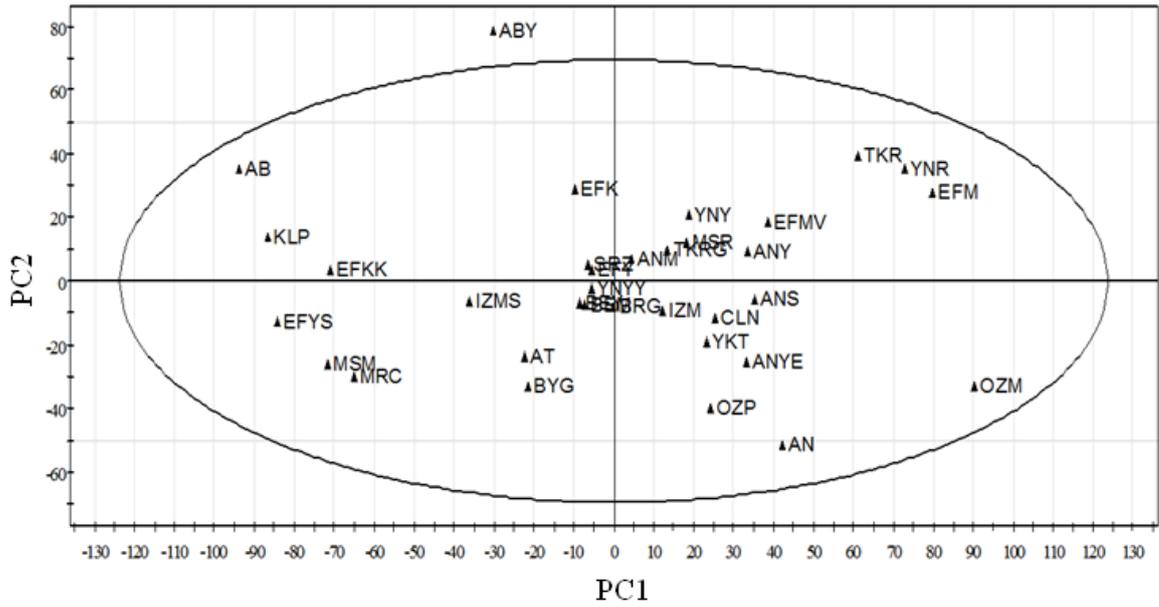


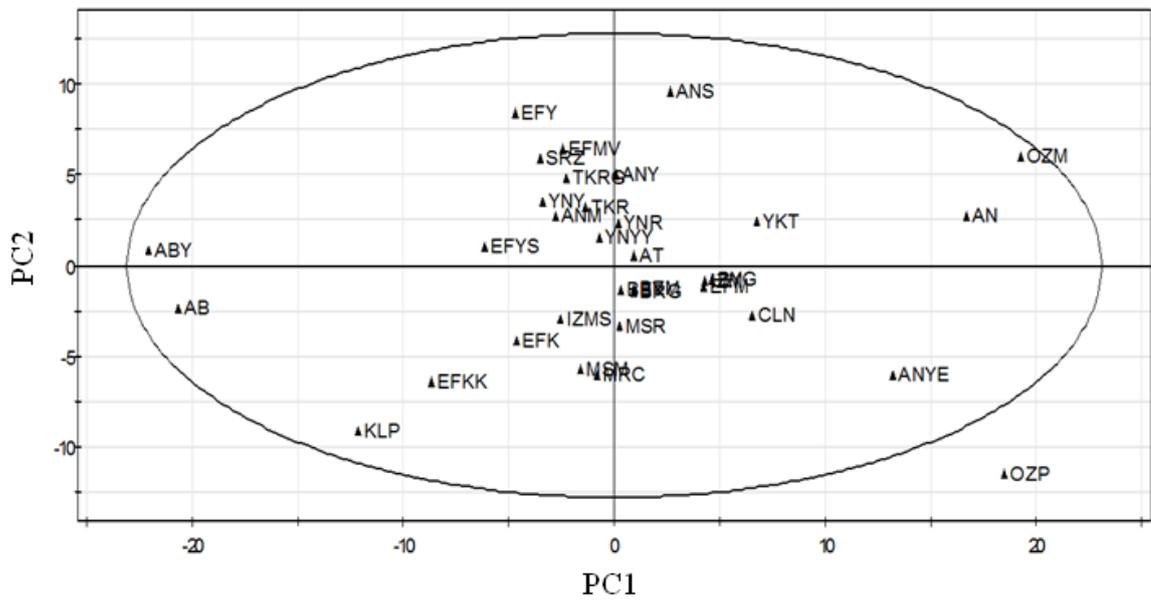
Figure 4.1. Typical FT-IR data spectra of all Rakı samples

4.2. Classification of Pure Rakı Using FT-IR Data

Rakı could be produced from fresh, dried or fresh and dried grapes. Distillation could be applied twice or three times during production. In addition, only suma or suma mixed with ethanol from agricultural sources could be used in Rakı production. Therefore, first aim in this study was to investigate if a classification is possible for Rakı samples according to their raw materials or type of production. For this purpose, PCA was used for the analysis of mid-IR spectroscopic data. Whole spectra or the selected range of spectra were used for the data analysis. Also, PCA was run with and without using spectral filter WCS. Both whole spectral and selected spectral ranges produced similar score plots. Score plots for the classification of Rakı for whole spectral range are shown in Figure 4.2 a and b. Figure 4.3.a and b show the score plots for the selected range.



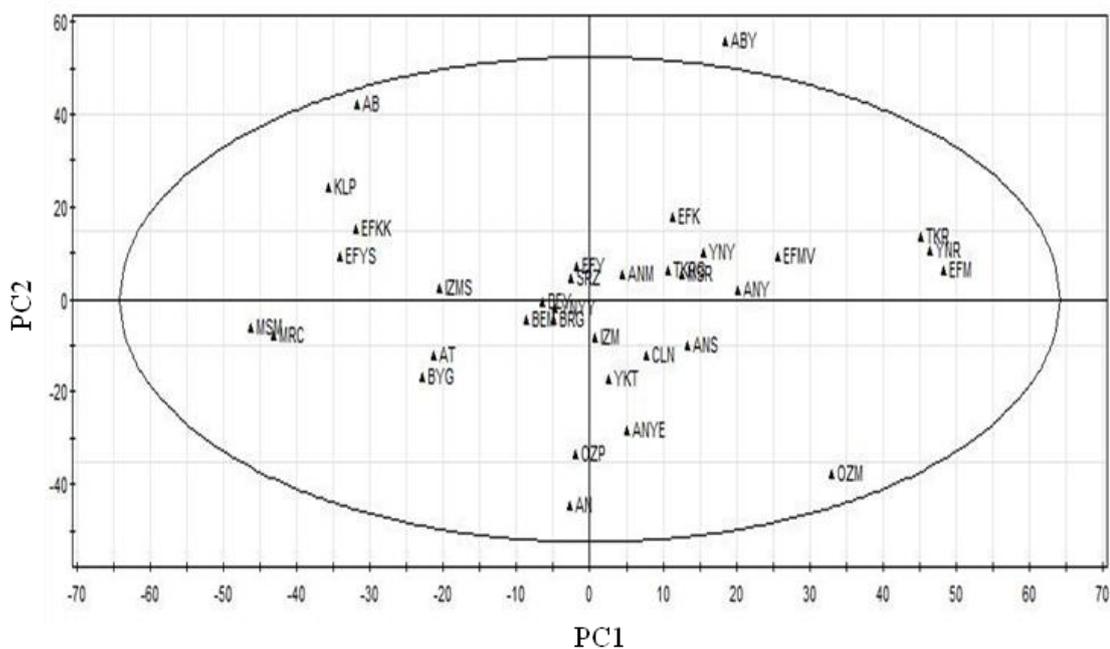
a.



