Classification of Virgin Olive Oils from Different Olive Varieties and Geographical Regions by Electronic Nose and Detection of Adulteration

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Pınar KADİROĞLU

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İZMİR

We approve the thesis of **Pınar KADİROĞLU**

Assist. Prof. Dr. Figen KOREL
Supervisor

Assist. Prof. Dr. Figen TOKATLI
Co-Supervisor

Assist. Prof. Dr. Banu ÖZEN
Co-Supervisor

Prof. Dr. Şebnem HARSA
Committee Member

Assoc. Prof. Dr. Durmuş ÖZDEMİR
Committee Member

3 January 2008
Date

Prof. Dr. Şebnem HARSA
Head of the Department of
Food Engineering

Prof. Dr. Hasan BÖKE
Dean of the Graduate School of
Engineering and Science

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ABSTRACT

CLASSIFICATION OF VIRGIN OLIVE OILS FROM DIFFERENT OLIVE VARIETIES AND GEOGRAPHICAL REGIONS BY ELECTRONIC NOSE AND DETECTION OF ADULTERATION

Extra virgin olive oils produced from fresh and healthy olive fruits have a delicate and unique flavor that makes them highly appreciated by consumers. Their taste and aroma are closely related to volatile and non-volatile compounds and determined by chromatographic and sensory analyses. However, these methods are expensive and time consuming to be used routinely in food industry. Electronic nose that can mimic the human sense of smell and provide low-cost and rapid sensory information is a new approach allowing the discrimination of aroma fingerprints of oils.

In this study, the aroma fingerprints of Turkish extra virgin olive oils produced from various olive varieties (Ayvalık, Gemlik, Memecik, Erkence, Domat and Nizip) and Ayvalık and Gemlik olive varieties growing in two different regions of West Turkey (İzmir and Edremit) and the commercial extra virgin olive oils obtained from Tariş Olive and Olive Oil Agricultural Sales Cooperatives Union during two consecutive harvest years were determined by an electronic nose. In addition, the electronic nose was proposed for the detection of adulteration of these oils with monovarietal olive oils and with other edible oils such as sunflower, corn, soybean and hazelnut oils. The data were analyzed using chemometric methods by soft independent modeling of class analogy (SIMCA) software.

As a conclusion, it was found that the electronic nose could provide good separation on some of the varieties and geographical regions. The electronic nose has been able to differentiate adulterated and non-adulterated extra virgin olive oils at higher than 10 % adulteration level successfully.

ÖZET

DEĞİŞİK ZEYTİN TİPLERİNDEN VE COĞRAFİ BÖLGELERDEN ELDE EDİLEN SIZMA ZEYTİNYAĞLARININ ELEKTRONİK BURUN İLE SINIFLANDIRILMASI VE TAĞŞİŞİN TESPİTİ

Taze ve sağlam zeytinlerden elde edilen naturel sızma zeytinyağlarının tüketiciler tarafından beğenilen kendisine özgü bir aroması vardır. Bu tat ve aroma birçok uçucu ve uçucu olmayan bileşikle ilişkilidir ve kromatografik ve duyusal analizlerle belirlenir. Fakat bu yöntemler gıda sanayinde rutin olarak kullanılmak için pahalı ve zaman alıcıdır. İnsan koku alma hissini taklit edebilen elektronik burun naturel sızma zeytinyağlarının aroma parmak izlerinin sınıflandırılmasında kullanılabilen düşük fiyatlı ve hızlı yeni bir yaklaşımdır.

Bu çalışmada birbirini takip eden iki hasat yılına ait Ayvalık, Gemlik, Memecik, Erkence, Domat ve Nizip gibi farklı türlerden elde edilen Türk zeytinyağları ile Türkiye'nin batı bölgesinin iki farklı yerinden (İzmir and Edremit) alınan Gemlik ve Ayvalık zeytinlerinden elde edilen zeytinyağları ve Tariş Zeytin ve Zeytinyağı Tarım Satış Kooperatifleri Birliği'nden alınan ticari naturel sızma zeytinyağlarının aroma parmak izleri elektronik burun ile belirlenmiştir. Buna ek olarak elekronik burun, bu yağların diğer naturel sızma zeytinyağları ve ayçiçek, mısır, soya ve fındık yağları gibi diğer yenilebilir yağlar ile tağşişinin belirlenmesi için kullanılmıştır. Elde edilen veriler kemometrik yöntemler ve SIMCA paket programı kullanılarak analiz edilmiştir.

Sonuç olarak, elektronik burunun bazı türler ve bölgeler üzerinde iyi bir ayrım sağladığı belirlenmiştir. Elektronik burun tağşişli ve tağşişli olmayan naturel sızma zeytinyağlarını % 10'un üzerinde bir tağşiş oranı ile başarılı bir şekilde ayırabilmiştir.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

EU	European Union
EVOO	Extra virgin olive oil
OPO	Olive-pomace oil
IOOC	International Olive Oil Council
PDO	Protected Denomination of Origin
NMR	Nuclear magnetic resonans
PCA	Principal component analysis
GC	Gas chromatography
GC/MS	Gas chromatography/Mass spectrometry
HPLC	High performance liquid chromatography
LDA	Linear discriminant analysis
CA	Canonical analysis
PLS	Partial least squares regression
PDO	Protected Denomination of Origin
LOX	Lipoxygenase pathway
HPL	Hydroperoxide lyase
ADH	Alcohol dehydrogenase
AAT	Alcohol acetyl transferase
FTIR	Fourier transform-infrared
NIR	Near infrared spectrometry
FID	Flame ionization detector
SAW	Surface acoustic wave
LLL	Low level of trinolein
ECN	Equivalent carbon number

MS	Mass spectrometry
HCA	Hierarchical cluster analysis
SIMCA	Soft independent modelling of class analogy
ANN	Artificial neural networks
PCR	Principal component regression
SEC	Standard error of calibration
SEP	Standard error of prediction
VOC	Volatile organic compounds
BPNN	Back Propagation Neural Networks
GRNN	General Regression Neural Network

CHAPTER 1

INTRODUCTION

Olive oil is an economically important product in the Mediterranean countries (Aparicio, et al. 1996). According to the recent estimations on olive oil markets, the European Union (EU) produces 78% of the world production followed by Turkey (6%), Syria (6%), Tunisia (3%) and Morocco (2%). The world consumption is dominated by EU (73%) while the rest of the production is absorbed by USA (8%), Japan (1%), Canada (1%) and Australia (1%). Spain, Italy and Greece are main producers with approximately 865, 590 and 375 thousands of tons reached in 2003, respectively (Rezzi, et al. 2005).

The quality of olive oil ranges from the high quality extra virgin olive oil (EVOO) to the low quality olive–pomace oil (OPO). EVOO is obtained from the olive fruit named *Olea europaea*. It is extracted by only mechanical procedure without application of refining process. It is one of the primary ingredients of the Mediterranean diet (Guimet, et al. 2005). Different factors such as cultivar, environment and cultural practices determine the quality and uniqueness of specific EVOOs (Cosio, et al. 2006). International Olive Oil Council (IOOC) have demonstrated the benefits of eating olive oil in cardiovascular diseases (Harwood and Aparicio 2000) and diabetes (Rodríguez-Villar, et al. 2004), as well as in bone and nervous system development (Puel, et al. 2004, Tuovinen 2004). In addition, it has been proved that it has antioxidant and anti-aging properties at cell and mitochondrial levels (Huertas, et al. 1999). Olive oil has also general favorable action on the nutrition and diet (Gómez-Ariza, et al. 2006). The pleasant taste and aroma with the health benefits of EVOO are important reasons for consumers to consume this product (Aparicio, et al. 1996).

One of the agricultural products designated with the Protected Denomination of Origin (PDO) is olive oil. An important European regulation allows the PDO labeling of some European EVOOs and this designation guarantees that the geographical origin of the product is closely in conjunction with the quality of the product (Cosio, et al. 2006). That's why several researches have been performed to characterize and classify olive oils using different techniques in recent years (D'Imperio, et al. 2007, Casale, et al. 2007). Authenticity and quality of olive oils can be often connected with the certain

geographical origin. Therefore, the development of methods for the classification of olive oils is very important (Ballabio, et al. 2006a).

In recent years, several attempts have been performed in order to authenticate the geographical origin of olive oils by appropriate chemical parameters, such as triglyceride and fatty acid profiles or by means of ^{1}H high field nuclear magnetic resonans (NMR) spectroscopy (Mannina, et al. 2001). Chemometrics have been often conducted for the classification and comparison of different vegetable oils (Brodnjak-Vončina, et al. 2005). The main purpose is the discrimination among cultivars and geographical origin including adulteration, and authentication (Rezzi, et al. 2005).

Today there is an increasing interest for a simple and fast technique called electronic nose for various applications (Ballabio, et al. 2006b). This technology has also been successfully used for the differentiation of olive oils on the basis of geographical origin (Casale, et al. 2007). An electronic nose is an instrument, which generally consists of an array of partially selective electronic chemical sensors and an appropriate pattern recognition method, to detect and discriminate simple or complex odors automatically (Fu, et al. 2007).

Due to the high value of olive oil, it is usually adulterated with other edible oils of lower commercial value. The most common adulterants found in virgin olive oil are refined olive oil, synthetic olive oil-glycerol products, seed oils and nut oils (Flores, et al. 2006). Several researches reported the use of an electronic nose for classification and determination of adulteration of oils. Sixteen different types of vegetable oils were characterized using a surface acoustic wave (SAW) detector based electronic nose by Gan et al. (2005). Hai and Wang (2006) used an electronic nose to detect adulteration of sesame oil with corn oil using an electronic nose and to predict the adulteration percentage in sesame oil adulterated with maize oil particularly applying principal component analysis (PCA) as a chemometric method.

The determination of the volatile aroma compounds of EVOOs were also done by using an electronic nose. Physical-chemical techniques such as gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), high performance liquid chromatography (HPLC) and sensory panel tests are the classical methods used for the determination of volatile compounds. Pattern recognition techniques such as PCA, linear discriminant analysis (LDA), canonical analysis (CA), partial least squares regression (PLS) were carried out on electronic nose, GC/MS and sensory analysis data (Cimato, et al. 2006).

Objectives of this study were to classify the extracted and commercial EVOOs according to their variety, geographical origin, and harvest year based on their aroma fingerprints using an electronic nose consisting of a SAW detector; to determine the differences in the organoleptic properties of the extracted olive oils of the same varieties harvested from different geographical origin; to determine the consumers' preferences for the extracted olive oils based on their color, odor, and taste attributes and their overall acceptabilities; and to detect and quantify olive oil adulteration with other edible oils based on their aroma fingerprints. Discrimination of the extracted and commercial EVOOs as well as the detection of the adulteration levels were performed using various chemometric methods, such as PCA and PLS.

CHAPTER 2

OLIVE OIL

2.1. The Olive Fruit and Olive Oil

The olive is one of the major products for the agriculture of the Mediterranean region particularly in the central and southern areas of Spain, Italy, and in Greece, Turkey, Tunisia and Morocco. There are thousands of olive cultivars. The olive has been cultivated since ancient times as a source of olive oil, fine wood, and olives for consumption (Harwood and Aparicio 2000).

It is important to evaluate and conserve the olive genetic diversity preserved from influence of the cultivation area. The high variability in the origin and the geographical distribution are still under investigation in the cultivated olive. Therefore, the significant point is the identification of particular cultivars and their genetic and sanitary certification processes in the improvement of olive oil production (Cimato, et al. 2006). The agronomic and technological factors may cause the chemical composition of olive oils to be discrete which demonstrates the importance of the characterization of each typical olive oil (Lanteri, et al. 2002).

Olive harvesting is an important process influencing the quality and commercial value of virgin olive oil. The organoleptic quality of virgin olive oil depends on the ripeness of olives and on the harvest period. If the olives are unripe and dark, a virgin olive oil will have an herbaceous odor and a bitter, pungent taste based on the variety. When the olives are ripe or overripe, it is characterized by ripe flavor and sweet taste. To obtain good quality olive oil, the olives should be healthy and picked from tree and processed immediately. The leaf removal and washing operations should also be performed to remove foreign vegetable or nonvegetable material that could be harmful to the machinery or contaminate the product (Harwood and Aparicio 2000).

2.1.1. The Designations and Definitions of Olive Oils and Olive Pomace Oils

Olive oil is the oil obtained only from the fruit of the olive tree (Olea europaea L.), not including oils obtained using solvents or reesterification processes. It is marketed according to the following designations and definitions:

Virgin olive oil is the oil obtained from the fruit of the olive tree only by mechanical or other physical conditions, peculiarly thermal conditions, that do not cause alterations in the oil, and which has not undergone any treatment other than washing, decantation, centrifugation, and filtration.

Virgin olive oils fit for consumption as they are include:

Extra virgin olive oil: free fatty acidity (expressed as oleic acid) of a virgin olive oil should not exceed 0.8 grams per 100 grams.

Virgin olive oil: virgin olive oil which has a free fatty acidity (expressed as oleic acid), of not more than 2 grams per 100 grams.

Ordinary virgin olive oil: virgin olive oil which has a free acidity (expressed as oleic acid), should not exceed 3.3 grams per 100 grams.

Virgin olive oil not fit for consumption as it is, designated lampante virgin olive oil, is virgin olive oil having a free acidity (expressed as oleic acid), more than 3.3 grams per 100 grams. It is intended for refining or for technical use.

Refined olive oil is the olive oil obtained from virgin olive oils by refining methods which do not alter in the initial glyceridic structure. It has a free fatty acidity (expressed as oleic acid), not more than 0.3 grams per 100 grams.

Olive oil is the oil consisting of a blend of refined olive oil and virgin olive oils fit for consumption. It has a free fatty acidity (expressed as oleic acid), not more than 1 gram per 100 grams.

Olive-pomace oil is the oil obtained by treating olive pomace with solvents or other physical treatments not including the oils obtained by reesterification processes and of any mixture with oils of other kinds. It is marketed in accordance with the following designations and definitions:

Crude olive-pomace oil is olive pomace oil is intended for refining for use for human consumption, or for technical use.

Refined olive pomace oil is the oil obtained from crude olive pomace oil by refining methods which do not alter in the initial glyceridic structure. It has a free fatty acidity (expressed as oleic acid), not more than 0.3 grams per 100 grams.

Olive pomace oil is the oil comprising the blend of refined olive pomace oil and virgin olive oils fit for consumption. Free fatty acidity of this oil should not exceed 1 gram per 100 grams (International Olive Council 2007).

2.1.2. Olive Oil Processing

The purpose of processing the olives is to obtain virgin olive oil as defined by the IOOC. Olive oil extraction is the process of separating the liquid phases (virgin olive oil and vegetation water) from the solid phase (pomace) (Harwood and Aparicio 2000).

2.1.2.1. Pressing Method

Olive crushing is the first step to obtain virgin olive oil. The pressure is applied onto the olives by using habitually big size millstones. The mixing step is performed in stainless steel semicylinderical or semispherical mixers. The olive paste generally stays under the stones for 20–30 minutes. After grinding, the olive paste is spread on fiber disks, which are stacked on top of each other, then placed into the press. Pressure is then applied onto the disk for further separation of the oil from the paste. The flow diagram of olive oil extraction by pressing method is given in Figure 2.1. The quality of the virgin olive oils obtained by the pressing system when compared with the quality of oils obtained by other systems is good if the machinery and factory are quite clean, healthy olives are processed, and the work is continuous even during the night (Harwood and Aparicio 2000).

The pressing systems have some advantages that the machinery do not need high investment, simple and reliable machinery is used, little electrical power is needed, therefore the energy consumption is low. The pomace is less wet and a small amount of vegetable water which contains little oil is produced in pressing systems (IOOC 1990).

The pressing systems have also these disadvantages that the machinery is massive; much effort is required and also the filtering mats can possibly be contaminated, the process is discontinuous and the working capacity is low (IOOC 1990).

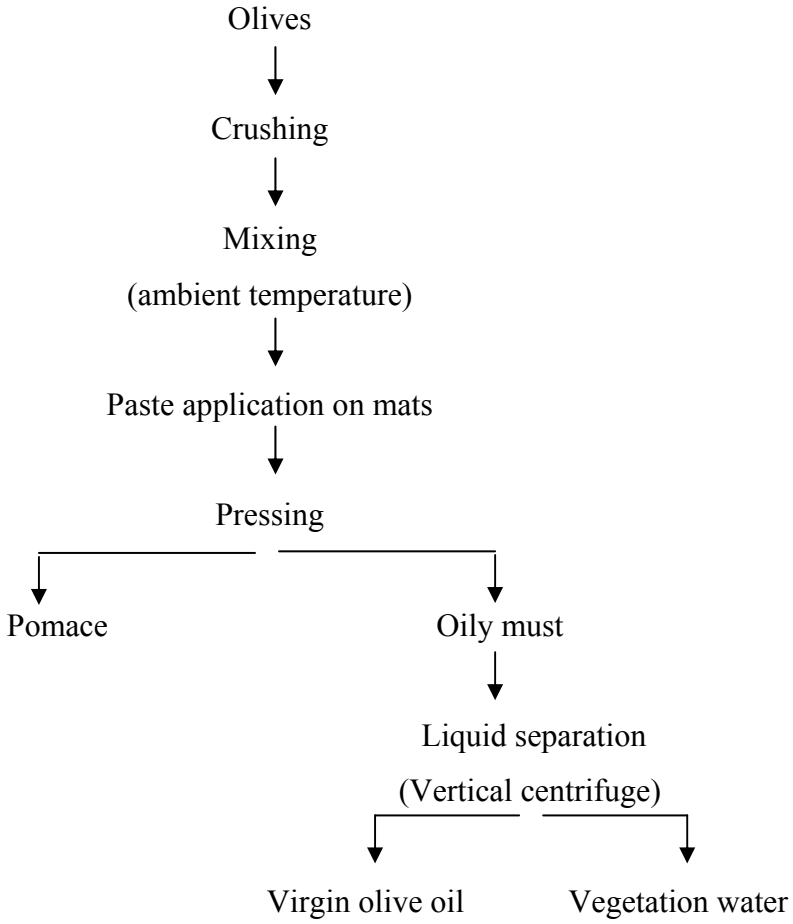

Figure 2.1. Flow diagram of olive oil extraction by pressing method
(Source: Harwood and Aparicio 2000)

2.1.2.2. Centrifugation Method

The modern method of olive oil extraction is the use of an industrial decanter to separate all the phases by centrifugation. When a centrifugation method is used, olive crushing can be carried out by the machines consisting of a metallic body and a high speed rotating 'hammer' of different shapes. The methods of olive crushing affect the volatile composition of the olive oil. The method of olive crushing with millstones gets higher content of volatile substances in particular, of (E)-2-hexenal. The malaxation time of the paste is 25 to 35 min to allow the small olive droplets to agglomerate. Then the mixed olive paste is pumped into a decanter where the liquid and solid phases will be separated by the centrifugal force. Lukewarm water is added to enable the extraction process with the paste. With the three phase decanter the high amount of water cause the polyphenols to be washed out and hence the stability of virgin olive oil during

storage decreases. The amount of vegetation water is also high (Harwood and Aparicio 2000). The flow diagram of the olive oil extraction by centrifugation method is given in Figure 2.2.

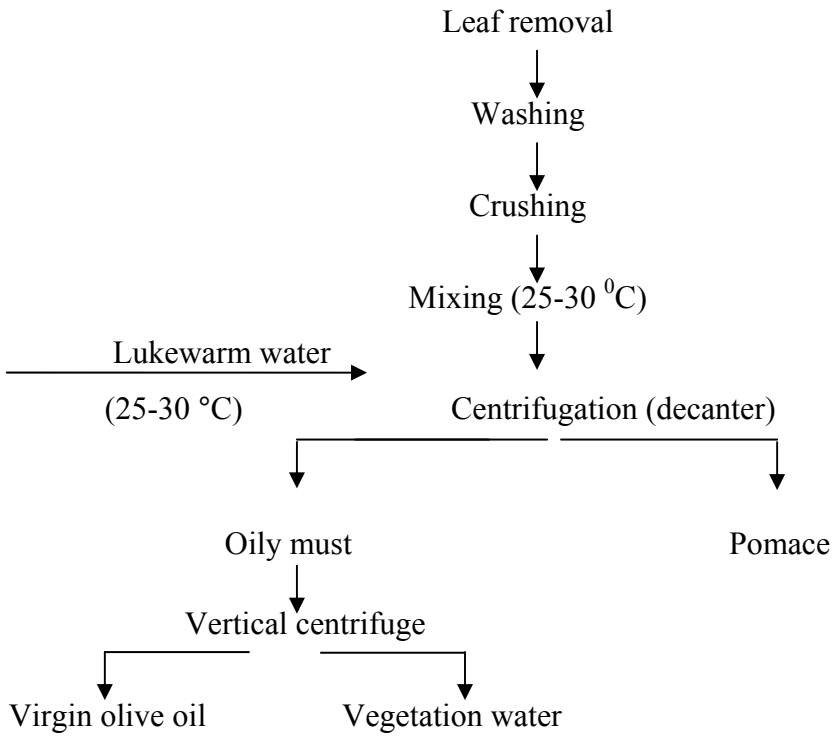

Figure 2.2. Flow diagram of olive oil extraction by centrifugation method (Source: Harwood and Aparicio 2000)

2.2. The Chemical Composition of Olive Oil

Olive oils are complex mixtures formed of two main groups of substances:

a) saponifiable substances which represent nearly 98% of the chemical composition, such as triglycerides, partial glycerides, esters of fatty acids or free non-esterified fatty acids;

b) unsaponifiable substances, which represent only 2% of all olive oil composition, such as sterols, hydrocarbons, pigments, phenols, flavonoids or volatile compounds with many different chemical structures (Aparicio and Aparicio-Ruíz 2000). Olive oil is basically formed of monounsaturated fatty acids. Primary fatty acids are oleic and linoleic acid with a small amount of linolenic acid. The minor constituents of olive oil have influence on sensory and biological properties. The main components of these constituents are squalene (e.g. terpenic hydrocarbons), triterpene alcohols (e.g.

24-methylene-cycloarthenol), sterols (e.g. β-sitosterol), tocopherols (e.g. α-tocopherol) and phenolic compounds (e.g. tyrosol, hydroxytyrosol, elenolic acid, gallic acid) (Harwood and Aparicio 2000). The volatile compounds identified in different kinds of virgin olive oils are given in Table 2.1 and the chemical structures of some of these volatile compounds are shown in Figure 2.3.