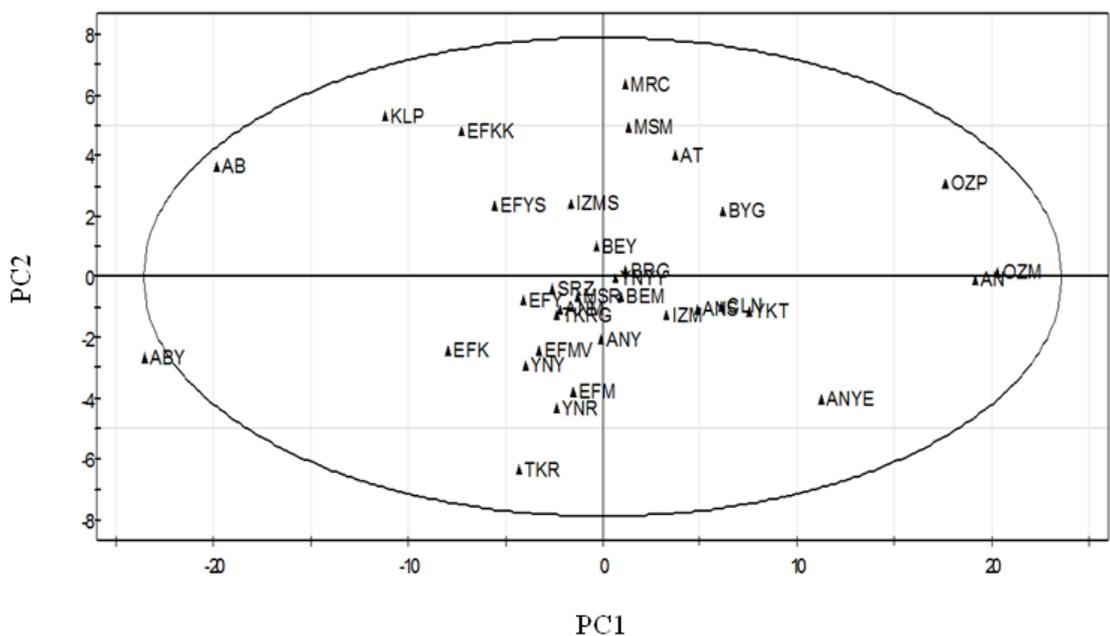
b.

Figure 4.2.a. Score plot based on FT-IR spectra of pure Rakı samples (Whole spectral range; $4000\text{-}650\text{cm}^{-1}$)
 b. Score plot based on WCS scaled FT-IR spectra of pure Rakı samples (Whole spectral range; $4000\text{-}650\text{cm}^{-1}$)

To build the model in Figure 4.2.a, 7 PCs were used and the first two components, PC1 and PC2, explained 88.61% of the variance whereas in Figure 4.2.b, 16 PCs explained total variance and the first two explained 66.16% of variance.



a.



b.

Figure 4.3. a. Score plot based on FTIR spectra of pure Raki samples (Selected ranges $965\text{-}1565\text{ cm}^{-1}$, $1700\text{-}1900\text{ cm}^{-1}$, and $2800\text{-}3040\text{ cm}^{-1}$)
 b. Score plot based on WCS FTIR spectra of pure Raki samples (Selected ranges $965\text{-}1565\text{ cm}^{-1}$, $1700\text{-}1900\text{ cm}^{-1}$, and $2800\text{-}3040\text{ cm}^{-1}$)

The first two components explained 97.13% of total variable variations in the model developed using the selected range without any filtering and the number of PCs used in classification was 5, while 9 PCs explained total variance for model with WCS filtered selected range and the first two components explained 92.64% of variance.

Score plots do not show any discrimination with regard to either raw material or production type. However, groupings according to alcohol content of Rakı were observed. AB, ABYE and KLP have alcohol contents of around 50%. These samples are located in the upper or left side of score plot. EFK and EFKK have 47% alcohol and are located closest to AB, ABYE and KLP compared to other samples. The differences between AB and KLP may arise from their anethole content since AB has more anethole than KLP (Gözen, 2005). Therefore, spread among the samples having the same alcohol content could be attributed to different levels of these volatile compounds. Samples having lower alcohol level (38-40%) OZP, OZM, AN, and ANYE are in the lower or right side of the plots. Most of the Rakı samples have around 45% alcohol and they are in the middle of the score plot while Rakı samples (YKT, ANS, CLN) having 43% alcohol are between middle and right or lower end. The concentration of some volatile compounds (high alcohols, ester, aldehydes) formed in fermentation is more for Rakı samples having high alcohol level compared to lower alcohol containing Rakı samples, this also could explain the distribution of Rakı samples in score plot (Gözen, 2005). According to the Turkish Food Codex (2005), Rakı is produced only from suma or suma mixed with agricultural ethanol. Although AB, ABYE, KLP, TKR, TKRG are produced only from suma, YNY, YNR, YNYY, EFK, EFKK, EFM, EFMV, EFY, EFYS, BRG are the samples having suma and agricultural ethanol (Mey Rakı, 2011; Efe Rakı, 2011). Rakı produced only from suma have higher content of alcohols and total volatile content than the Rakı produced from suma and agricultural ethanol, this could also has an effect on distribution in the score plot. In the study of Cabaroglu, et al. (2011), Rakı samples were analyzed for their volatile contents with GC-MS-FID and significant differences were observed between Rakı produced from only suma or suma mixed with ethanol using PCA. When comparing these two types of Rakı; total volatiles, methanol, trans-anethole, estragole and sugar values were found higher in the samples made from only suma.

4.3. Results of Chemical Analysis

Several analytical parameters of various Rakı samples produced in Turkey were measured in this study. Having different grape varieties as the raw material, distillation process and alcohol content lead to different chemical properties. All the results with regard to the determination of pH, brix, sugar content, total phenol content and electrical conductivity of Rakı samples are listed with mean values and standard deviations in Table 4.1

pH value of Rakı samples ranged from 5.63-8.97 but most samples have pH values around 6. However, pH values of OZP and IZMS were higher compared to others and SRZ had the lowest pH value of 5.63. Differences in the raw materials (grape varieties, fresh or dried grapes), process conditions, volatile compounds and additives can cause the differences among the pH values. IZMS is produced like other Turkish Rakı samples except adding mastic, therefore, the high pH value can arise from mastic. Ouzo types were produced with a mixture of ethanol, sugar, different anise and other flavorful seeds, also volatile compounds released during fermentation are different (Anlı, et al. 2010). Hence, these differences might also affect pH value.

Brix values of tested samples differed between 14.09 and 17.63. Use of different grape varieties could be one of the sources of variation.

Electrical conductivity results of Rakı samples ranged from 5.05 to 155.46 $\mu\text{S cm}^{-1}$, and the largest variance of the values belonged to OZP. Measuring electrical conductivity was indicated as a practical method to identify fraud distilled spirits (Lachenmeir, et al. 2008). The conductivity of Rakı is related to its content of inorganic anions. Co-linearity between the anionic content of spirit and its conductivity contribute to detection of the Rakı authenticity. The conductivity of spirits is very stable between different batches of bottle filling of the same brand. However, it shows large differences between different brands. Cheap and counterfeit factitious distilled spirits have higher conductivity than original products. During production process, a specific water treatment or different additives for the adjustment of alkalinity (e.g. alkalisation with sodium bicarbonate, neutralization with 0.1M hydrochloric acid) can affect the conductivity, which also enhance taste (Jones, et al. 2002; Lachenmeier, et al. 2008).