Table 2.1. Volatile compounds identified in different kinds of virgin olive oils (Source: Harwood and Aparicio 2000)

Aldehydes	**Alcohols**	**Esters**
Acetaldehyde	Methanol	Methyl acetate
2-Methylbutanal	Ethanol	Ethyl acetate
3-Methylbutanal	2-Methyl-1-butanol	Butyl acetate
2-Methyl-2-butenal	3-Methyl-1-butanol	2-Methylbutyl acetate
Pentanal	2-Methyl-3-butenol	Isopentyl acetate
(E)-2-Pentenal	1-Pentanol	Hexyl acetate
(Z)-2-Pentenal	3-Pentanol	2-Hexenyl acetate
Hexanal	1-Hexanol	3-Hexenyl acetate
2-Hexenal	1-Penten-3-ol	(Z)-3-Hexenyl acetate
(E)-2-Hexenal	3-Hexen-1-ol	Octyl acetate
(Z)-2-Hexenal	(E)-3-Hexen-1-ol	2-Ethylphenyl acetate
3-Hexenal	(Z)-3-Hexen-1-ol	Benzyl acetate
(Z)-3-Hexenal	2-Hexen-1-ol	Phenethyl acetate
2,4-Hexadienal	(E)-2-Hexen-1-ol	Ethyl propanoate
Heptanal	(Z)-2-Hexenol	Propyl propanoate
(E)-2-Heptenal	4-Hexen-1-ol	Ethyl 2-methylpropanoate
(Z)-2-Heptenal	1-Heptanol	Propyl 2-methylpropanoate
2,4-Heptadienal	1-Octanol	Methyl butanoate
Octanal	1-Octen-3-ol	Ethyl butanoate
(E)-2-Octenal	2-Octen-1-ol	Methyl 2-methylbutanoate
Nonanal	1-Nonanol	Ethyl 2-methylbutanoate
(E)-2-Nonenal	1-Decanol	Methyl 3-methylbutanoate
2,4-Nonadienal	Lavandulol	Ethyl 3-methylbutanoate
(E)-2-Decenal	Linalool	Butyl 3-methylbutanoate
2,4-Decadienal	Benzyl alcohol	Methyl pentanoate
(E)-2-Undecenal	2-Phenylethanol	Methyl hexanoate
Benzaldehyde	α-Terpineol	Ethyl hexanoate
	2-Penten-1-ol	Methyl heptanoate
		Methyl octanoate

(cont. on next page)

Table 2.1. Volatile compounds identified in different kinds of virgin olive oils (Source: Harwood and Aparicio 2000) (cont.)

Hydrocarbons	**Ketones**
2-Methylbutane	2-Butanone
2-Methylpentane	3-Methyl-2-butanone
3-Methylpentane	3-Pentanone
Hexane	4-Methyl-2-pentanone
Hexene	1-Penten-3-one
Heptane	2-Hexanone
Octane	2-Heptanone
1-Octene	6-Methyl-5-hepten-one
Nonane	2-Octanone
Tridecene	3-Octanone
Pentene dimers	2-Nonanone
Methyl benzene	Acetophenone
Styrene	
	Sulfur Compounds
Phenols	3-Isopropenylthiophene
Anisole	2,5-Diethylthiophene
	2-Ethyl-5-hexylthiophene
	Furans
	Ethylfuran
	2-Propylfuran
	3-Propylfuran
	3-Methyl-2-penthylfuran
	2-Propyldihydrofuran
	3,4-Methyl-3-pentenyl furan
	Ethers
	Diethyl ether
	1,8-Cineole

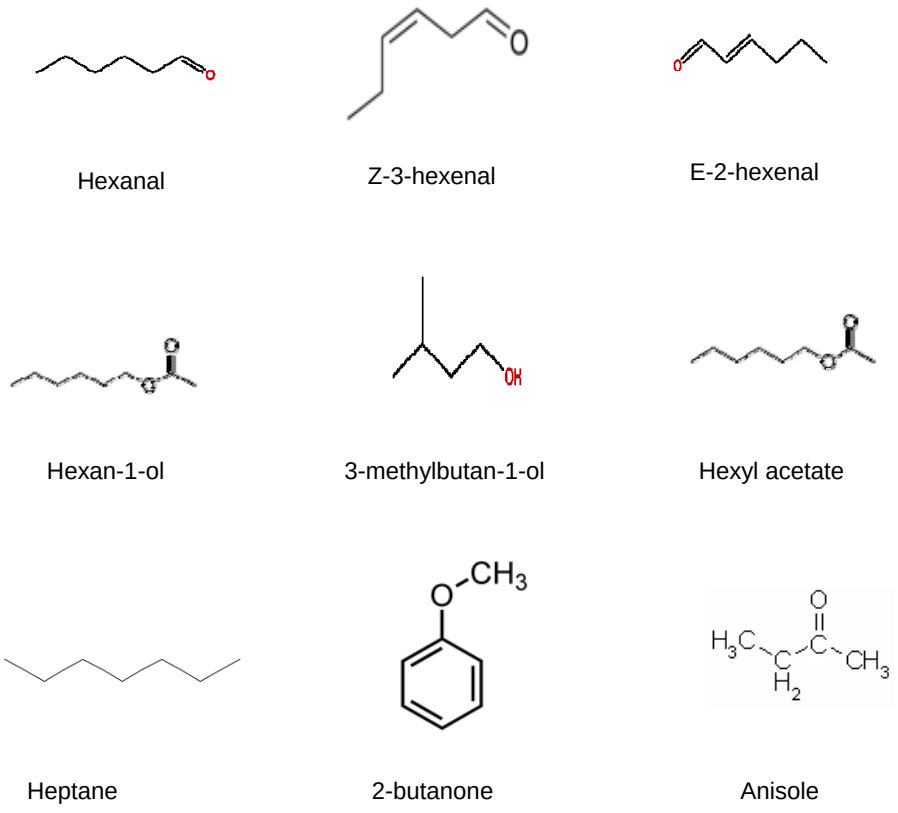

Figure 2.3. Chemical structures of some of the volatile compounds found in virgin olive oils (Source: Griffin 1986, International Programme on Chemical Safety 2007)

2.2.1. Characterization of Monovarietal Virgin Olive Oils

There are many varieties of cultivated olive trees in the world. Because of the fondness of the farmers to their own cultivars between numerous varieties of cultivars, it has been focused on varietal characterization of the virgin olive oils in the literature (Harwood and Aparicio 2000).

Monovarietal characterization of the quality and uniqueness of specific EVOOs based on their chemical and sensory properties is influenced by different factors such as climate, agronomic factors, extraction methods, and processing techniques and can vary by growing location. European Protected Denomination of Origin (PDO) was maintained for the labeling of some European EVOOs with the names of the areas where they are produced. This designation guarantees that the product quality is closely

linked to its geographical origin. PDO olive oils are the best among EVOOs used as indicator of authenticity and quality (Brescia, et al. 2003, Cosio, et al. 2006).

Specific olive cultivars, cultural practices, identical geographical production areas, chemical and sensorial properties are essential to obtain the PDO label. Therefore, it is important to develop methods for the classification of oils for the assignment of a "denomination of origin" trademark. Since the official analysis of virgin olive oils consists of series of several determinations of chemical and physical constant they will be mostly used in the geographical certification of the oil samples. Therefore, reliable methods are required for geographical origin authentication of olive oil (Cosio, et al. 2006). Because these olive oils have high commercial value, there is a great interest for fraud by marketing non-authentic or adulterated PDO oils (Bianchi, et al. 2001).

CHAPTER 3

OLIVE OIL AROMA AND ITS IMPORTANCE

3.1. The Virgin Olive Oil Volatile Compounds

The flavor and aroma of virgin olive oil are formed by some nonvolatile compounds and a complex mixture of volatile compounds (Cimato, et al. 2006). Non-volatile compounds such as phenolic compounds stimulate the tasting perception of bitterness, the latter pungency, astringency and metallic attributes (Morales and Tsimidou 2000). Volatile compounds including aldehydes (hexanal, trans-2-hexenal, acetaldehyde), alcohols (methanol, hexan-1-ol, 3-methylbutan-1-ol), ketones (2-butanone, 3-methyl-2-butanone, 3-pentanone), hydrocarbons (2-methylbutane, hexane, nonane) and esters (methyl acetate, ethyl acetate, hexyl acetate) stimulate the olfactory receptors and they are responsible for the whole aroma of virgin olive oil (Angerosa, et al. 2004, Cimato, et al. 2006). Volatiles and other minor compounds are retained by virgin olive oils during their mechanical extraction process from olive fruits (*Olea europaea* L.) (Angerosa, et al. 2004, Aparicio and Morales 1998). The delicate taste and aroma of the virgin olive oil are related to these non-volatile and volatile minor compounds that increase the fragrant and delicate flavor important for the consumers since ancient times (Cimato, et al. 2006, Luna, et al. 2006). The extraction methods performed to process olives affect the volatile substances compositions that characterize the virgin olive oil aroma. The results obtained by pressing and centrifugation methods demonstrated that some compounds such as n-octane, isoamyl alcohol, isobutyl alcohol, acetic acid and ethyl acetate are present at higher quantity in oils obtained by pressing methods (Harwood and Aparicio 2000). In order to satisfy consumer expectations, oil from a certain producer must be easily differentiated and identified by presenting the same smell as well as the same taste and color (Cimato, et al. 2006).

Volatile compounds characteristics responsible for virgin olive oil aroma are as follows:

- Low molecular weight (<300 Da);

- High volatility so that a appropriate number of molecules can reach the olfactory epithelium as molecular dispersion, transported by the air streams due to inhalation and expiration;
- Sufficient hydrosolubility to diffuse into the mucus that covers the sensitive olfactory cells;
- Fair liposolubility to dissolve in membrane lipids contiguous to proteins of receptors;
- Chemical features to bond specific proteins (Angerosa 2002).

3.1.1. The Factors Affecting the Volatile Composition of Olive Oil

Cultivar, geographical region, fruit maturity, and processing methods and parameters influence the volatile composition of olive oil. These factors affecting the characterization of monovarietal virgin olive oils can be classified into four main groups:

- Environmental (soil, climate);
- Agronomic (irrigation, fertilization);
- Cultivation (harvesting, ripeness);
- Technological factors (post-harvest storage and extraction system).

Cultivars which do not always grow at the same altitude and the climatic conditions such as rainfall, temperature, humidity can obviously be quite different from each other. As a consequence, this has an effect on chemical and sensory profiles of olive oil (Aparicio and Luna 2002). The olive of different cultivars grown under the same environmental conditions produce oils having different volatile compounds, as does fruit of the same cultivar olive grown in different geographical regions (Benincasa, et al. 2003)

The organoleptic quality of virgin olive oil is related to the ripeness of olives and on the period of harvest (Aparicio and Morales 1998). During the ripening period, several metabolic processes take place in olives with the variation on profiles of some compounds. These changes have influence on the quality grade, sensorial characteristics, oxidative stability and/or nutritional value of the olive oil. Polyphenols, tocopherols, chlorophyllic pigments and carotenoids are examples of these compounds with the fatty acid and sterol compositions (Matos, et al. 2007). Olive harvesting is an important process that has significant contribution to the virgin olive oil quality and its

commercial value. Olives are picked from the plant by hand, by shaker machines, or by facilitating machines, or they are picked from the ground by manual tools or aspirators (Harwood and Aparicio 2000). Apart from the condition of the fruit at harvest, differences in post-harvest handling of the fruit produce different volatile profiles of olive oils. Extraction methods and conditions especially the malaxation time and temperature make olive oil flavors discrete (Angerosa, et al. 1998, Di Giovacchino, et al. 2002, Ranalli, et al. 2001, Ranalli, et al. 2003). After the production of virgin olive oil in the olive oil mill, it should be properly stored in large underground vats or in metallic tanks. The storage buildings must be free from unpleasant sources of smell. In order to avoid the defect of muddy or putrid sediment, virgin olive oil should be separated from the sediment quickly by pouring or filtering it through hydrophilic materials. The oxidation process can be delayed by preventing the exposure of virgin olive oil from light, contact with air, ambient high temperature (higher than 30 °C) and high contents of metals (especially copper and iron) (Harwood and Aparicio 2000). Storage of the fruit after harvesting and of the oil before reaching the consumer changes the volatile composition of olive oil such as decreasing the aldehyde and ester content that is responsible for the positive aroma and producing volatile compounds that are responsible for off-flavours (Kiritsakis 1998, Koprivnjak, et al. 2000). C6 and C5 compounds are enzymatically produced from polyunsaturated fatty acids through the so-called lipoxygenase (LOX) pathway (Angerosa, et al. 2004). The absence of the C6 aldehydes, alcohols and esters from the lipoxygenase pathway and the presence of many aldehydes from chemical oxidation, including hexanal from both chemical and enzymatic reactions, characterize the off-flavor of olive oil. The off-flavour compounds are potentially toxic and have low odor thresholds (Angerosa, et al. 2000).

3.1.2. Formation of Volatile Compounds

Olive oil harvested at the appropriate ripeness and produced by proper technological extraction methodologies, have a volatile fraction fundamentally formed by compounds which are common participants of the aroma of many fruits and vegetables (Angerosa 2002). Approximately one hundred and eighty compounds of several chemical classes were separated from the volatile fractions of different quality virgin olive oils (Angerosa 2002). One of the most important aspects of variety related to oil flavor is the specific composition and quantity of the polyphenols and aromatic compounds. The watery portion of the cell surrounding the globules of oil contains all

the water-soluble and semi-water-soluble compounds, such as the polyphenols, tocopherols, glucosides, aldehydes, ketones, esters, organic acids, aromatic hydrocarbons, and pigments like chlorophyll and the carotenoids. The polyphenols and glucosides give the taste of bitterness, pungency and its antioxidant property of tocopherols (Vossen 2007).

C6 and C5 compounds are the major components of virgin olive oil headspace, which make high contribution to the volatile compounds and for the green odor of olive oil aroma (Angerosa, et al. 2004). Aparicio and Morales (1998) have found (Z)-3-hexen-1-ol, (E)-2 hexen-1-ol, hexan-1-ol at high concentrations which is affected by the variety and the stage of olives ripeness. The high quality of virgin olive oils is characterized by these compounds and preferred by consumers. These volatile compounds are formed from polyunsaturated fatty acids through the enzymatic oxidation of linolenic and linoleic acids, the lipoxygenase (LOX) pathway which is shown in Figure 3.1. The aroma of the oil is determined by the relative activity of the enzymes involved in the pathway (Harwood and Aparicio 2000).

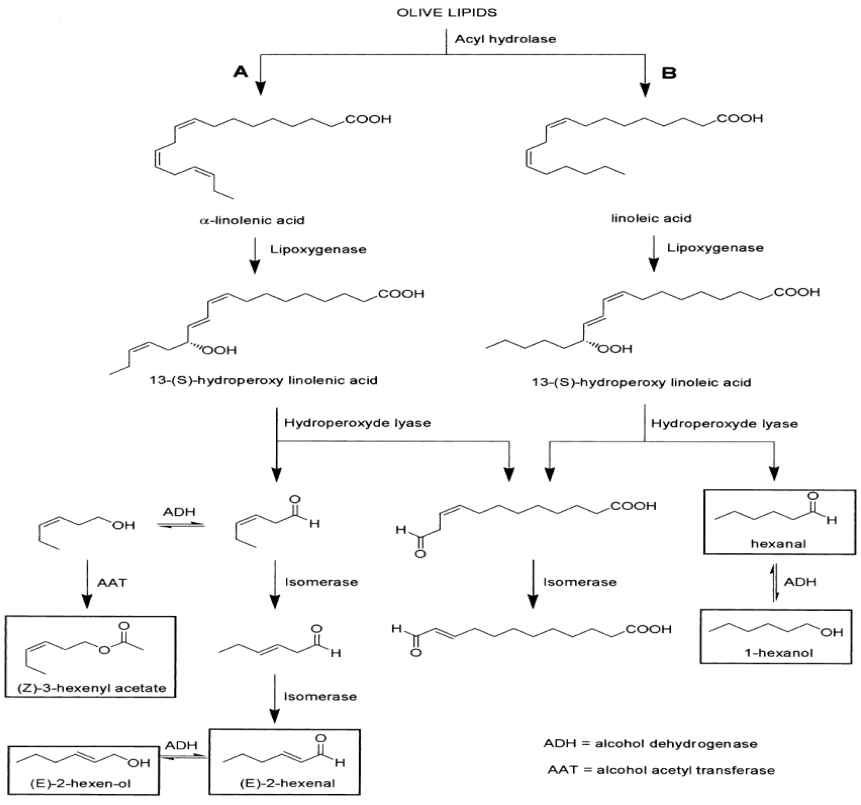

Figure 3.1. Lipoxygenase pathways for the formation of major volatile compounds (Source: Benincasa, et al. 2003)

The LOX pathway starts with the production of 9- and 13-hydroperoxides of linoleic (LA) and linolenic (LnA) acids mediated by LOX. The subsequent cleavage of 13-hydroperoxides is catalysed by very specific hydroperoxide lyases (HPL) and leads to C6 aldehydes. The unsaturated ones of C6 aldehydes can isomerize from *cis*-3 to the more stable *trans*-2 form. The mediation of alcohol dehydrogenase (ADH) reduces C6 aldehydes to corresponding alcohols, which can produce esters because of the catalytic activity of alcohol acetyl transferases (AAT). An additional branch of the LOX pathway is active when the substrate is LnA. LOX would catalyse, besides the hydroperoxide formation, also its cleavage via an alkoxy radical increase the formation of stabilized 1,3-pentene radicals. These last can dimerize leading to C10 hydrocarbons (known as pentene dimers) or couple with a hydroxy radical present in the medium producing C5 alcohols, which can be enzymatically oxidated to corresponding C5 carbonyl compounds (Angerosa, et al. 2004).

This pathway includes the actuation of different enzymes that increase the different amounts of aldehydes, alcohols, and hexyl acetates which have sensory properties and contribute to the overall flavor (Aparicio and Morales 1998). It has been demonstrated that the LOX pathway improve the formation of C6 and C5 volatile compounds against C9 volatile compounds and a great amount of volatile compounds for green sensory notes can be found in fresh and high quality virgin olive oils (Aparicio and Morales 1998, Morales, et al. 1994). The formation of C6 and C5 compounds through the enzymatic oxidation of linoleic and linolenic acids is affected by the cultivar, the degree of ripeness of fruits and by their processing conditions (Angerosa 2002). This reveals the importance of the biochemical pathways for the particular profiles of the monovarietal virgin olive oils (Aparicio and Luna 2002). Some volatile compounds that are affected by organoleptic defects reach high concentrations in the aroma of virgin olive oils (Angerosa, et al. 2004). The volatile components can be used to determine the quality of an olive oil (Angerosa 2002), to detect an adulteration (Lorenzo, et al. 2002), to detect a possible rancidity (off-flavors) (Morales, et al. 1997) or to determine the variety of olive used (Lorenzo, et al. 2002).

3.2. Olive Oil Aroma Analysis Techniques

Many analytical procedures such as physicochemical techniques (GC, GC-MS, HPLC), chemical and sensory analyses have been applied for the identification and quantification of the volatile components that characterize olive oil aroma (Angerosa 2002). The analytical methods such as GC and HPLC are widely used for the determination of individual fatty acid content, sterols or pesticide residues in oils and the identification, quality control and detection of adulteration with other edible oils (Harwood and Aparicio 2000). However, these techniques are usually time-consuming, expensive and sample preparation and a qualified staff are required (Cosio, et al. 2006).

Fourier transform-infrared (FTIR) or Fourier transform-Raman spectroscopy has been used to provide data on fatty acids and composition in a short period of time. Recently, implementation of near infrared spectrometry (NIR) to oils and fats has increased in quality and composition studies (Armenta, et al. 2007). Several attempts have been performed to confess the geographical origin of olive oils by suitable chemical parameters, such as triglyceride and fatty acid profiles or by ^{1}H NMR spectroscopy (Mannina, et al. 2001). The geographical origin of olive oil was studied by chemometric methods using the data of the chemical composition of olive oils (Lanteri, et al. 2002). Consequently, there is a need for quick and simple methods to classify the extra virgin olive oils based on their geographical origin (Cosio, et al. 2006).

3.2.1. Gas Chromatography

Chromatographic methods have been mostly carried out in analyses of edible oils, such as olive oil in recent years. Usually performed techniques for volatile analysis by GC are static headspace, dynamic headspace and direct injection. Dynamic headspace techniques have been mostly used for the olive oil oxidation studies. Although, these techniques provide high sensitivity and accuracy, they are also time consuming and expensive (Harwood and Aparicio 2000). Approximately one hundred and eighty compounds were found in virgin olive oil aromas. The structures of these compounds were assessed by means of GC-MS. Gas chromatographic profiles of a good quality virgin olive oil are shown in Figure 3.2 (Angerosa 2002). Several studies have been performed to characterize virgin olive oils by quantification of the volatile

compounds. In one of these studies, thirty-nine single cultivar virgin olive oils cultivated in the same orchard under the same agronomic and pedoclimatic conditions were characterized by 64 volatile compounds quantified by dynamic headspace-gas chromatography (Luna, et al. 2006).

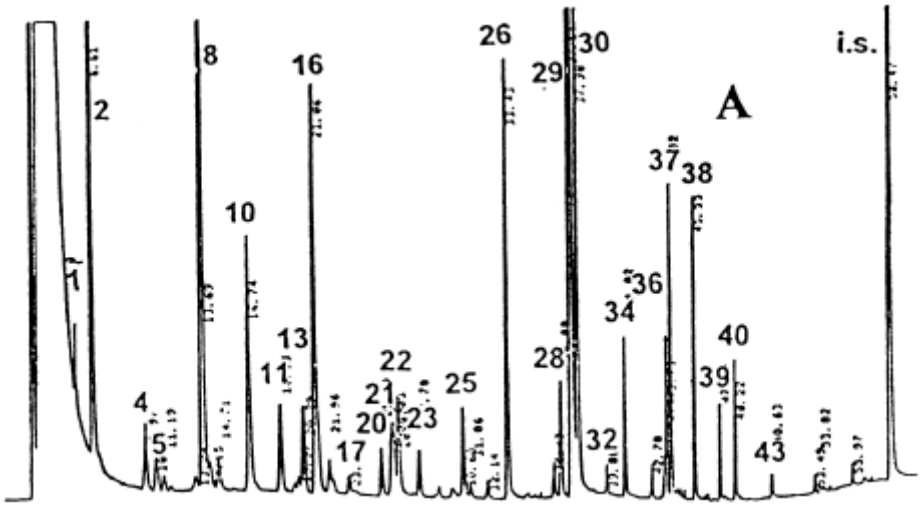

Figure 3.2. Gas chromatographic profiles of a good quality virgin olive oil A) Peaks: 1: octane; 2: acetone; 4: ethyl acetate; 5: methanol; 8: ethanol; 10: pentan-3-one; 11: pentene dimer; 13: pentene dimer; 16: 1-penten-3- one; 17: propan-1-ol; 20: pentene dimer; 21: pentene dimer; 22: pentene dimer + hexanal; 23: 2- methyl propan-1-ol; 25: 2- pentenal; 26: 1 penten-3-ol; 28: 3-methyl butan-1-ol; 29: *trans*-2-hexenal; 30: unknown; 32: pentan-1-ol; 34: hexyl acetate; 36: *cis*-3-hexenyl acetate; 37: *cis*-2-penten-1-ol; 38: hexan-1-ol; 39: *cis*-3- hexen-1-ol; 40: *trans*-2-hexen-1-ol; 43: acetic acid; i.s.: nonan-1-ol (internal standard) (Source: Angerosa 2002)

3.2.2. Electronic Nose

In recent years, a great demand for a rapid, cheap, and effective electronic instrument that can mimic the human sense of smell and provide low-cost and rapid sensory information has been increased for quality control of EVOOs (Cosio, et al. 2006, Gan, et al. 2005). The term ‘electronic nose’ was used at a conference almost twenty years ago. Gardner and Bartlett (1994) defined an electronic nose as ‘an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odor’.

Basically, the principle of the instrument is the transfer of the total headspace of a sample to a sensor array. Each sensor has partial specificity to a wide range of aroma molecules. In the electronic nose the signal pattern from the sensory array is collected and handled by a computer, where the data are processed by pattern recognition software (Benedetti, et al. 2004). The comparison of human sensing process and electronic nose sensing process is shown in Figure 3.3.