Total phenol content results of Rakı samples were between 36 and 122.5 mg gallic acid/L. Compared to distilled or only fermented spirits, total phenol values of

Rakı samples are lower (Ávila-Reyes, et al. 2010; Goldberg, et al. 1999; Hosry, et al. 2009). Phenolic compounds play an important role in some alcoholic beverages such as wine since they contribute to their organoleptic characteristics such as colour, astringency, bitterness, and aroma, also phenolic compounds have antioxidant properties (Boulton, 2001; Go´mez-Alonso, et al. 2007). A large difference from most of other spirits is that Rakı produced with two distillation processes, thus it is colorless and contain less phenolic compounds.

The results of sugar content (in terms of amount of sucrose) of Rakı samples ranged from 2410 to 24097 ppm. Sucrose is added to Rakı during processing and these significant differences may be resulted from added sucrose amount as well as the grape varieties used in production. Only OZP and OZM contained more than 10 g sugar. On the other hand, sugar contents of other Rakı samples were found below the limits specified in the Turkish Food Codex (2005).

Table 4.1 The chemical parameters of Rakı samples

Rakı Code	pH	Brix (%)	Electrical Conductivity ($\mu\text{S cm}^{-1}$)	Total Phenol Content (mg gallic acid/L)	Sugar* Content (ppm sucrose)
AB	6.74±0.69	17.59±0.07	5.55±0.19	65±0.005	8095
AN	6.40±0.53	14.86±0.08	13.25±0.20	72.91±0.031	3376
ANM	6.79±0.20	16.14±0.21	11.8±0.14	59.37±0.015	5141
ANS	6.63±0.38	15.87±0.15	10.6±0.42	53.12±0.014	5371
ANY	6.70±0.34	16.17±0.20	9.4±0.28	60.62±0.01	4760
AT	6.64±0.35	15.94±0.26	5.05±0.07	84.37±0.02	2410
BEM	6.33±0.028	16.27±0.075	8.75±0.07	91.87±0.01	4199
BEY	6.15±0.63	16.02±0.15	6.95±0.07	76.25±0.008	2630
BYG	7.34±0.007	15.64±0.09	8.75±0.21	62.5±0.01	2795
BRG	6.78±0.39	16.09±0.08	14.95±0.21	106.87±0.02	2958
CLN	6.94±0.54	15.72±0.15	16.05±0.63	83.12±0.025	5067
EFKK	6.63±0.71	17.09±0.06	19.92±0.57	101.25±0.01	9954
EFK	6.19±0.08	16.69±0.02	22.85±0.49	50±0.004	5118
EFM	6.08±0.12	16.24±0.04	18.25±0.35	80.41±0.01	4693
EFMV	6.55±0.49	16.15±0.15	12.7±0.37	66.25±0.01	4125
EFY	6.58±0.46	16.21±0.06	12.17±0.38	68.75±0.003	4395
EFYS	6.40±0.53	16.31±0.04	12.5±0.14	85±0.018	4843
IZM	6.09±0.34	16.02±0.14	8.2±0.21	81.25±0.02	6297

(Cont. on the next page)

Table 4.1. (Cont.)

Rakı Code	pH	Brix (%)	Electrical Conductivity ($\mu\text{S cm}^{-1}$)	Total Phenol Content (mg gallic acid/L)	Sugar* Content (ppm sucrose)
IZMS	8.04±0.07	16.43±0.15	64.4±1.97	80.62±0.01	7670
KLP	7.12±0.014	17.58±0.11	5.95±0.07	64.37±0.007	8734
MRC	6.86±0.035	16.33±0.04	14.55±0.49	47.5±0.007	5535
MSM	6.89±0.014	16.34±0.07	9.9±0.14	36.25±0.004	8052
MSR	6.59±0.007	16.40±0.07	10.15±0.21	75.62±0.008	7767
OZM	6.055±0.16	15.12±0.33	5.25±0.49	44.37±0.008	14708
OZP	8.97±0.64	16.18±0.014	155.46±8.5	40.62±0.006	24097
SRZ	5.63±0.049	16.41±0.02	14.75±0.77	122.5±0.02	7282
TKR	6.51±0.21	16.31±0.15	10.7±0.28	116.87±0.03	7111
TKRG	6.87±0.09	16.45±0.04	17.05±0.49	73.12±0.007	3286
YKT	6.79±0.48	15.84±0.09	9.35±0.35	57.5±0.007	5970
YNR	6.50±0.47	16.30±0.08	7.7±0.2	74.5±0.01	5977
YNY	5.93±0.23	16.40±0.007	7.05±0.07	41.25±0.0007	6277
YNY Y	6.57±0.44	16.37±0.04	6.4±0.14	49±0.007	5932
ABY	6.66±1.32	17.63±0.01	6.9±0.07	68±0.0035	7974
ANYE	6.06±0.31	14.09±0.02	13.25±0.07	45±007	3388

*HPLC measurements are done only once, so there are no standard deviations among Rakı samples for sugar content.

4.4. Prediction of Quality Parameters of Rakı Samples from FTIR Spectra

Determination of chemical parameters of distilled spirits ensures not only controlling of production process but also producing optimal characteristics and a high quality product (Lachenmeier, 2007; Patz, et al.2006). Although several measurement techniques such as GC, HPLC, VIS, NIR, NMR, ICP and AAS have been used in different studies to obtain information on the chemical composition and to detect the changes in the chemical composition of the product, FTIR is emerging as an alternative tool for the determination of the quality parameters in production control, quality management and authenticity assessment due to its advantages of being a fast, accurate and convenient method (Bauer et al. 2008; Fernandez, et al. 2007; Lachenmeier, 2007; Saurina, 2010; Soriano, et al. 2007; Patz, et al. 2004; Pontes, et al. 2006). FTIR spectroscopy was utilized in this study to determine the several quality parameters of Rakı samples within a short time. FTIR spectroscopy, combined with PLS was used to

develop calibration models based on full infrared spectral data. By using PLS regression technique, the relationship between FTIR data and the results of chemical analysis were established; and the models were also validated using a separate data set.

All the chemical results including pH value, brix value, electrical conductivity, total phenol content and sugar content were analyzed separately. Rakı samples were divided into two groups namely calibration set and validation set. 2/3 of the Rakı samples were randomly selected for calibration models and 1/3 of the samples for validation models. Orthogonal signal correction in combination with wavelet (OSWC) method was used as a spectral filter. This filter improved the model; besides, the model constructed using whole spectra had better results compared to the selected range. Goodness of the PLS regression models was determined by calculating 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). All these parameters are listed in Table 4.2.

Table 4.2. Summary of statistical results for PLS analysis of Rakı samples

Parameter	number of PCs	R^2 (cal)	R^2 (val)	RMSEC	RMSEP	RPD
pH (pH unit)	3	0.986	0.924	0.074	0.220	2.725
Brix (%)	2	0.991	0.985	0.067	0.099	6.464
electrical conductivity ($\mu\text{S cm}^{-1}$)	3	0.989	0.756	3.120	8.307	2.005
Total phenol (mg/L)	3	0.991	0.968	1.965	4.494	5.417
Sugar (ppm)	3	0.986	0.983	523.6	464.7	7.059

Accuracy of the PLS models were assessed using R^2 , RPD and slope of the equations determined for the calibration sets, and Table 4.3 shows the criteria for evaluating the prediction models. In addition to these criteria; the absolute values of RMSEC and RMSEP and differences between them should be small to construct a good model.

Table 4.3. The criteria used for evaluation of the prediction models

(Source: Tamaki, et al. 2011; Pizarro, et al. 2011; Zornoza, et al. 2008; Saeys, et al. 2005)

R ²	between 0.66-0.80 approximate predictions 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 was applied to establish a relation between FTIR data as X variables and chemical parameters obtained by analytical methods as Y variables. Total number of Rakı samples was 34. 23 Rakı samples were randomly selected for calibration set and 11 Rakı samples were used as validation set.

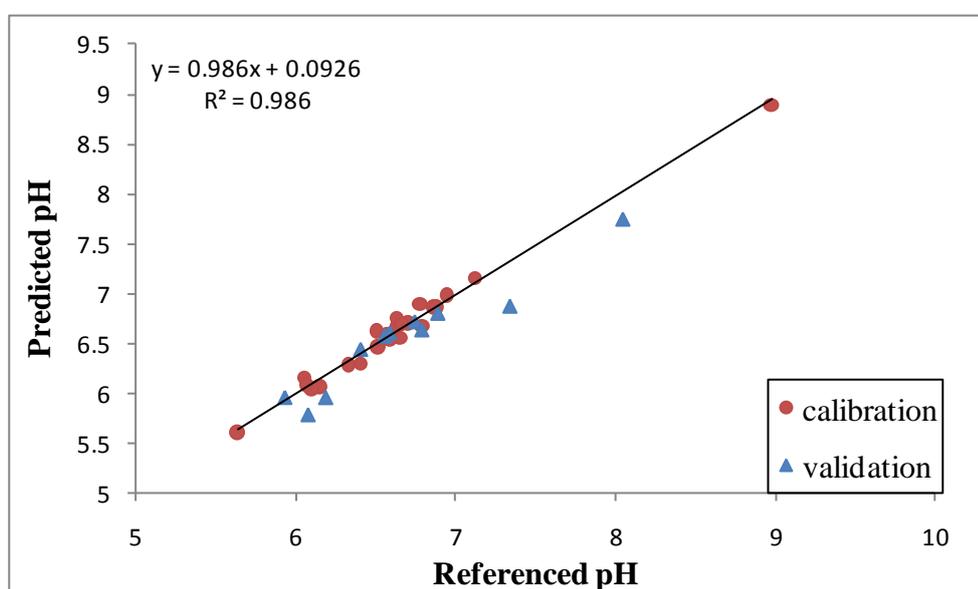


Figure 4.4. PLS regression of referenced vs. predicted pH value of calibration and validation sets

pH value prediction model from FTIR data constructed with PLS regression was explained with 3 PCs (Figure 4.4). The regression coefficient of the model was found as 0.98 for calibration set and 0.92 for validation set. RPD value (2.72) indicated a good prediction. Since slope of the regression line was 0.98 the model is considered as very reliable according to Table 4.3.

PLS analysis for prediction of brix value is shown in Figure 4.5. Highly good regression correlation (R^2 0.99) was obtained using calibration set, and also regression coefficient of validation set was 0.98. Two PCs were enough for the prediction model. Slope of the brix prediction model was 0.99 and RPD value was calculated as 6.46. Therefore, according to all criteria brix model could be considered to provide excellent prediction.

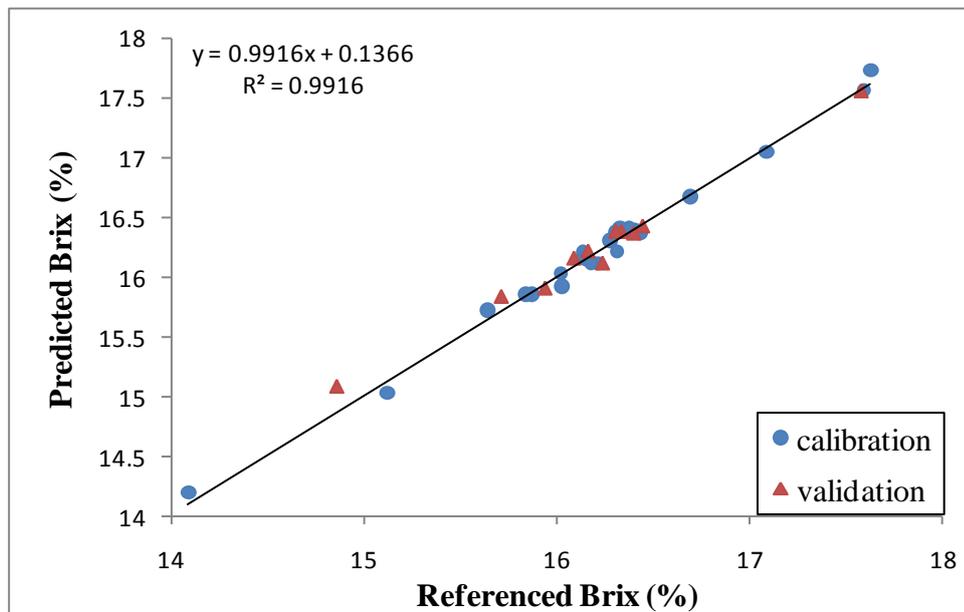


Figure 4.5. PLS regression of referenced vs. predicted brix value of calibration and validation sets

Prediction of electrical conductivity from FT-IR spectral data with PLS analysis was carried out with 3 PCs. Correlation coefficients for calibration and validation sets are 0.98 and 0.75, respectively. RPD value was obtained as 2. Although the slope of the electrical conductivity prediction model was 0.98, the other results indicate a model with sufficient prediction (Figure 4.6). The values of electrical conductivity have big differences between each other, thus small value of RMSEC compared with the value of RMSEP could indicate an overfitting of PLS model.

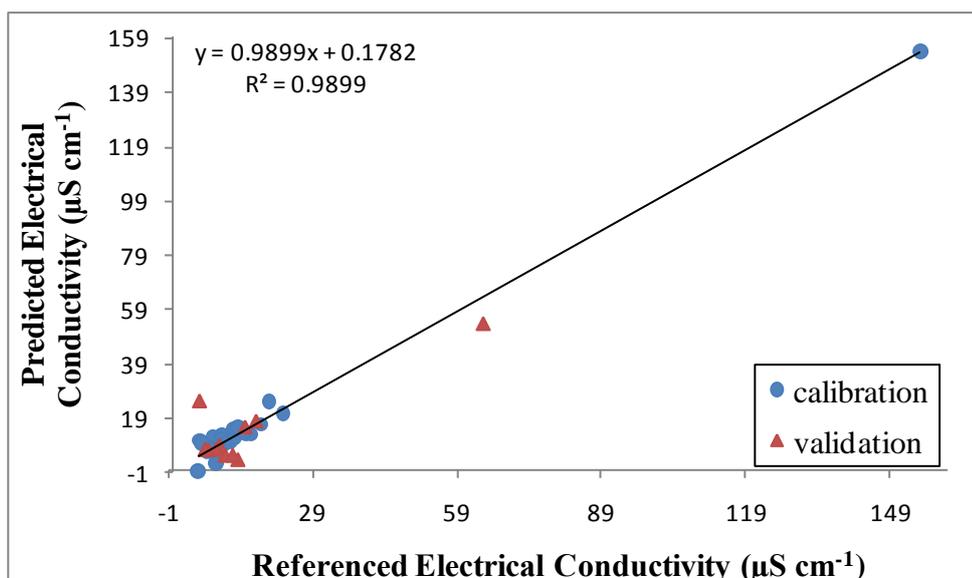


Figure 4.6. PLS regression of referenced vs. predicted electrical conductivity value of calibration and validation sets