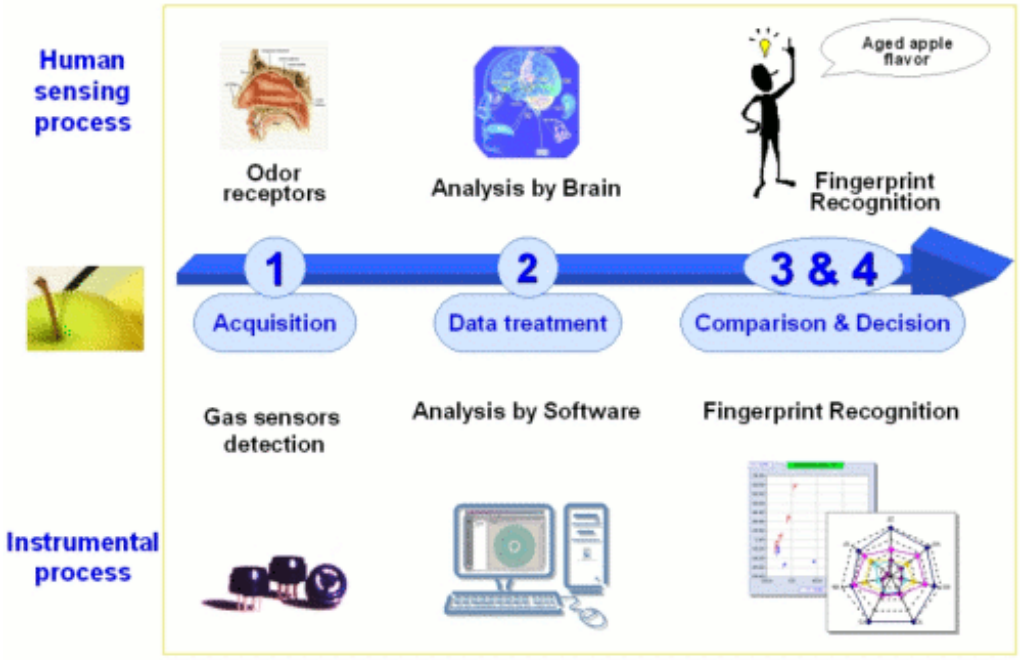

Figure 3.3. Comparison between human sensing and instrument sensing processes (Source: Anon 2007a)

The main steps of odor recognition can be summarized as follows:

- Heating the sample for a certain time generates volatile compounds.
- The gas phase is transferred to a detection device which reacts to the presence of molecules.
- The difference in sensor reactions is revealed using different statistical calculation techniques to classify the odors. From this pattern and from previous human input (human training from sensory panels), the system predicts the mostly likely human response to the new pattern (Anon 2007a).

The sample preparation before introducing to the electronic nose is very simple. The sample is transferred into a vial and then the vial is heated for a precise length of time and temperature. The headspace is injected into a carrier gas of the instrument (air) (Anon 2007b).

There are various technologies available for volatile compounds/flavor detection:

- Gas Sensor Arrays
- Fingerprint Mass Spectroscopy
- Ultra Fast Gas Chromatography

Gas sensor arrays: They are non-specific electrochemical devices. Gas sensors can be classified into three categories:

- metal oxide sensor
- conducting polymer sensor
- quartz crystal microbalance sensor

The main features of these sensors are described below:

Metal oxide sensors operate at high temperatures, around 400 °C. Chemisorbed oxygen reacts with odor molecules irreversibly liberating the electrons and lowering the measured resistance of the sensor. Metal oxide sensors are inorganic. They are sensitive to combustible materials such as alcohols, but less sensitive at detecting nitrogen and sulfur based odors (Anon 2007b, Korel and Balaban 2003).

Conducting polymer sensors are made of conducting materials which show variation in conductivity for the detection of different gases and vapors. The sensors tend to swell in the presence of odor molecules and thus change resistance. Conducting polymers are nonspecific. These sensors are small and operate at room temperature. A less desirable feature of conducting polymers is their sensitivity to water and humidity. They are also less sensitive than metal oxides. Because of their organic nature, they tend to drift and destabilize (Anon 2007b, Korel and Balaban 2003).

Quartz Crystal Microbalance: The sensor element is a quartz resonator coated with an organic material similar to the stationary phase of a GC column. The sensor has a resonant frequency of the sensor changes as aroma adsorbs and desorbs from the coating, changing the mass of the resonator, and hence its frequency. Again this sensor depends on an organic interface. Like all sensors, by the material from which it is made will determine its lifetime and drift characteristics (Anon 2007b, Korel and Balaban 2003).

Fingerprint mass spectrometry: The quadrupole instruments for electronic olfaction, called "Fingerprint Mass Spectrometry", have similarity in design with the technology used for GC/MS. The only difference is the absence of the GC module which separates the volatile molecules prior to their detection by the mass spectrometer. In fingerprint mass spectrometry, the entire aroma enters the quadrupole module without separation. The resulting fingerprint explains the entire aroma, as it is given to a human being (Anon 2007b).

A mass spectrometer includes an ion source to create gas-phase ions, a mass-selective analyzer for the separation of the ions based on their mass-to-charge ratio, and an ion detector to measure the quantity of ions of each mass-to-charge ratio (Anon 2007b).

Ultra Fast Gas Chromatography: Volatile compounds analysis is carried out by flash GC in less than 60 seconds. The sample (liquid or headspace) is injected simultaneously on 1 or 2 short columns of different polarities, and detection is conducted by 2 flame ionization detector (FID) or surface acoustic wave (SAW) detectors. Including a trap (Carbosieve or Tenax support), a very low sensitivity detection is reached. Chromatograms are treated globally and converted into a chemical fingerprint by using several chemometric methods (Anon 2007b).

Electronic nose has been used in food science for a variety of applications; such as assessment of food properties (Brezmes, et al. 2001, Garcìa-Gonzàlez and Aparicio 2003, Guadarrama, et al. 2000), detection of adulteration (Oliveros, et al. 2002), sensory properties prediction (Buratti, et al. 2007). They are preferred to routine laboratory analysis since they have several advantages such as cheapness, quickness, simplicity, little or no prior sample preparation. On the contrary to traditional analytical methods, electronic nose sensor responses do not provide information on the nature of the

compounds. It only gives a digital fingerprint of the food product which could be investigated by chemometric methods (Ballabio, et al. 2006b).

Recently, a new approach has become available commercially: zNose™. This instrument is based on fast GC and a single SAW sensor. The technique used in zNose™ is a fast GC technique, which allows identification and fingerprinting of aroma as with GC. On the other hand, it operates at the speed of an electronic nose (Gan, et al. 2005). Different from other analytical chemical instruments such as, GC/MS, the electronic nose does not detect and identify single volatiles, but distinguishes smell patterns of vapor mixtures by using pattern recognition algorithms (Li, et al. 2007).

The zNose™ simulates a virtual sensor array containing orthogonal sensors. Even though one physical sensor is only used, sensor space is determined mathematically by assigning unique retention time slots to each sensor. The use of a single sensor has the great advantage of reducing the drift errors. In addition, sensitivity is quite high with part per billion levels which is typical for volatile organics in air or water (Gan, et al. 2005). There has been a lot of success using electronic nose technology for the differentiation of olive oils on the basis of geographical origin (Guadarrama, et al. 2001, Cosio, et al. 2006). In previous studies, there are several examples that denote the using of an electronic nose for the vegetable oil characterization (Oliveros, et al. 2002) and for the quality control of olive oil aroma (Guadarrama, et al. 2001). For example; electronic nose and chemometric analysis were successfully applied by Oliveros et al. (2005) to discriminate the different aromas of olive oils from five Mediterranean areas. An electronic nose has also been used to characterize the geographical origin of Garda EVOOs by means of multivariate statistical analysis (Cosio, et al. 2006). Gan et al. (2005) applied a SAW detector based electronic nose to characterize 16 different types of vegetable oils.

3.2.3. Sensory Analysis

Sensory analysis has been defined as a scientific discipline carried out by a panel of trained tasters. The simplest sensory test is performed to answer whether any difference exists between two products. These are the discrimination tests. The second major class of sensory test methods is the descriptive tests which quantify the perceived intensities of the sensory characteristics of a product. The third main classes of sensory

tests called hedonic or affective test, are used to quantify the degree of liking and disliking of a product (Lawless and Heymann 1998). In general, the sensory quality of a food demonstrates the acceptability and desirability of that product. Color, taste and aroma are the main variables for the definition of the quality of olive oils (Harwood and Aparicio 2000). Sensory analysis is used to differentiate the olive oil based on the region of provenience, variety, ripeness and extraction techniques (Cimato, et al. 2006). Aroma is a principal parameter in the sensory quality assessment procedures for virgin olive oil (Garcìa-Gonzàlez and Aparicio 2002). A panel of trained tasters is used in the analysis (Cimato, et al. 2006).

Sensory descriptors of olive oil can be classified into "positive attributes", such as fruity, bitter and pungent, and "negative attributes", such as fusty, mustiness, muddy sediment, vinegary, metallic, rancid (Escuderos, et al. 2007). Recently, the intensity of defects and positive attributes of virgin olive oil is quantified by trained assessors using the panel test. This sensorial analysis is regulated by IOOC trade norm and the EC Regulation (López Feria, et al. 2007). Specific vocabulary of virgin olive oil proposed by International Olive Council (2007) and given in Table 3.1. However, this method is lengthy and expensive because it requires panelists' training and the specific vocabulary (Gan, et al. 2005).

Cimato et al. (2006) carried out the sensory analysis with other physical-chemical techniques (GC, GC/MS, HPLC) and electronic nose for the analysis of single-cultivar EVOOs. They tried to relate the electronic nose results with the sensory profile of the olive oils. It was found that the volatile compounds hexanal and 2-hexenal were significantly related with the sensory attributes of ripe olive, green olive.

Table 3.1. Specific vocabulary for virgin olive oil
(Source: International Olive Council 2007)

Positive Attributes	**Negative Attributes**
Fruity	Fusty/muddy sediment
Bitter	Musty/humid
Pungent	Winey-vinegary
	Metallic
	Rancid

CHAPTER 4

ADULTERATION

In recent years, there has been a great interest for the certification of the geographical origin of food products since authenticity and quality issues can be often related with a given geographical origin (Ballabio, et al. 2006a).

PDO labeling is an important act of EU that protects the names of the foods with the names of the areas they are produced. The designation of PDO labeling of EVOOs guarantees that the quality of an olive oil is closely related to its geographical origin. According to their authenticity and specified organoleptic characteristics these olive oils are the best among the other EVOOs. As a result, they have high commercial value and these EVOOs are commonly subjected to fraud. Therefore, the development of methods for the classification of oils is very important for the assignment of a "denomination of origin" trademark (Cosio, et al. 2006). Moreover, it is essential to authenticate the origin with reliable techniques, because official analysis of virgin olive oil consists of a series of several determinations of chemical and physical parameters that will be commonly used in the geographical certification (Ballabio, et al. 2006a).

In food industry, the determination of food authenticity and the detection of adulteration are important. Virgin olive oils are often adulterated with other edible oils of lower commercial value (Papadopoulos, et al. 2002). Adulteration of a food product is not only a main economic fraud, but can also have major health implications for consumers. In the 1980s, more than 400 deaths and 20,000 casualties occurred from the disease known as 'Spanish toxic syndrome,' caused by the consumption of adulterated oil (Guimet, et al. 2005). Due to the health benefits, olive oil is one of the most consumed edible vegetable oils and it is particularly expensive, which may cause producers to adulterate it with other vegetable oils of lower quality and commercial value (Papadopoulos, et al. 2002, Cercaci, et al. 2003). Commonly used edible oils include olive-pomace oil, corn oil, peanut oil, cottonseed oil, sunflower oil, soybean oil and poppy seed oil (Aparicio, et al. 1996, Kiritsakis 1998).

In recent years, the food authenticity determination and the detection of adulterants are of increasing importance in the food industry (Lorenzo, et al. 2002, Tay,

et al. 2002). Authenticity and quality of EVOO can be associated with a given geographical origin which is used for the certification of this product (Ballabio, et. al. 2006a). Owing to its higher price, the most common adulterations of olive oil carried out with sunflower oil, maize oil, and even with hazelnut oil on account of their similar composition as regards triacylglycerol, total sterol and fatty acid profile (Hai and Wang 2006, Lorenzo, et al. 2002). Due to its high quality, the extra virgin olive oil is the most expensive oils among the other vegetable oils. Therefore, the mislabeling and adulteration could sometimes be observed. Mislabeling often consists in false labeling involving the geographical origin or the oil variety of an olive oil (Aparicio, et al. 1997). The situation is especially significant for hazelnut oil adulteration due to its high similarity in chemical composition with olive oil. This fact makes the use of triacylglycerols (TAGs), which are considered to be good fingerprints for adulteration detection purposes, difficult (Aparicio and Aparicio-Ruíz 2000).

4.1. Adulteration Detection Methods

4.1.1. Sterol Composition

Sterols are characteristic and major proportion of the unsaponifiable matter of vegetable oils. Rapeseed oils contain significant levels of brassicasterol (100–1100 mg/kg for canola), while olive oil has high levels of β-sitosterol (683–2610 mg/kg) and Δ^5-avenasterol (34–266 mg/kg), and safflower oils and sunflower seed oils have high levels of Δ^7- stigmastenol (300–550 and 150–500 mg/ kg, respectively). By these differences, the botanical origin of oils and the adulteration among vegetable oils can be determined (Harwood and Aparicio 2000).

4.1.2. Triacylglycerol

In food industry, triaclyglycerol analysis has always been commonly performed. Fatty acids are distributed on glycerol molecules for the certain position specific patterns and thus, triglycerids are good fingerprints for the detection of adulteration (Aparicio and Aparicio-Ruíz 2000). The low level of trinolein (LLL) in the olive oil has

been used for the authentication and detection of adulteration. The equivalent carbon number (ECN) determination is essential to perform the triaclyglycerol analysis. This method depends on the triaclyglycerol separation for the ECN by HPLC. Olive oil is characterized by four major peaks with ECNs of 44, 46, 48 and 50. All the common edible oils rich in linoleic acid (corn, sunflower and soybean) are characterized by a large HPLC peak with an ECN of 42 while it is in trace amounts in olive oil (Harwood and Aparicio 2000).

4.1.3. Waxes

Wax esters determination is used to detect olive-pomace oil in olive oil. Virgin olive oil can be differentiated from refined olive oil and olive-pomace oil because the virgin olive oil has a higher content of C_{36} and C_{38} waxes than of C_{40}, C_{42}, C_{44} and C_{46} while the other oils have an inverse relation. The most common methods are based on separation by HPLC and GC analysis (Aparicio and Aparicio-Ruíz 2000).

4.1.4. Other Methods

Developing reliable analytical techniques to detect olive oil adulteration for the inspection of geographical origin and determination of uniqueness of the product are required (Guimet, et al. 2005, Rui Alves, et al. 2005). Recently, various analytical techniques have been used for the authentication of vegetable oils, including GC and GC/MS analysis (Caruso, et al. 2000, Cert, et al. 2000, Webster, et al. 1999), NMR (Sacco, et al. 2000), and NIR spectroscopy (Lai, et al. 1994). However, some of these techniques are costly and time consuming. It is difficult to interpret the information obtained with these techniques. Thereby, chemometric methods have been applied to provide grouping of samples with similar properties as well as discrimination between different oils (Bertran, et al. 2000, Bucci, et al. 2002, Kupper, et al. 2001, Mignani, et al. 2003). The most common chemometric methods applied to the adulteration studies were exploratory methods of pattern recognition, such as PCA, hierarchical cluster analysis (HCA), and classification methods, such as soft independent modelling of class analogy (SIMCA), PLS, LDA, and Artificial Neural Networks (ANNs) (Bianchi, et al. 2001).

In recent years, the techniques for the characterization of olive oils are based on the generation of the headspace (Pena, et al. 2005). This system gives the chemical signature of the odor which is called visual aroma pattern, a VaporPrint^{TM}, by analyzing the volatile composition of the olive oil in the similar way to the human olfactory system (Oliveros, et al. 2002, Pena, et al. 2005, Biswas, et al. 2004). This technique enables to obtain a rapid and efficient odor classification between adulterants, a decrease in time and the cost of the analysis is observed (Oliveros, et al. 2002). Several applications of the electronic nose can be found in the literature which shows the successful quality evaluation of olive oil (Oliveros, et al. 2002). Oliveros et al. (2002) reported that the electronic nose with a selected array of sensors could be used to detect the adulterations of olive oils based on the multivariate chemometric methods, LDA. Christy et al. (2004) have detected and quantified adulteration in olive oil by NIR spectroscopy and using chemometric techniques: PCA, PLS, and applied methods for data pretreatments such as multiplicative signal correction. Hai and Wang (2006) reported the use of an electronic nose based on ten metal oxide semiconductor sensors for the detection of adulteration in sesame oil and camellia seed oil with maize oil. The results were acceptable for adulteration of both camellia seed oil and sesame oil in the process of canonical discriminant analysis.

CHAPTER 5

CHEMOMETRIC METHODS FOR DETERMINING AUTHENTICITY OF OLIVE OILS

Chemometric methods are commonly used for the determination of olive oil authentication (Brodnjak-Vončina, et al. 2005). The main purposes are the discrimination between cultivars and geographical origin and identification and quantification of adulteration (Rezzi, et al. 2005). As an example, Christy et al. (2004) have detected and quantified adulteration in olive oil by NIR spectroscopy and they analyzed the multivariate data by methods such as: PCA and PLS.

5.1. Principal Component Analysis

PCA is a projection and dimension reduction method by transforming the original measurement variables into new, uncorrelated variables called principal components (PCs). These PCs retain as much as possible of the information present in the original data (Rezzi, et al. 2005). By using PCA, a data table is modelled as:

$$X = 1 * \overline{x'} + T * P' + E \qquad (5.1.)$$

Where X is the original data matrix consisting *n* rows (samples or objects) and *k* columns (variables or features). $1 * \overline{x'}$ demonstrates the variable averages and originates from the preprocessing step. The second term, the matrix product, $T * P'$, models the structure which includes *T*, the scores, which have as many rows as the original data matrix, *P* are the loadings and have the same number of columns with the original data matrix and the third term, E, is an error matrix. Two PCs together define a plane, a window into the K dimensional variable space. The first principal component explains the maximum amount of variation possible in the data set in one direction. The coordinate values of the observations on this plane are called scores, therefore, the

plotting of such a projected configuration is known as a score plot. The PC loadings give the knowledge of the influential variables and also how the variables are correlated (Erikkson, et al. 2001).

SIMCA was first demonstrated by S. Wold in the early 1970s. SIMCA uses PCA to model the shape and the position of the object formed by the samples in row space for class definition. A multidimensional box is constructed for each class and the classification of future samples is performed by determining within the box (if any) the sample lies (Beebe, et al. 1998). Class modeling techniques build a class space whose boundary discriminates between samples fitting the class model and samples that do not belong to the studied class. In order to define the class boundary that involves the class space, it is necessary to determine the mathematical model of the class and to develop some kind of confidence interval around it. The significant PCs of each category build the class model, which is computed after a separate scaling for each category (Lanteri, et al. 2002).

As an example, SAW sensing electronic nose (zNose^{TM}) for flavor analysis was performed to characterize 16 different types of vegetable oils. A chemometric method, particularly PCA, was applied for electronic nose data processing and identification. Analysis of the score plot of the PCA for the zNose^{TM} measurement showed that 97% of the total variance in the data was described by PC1 and PC2 (Gan, et al. 2005).

5.2. Partial Least Squares Regression Analysis

PLS is often the main regression technique for multivariate data. This method is performed to relate the information in two blocks of variables, X and Y to each other (Eriksson, et al. 2001). The principle of PLS is to find the components in the input matrix (X) that describe as much as possible of the relevant variations in the input variables, and at the same time have maximal correlation with the target value in Y, but without including the variations that are irrelevant or noisy (Rezzi, et al. 2005). The objectives are to model X and Y and to predict Y from X.

$$X = 1 * \overline{x'} + T * P' + E \tag{5.2}$$

$$Y = 1 * \overline{y'} + U * C' + F \tag{5.3}$$

In these expressions, the first terms $1*\overline{x'}$ and $1*\overline{y'}$ shows the variable averages and originates from the pre-processing step. The information related to the observations is given by the scores matrices *T* and *U*; n the information related to the variables is stored in the X- loading matrix *P'* and Y-weight matrix *C'*. The variation in the data that is left out of the modelling forms the E and F residual matrices (Eriksson, et al. 2001).

Powerful statistical software packages are useful for the user since a series of very complicated calculations can be performed in a fast and comfortable way. These packages make the calculations very easy to apply sophisticated algorithms to almost any kind of data, without the need for special mathematical background. This includes reduction of dimensionality by PCA with cross-validation of the number of components, followed by the use of canonical variate predictive biplots for model development and canonical variate interpolative biplots for approximate classification of monovarietal and PDO olive oils (Rui Alves, et al. 2005)

Pena et al. (2005) developed a new methodology to detect and quantify adulteration of virgin olive oil and olive oil with hazelnut oil through direct analysis of oil samples by headspace-mass spectrometry and various multivariate pattern-recognition and regression techniques for data treatment: CA, SIMCA, PLS, and PCR.

CHAPTER 6

MATERIALS AND METHOD

6.1. Materials

6.1.1. Extracted Extra Virgin Olive Oil Samples

Extracted EVOO samples were obtained from different varieties and different geographical regions. The olive samples used in this study, Ayvalık, Domat, Erkence, Gemlik, Memecik, Nizip, were obtained from Olive Research Institute in İzmir, Turkey and Ayvalık and Gemlik varieties were also obtained from Olive Nursery in Edremit, Turkey in 2005-2006 (1.) and 2006-2007 (2.) harvest years. The olives were harvested in October till November of each harvest year. About 15-25 kg olives from each variety were divided to 5 kg batches and pressed with a laboratory scale mill (TEM Spremoliva, Italy). At least two different batches of oil were obtained from each variety and stored in dark brown bottles at 8°C for further analyses. The oil samples are listed in Table 6.1.

Table 6.1. Names and codes of the extracted EVOO samples obtained in the 1. and 2. harvest years

Sample Name	Sample Code
Memecik	M
Erkence	E
Gemlik	G
Ayvalık	A
Domat	D
Nizip	N
Gemlik-Edremit	GE
Ayvalık-Edremit	AE

6.1.2. Commercial Extra Virgin Olive Oil Samples

Total of 22 and 26 commercial EVOO samples were obtained in the 1. and 2. harvest years, respectively, from Tariş Olive and Olive Oil Agricultural Sales Cooperatives Union in İzmir, Turkey. These oil samples were obtained from different locations of the North and South of Aegean region is shown in Figure 6.1. The names and codes of oil samples of the 1. and 2. harvest years are given in Table 6.2. Ayvalık and Memecik are the dominant varieties of North and South Aegean regions, respectively. Approximately 500-1000 ml were obtained for each oil sample and stored in dark brown bottles at 8°C for further analysis.