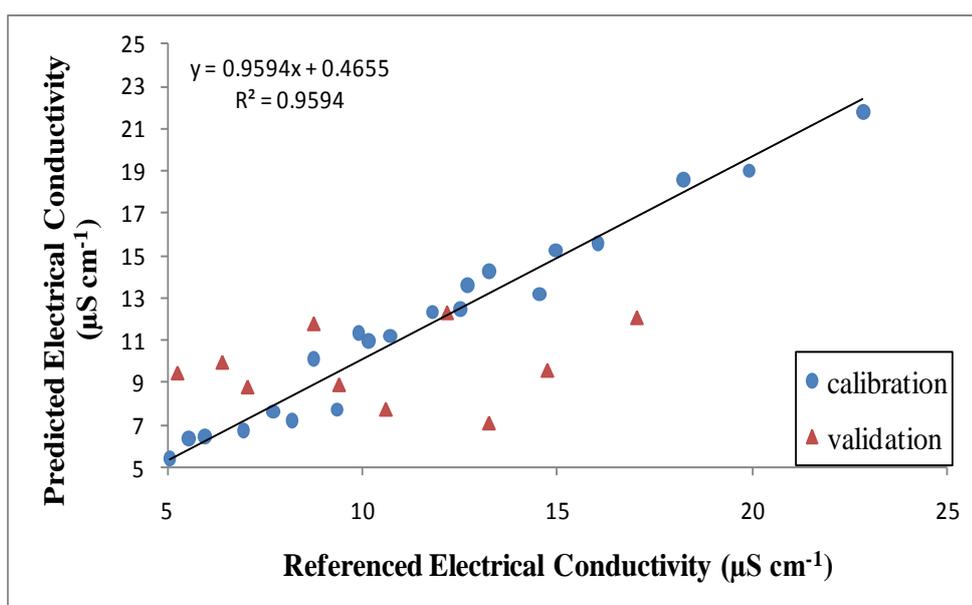


Figure 4.7. PLS regression of referenced vs. predicted electrical conductivity value of calibration and validation sets except for OZP and IZMS

In Figure 4.7, prediction model of electrical conductivity except OZP and IZMS was constructed. As distance from other result, correlation coefficients for calibration and validation sets are 0.98 and 0.24, respectively. RPD value was obtained as 0.96 and slope was 0.95. Also, small value of RMSEC (0.99) and RMSEP (3.95) were calculated. Therefore, the model got worse with the exclusion of two data.

The model created for total phenol prediction composed of 3 PCs. $R^2_{cal} = 0.99$, $R^2_{val} = 0.96$, slope = 0.99, and RPD = 5.41 values indicated a good predictive model for total phenol content according to criteria given in Table 4.3. RMSEC and RMSEP values were not very large and were not very different from each other. Referenced vs predicted curve for total phenol content is provided in Figure 4.8.

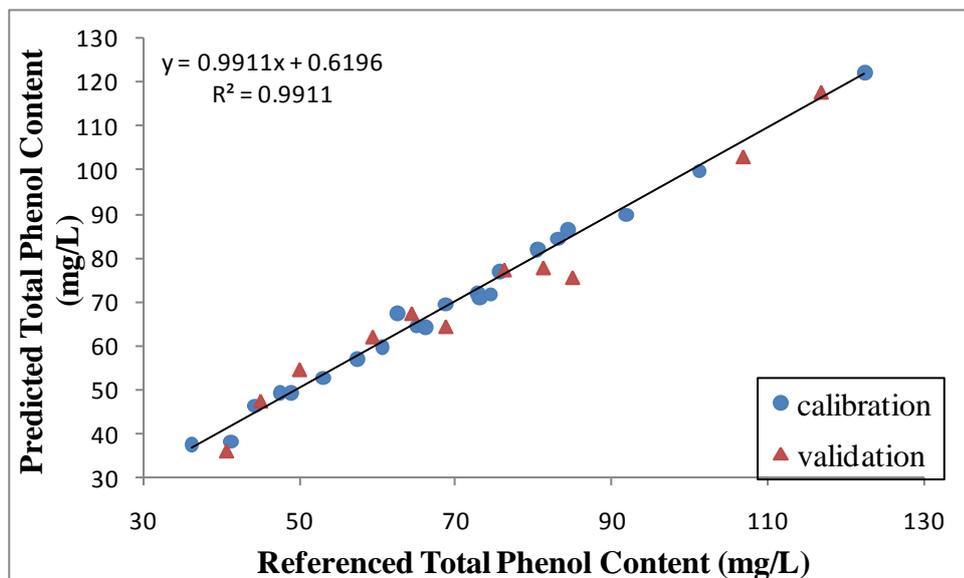


Figure 4.8. PLS regression of referenced vs. predicted total phenol value of calibration and validation sets

Excellent prediction of sugar content was obtained as a result of correlating FT-IR data with values obtained analytically (Figure 4.9). The first criterion of slope for prediction model is 0.98 which is between 0.9-1.1 and indicates a very reliable model. Secondly, R^2_{cal} and R^2_{val} values of prediction model mean high reliability because the results are not only higher than 0.9 but also close to each other. Lastly, according to RPD value of 7.05 which is higher than 3, model has very good prediction.

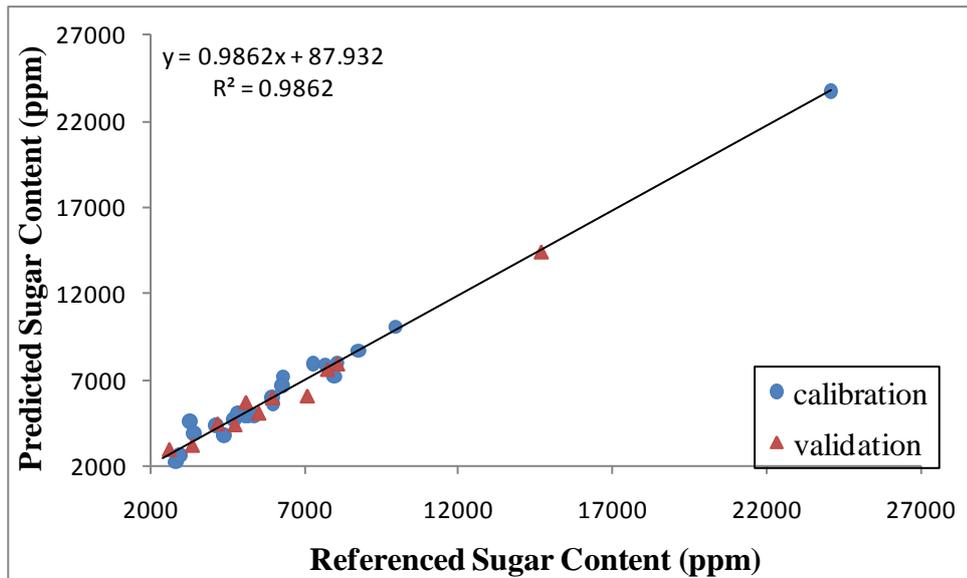


Figure 4.9. PLS regression of referenced vs. predicted sugar value of calibration and validation sets