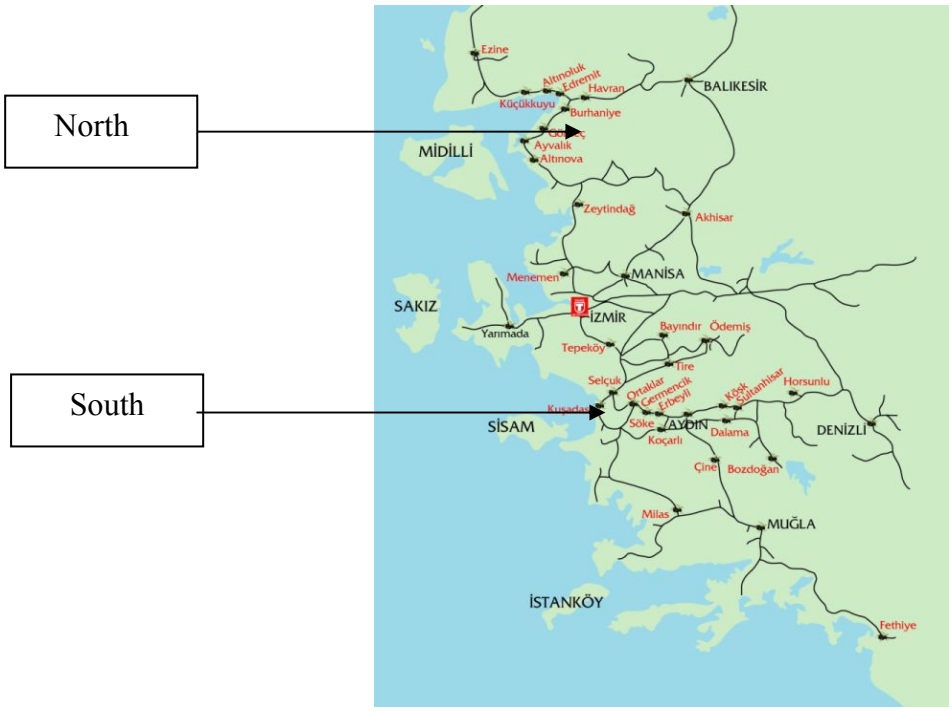

Figure 6.1. Commercial EVOO samples obtained from North and South of Aegean region (Source: Tariş Zeytinyağı 2007)

Table 6.2. Names and codes of commercial EVOO samples obtained in the 1. and 2. harvest years

1. harvest year		2. harvest rear	
Name	**Sample Code**	**Name**	**Sample Code**
Ezine (N)	Ez	Ezine (N)	Ez
Ezine Gülpınar Organik (N)	Ez-org	Küçükkuyu (N)	KucKuy
Küçükkuyu1 (N)	KucKuy1	Altınoluk (N)	Altol
Küçükkuyu2 (N)	KucKuy2	Edremit (N)	Edr
Altınoluk (N)	Altol	Havran (N)	Hav
Altınoluk-Sulubaskı (N)	Altol-sulbas	Burhaniye (N)	Bur
Edremit (N)	Edr	Gömeç (N)	Gom
Havran (N)	Hav	Ayvalık (N)	Ayv
Burhaniye (N)	Bur	Altınova (N)	Altova
Gömeç (N)	Gom	Zeytindağ (N)	Zey
Ayvalık (N)	Ayv	Tepeköy (S)	Tep
Altınova (N)	Altova	Bayındır (S)	Bay
Zeytindağ (N)	Zey	Ödemiş (S)	Ode
Akhisar (S)	Akh	Tire (S)	Tire
Menemen (S)	Men	Selçuk (S)	Sel
Tepeköy (S)	Tep	Kuşadası (S)	Kus
Bayındır (S)	Bay	Germencik (S)	Ger
Selçuk (S)	Sel	Aydın (S)	Ayd
Aydın (S)	Ayd	Ortaklar (S)	Ort
Ortaklar (S)	Ort	Köşk (S)	Kosk
Koçarlı (S)	Koc	Dalaman (S)	Dal
Milas (S)	Mil	Koçarlı (S)	Koc
		Erbeyli (S)	Erb
		Çine (S)	Cine
		Milas (S)	Mil
		Karaburun (S)	Karbur

(N): North
(S): South

6.1.3. Adulterated Extra Virgin Olive Oil Samples

For monovarietal olive oil adulteration Ayvalık-Edremit or Erkence oils were adulterated with Nizip oil at the percentages of 5, 10, 15 and 20 % (v/v). Commercial olive oils were also adulterated with other edible oils such as sunflower, corn, soybean, and hazelnut oils. Commercial extra virgin olive oil samples were obtained from Tariş Olive Oil Company (İzmir, Turkey) and sunflower, corn, soybean, and hazelnut oils were purchased from a local supermarket in İzmir. Commercial olive oil obtained from the North Aegean region, which was mainly belonging to Ayvalık variety, was mixed with one of the edible oils (sunflower, corn or soybean oils) at 7 different levels ranging from 5 to 50% (v/v). Two North Aegean region (Zeytindağ and Küçükkuyu – mainly belonging to Ayvalık variety), two South Aegean region (Milas and Selçuk – mainly belonging to Memecik variety), and Erkence oils were blended with hazelnut oil at 7 different levels varied from 5% to 50% (v/v). The adulterated samples were prepared prior to analysis.

6.2. Methods

6.2.1. Electronic Nose Analysis

The aroma fingerprints of extracted and commercial EVOO samples and adulterated oil samples were obtained using an electronic nose (zNose^{TM} 7100 vapor analysis system, Electronic Sensor Technology, CA, USA). The zNose^{TM} consists of 1 m DB-5 column and a surface acoustic wave (SAW) detector with a parts per billion sensitivity. The SAW detector, a small vapor sensor, is used to detect volatile organic compounds (VOCs). The SAW detector is an uncoated piezo-electric quartz crystal which is only specific to vapor pressure. The specificity of the detector depends on the crystal surface temperature and the vapor pressure characteristics of the condensate itself. The crystal is in contact with a thermoelectric heating and cooling element, which controls the temperature for heating during the cleaning of the crystal and especially cooling during vapor adsorption. The crystal operates by maintaining highly focused and resonant surface acoustic waves (500 MHz) on its surface. The volatiles adsorbed on the surface of the SAW detector alter the frequency of the SAW and this affects the

detection signal and allows the detection of the volatile compound (Staples 1998; Staples 2001). The SAW detector is shown in Figure 6.2.

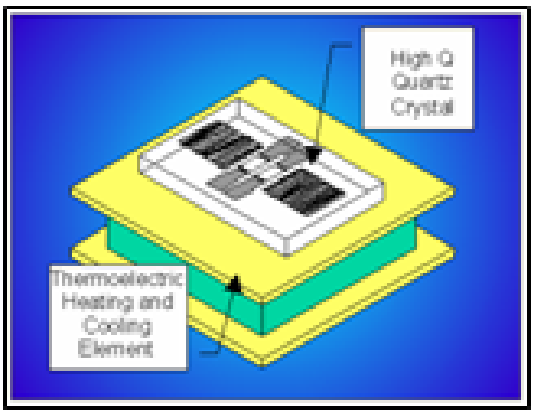

Figure 6.2. SAW detector
(Source: EST 2002)

For the zNose™ measurements, 10 ml of each oil sample was transferred into a 40 ml septa-sealed vial and left overnight at room temperature prior to analysis. The vials were then placed into a water bath at 30 °C for 15 min. During this time, the oil samples were allowed to equilibrate with the headspace in the vial and then the sample's vapor was pumped into the zNose™ with a side-ported sampling needle through the septa. While the samples were in the water bath, the system was calibrated with n-alkane solution (C6-C14). After calibration, the samples were measured one at a time with the zNose™. For each oil sample at least 3 vials were prepared and 4-6 readings were taken from each vial.

For each measurement, there were 3 phases, the sampling phase, the injection phase and the analysis. During the sampling phase, the system analyzed compounds by drawing an air sample via a pump into the inlet. The sampling mode was set to 10 sec and the inlet temperature was 200 °C. The sample passed through the valve where the compounds were adsorbed onto the trap tube. This sampling phase is illustrated in Figure 6.3. The valve (165 °C) was then rotated to put the trap in line with the column for injection phase.

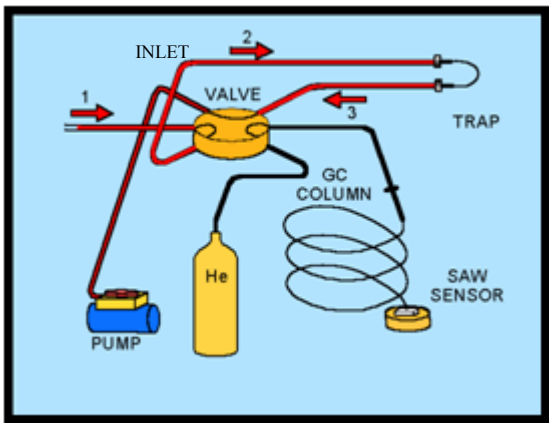

Figure 6.3. Sampling phase

(Source: EST 2002)

During the injection phase, the trap was heated (280 °C) to vaporize the adsorbed compounds. The carrier gas (helium) was transported the compounds to the DB-5 capillary column (4.0 cm^3). The column was heated from 40 °C to 180 °C at a rate of 7 °C/sec and the compounds were separated. Column separation was achieved by means of an internal coating of a bonded liquid phase. The solubility of a compound in the liquid phase determined the time required for a compound to travel down the column which was shown as retention time. In the analysis phase, the separated compounds sequentially exit the column and stick on the SAW detector. The SAW detector was operated at 20 °C. The added mass of the compound caused the frequency of the SAW crystal to shift. The identification and quantification of the material were determined due to the change of SAW crystal frequency (EST 2002, Gan, et al. 2005). The injection and analysis phases are illustrated in Figure 6.4.

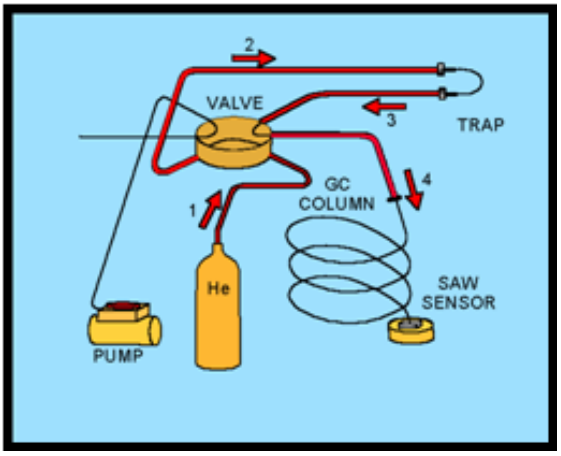

Figure 6.4. Injection and analysis phases

(Source: EST 2002)

The data were collected every 0.02 sec. After each data sampling period the sensor was shortly heated to 150°C for 30 sec. During this baking period, the temperature conditions of the inlet, column, and sensor were reset to the initial conditions. The n-alkane solution was run to ensure cleaning of the system and a stable baseline in between each measurement.

The zNose™ utilized the MicroSense software which was used to interpret the results of the analysis. The data arrived at the system controller as digital information in the form of frequency and time. The frequency was plotted as a function of time. The frequency information as read directly from SAW detector was shown in the lower window in Figure 6.5. This data was differentiated to produce the derivative plot which was shown in the upper window. The upper derivative window represented the compounds exiting the column in a traditional gas chromatogram style. Each peak found in the derivative plot was listed in the peaks window. After a peak was detected, it is quantified by determining its peak area. This is the summation of the area underneath a peak in the derivative window. The area which was bounded by a line drawn from a start point to the stop point and the peak curve, determined the peak area. This value was the same as the actual frequency change occurred as the compound deposited on the SAW detector. The peak area was correlated to the compound concentration and was expressed in counts (cts) (EST 2002).

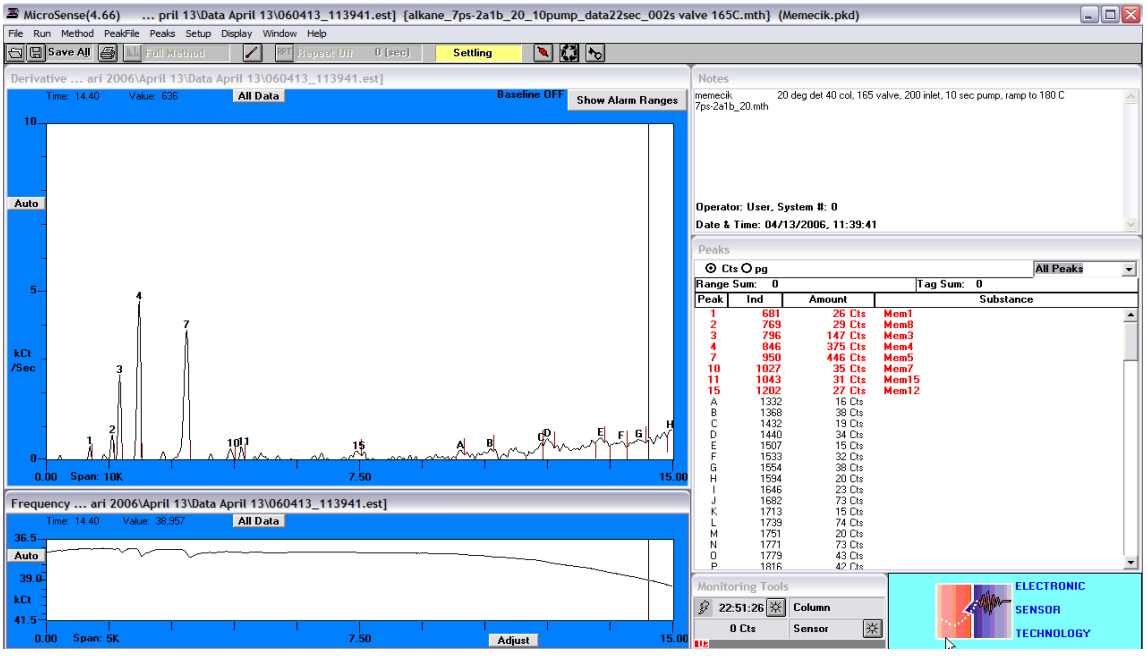

Figure 6.5. Results window illustrated by Microsense software

6.2.2. Sensory Analysis

6.2.2.1. Same-Different Test

For the 1. and 2. harvest years the same cultivars obtained from different regions, Ayvalık-Edremit (AE), Ayvalık (A) and Gemlik-Edremit (GE), Gemlik (G) were included in order to perform the same-different test. (Ayvalık and Gemlik cultivars were obtained from İzmir.) The test was carried out with 30 untrained panelists who consume olive oil. One pair contained identical samples (A-A, AE-AE, G-G or GE-GE), and the second pair contained the samples that differ in the geographical region were evaluated by the panelists. Each sample was assigned a three-digit random number. They could taste the samples as many times as they wished, the only limitation being the amount of olive oil sample provided (approximately 5 ml). The panelist was required to state whether the two olive oil samples were same or different and to mark their response on the ballot for same-different test. Panelists were allowed to palate cleansing with water and unsalted bread.

Table 6.3. The same-different test ballot

SAME / DIFFERENT TEST Test No.
Panelist No. ____ Name: _____ Age: _____ Date: _____ Type of sample: Extra Virgin Olive Oil
Instructions 1. Taste the samples from left to right. 2. Determine if samples are the same / identical or different. 3. Mark your response below. Note that some of the sets consist of two identical samples.
Sample codes ____ Products are the same. ____ Products are different.

6.2.2.2. Acceptance Test

The acceptance test was conducted in order to establish the panelists' preference between 8 different EVOO samples (Ayvalık, Domat, Erkence, Gemlik, Memecik, Nizip, Ayvalık-Edremit, Gemlik-Edremit produced from different cultivars and different geographical regions for the 1. and 2. harvest years. The color, odor, taste of the olive oils were evaluated by 20 untrained panelists who were selected from a group of people consuming olive oil. The 4 different olive oil samples at about 5 ml were presented to the panelists and asked them to define their preferences based on color, odor, taste, and overall acceptance according to the categorical scale ranging from excellent (1) to very bad (5) and their opinions. The ballot used for the acceptance test is given in Table 6.4. Panelists were allowed to clean the palates with water and unsalted bread. The same procedure was applied to the other set which consisted of 4 different olive oils on different date.

Table 6.4. The acceptance test ballot

Panelist No. ____ Name: _____ Age: _____ Date: _____
Product: Extra Virgin Olive Oil

Taste the samples from left to right. Thank you.

How often do you buy olive oil?

____ Never
____ Less than one per year
____ 1-2 times per year
____ 3-5 times per year
____ Less than 1 time per month
____ 1 time per month
____ 2-3 times per month

How often do you consume olive oil?

____ One or less per year
____ Less than 1 time per year
____ 1-3 times per month
____ 1 time per week
____ 2-4 times per week
____ 1 time per day
____ More than 1 time per day

Sample No:	Evaluation criterions				Opinions
	Color	Odor	Taste	Overall acceptance	
Excellent (1)					
Good (2)					
Neither good or bad (3)					
Bad (4)					
Very bad (5)					

6.3. Data Analysis

The sensory analysis data were analyzed using the chi-square test and analysis of variance (ANOVA) by MINITAB® release 13 (Minitab Inc., State College, USA). The discrimination of extracted EVOOs, commercial EVOOs, adulterated oils and the prediction of the adulteration levels were demonstrated by using multivariate statistical methods which are PCA and PLS using soft independent modelling of class analogy (SIMCA) software (Umetrics, Sweden).

6.3.1. Chi-Square Test

The results of the sensory evaluation of the Ayvalık and Gemlik EVOO samples obtained from two different regions (Bornova and Edremit) for the 1. and 2. harvest years were evaluated by chi-square test. The chi-square test is a useful statistical distibution for comparing frequencies of events classified in a table of categories. If each observation can be classified by two or more variables, it enters into the frequency count for a part of a matrix or classification table, where rows and columns represent the levels of each variable (Lawless and Heymann 1998).

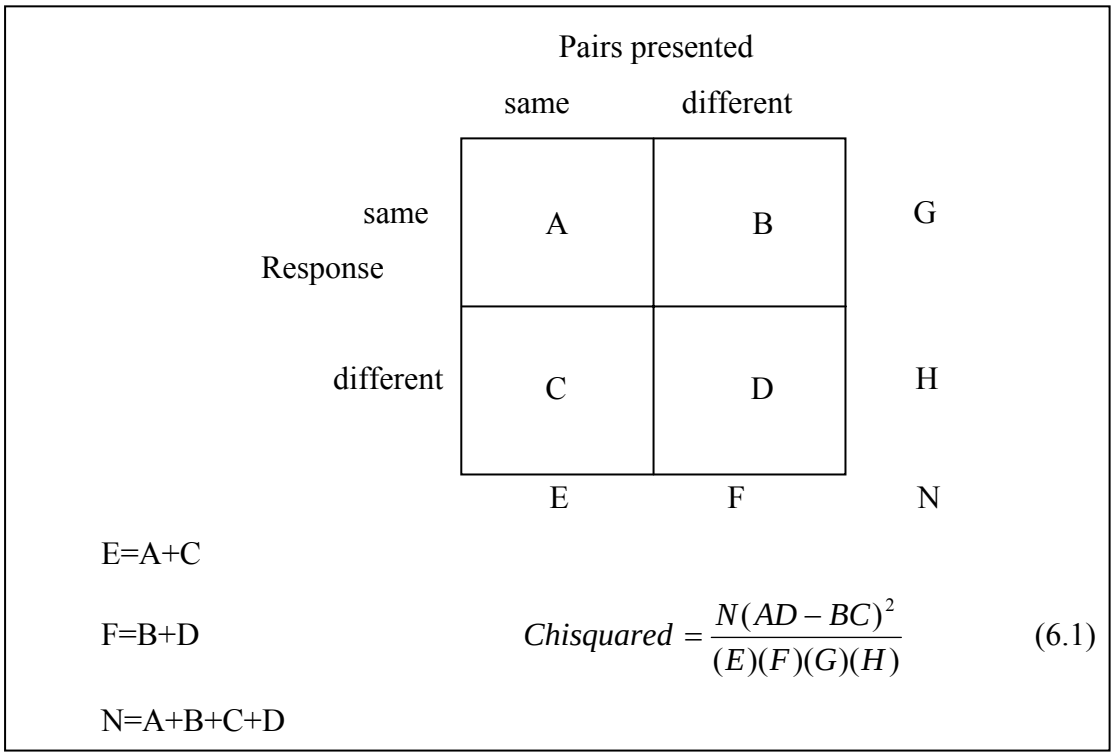

Figure 6.6. The use of chi-square distribution for the same-different test
(Source: Lawless and Heymann 1998)

6.3.2. Analysis of Variance

ANOVA and Fisher significance test was conducted for the evaluation of the acceptance test results of the extracted EVOO samples. Significance was accepted at $p<0.05$. ANOVA is commonly used statistical technique used for analyzing measurements based on several types of effects to decide which kinds of effects are significant and to estimate the effects which tests the difference between the means of two or more groups. In analysis of variance, the ratio of the factor variance to the error variance gives the distribution of an F-statistic. A significant F-ratio for a given factor means that at least one of the indiviual comparisons among means is significant for that factor. The null hypothesis for ANOVA is that the means for the treatment levels are equal in the main population (Lawless and Heymann 1998).

6.3.3. Principal Component Analysis

PCA was carried out on the discrimination of extracted and commercial EVOO samples based on their aroma fingerprints obtained by the electronic nose. Before the analysis of the electronic nose data of each extra virgin olive oil sample, the averages of readings belong to the same vial were calculated. PCA is a multivariate projection method to visualize data. It converts a dataset of correlated variables into a new set of uncorrelated (i.e. orthogonal) variables called PCs (Focardi, et al. 2006). The main aim of PCA is to reduce the number of variable dimensionality to a much smaller number of principal components (PCs). This is done by effective visualization, regression and classification of multivariate data (Poulli, et al. 2005). PCA gives general information about the relation of the observations and if there are any deviating observations or groups of observations in the data. Two PCs have been used to define a plane into the K-dimensional variable space. It is possible to examine the structure of the data set by projecting all the observations. The plotting of this projected configuration is called a score plot. Coomans' plot is constructed using the PCA class model and this model is used as a graphical display of the classification of each classes. The significant principal components of each category build the class model after a separate scaling of each category. In Coomans' plot class distances for two classes are plotted against each other

in a scatter plot (Eriksson, et al. 2001). The methods were performed using soft independent modelling of class analogy (SIMCA) (Umetrics, Sweden) software.

6.3.4. Partial Least Squares Regression Analysis

In adulteration studies, the quantification of the concentration of the sample in the adulteration mixtures was performed using partial least squares regression analysis (PLS). The average values of the readings of the same vial were calculated. The data were divided into two groups: The prediction set was formed by choosing approximately one third of the total samples randomly and the other samples were used for the calibration model. PLS was conducted for modelling the association between the electronic nose data of the adulterated samples with the adulteration percentages of the samples.

The principle of PLS is to find the components in the input matrix (*X*) that describe as much as possible of the relevant variations in the input variables both with the maximal correlation with the target value in *Y*, but without including the irrelevant or noisy variations (Rezzi, et al. 2005). The calibration models were validated by excluding selected samples randomly and developing a number of parallel models from the reduced data. Then, the omitted data is predicted by the different models and finally compared with the actual values (Eriksson, et al. 2001). The standard error of calibration (SEC) is calculated to measure how well models with different number of variables fit the calibration data. The standard error of prediction (SEP) accounts for the predictive ability of the model (Beebe, et al. 1998). SEP involves the prediction of external samples by using the model and depends on the number of factors used for the calibration. SEC and SEP were calculated by means of the following expressions:

$$SEC = \sqrt{\frac{\sum_{i=1}^{m}(c_i - \hat{c}_i)^2}{m-2}} \qquad SEP = \sqrt{\frac{\sum_{i=1}^{m}(c_i - \hat{c}_i)^2}{m}} \tag{6.2}$$

Where c_i is the actual, $\hat{c}_i$ is the predicted concentration of the *i*th sample of i^{th} for m number of samples (Hajimahmoodi, et al. 2005, López-Feria, et al. 2007).