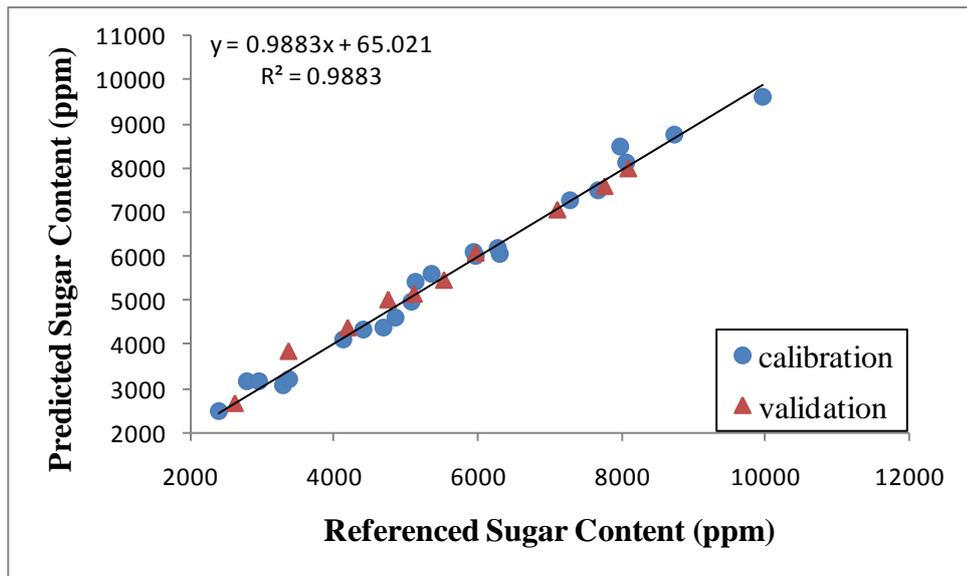


Figure 4.10. PLS regression of referenced vs predicted sugar value of calibration and validation set except for Ouzo types

In Figure 4.10, OZP and OZM, which have higher sugar content than Turkish Rakı, were omitted and new prediction model were constructed. R^2_{cal} (0.98), R^2_{val} (0.99), slope (0.98), RMSEC (228) and RMSEC (200) values showed that the model was better than before.

There are several studies in literature that aim to predict various quality parameters of alcoholic beverages with IR data using chemometric techniques. Pizarro, et al. (2011) applied PLS regression methods for calibration based on full infrared spectra and selected efficient wavenumbers for red wine samples. The high quality of the calibration models were obtained for both total acidity (22 significant wavenumbers) and volatile acidity (11 selected predictor variables). Besides; the study about young red wines from Spain used PLS regression techniques to establish calibration models for several wine quality parameters such as anthocyanin content (Soriano, et al. 2007). Prediction of anthocyanins from FTIR spectra was successful using HPLC as a reference method. In another study, Maderia wines were investigated in terms of their volatile and phenolic composition by using UV-vis, GC-MS and HPLC (Pereira, et al. 2011). The multivariate prediction models for volatile and phenolic composition of wines were established with PLS regression and they had good accuracies according to RMSEP criterion. Also Fernandez, et al. (2007) investigated tannin concentration of the red wines by using FT-IR spectroscopy combined with PLS regression analysis. Accurate calibration model for tannin concentration was obtained using protein precipitation and phloroglucinolysis as analytical reference methods (RMSEC = 2.6%, RMSEP = 9.4%, $r = 0.995$). Furthermore, FTIR spectra and PLS method were proposed as the quality control and authenticity assessment tools for spirit drinks and beer (Lachenmeier, 2007). Strong correlation with reference methods and great accuracy were found for spirit parameters like density, ethanol, methanol, ethyl acetate, propanol-1, isobutanol and 2-/3-methyl-1-butanol contents ($R^2 = 0.90-0.98$), for beer parameters of ethanol content, density, original gravity and lactic acid content ($R^2 = 0.97-0.98$). On the other hand, beer parameters such as pH, bitterness unit, and EBC colour ($R^2 = 0.63-0.75$) showed lower correlation and accuracy. In another study, wine quality parameters of alcohol, relative density, extract, sugar-free extract, refraction, conductivity, glycerol, total phenols, reducing sugar, fructose, glucose, sucrose, total acid, pH value, volatile acid, total SO₂ and tartaric acid, malic acid, lactic acid and citric acid were tried to be predicted from IR data using PLS regression in the study of Patz, et al (2004). Constructed models have good prediction with high regression correlation coefficient and appropriate RMSEP values.

Partial least squares (PLS) regression technique using FTIR spectral data ensured successful results for prediction of several chemical parameters of Rakı. As a result, a relationship between FTIR spectra and some chemical analyses was obtained;

therefore, desired chemical parameters could be determined in a short time using FTIR as an alternative method.

4.5. Adulteration of Rakı

In order to detect methanol adulteration of Rakı and also to determine the methanol content of adulterated Rakı, PCA and PLS methods were applied in combination with FTIR spectral data. Figure 4.11 shows the mid-IR spectral differences between adulterated and pure Rakı. As can be seen from spectra, distinctness arising from methanol content could be determined by comparing several regions. The first big difference corresponds to region around 3500 to 3200 cm^{-1} that can be attributed to O-H stretching. Other peak at 2900-2800 cm^{-1} region arises from C-H stretching and its source could be methyl groups. The other distinction between pure and adulterated Rakı is in the fingerprint area (between 1565 to 965 cm^{-1}), and this difference could be attributed to C=C stretch (Fernandez, et al. 2007; Ismail, et al. 1997; Lachenmeier, et al. 2007).

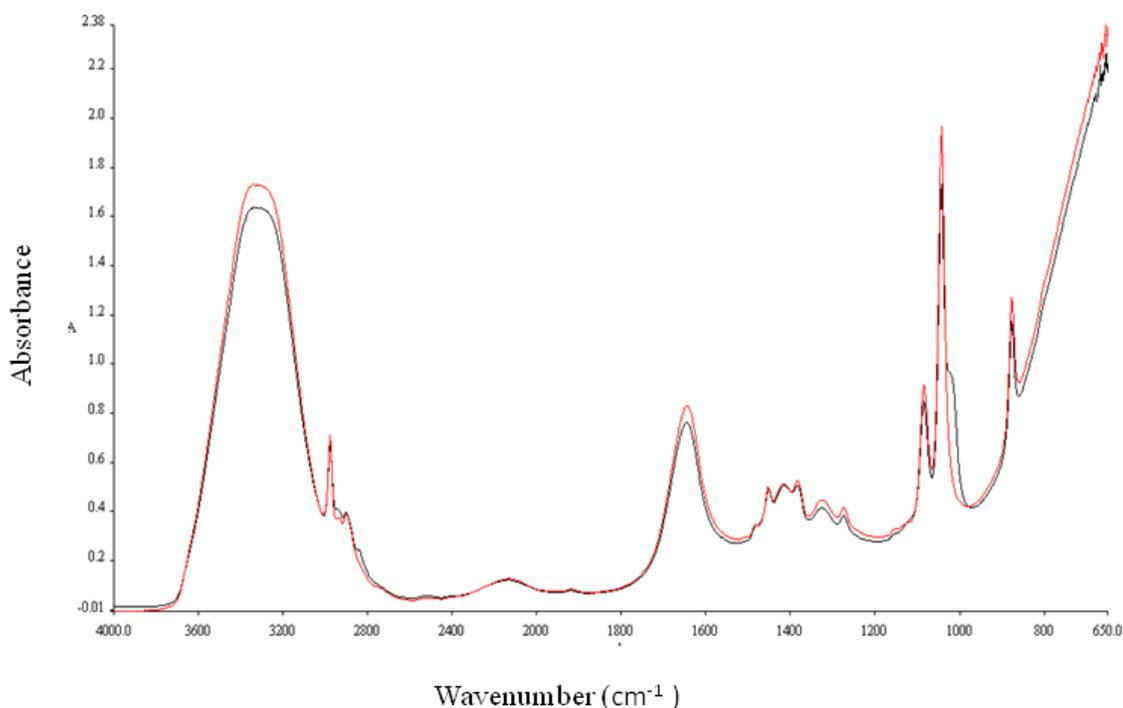


Figure 4.11. Typical FT-IR data spectra of pure and adulterated Rakı (red color represents pure Rakı, black one represents adulterated with the level of 10% of methanol)

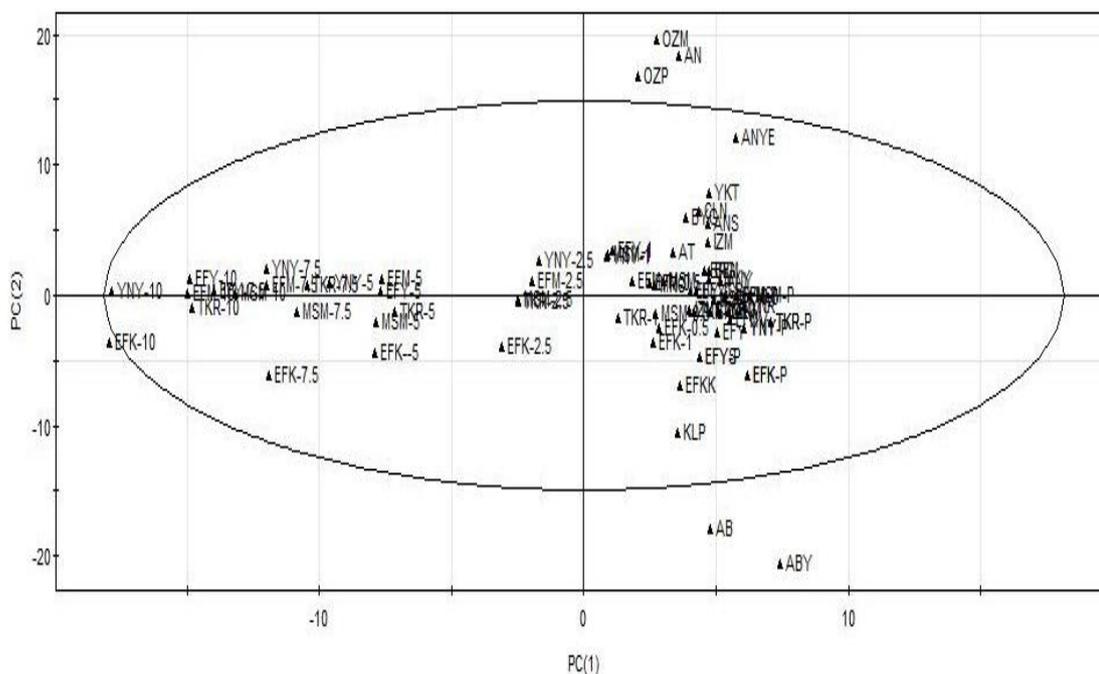


Figure 4.13. Score plot based on WCS FTIR spectra of pure Rakı samples (selected ranges 1565-965 cm^{-1} , 1900-1700 cm^{-1} , and 3040-2800 cm^{-1})

Coomans' plot for the classification of pure and adulterated Rakı samples shows good separation of adulterated Rakı with the ratio of 0.5 to 10% from pure samples (Figure 4.14). Using FTIR spectroscopy combined with a chemometric technique was successful in detecting the adulteration with methanol at levels 0.5% and above (Figure 4.14). By using whole spectral range (4000-650 cm^{-1}); 12 PCs and 16 PCs were used to construct class models. The differentiation of pure and adulterated Rakı samples can be seen clearly as shown in Figure 4.14. Pure Rakı samples colored with black were located in the upper side of the plot and separated from adulterated Rakı samples with red color and there were no samples located in the common region. In addition, samples according to their adulteration ratio grouped together. Detection of adulteration in alcoholic beverages was investigated in some studies. In the study of Pontes, et al. (2006); 69 samples of alcoholic beverages (whiskey, brandy, rum and vodka) were investigated to verify adulteration with water, ethanol or methanol by using near-infrared spectroscopy and chemometric methods. Another studies by Hang, et al. (2008) investigated the methanol levels in the grappa which is a traditional Italian spirits. They measured the methanol, ethanol, higher alcohols and volatile acids by HPLC. The results showed that five New York grape varieties used in the production of grappa with a methanol level in the United States legal limit.

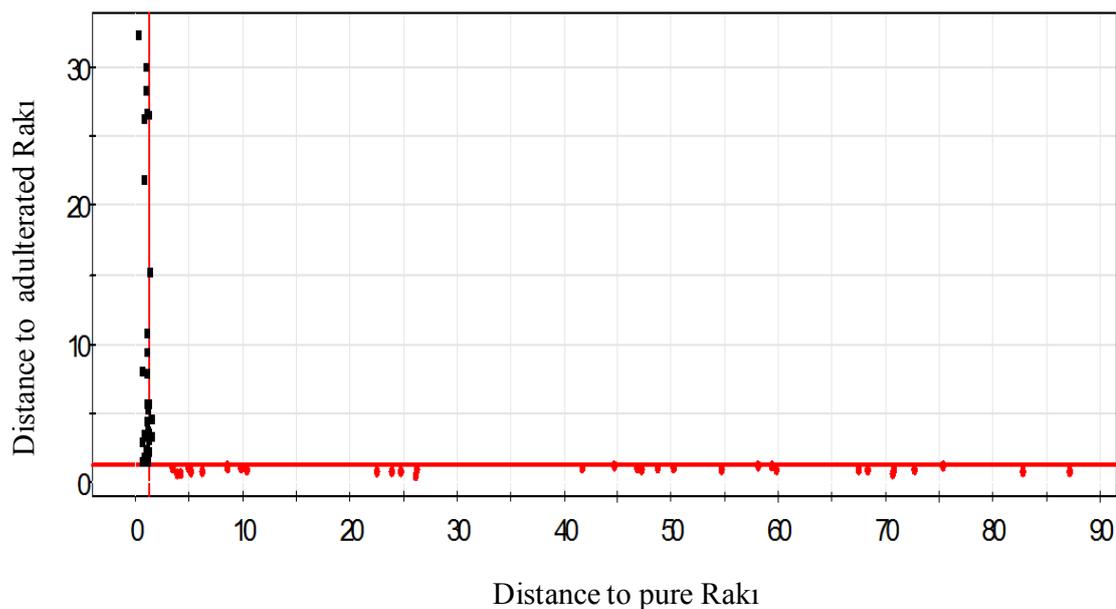


Figure 4.14. Coomans' plot for the classification of pure and adulterated Rakı samples using spectral FT-IR data (pure samples and adulterated Rakı samples are represented with black and red colors, respectively) (whole spectral range; $4000\text{-}650\text{cm}^{-1}$)

4.5.2. Prediction of Adulteration Ratio

FTIR in association with multivariate chemometric techniques was employed to identify Rakı samples adulterated with methanol. Results indicated that PLS model based on IR data was proven suitable as a practical analytical method for predicting adulterant content in Rakı samples in the volume fraction range from 0.5 to 10%. RPD, slope and R^2 values were calculated for calibration and prediction sets to test the accuracy of the model.