CHAPTER 7

RESULTS AND DISCUSSION

7.1. Classification of Extra Virgin Olive Oil Samples Based on Their Aroma Fingerprints

Extracted EVOO samples were obtained from different varieties in 2005-2006 (1.) and 2006-2007 (2.) harvest years to study the effect of variety and harvest year on the aroma fingerprints of the EVOO samples. Nizip olive oil sample was different than the other samples since this cultivar belongs to the Southeast region of Turkey but obtained from İzmir. Ayvalık and Gemlik olive varieties were also collected from two different regions (İzmir and Edremit) to investigate the effect of geographical region on the same variety of EVOO.

Total of 22 and 26 commercial extra virgin olive oil samples were obtained in 2005-2006 (1.) and 2006-2007 (2.) harvest years, respectively. These oil samples were obtained from different locations of North and South of Aegean region to investigate the importance of geographical regions on the aroma fingerprints of these olive oil samples.

The aroma fingerprints of extracted and commercial extra virgin olive oil samples were obtained using an electronic nose consisting of a SAW detector. The zNose^{TM} was calibrated with a n-alkane solution (C6-C14) before the analysis of the oil samples. The electronic nose chromatogram of the alkane mixture is given in Figure 7.1.

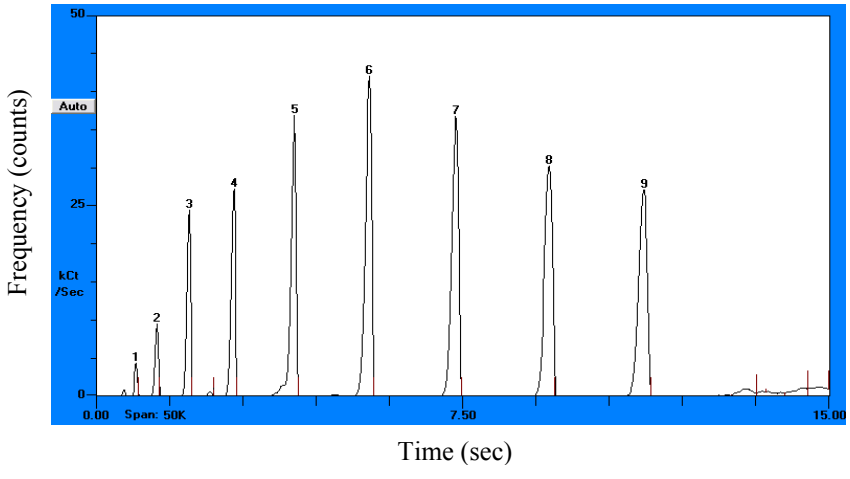

Figure 7.1. The electronic nose chromatogram of the n-alkane solution

7.1.1. Classification of Extracted Extra Virgin Olive Oil Samples of the 1. Harvest Year

For the 1. harvest year the electronic nose responses of 8 different monovarietal EVOO samples were analyzed using PCA to see the discrimination of the samples according to variety. PCA is a very powerful multivariate statistics method used to find the linear combinations of the variables that contribute to the differentiation of the samples. PCA of the electronic nose data of the 8 samples resulted in four PCs explaining 75.2% of the total variation. PCA score plot of the 8 EVOOs of the 1. harvest year is given in Figure 7.2. The figure shows the two dimensional score plot of the first two components (PC1 and PC2) which reflect 54.3 % of the total variation. Most of the Nizip, Erkence, Gemlik-Edremit and Ayvalık-Edremit varieties clustered and distinguished from the other olive oil samples.

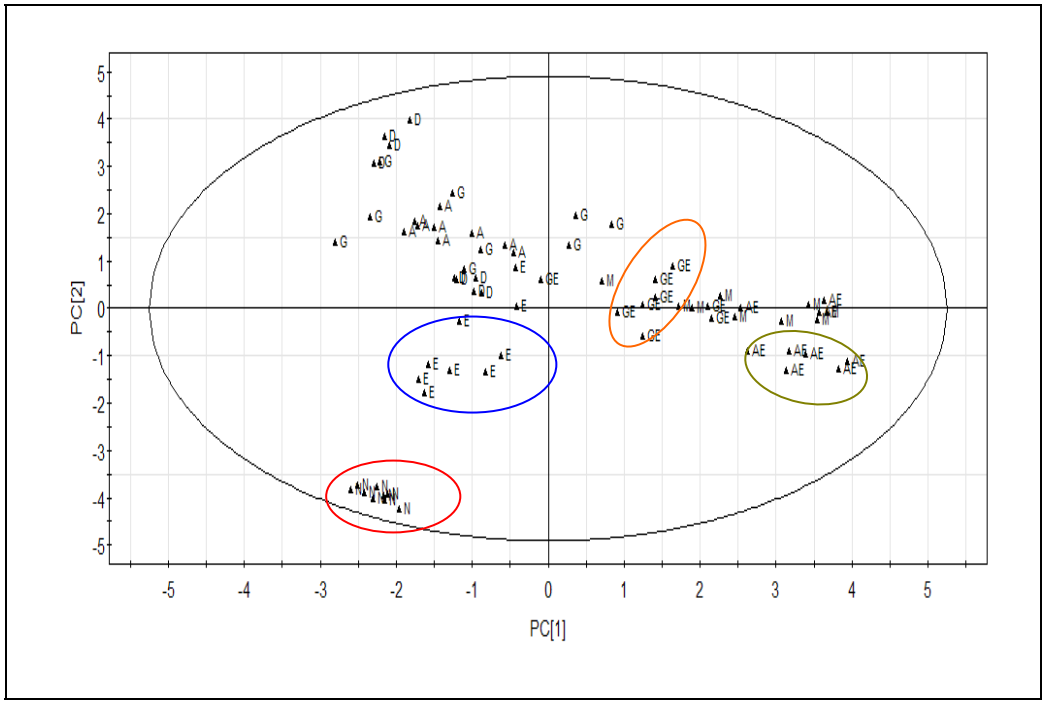

Figure 7.2. PCA score plot of the 8 different EVOOs of the 1. harvest year

To see the differentiation among the monovarietal olive oil samples clearly, using PCA class model the Coomans' plot was constructed for the classification of Nizip and Ayvalık olive oils is shown in Figure 7.3. The general statistics of PCA class model is given in Table 7.1. R^2 cumulative values in Table 7.1 were calculated to show

how the data fits the model. The x-axis of the Cooman's plot corresponds to the SIMCA distance of the Ayvalık class model and the y-axis of the plot shows the SIMCA distance of the Nizip class model. Ayvalık was successfully discriminated from Nizip by PCA class model. Erkence and Gemlik Edremit olive oil samples clustered on the region that contained the samples which did not fit any of these class models. Lorenzo et al. (2002) reported that the headspace-mass spectrometry as an alternative to the conventional methodology had afforded better results for the differentiation of the monovarietal olive oils.

Table 7.1. General statistics of PCA class model

Sample codes	Class no	Number of PCs	R^2X(cum)
M	1	2	0.636
E	2	2	0.607
G	3	5	0.932
A	4	3	0.871
D	5	3	0.966
N	6	4	0.976
GE	7	2	0.507
AE	8	2	0.805

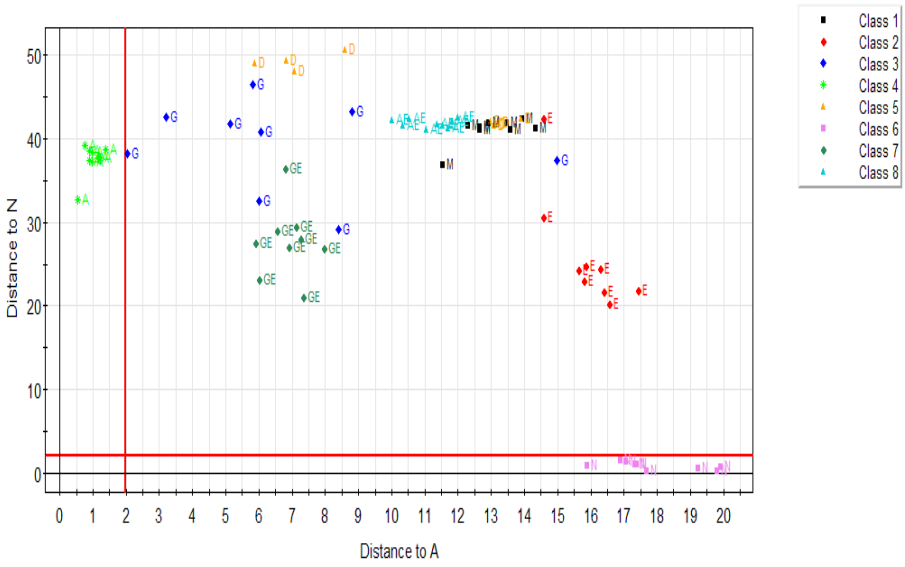

Figure 7.3. Coomans' plot with the distance to the Ayvalık (A) model plotted versus distance to the Nizip (N) model.

7.1.2. Aroma Fingerprints of Extra Virgin Olive Oil Samples of the 2. Harvest Year

PCA was also carried out on the electronic nose results of the 8 different monovarietal EVOO samples which were produced in 2. harvest year to classify the olive oil samples according to the variety. The score plot of PC1 versus PC2 is presented in Figure 7.4. The PC1 and PC2 factors resulted in a model that described 44.5 % of the total variance in the data. It was observed that there was no distinct separation between the samples. Only Erkence olive oil samples seemed to be grouped together in the score plot.

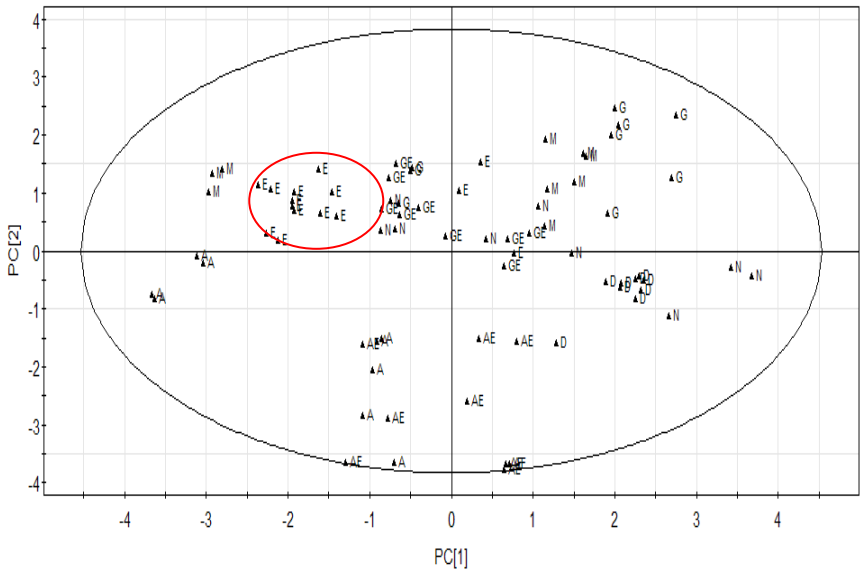

Figure 7.4. PCA score plot of the 8 EVOOs of the 2. harvest year

Coomans' plot with the distance to the Ayvalık-Edremit (AE) class model plotted versus distance to the Nizip (N) class model is presented in Figure 7.5. General statistics of PCA class model is given in Table 7.2. The class models of Nizip and Ayvalık-Edremit samples were discriminated successfully, but Domat and some of the Erkence olive oil samples could not be separated from Nizip class model.

Table 7.2. General statistics of PCA class model

Sample codes	Class no	Number of PCs	R^2X(cum)
M	1	3	0.919
E	2	3	0.878
G	3	3	0.941
A	4	3	0.961
D	5	3	0.941
N	6	3	0.927
GE	7	3	0.939
AE	8	4	0.943

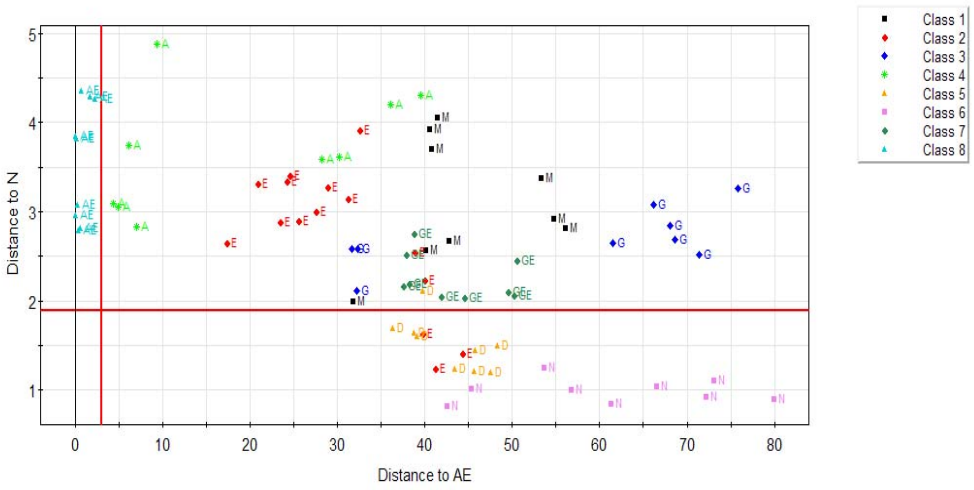

Figure 7.5. Coomans' plot with the distance to the Ayvalık-Edremit (AE) model plotted versus distance to the Nizip (N) model.

The Coomans' plot was also constructed to see the differentiation of the Gemlik monovarietal EVOO samples which were obtained from two different regions (İzmir and Edremit). General statistics of PCA class model is shown in Table 7.3. The Coomans' plot with the distance to the Gemlik-Edremit model (Class 2) versus distance to the Gemlik model (Class 1) is given in Figure 7.6. Two class models were

discriminated accurately by PCA. On the other hand in the lower-left hand part of the plot, some of the Gemlik-Edremit (GE) samples were located on the region that contains the samples fitted both models.

Table 7.3. General statistics of PCA class model

Sample codes	Class no	Number of PCs	R^2X(cum)
G	1	5	0.926
GE	2	4	0.818
A	1	3	0.841
AE	2	5	0.969

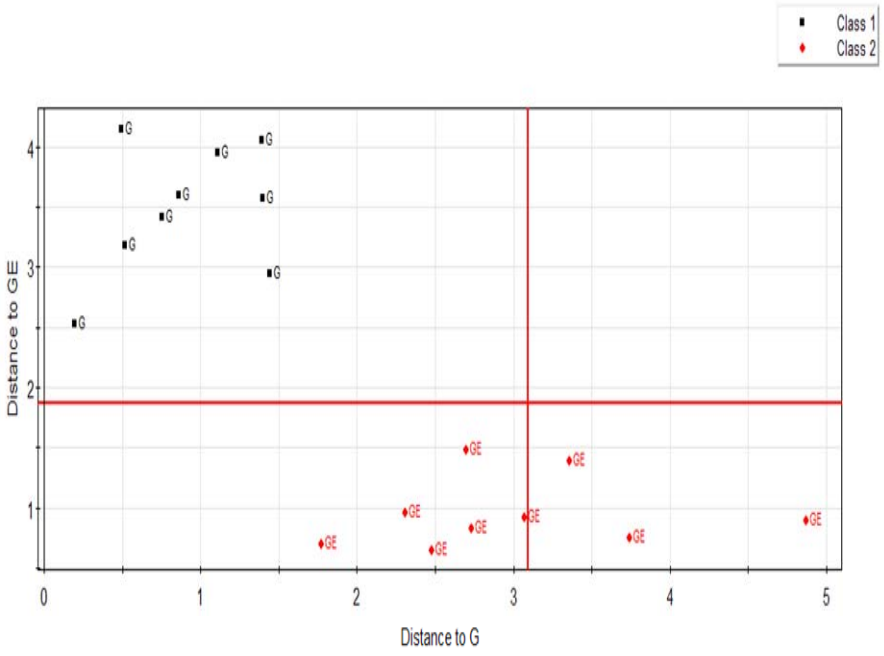

Figure 7.6. Coomans' plot of the Gemlik (Class 1) and Gemlik-Edremit (Class 2) class models

The Coomans' plot of the Ayvalık olive oil obtained from the same variety of olives collected from two different regions is presented in Figure 7.7. General statistics of PCA class model is given in Table 7.4. The x and y axis of the Coomans' plot demonstrated the SIMCA distance of the Ayvalık (A) and Ayvalık- Edremit class models with two PCs for each class model. The right-lower hand part of the plot

demonstrated the region where there were observations predicted to fit the Ayvalık-Edremit model and the left–upper part of the plot showed the observations that fitted the Ayvalık model. The two models were classified successfully by PCA class model and is shown in Figure 7.7.

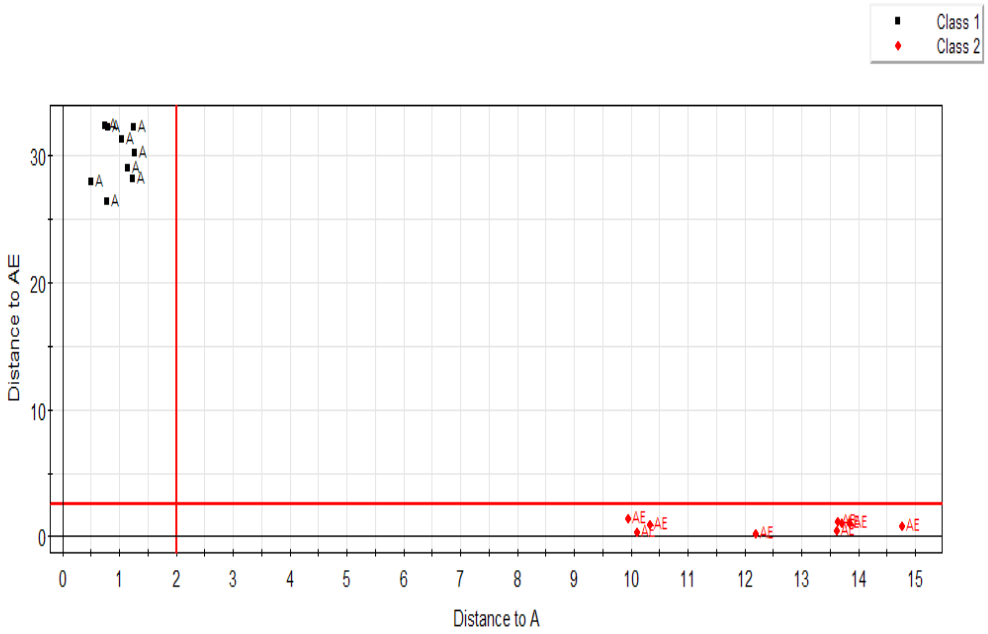

Figure 7.7. Coomans' Plot of the Ayvalık (Class 1) and Ayvalık Edremit (Class 2) class Models

The Coomans' plot of the Gemlik monovarietal extra virgin olive oil sample obtained from two different regions (İzmir and Edremit) was also demonstrated to observe the discrimation among the regions. The Coomans' plot with the distance to the Gemlik-Edremit class model (Class 2) versus distance to the Gemlik class model (Class 1) is shown in Figure 7.8. General statistics of this class model is presented in Table 7.4. The two models were discriminated from each other accurately. In previous works, Guadarrama et al. (2001) successfully applied PCA to the electronic nose data to discriminate the similar types of olive oils obtained from different geographical origins. The results revealed that the samples of EVOOs which had similar organoleptic characteristics with different geographical origins could be differentiated by using PCA.

Table 7.4. General statistics of PCA class model

Sample codes	Class no	Number of PCs	R^2X(cum)
G	1	3	0.939
GE	2	4	0.977
A	1	2	0.738
AE	2	2	0.733

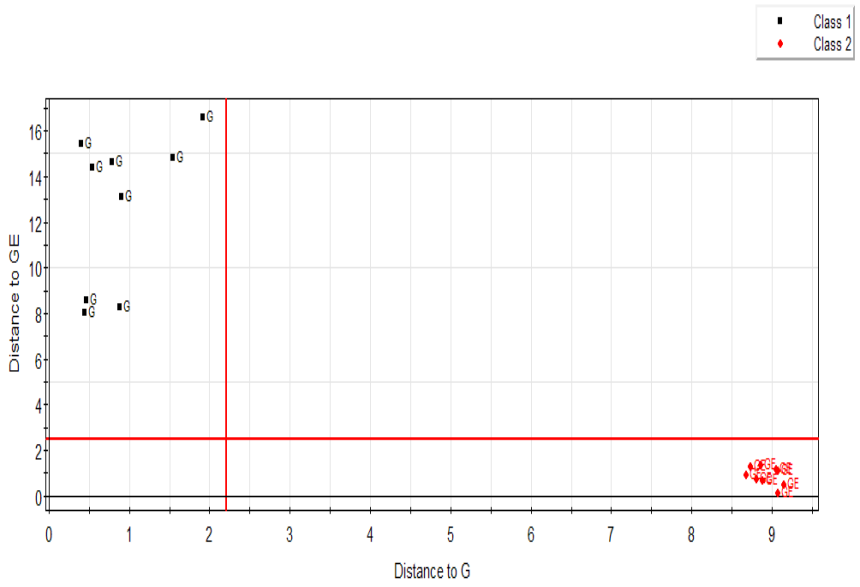

Figure 7.8. Coomans' plot of the Gemlik (Class 1) and Gemlik-Edremit (Class 2) class models

The Coomans' plot of the Ayvalık olive oil obtained from the same variety of olives collected from two different regions is shown in Figure 7.9. The x-axis showed the SIMCA distance to the Ayvalık (A) model, the y-axis showed the SIMCA distance to the Ayvalık-Edremit (AE) model. The right-lower hand part of the plot demonstrated the region where there were observations predicted to fit the Ayvalık-Edremit class model and the left–upper part of the plot showed the observations that fitted the Ayvalık

class model. The Ayvalık samples were separated successfully using PCA class model based on their geographical regions and is shown in Figure 7.9.

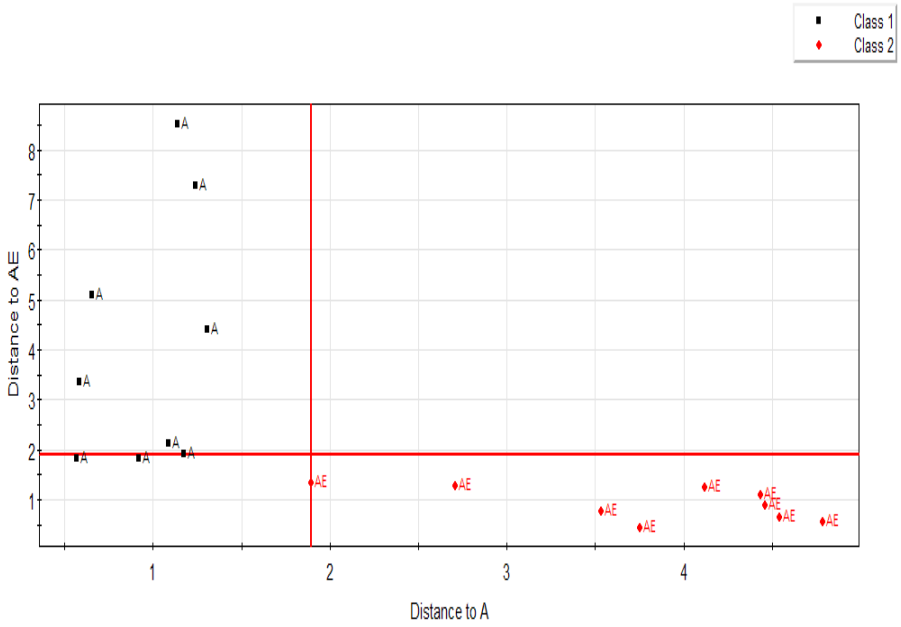

Figure 7.9. Coomans' plot of the Ayvalık (Class 1) and Ayvalık-Edremit (Class 2) class models

7.1.3. The Comparison of Aroma Fingerprints of Extra Virgin Olive Oil Samples of the 1. and 2. Harvest Years

The comparison of the extracted EVOO samples of the 1. and 2. harvest years were obtained by applying PCA and Coomans' plot to the electronic nose data. PCA score plot is shown in Figure 7.10. It was observed that the 1. and 2. harvest years extra virgin olive oil samples were separated along PC1. Nizip and Erkence EVOO samples that were obtained in the 1. harvest year were clustered closer to the extra virgin olive oils of the 2. harvest year.

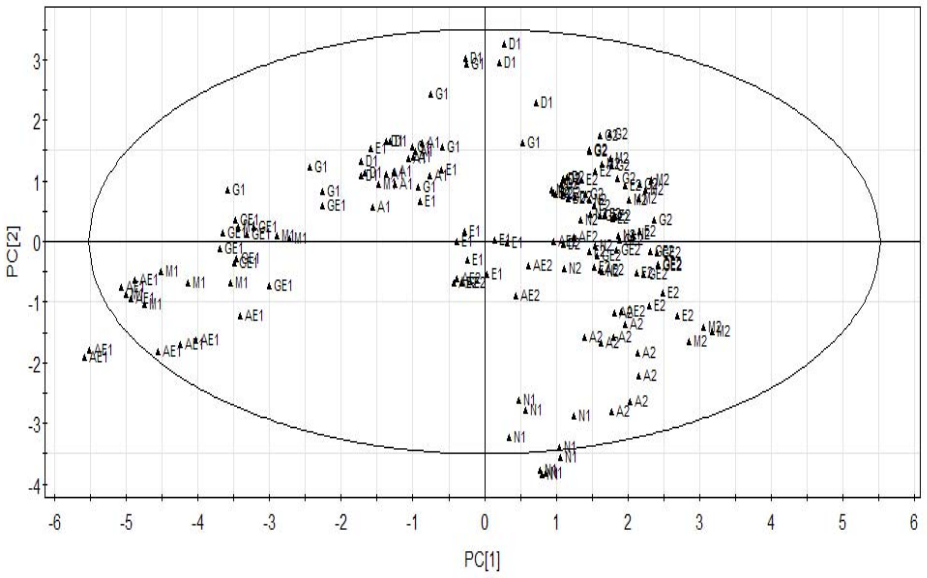

Figure 7.10. PCA score plot of the EVOO samples of the 1. and 2. harvest years

The similarity and dissimilarity of the EVOO samples of both harvest years were also compared by the Coomans' plot as shown in Figure 7.11. The x-axis of the Coomans' plot was corresponded to the SIMCA distance to the 1. harvest year, the y-axis showed the SIMCA distance to the 2. harvest year. In the right-lower hand part of the plot the EVOO samples of 2. harvest year were grouped. Some of the Erkence and Ayvalık olive oil samples were found in the common region. In the left-upper hand part of the plot the EVOO samples of 1. harvest year were clustered. One of the Gemlik oil sample did not fit any of the models. Garcìa-Gonzàles and Aparicio (2004) reported that a large set of single varietal olive oils from different geographical origins could be classified correctly based on metal-oxide sensors and a mathematical model.

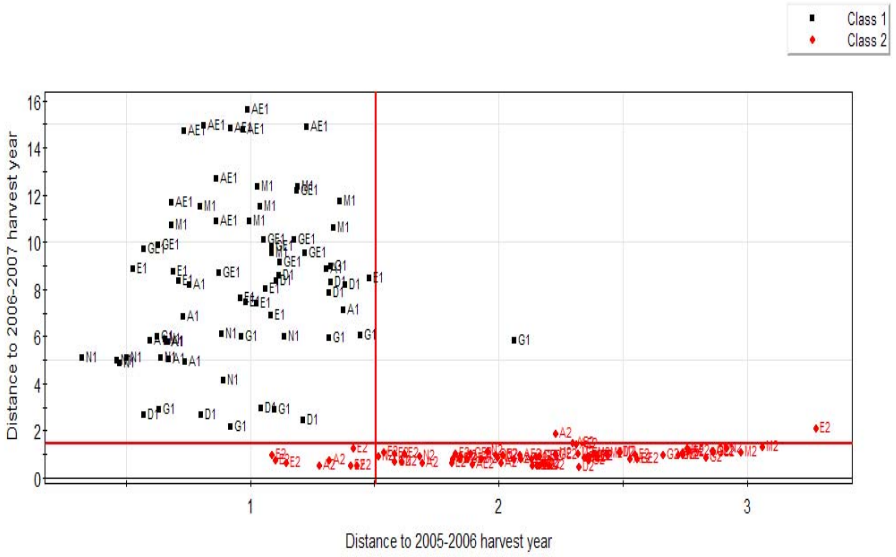

Figure 7.11. Coomans' plot of the EVOO samples of the 1. and 2. harvest years

7.2. Sensory Analyses of the EVOOs Produced in the 1. and 2. Harvest Years

7.2.1. Same-Different Test Results

For the sensory evaluation of the extracted olive oil samples, the same-different test was performed to determine whether the same cultivars grown in different regions (Ayvalık-Edremit, Ayvalık and Gemlik-Edremit, Gemlik) could be distinguished by the untrained panelists. The results of the panelists' responses of the Ayvalık olive oil sample of the 1. and 2. harvest years are given in Tables 7.5 and 7.6, respectively. The responses of the panelists were evaluated by Chi-Square test procedure. This test was applied to determine the relationship between the classification variables. The results showed that there were no differences distinguished between the EVOO samples obtained from the olives Ayvalık and Gemlik cultivated in different geographical regions (İzmir and Edremit) based on the panelists' responses.

Table 7.5. The panelist responses for Ayvalık olive oil of the 1. harvest year

		Subjects received		
		Matched pair (Ayv and Ayv or Ayv-Edr and Ayv-Edr)	Unmatched pair (Ayv and Ayv-Edr)	Total
Subjects responses	Same	11	12	23
	Different	19	18	37
	Total	30	30	60

Ayv: Ayvalık (İzmir)
Ayv-Edr: Ayvalık-Edremit

$$\text{Chi-squared} = \frac{60.(11.18 - 12.19)^2}{30.30.23.37} = 0.07$$

$df = 1 \propto = 0.05 \;\; X^2 = 3.84$

Since $0.07 < 3.84$

Table 7.6. The panelist responses for Ayvalık olive oil of the 2. harvest year

		Subjects received		
		Matched pair (Ayv and Ayv or Ayv-Edr and Ayv-Edr)	Unmatched pair (Ayv and Ayv-Edr)	Total
Subjects responses	Same	16	14	30
	Different	14	16	30
	Total	30	30	60

Ayv: Ayvalık (İzmir)
Ayv-Edr: Ayvalık-Edremit

$$\text{Chi-squared} = \frac{60.(16.16 - 14.14)^2}{30.30.30.30} = 0.26$$

$df = 1 \propto = 0.05 \;\; X^2 = 3.84$

Since $0.26 < 3.84$

The same-different test was also applied for the differentiation of the Gemlik olive oil sample. The chi-squared values were calculated by using the panelists' responses for the Gemlik varieties cultivated in different regions for the 1. and 2. harvest years. The results are given in Tables 7.7 and 7.8 and it was found that there were no differences related to the effect of environmental and location conditions on the sensory quality of these olive oils distinguished between the olive oils cultivated in different regions (Ayvalık and Gemlik) by the panelists.

Table 7.7. The panelist responses of Gemlik olive oil of the 1. harvest year

		Subjects received		
		Matched pair Gem and Gem or Gem-Edr and Gem-Edr)	Unmatched pair (Gem and Gem-Edr)	Total
Subjects responses	Same	11	12	23
	Different	19	18	37
	Total	30	30	60

Gem: Gemlik (İzmir)
Gem-Edr: Gemlik-Edremit

$$\text{Chi-squared} = \frac{60.(11.18 - 12.19)^2}{30.30.23.37} = 0.07$$

$df = 1 \propto = 0.05 \quad X^2 = 3.84$

Since $0.07 < 3.84$

Table 7.8. The panelist responses of Gemlik olive oil of the 2. harvest year

		Subjects received		
		Matched pair Gem and Gem or Gem-Edr and Gem-Edr)	Unmatched pair (Gem and Gem-Edr)	Total
Subjects responses	Same	16	12	28
	Different	14	18	32
	Total	30	30	60

Gem: Gemlik (İzmir)
Gem-Edr: Gemlik-Edremit

$$\text{Chi-squared} = \frac{60.(16.18 - 12.14)^2}{30.30.28.32} = 1.071$$

$df = 1 \propto = 0.05 \quad X^2 = 3.84$

Since $1.071 < 3.84$

7.2.2. Acceptance Test Results

Acceptance test was conducted in order to establish the panelists' preferences among the 8 different EVOO samples each of which was evaluated from the aspect of some sensorial properties. The color, odor, taste of the olive oils were evaluated by 20 untrained panelists. The results were evaluated by using ANOVA. There were no significant differences among the samples based on their color, odor and taste attributes (Table 7.9). Only Nizip showed different odor property among the other olive oil samples. When the overall acceptance results were compared, significant differences between the samples were observed among the samples according to the panelists' scores ($p<0.05$). Erkence and Ayvalık-Edremit olive oil samples were preferred by the panelists for their color and odor, respectively. Gemlik-Edremit olive oil sample was liked for its taste and also mostly preferred among other olive oil samples. Caporale et

al. (2006) worked with a panel of consumers familiar with several typical EVOOs to assess the impact of information about the origin of the product on the sensory profile perception and it was shown that the origin affected the expectations based on the specific sensory attributes in familiar consumers with EVOO.

Table 7.9. Sensory scores for the EVOOs of the 1. harvest year

Olive oil samples	Sensory scores			
	Color	Odor	Taste	Overall acceptance
Memecik	2.05±0.60	2.45±0.76[a]	2.80±1.01	2.68±0.67[ab]
Erkence	2.00±1.08	2.45±0.69[a]	2.95±1.19	2.84±1.01[bc]
Gemlik	2.30±0.80	2.60±0.94[a]	3.00±0.97	2.89±0.81[bc]
Ayvalık	2.15±0.75	2.50±1.00[a]	3.25±1.21	2.95±0.94[b]
Domat	2.30±0.57	2.30±0.73[a]	2.55±0.76	2.40±0.68[ac]
Nizip	2.50±0.83	3.20±1.32[b]	2.80±1.47	3.05±1.31[b]
Gemlik-Edremit	2.05±0.60	2.60±0.95[a]	2.40±0.82	2.33±0.69[a]
Ayvalık-Edremit	2.15±0.67	2.25±0.72[a]	2.50±0.89	2.35±0.61[ac]

a-c: Column means having different letter or letters differ ($p<0.05$)

Total of 8 different extra virgin olive oil samples for the 2. harvest year were also evaluated from the aspect of color, odor, taste and overall acceptance. The ANOVA was applied to the obtained scores and significant differences ($p<0.05$) were observed based on color, taste and overall acceptance of the samples and is given in Table 7.10. There was no significant difference between the odors of the samples recognized by the panelists. Erkence olive oil sample was mostly liked according to its color, Gemlik olive oil was preferred for its odor and Ayvalık was mostly liked by the panelists especially for its taste.

Table 7.10. Sensory scores for the EVOOs of the 2. harvest year

Olive oil samples	Sensory scores			
	Color	Odor	Taste	Overall acceptance
Memecik	2.10±0.45^{ab}	2.40±0.82	2.50±0.76^{a}	2.47±0.77^{ab}
Erkence	1.85±0.87^{a}	2.50±1.19	3.45±1.14^{b}	3.10±1.15^{b}
Gemlik	2.15±0.67^{ab}	2.20±0.69	2.40±0.82^{a}	2.37±0.68^{a}
Ayvalık	2.20±0.52^{ab}	2.50±0.68	2.30±0.57^{a}	2.25±0.44^{a}
Domat	3.15±0.81^{c}	2.65±0.93	2.75±0.72^{a}	2.79±0.63^{ab}
Nizip	1.90±0.78^{a}	2.74±0.87	2.95±1.09^{a}	2.68±1.06^{ab}
Gemlik-Edremit	2.05±0.39^{ab}	2.40±0.82	2.60±0.99^{a}	2.53±0.90^{ab}
Ayvalık-Edremit	2.40±0.94^{ab}	2.40±0.94	2.55±0.76^{a}	2.53±0.69^{ab}

a-c: Column means having different letter or letters differ ($p<0.05$)

7.3. Classification of Commercial Extra Virgin Olive Oil Samples

The electronic nose aroma fingerprints of 22 commercial EVOO samples obtained from Tariş for the 1. harvest year and 26 commercial EVOO samples for the 2. harvest year were obtained. The electronic nose responses were analyzed using multivariate statistical analysis by SIMCA software. PCA was utilized to discriminate North and South regions of West Turkey based on the aroma fingerprints of these commercial EVOO samples.

To observe the effect of geographical origin on the olive varieties, Coomans' plot was constructed for the classification of the North (Class 1) and South (Class 2) model classes. General statistics of the class model is given in Table 7.11. Figure 7.12 represents the Coomans' plot of North and South model for olive oil samples of the 1. harvest year. The x-axis showed the SIMCA distance to the North class while the y-axis showed the SIMCA distance to the South class. The aroma fingerprints of most of the EVOO samples were classified correctly according to their geographical regions. The distances in the Coomans' plot demonstrated that Akhisar and Menemen olive oil samples were closer to the North class and also the Havran and Küçükkuyu olive oil sample was the closest to the South class.

There have been a lot of successful applications of electronic nose technology for the differentiation of olive oils on the basis of geographical origin. Oliveros et al. (2005) successfully applied electronic nose and chemometric analysis to discriminate the different aromas of olive oils from five Mediterranean areas and the results indicated that the different aromas of olive oils coming from several geographical areas could be discriminated with a mean prediction ability of 80% after feature selection. An electronic nose with multivariate analysis have also been used to verify the geographical origin of extra virgin olive oils by Casale et al. (2007) and good results were obtained in classification of 46 oil samples from three different areas of Liguria by the application of LDA.

Table 7.11. General statistics of PCA class model

Samples	Class no	Number of PCs	R^2X(cum)
North	1	4	0.811
South	2	5	0.913

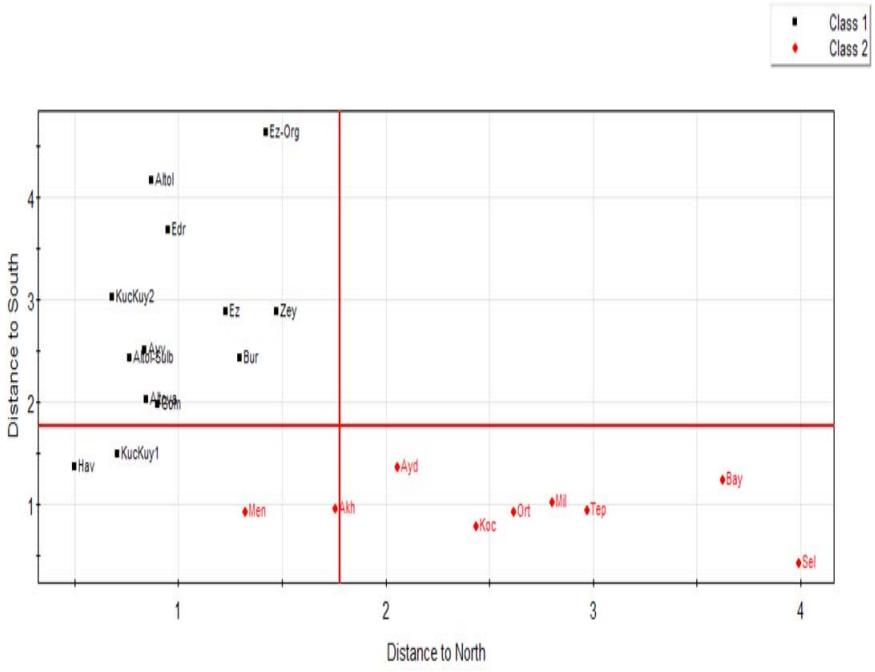

Figure 7.12. Coomans' plot of North (Class 1) and South (Class 2) class models using commercial EVOO aroma fingerprints of the 1. harvest year

PCA allows visualizing the information of the data set in a few PCs retaining the maximum possible variability within that set. The score plot of the electronic nose data of the 22 commercial olive oil samples is given in Figure 7.13. The two PCs explained 50.7 % of the data matrix variance. The North class had higher positive scores in PC1. Tepeköy olive oil sample had almost zero value of PC1 among South class. Altınoluk-Sulubaskı olive oil sample had higher negative value along PC1 and higher negative score along PC2. Küçükkuyu and Havran olive oil samples had negative values in the PC1 among North class.

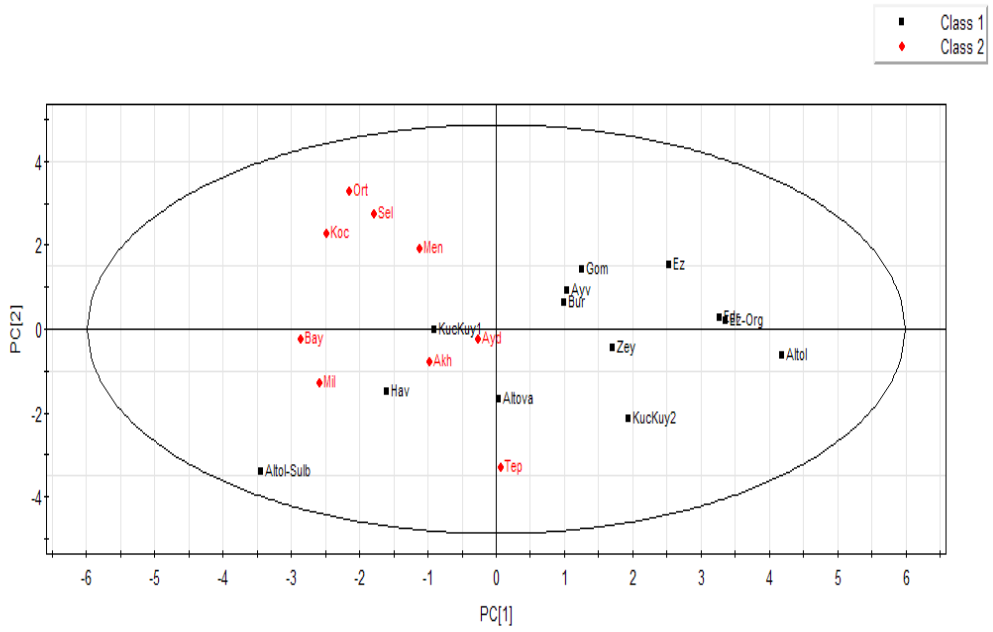

Figure 7.13. PCA (score plot) of the electronic nose data of commercial EVOO samples of the 1. harvest year.

The Coomans' plot of the North and South classes of the commercial EVOO samples of the 2. harvest year is demonstrated in Figure 7.14. The statistics of this class model is shown in Table 7.12. The PCA class model was applied successfully for the discrimination of the North and South classes. Altınova olive oil sample that belongs to the North region was only located on the region that contained the samples that fitted both class models.

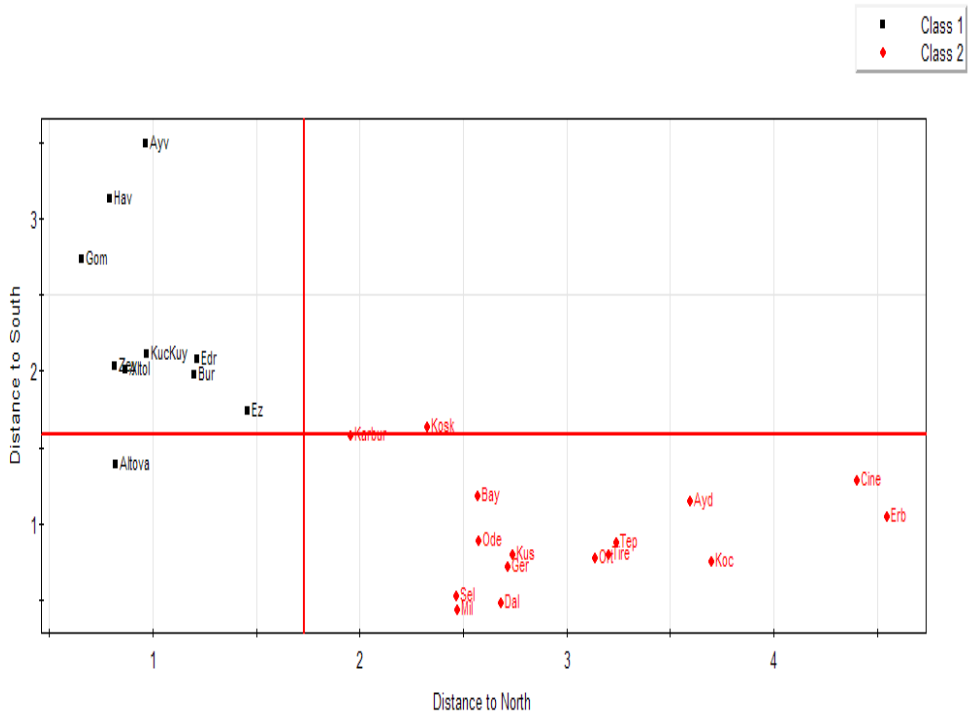

Figure 7.14. Coomans' plot of the North (Class1) and South (Class 2) class of commercial EVOO samples of the 2. harvest year

Table 7.12. General statistics of PCA class model

Samples	**Class no**	**Number of PCs**	**R^2X(cum)**
North	1	3	0.806
South	2	3	0.693

Figure 7.15 gave the ability of PCA analysis to reveal the degree of classification of the North and South classes of the commercial EVOO samples of the 2. harvest year. Most of the olive oil samples of North class had higher positive values in PC1. Ayvalık and Havran olive oil samples that separated from the North class had negative values along PC1. 53.9% of the total variance was explained with PC1 and PC2.