The accurate and similar prediction results were obtained by PLS using both whole and selected spectral ranges; for using whole spectral range $R^2_{\text{cal}} = 0.9838$, $R^2_{\text{val}} = 0.9914$, RMSEC = 0.44, RMSEP = 0.35, RPD = 8.35, slope = 0.9 values were obtained and for using selected range $R^2_{\text{cal}} = 0.9865$, $R^2_{\text{val}} = 0.9874$, RMSEC = 0.40, RMSEP = 0.38, RPD = 8.29, slope = 0.99 were found (Figure 4.15 and Figure 4.16).

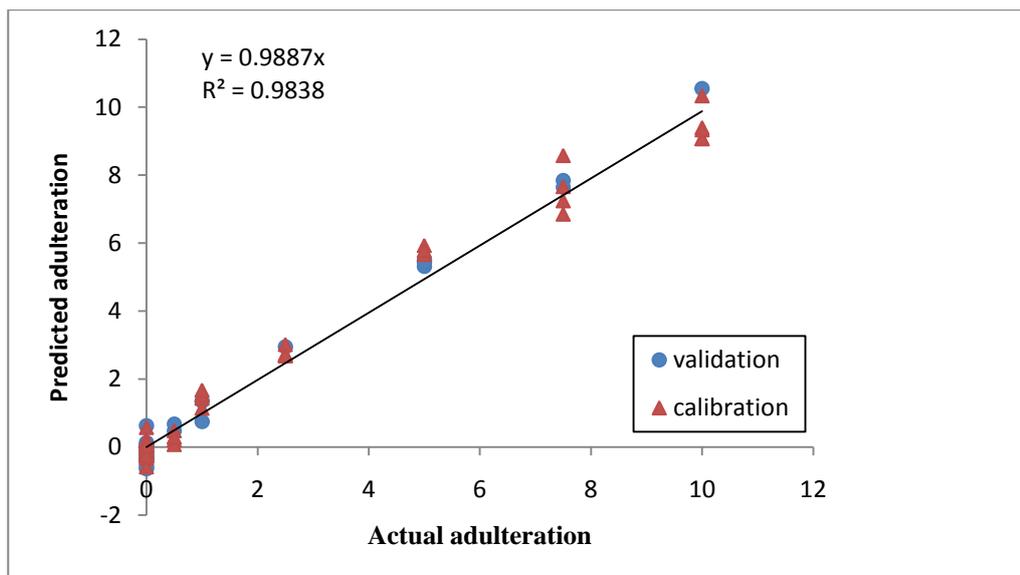


Figure 4.15 OSWC PLS regression of referenced vs. predicted adulteration of calibration and validation sets (whole spectral range; 4000-650 cm^{-1})

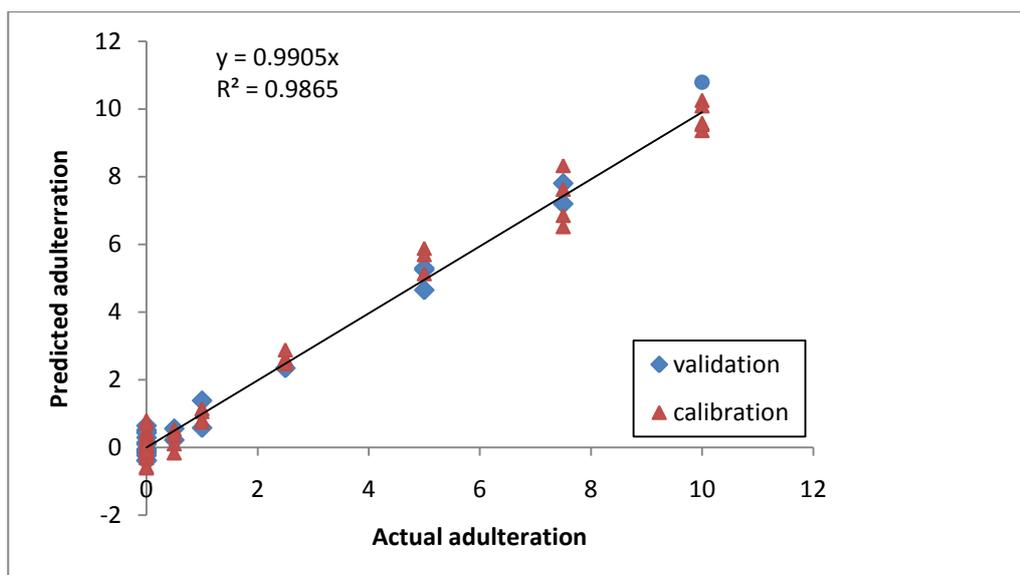


Figure 4.16. OSWC PLS regression of referenced vs. predicted adulteration of calibration and validation sets (selected ranges 1565-965 cm^{-1} , 1900-1700 cm^{-1} and 3040-2800 cm^{-1})

According to comparison of RPD and slope values with Table 4.3, PLS regression of FTIR spectral data provides good prediction for the determination of methanol adulteration ratio for Rakı samples. Besides, small RMSEC and RMSEP values and small differences between these values indicated that the created model provide satisfactory prediction. Consequently, it can be used to predict the adulteration

ratio of new samples quickly. The spectra of new samples only need to be collected and introduced as input into the established FTIR spectral model.

CHAPTER 5

CONCLUSION

In this study, the classification of Rakı samples, detection of methanol adulteration in Rakı and determination of some quality parameters were investigated using FTIR spectroscopy technique combined multivariate data analysis techniques of PCA and PLS.

For classification, a total of 34 pure samples were analyzed with FTIR spectra in combination with PCA. The distinct grouping between Rakı samples could not be acquired according to grape types and production process; however, distribution according to alcohol level was observed because alcohol content has significant influence on the IR spectra.

Distinction between methanol adulterated and pure Rakı samples was established via Coomans' plot. The performance of rate of adulteration detection was successful by using PLS models based on FTIR spectral data. The R^2 , RPD and slope values indicated the accuracy of the model ($R^2 = 98$, RPD = 8.35).

Determination of Rakı characteristics including pH, brix, electrical conductivity, total phenol and sugar content from FTIR spectra was also performed using PLS models by correlating experimental results with FTIR spectra. Excellent correlation coefficient and predictive ability were obtained for brix ($R^2 = 0.99$, RPD = 6.46) and sugar content ($R^2 = 0.98$, RPD = 7.05). Moreover, good prediction of total phenol content ($R^2 = 0.99$, RPD = 5.42) and pH value ($R^2 = 0.98$, RPD = 2.72) were achieved. Although electrical conductivity results ($R^2 = 0.98$, RPD = 2) provide approximately adequate prediction for Rakı.

Considering all the results, this study indicated that using FTIR spectroscopy in combination with multivariate techniques provided useful information related to relevant quality parameters of Rakı, thus prediction of this parameters could be successfully performed in a short time without the necessity of large amount of samples and chemicals, which can be an alternative to commonly used methods in industrial and research application. In addition, FTIR spectroscopy in combination with multivariate

analysis can be an effective and a rapid method to detect the methanol adulteration ratio level at 0.5% for authenticity assessment.

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