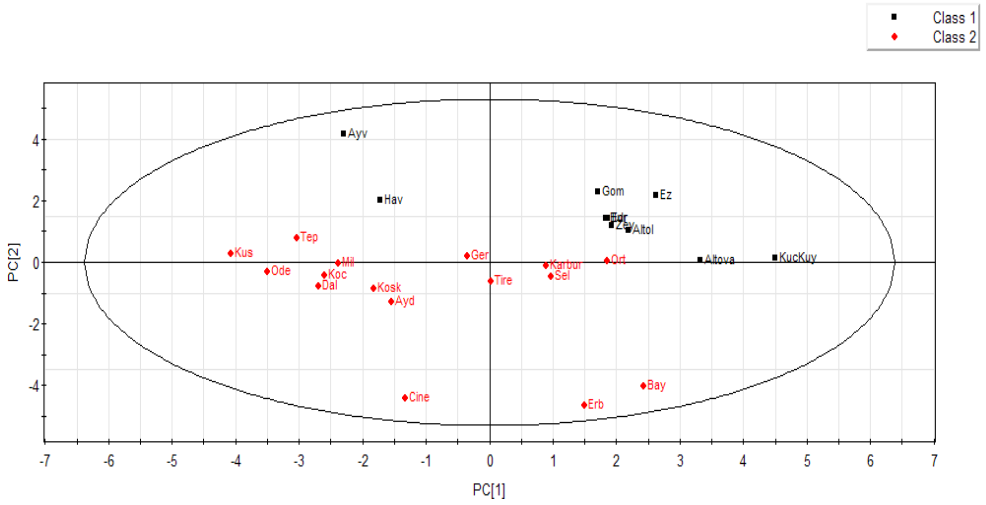

Figure 7.15. PCA of the North and South class of commercial EVOO samples of the 2. harvest year

Coomans' plot for the classification of commercial olive oil samples of the 1. and 2. harvest years is given in Figure 7.16. The x-axis shows the distance to the North class, the y-axis showed the South class of the 1. harvest year. The North and South classes of the 2. harvest year were also plotted in Figure 7.16. The Coomans' plot of the North and South classes of the 1. harvest year were discriminated clearly. Altınova, Küçükkuyu, Havran and Menemen olive oil samples were found in the region that fitted the both models. The North and South classes of the 2. harvest year were quite different than the 1. harvest year.

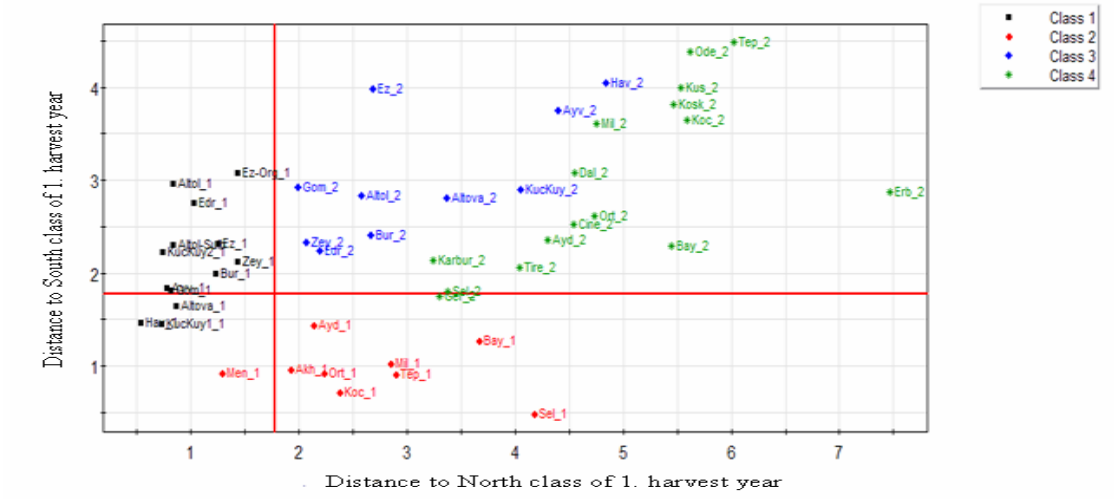

Figure 7.16. Coomans' plot for the classification of commercial EVOO samples of the 1. and 2. harvest years

7.4. Adulteration of Olive Oils

7.4.1. Monovarietal Olive Oil Adulteration

7.4.1.1. Adulteration of Ayvalık Olive Oil with Nizip Olive Oil

The monovarietal olive oil adulteration study was performed to denominate the importance of determination of adulteration of EVOOs with olive oils of different variety or lower commercial value. The adulterated olive oil samples were prepared using Ayvalık-Edremit and Nizip olive oils. Ayvalık-Edremit olive oil samples were mixed with Nizip olive oil at the percentages of 5, 10, 15 and 20. Nizip olive oil was used as an adulterant since the aroma of Nizip olive oils were different than the other oils produced from the olives harvested in İzmir and Edremit orchards. Nizip olive oil is mostly produced from the olives grown in Southeast part of Turkey and thus, it differed from the other EVOO samples. The aroma fingerprints of Nizip and Ayvalık-Edremit olive oil samples were obtained by an electronic nose and the chromatogram of these samples are shown in Figure 7.17. The Coomans' plot was constructed to determine the discrimination of the pure Ayvalık-Edremit olive oil, pure Nizip olive oil and the adulterated samples and it is given in Figure 7.18. The figure pointed out the clear separation of Ayvalık-Edremit and Nizip class models from the adulterated samples.

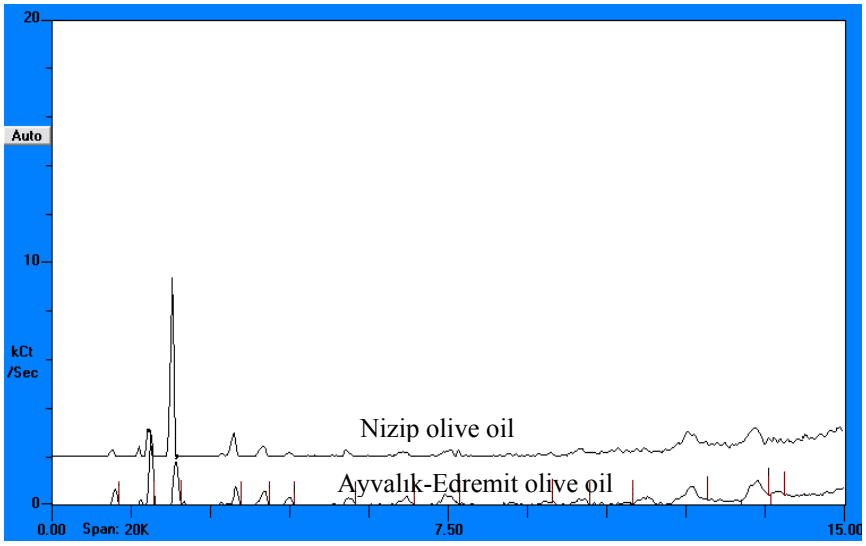

Figure 7.17. The electronic nose chromatogram of Nizip and Ayvalık-Edremit olive oil

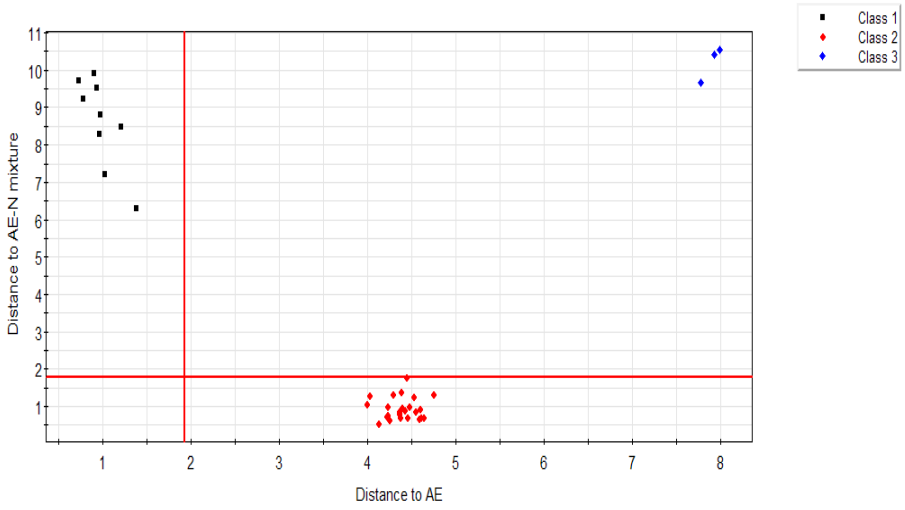

Figure 7.18. Coomans' plot for the classification of pure Ayvalık-Edremit EVOO (Class 1), adulterated olive oil (Class 2) and the pure Nizip olive oil samples (Class 3)

PLS analysis is a regression extension of PCA. It is used to connect the information in two blocks, X and Y to each other. The electronic nose data were evaluated using the PLS algorithm. PLS was used for the quantification of the adulteration percentage of Nizip olive oil in Ayvalık-Edremit olive oil. The X block in this model was the electronic nose data obtained for the adulteration mixtures of Ayvalık-Edremit and Nizip olive oils. The percentages of the Nizip olive oil in the adulteration mixtures formed the Y-block. Figure 7.19 shows the actual concentration values of Nizip olive oil versus the predicted concentration of Nizip olive oil in Ayvalık-Edremit olive oil samples. The data were divided into two data sets; a calibration subset containing two thirds of all data and a validation subset containing the remaining data (one–third). Therefore, 24 of the 36 samples were used to build the calibration set and the remaining 12 samples were reserved for prediction set to test the performance of the models. The R^2 value of actual versus predicted graph was 0.9646 and is given in Figure 7.19. Standard error of calibration (SEC) and standard error of prediction (SEP) are given in Table 7.13. The results showed that the detection of adulteration of Ayvalık-Edremit olive oils with Nizip olive oils as low as 5% could be possible using the electronic nose. Cheman et al. (2005) also reported that the detection of lard (as low as 1%) as an adulterant in refined, bleached, deodorized (RBD) palm olein using the SAW sensing electronic nose was possible.

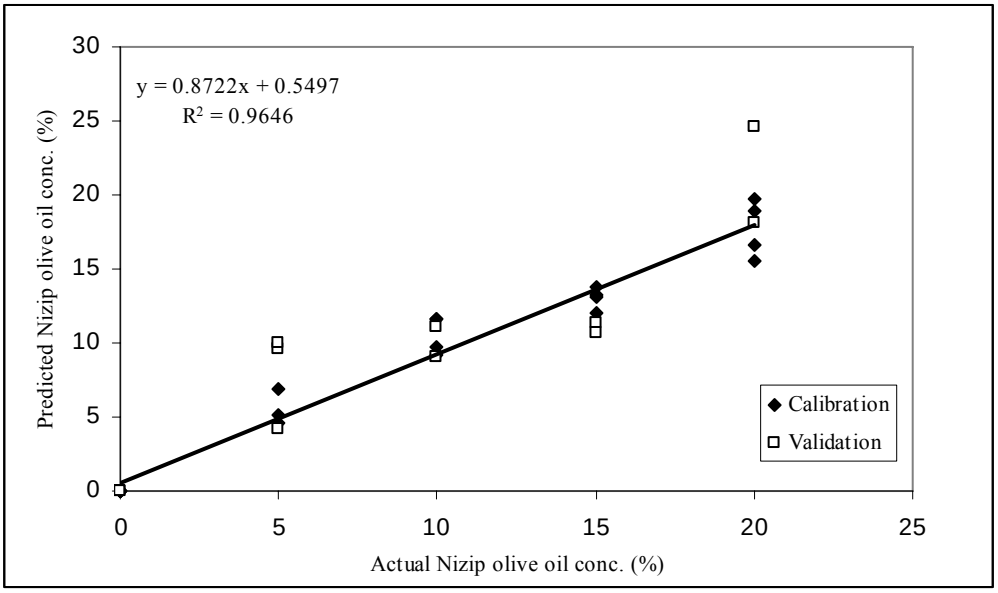

Figure 7.19. Concentration values for adulteration obtained from the PLS Model versus the actual concentration of Nizip olive oil

Table 7.13. The SEC and SEP values for the adulteration of Nizip and Ayvalık-Edremit olive oils

	Calibration set			**Validation set**	
	Niz. conc. in Ayv-Edr. (v/v %)			**Niz. conc. in Ayv-Edr. (v/v %)**	
Sample no	**Actual Niz conc. in Ayv-Edr.**	**Pred. Niz. conc. in Ayv-Edr.**	**Sample no**	**Actual Niz conc. in Ayv-Edr.**	**Pred. Niz. conc. in Ayv-Edr.**
1	0	0.003	1	0	0.000
2	0	0.002	2	0	0.000
3	0	0.000	3	0	0.000
4	0	0.007	4	5	9.568
5	0	0.000	5	5	10.035
6	0	0.000	6	5	4.227
7	5	6.827	7	10	11.117
8	5	5.091	8	10	9.030
9	5	4.607	9	15	11.347
10	10	11.651	10	15	10.636
11	10	9.161	11	20	18.139
12	10	11.649	12	20	24.563
13	10	9.710			
14	15	13.797			
15	15	13.043			
16	15	13.223			
17	15	12.073			
18	20	16.564			
19	20	18.972			
20	20	19.761			
21	20	15.489			
SEC (v/v %)		**1.77**	**SEP (v/v %)**		**2.967**

7.4.1.2. Adulteration of Erkence Olive Oil with Nizip Olive Oil

Erkence olive oil sample was mixed with Nizip olive oil at different adulteration levels ranging from 5-20%. The electronic nose chromatogram of Nizip and Erkence olive oil samples are presented in Figure 7.20. The discrimination ability of the electronic nose on the adulteration studies were examined by using Coomans' plot. Figure 7.21 represents the Coomans' plot that marks the separation among pure Erkence olive oil and Nizip olive oil adulterated samples and pure Nizip olive oil. It was observed that the adulterated samples could be differentiated from Erkence and Nizip olive oils successfully.

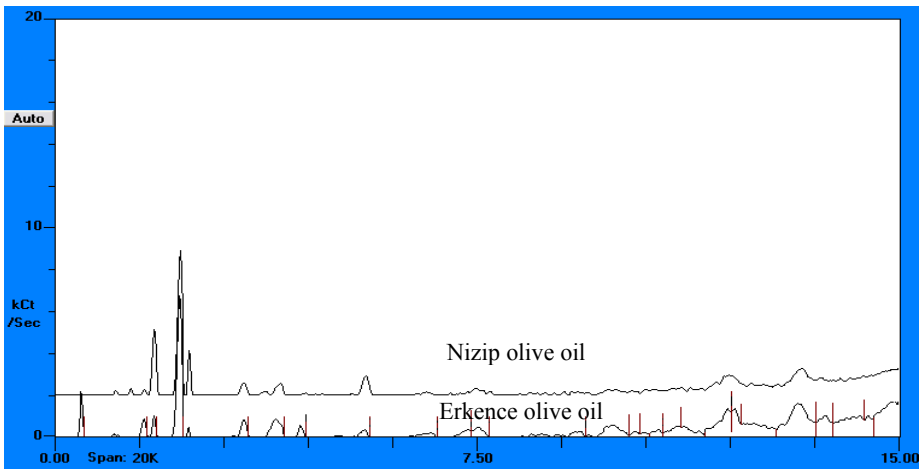

Figure 7.20. The electronic nose chromatogram of Nizip and Erkence olive oils

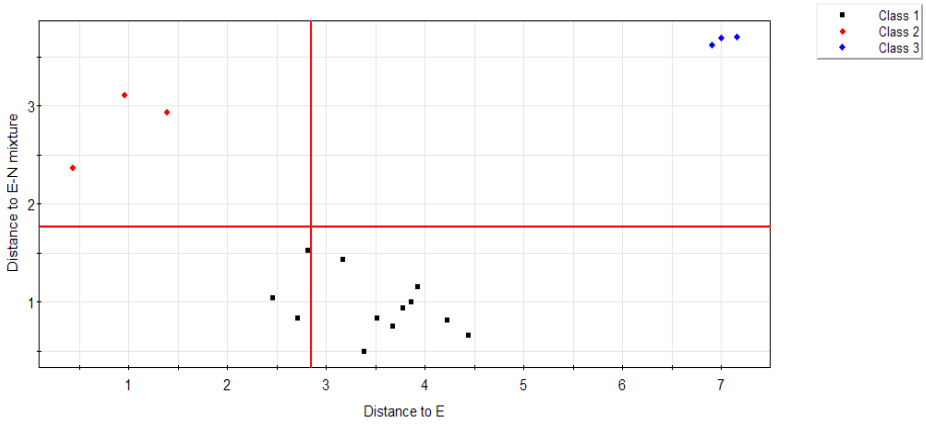

Figure 7.21. Coomans' plot for the classification of pure Erkence olive oil (Class 2), adulterated olive oils (Class 1) and pure Nizip EVOO (Class 3)

The PLS regression analysis was performed to determine the percentages of adulteration in the olive oil samples. A calibration set of 12 samples were used for modeling after randomly selection of 6 samples for the prediction model. The R^2 value of actual versus predicted concentration of Nizip olive oil in Erkence oil was found as 0.8999 and is represented in Figure 7.22. The results showed that the adulterated samples at least 5% to 20% could be quantified by PLS model with the electronic nose data. The calibration and prediction models were attempted to see the goodness of the model. The SEC and SEP were determined and they are given in 7.14.

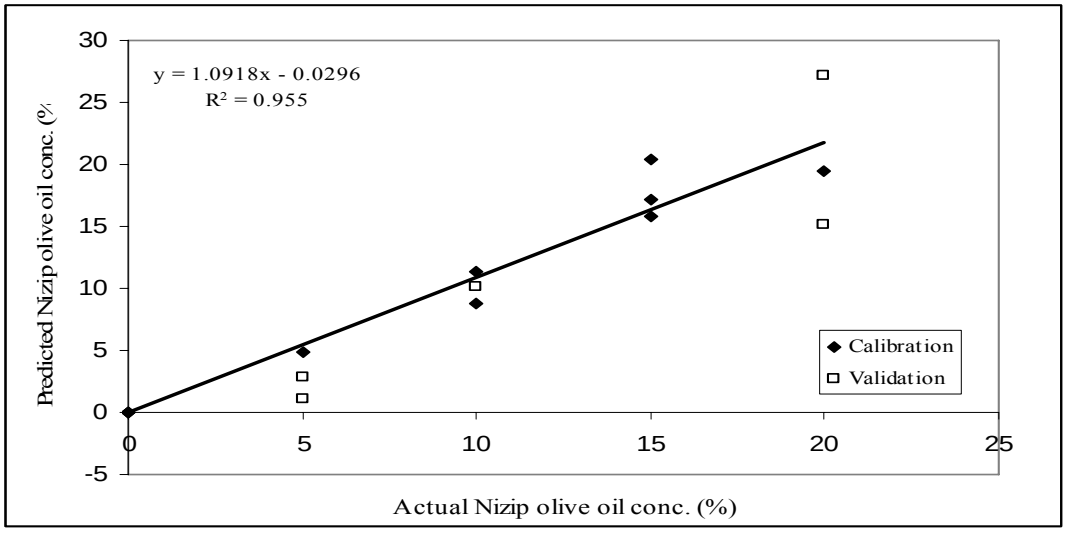

Figure 7.22. Concentration values for adulteration obtained from the PLS model versus the actual concentration of Nizip olive oil

Table 7.14. The SEC and SEP values for the adulteration of Nizip and Erkence olive oils

Sample no	Calibration set		Sample no	Validation set	
	Niz. conc.in Erk. (v/v %)			Niz. conc.in Erk. (v/v %)	
	Actual Niz conc. in Erk.	**Pred. Niz. conc. in Erk.**		**Actual Niz conc. in Erk.**	**Pred. Niz. conc. in Erk.**
1	0	0	1	5	1.105
2	0	0	2	5	2.811
3	0	0.012	3	10	10.167
4	5	4.908	4	20	27.13
5	10	8.829	5	20	15.184
6	10	11.348	6		
7	15	15.845	7		
8	15	20.395	8		
9	15	17.16	9		
10	20	19.467	10		
SEC (v/v %)		**2.17**	**SEP (v/v %)**		**4.336**

7.4.2. Adulteration of Olive Oils with Other Edible Oils

7.4.2.1. Adulteration of Olive Oils with Sunflower, Corn, and Soybean Oils

The olive oil is often adulterated with other cheaper edible oils. Some of these are sunflower, corn and soybean oils. In this adulteration study, the ability of the electronic nose to detect the adulterations of extra virgin olive oils with sunflower, corn and soybean oils was evaluated. The typical electronic nose chromatogram of these edible oils and olive oil is shown in Figure 7.23. The adulteration level of these edible oils and olive oil mixtures ranged from 5-50%. The actual versus predicted concentrations of sunflower, corn and soybean oils are illustrated in Figures 7.24, 7.25, and 7.26, respectively. The R^2 values of the actual versus predicted graphs of sunflower, corn and soybean oils were 0.9876, 0.9899 and 0.9835, respectively. Higher than 98% of the variance could be explained with the model constructed to predict the adulteration percentages of these edible oils. In order to prepare calibration models, 18 of these 27 samples were used to build calibration set and the 9 samples were used for prediction set to test the performance of the models. The SEC and SEP values are shown in Tables 7.15. and 7.16, respectively. The results showed that electronic nose could be applied to detect the adulteration levels of olive oils with other edible oils within these concentrations. Hai and Wang (2006) used an electronic nose based on 10 metal oxide semiconductor sensors to classify sesame oils with different adulteration levels, and predict the percentages of adulteration. Excellent results were obtained in the prediction of percentage of adulteration in sesame oil by back propagation neural networks (BPNN) and general regression neural network (GRNN). The electronic nose has also been used by Oliveros et al. (2002) for the detection of adulterations of virgin olive oil. Multivariate data analysis techniques such as LDA, QDA and ANN were applied for the detection of adulterations. The models generated with the discriminant analysis provided very satisfactory results, with prediction percentages higher than 95%, and in some cases almost 100%.

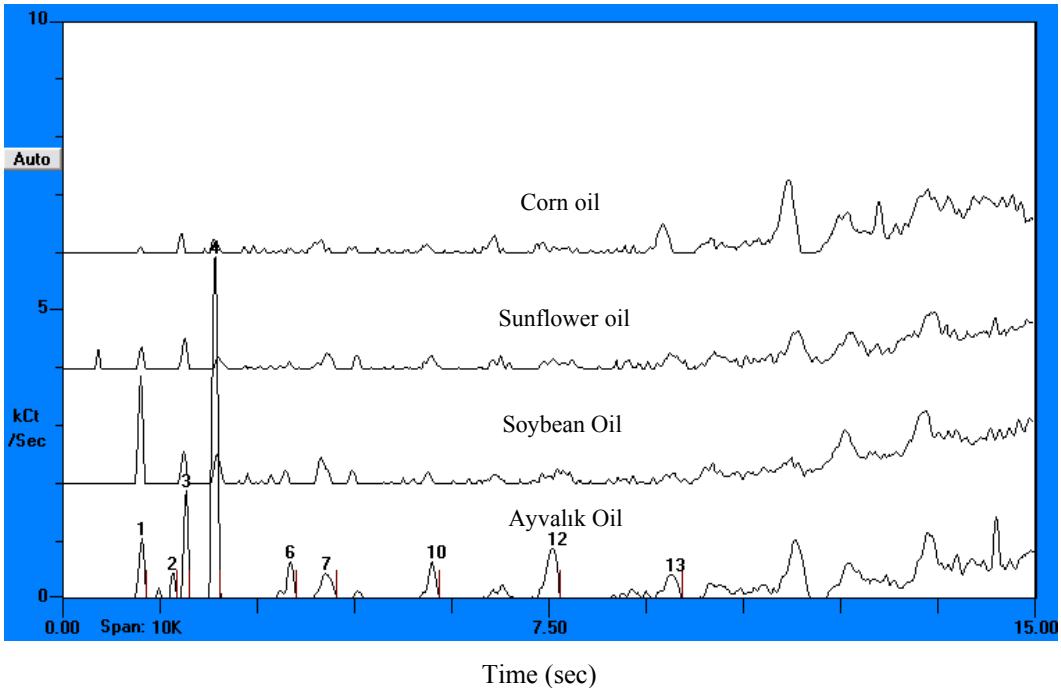

Figure 7.23. The electronic nose chromatogram of the sunflower, corn, soybean oils and Ayvalık olive oil

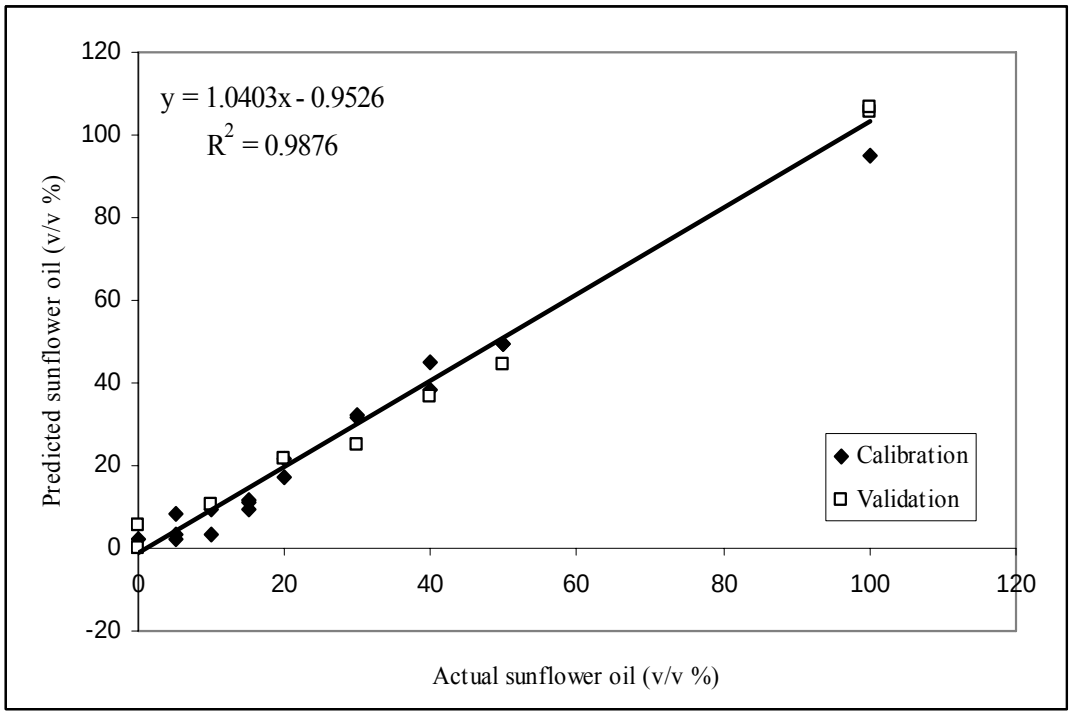

Figure 7.24. Actual versus predicted concentrations of sunflower oil

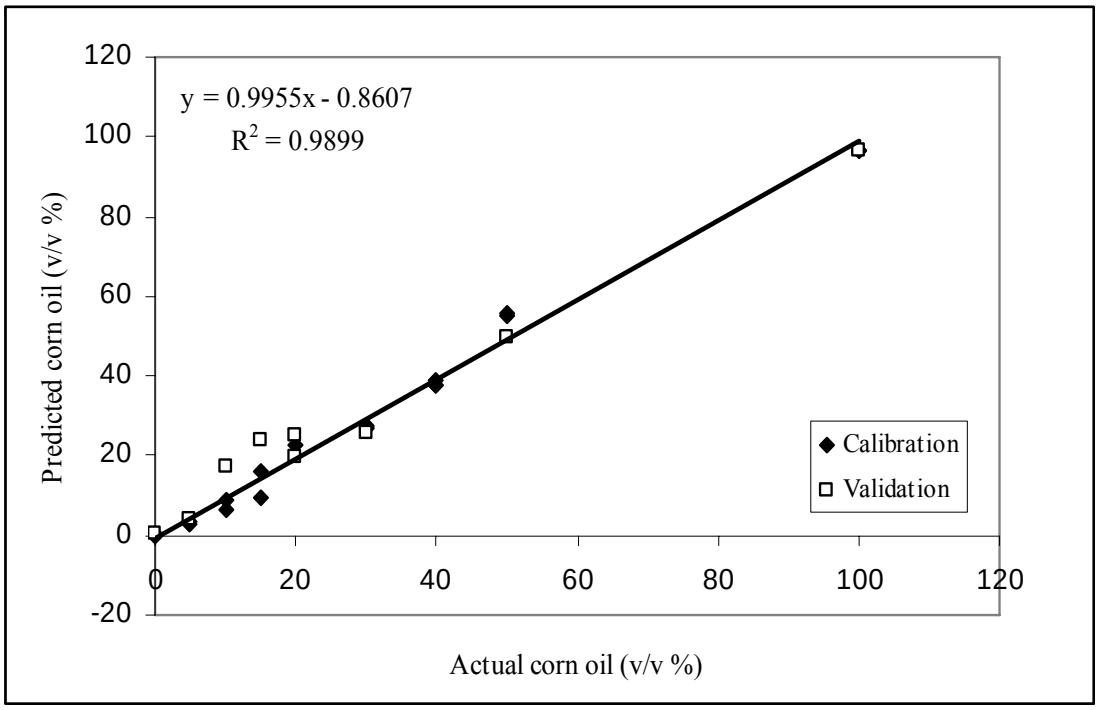

Figure 7.25. Actual versus predicted concentrations of corn oil

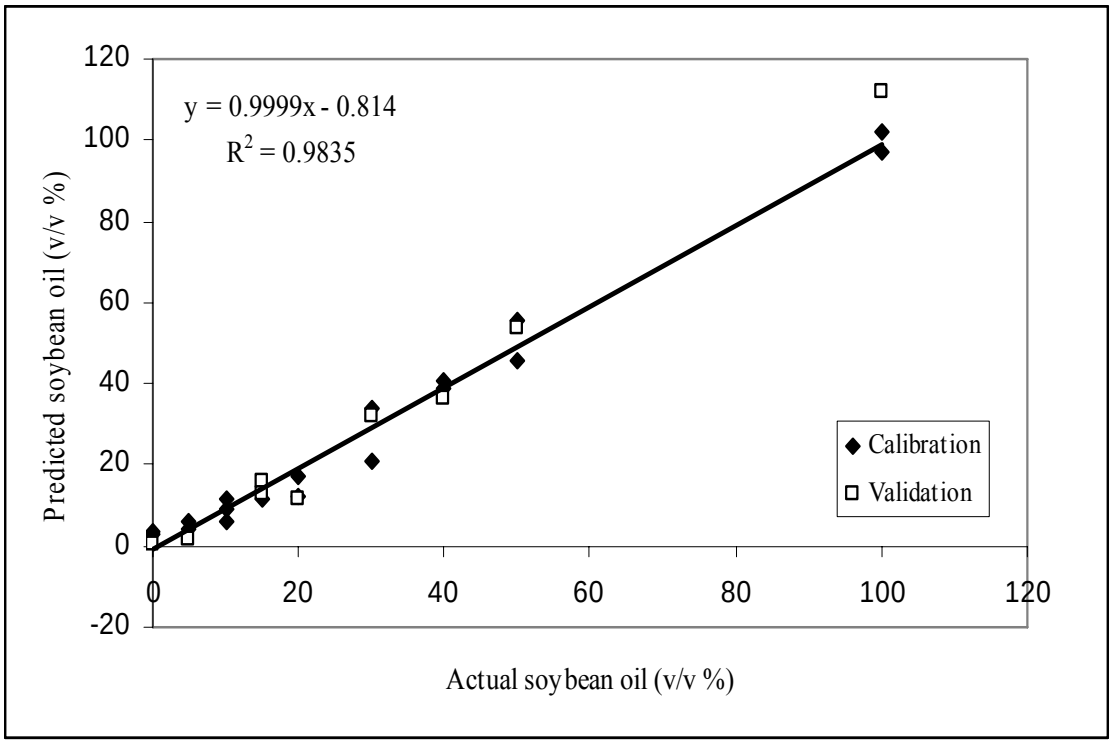

Figure 7.26. Actual versus predicted concentrations of soybean oil

Table 7.15. Results of calibration sets for sunflower, corn and soybean oils adulterated with EVOO determined with SEC

Sample no	Calibration set					
	Sunflower oil conc. (v/v %)		Corn oil conc. (v/v %)		Soybean oil conc. (v/v %)	
	Actual	Pred.	Actual	Pred.	Actual	Pred.
1	0	2.29	0	0.14	0	3.45
2	5	2.43	0	0.03	0	3.01
3	5	8.49	5	2.90	5	4.08
4	5	3.55	5	3.27	5	5.97
5	10	3.10	10	6.56	10	8.83
6	10	9.37	10	8.58	10	11.33
7	15	11.49	15	9.16	10	6.27
8	15	9.38	15	16.08	15	11.86
9	15	10.85	20	22.73	20	17.20
10	20	21.45	30	27.74	20	12.50
11	20	17.44	30	26.84	30	33.74
12	30	31.80	40	37.46	30	21.04
13	30	32.13	40	37.88	40	41.00
14	40	38.24	40	38.60	40	39.16
15	40	45.08	50	55.21	50	55.79
16	50	49.17	50	55.56	50	45.93
17	50	49.61	100	96.53	100	101.98
18	100	95.24	100	96.68	100	97.17
SEC (v/v %)		**3.57**		**3.28**		**4.12**

Pred.:Prediction

Table 7.16. Predicted sunflower, corn and soybean oil concentrations in EVOO in the prediction set determined with SEP

Sample no	Validation set					
	Sunflower oil conc. (v/v %)		Corn oil conc. (v/v %)		Soybean oil conc. (v/v %)	
	Actual	Pred.	Actual	Pred.	Actual	Pred.
1	0	0.00	0	0.26	0	0.42
2	0	5.69	5	4.05	5	1.61
3	10	10.32	10	17.42	15	16.19
4	20	21.51	15	24.04	15	12.60
5	30	24.73	20	19.82	20	11.79
6	40	36.48	20	25.11	30	32.34
7	50	44.39	30	25.72	40	36.67
8	100	105.52	50	49.68	50	53.43
9	100	106.88	100	96.63	100	112.14
SEP (v/v %)		**4.52**		**4.63**		**5.39**

Pred.:Prediction

7.4.2.2. Adulteration of Olive Oils with Hazelnut Oil

PLS regression analysis was applied to the adulteration study of hazelnut oil and different extra virgin olive oil samples. Zeytindağ and Küçükkuyu extra virgin olive oils were analyzed as the North region olive oil. Selçuk and Milas EVOOs were analyzed as South region olive oils. The plots of actual versus predicted concentrations of hazelnut oil adulterated with Erkence, North (Zeytindağ-Küçükkuyu) and South (Selçuk-Milas) region olive oils are given in Figures 7.28, 7.29, and 7.30, respectively. In this study, for the hazelnut oil and Erkence olive oil adulteration, 18 samples, for the North and South region olive oil samples 36 samples were chosen for the calibration model and for the Erkence olive oil sample 9 samples and for the North and South region olive oils 18 samples were randomly chosen for the prediction model. The SEC and SEP values are given in Tables 7.17 and 7.18, respectively. These results meant that there was no such clear discrimination of samples with an adulteration up to 20 %. Pena et al. (2005) established the detection of adulteration of virgin olive oils with hazelnut

oil by means of its analysis by a headspace autosampler directly coupled to a mass spectrometer used as detector (ChemSensor) applying PLS and PCA.

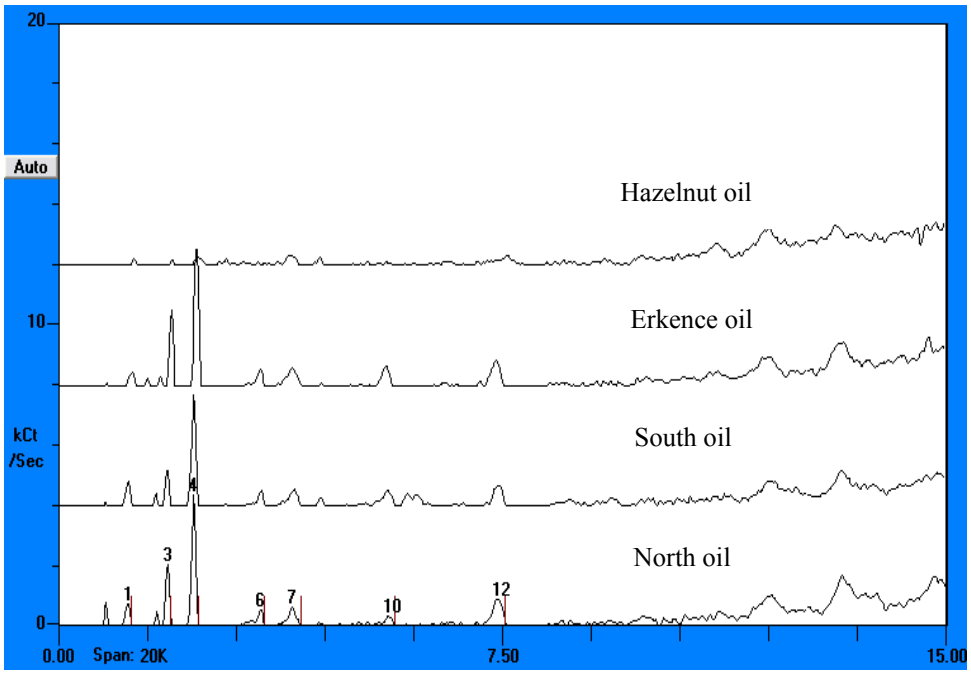

Figure 7.27. The electronic nose chromatogram of the hazelnut oil and Erkence, South and North olive oils

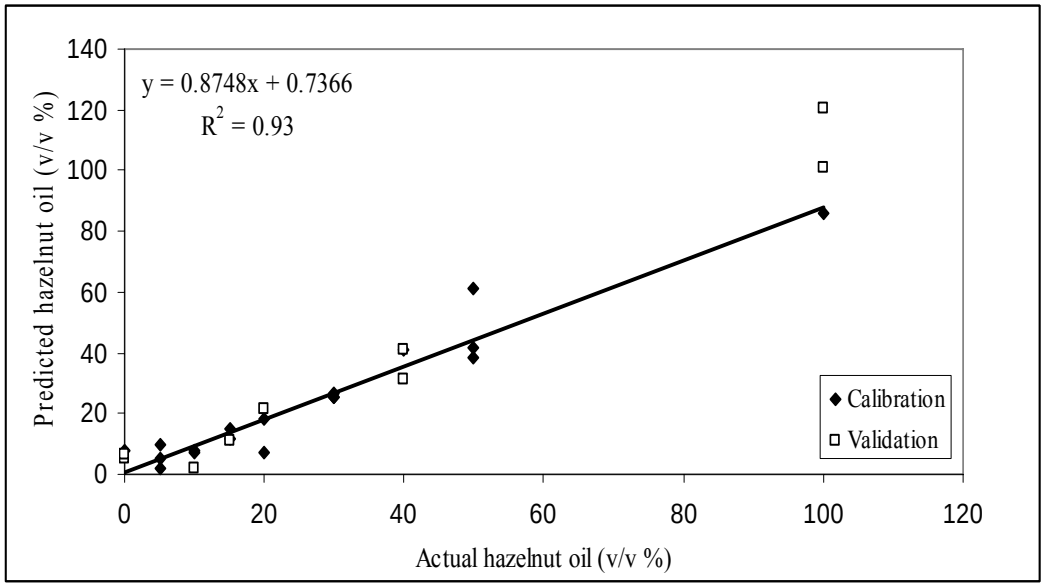

Figure 7.28. Actual versus predicted concentrations of hazelnut oil in Erkence olive oil

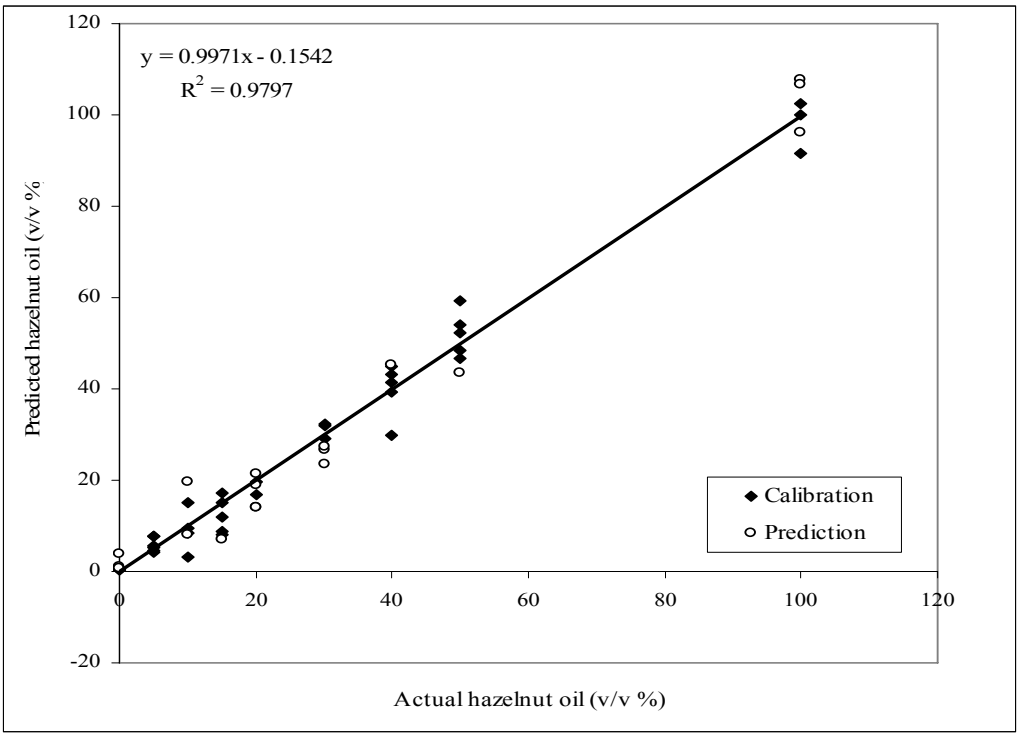

Figure 7.29. Actual versus predicted concentrations of hazelnut oil in North olive oil

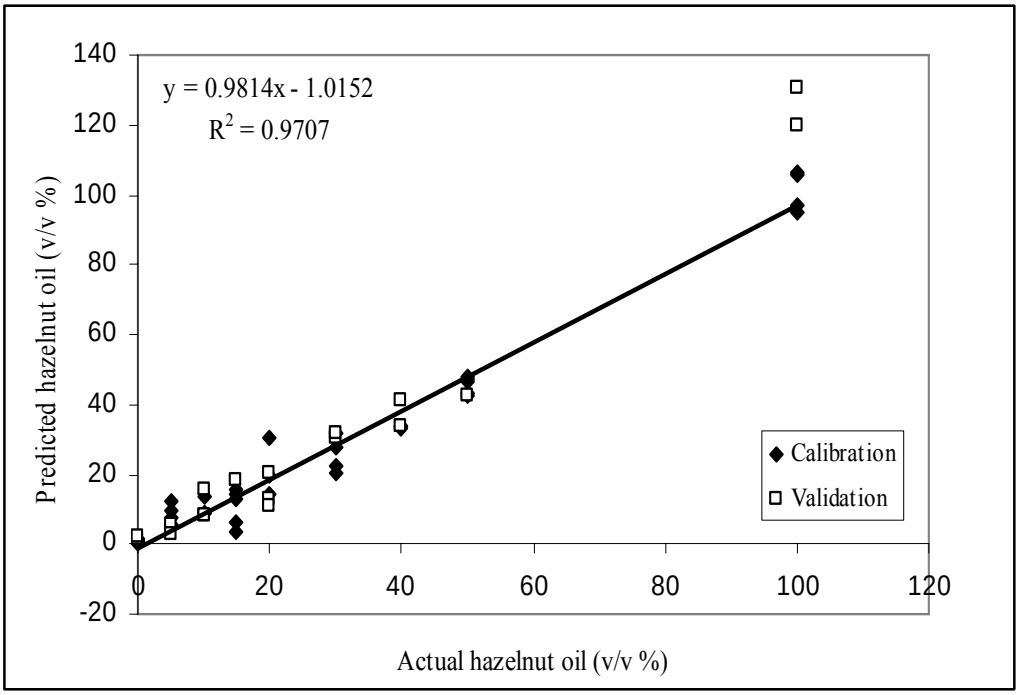

Figure 7.30. Actual versus predicted concentrations of hazelnut oil in South olive oil

Table 7.17. Results of calibration sets for Erkence, North (Zeytindağ-Küçükkuyu) and South (Selçuk-Milas) olive oils adulterated with hazelnut oil determined with SEC

Sample no	Calibration Set					
	Erkence (v/v %)		North (Zeytindağ-Küçükkuyu) (v/v %)		South (Selçuk-Milas (v/v %)	
	Actual	Pred.	Actual	Pred.	Actual	Pred.
1	0	7.56	0	0.75	0	0.68
2	0	6.61	0	0.80	0	0.24
3	5	1.73	0	0.05	0	0.49
4	5	10.08	0	1.22	5	4.47
5	10	1.99	5	12.43	5	5.15
6	10	7.39	5	7.81	5	7.76
7	15	15.25	5	9.74	5	5.58
8	15	11.03	5	5.49	5	7.78
9	20	7.19	10	13.51	5	4.09
10	20	17.91	10	9.20	10	3.33
11	20	21.38	10	9.09	10	8.40
12	30	25.11	15	15.89	10	9.41
13	30	25.54	15	13.10	10	15.20
14	40	40.82	15	14.13	15	17.14
15	50	41.77	15	3.74	15	14.94
16	50	61.44	15	6.54	15	12.06
17	100	101.08	20	30.54	15	8.23
18	100	120.67	20	14.41	15	8.76
19			20	19.89	20	16.78
20			30	28.03	20	19.68
21			30	20.27	30	32.08
22			30	22.22	30	32.34
23			30	31.90	30	29.07
24			40	33.45	40	44.87
25			40	33.75	40	29.76
26			40	34.03	40	39.14
27			40	33.23	40	41.33
28			50	43.35	40	43.11
29			50	48.07	50	46.58
30			50	47.54	50	59.15
31			50	46.86	50	53.91
32			50	42.45	50	52.31
33			100	105.75	50	48.50
34			100	95.29	100	100.06
35			100	106.07	100	91.57
36			100	97.11	100	102.53
SEC (v/v %)		**7.40**		**5.41**		**4.01**

Pred.:Prediction

Table 7.18. Predicted hazelnut oil concentration in Erkence, North (Zeytindağ-Küçükkuyu) and South (Selçuk-Milas) olive oils in the prediction set determined with SEP

Sample no	Validation Set					
	Erkence (v/v %)		**North (Zeytindağ-Küçükkuyu) (v/v %)**		**South (Selçuk-Milas) (v/v %)**	
	Actual	Pred.	Actual	Pred.	Actual	Pred.
1	0	4.99	0	1.21	0	1.15
2	0	6.61	0	2.20	0	3.74
3	10	1.99	5	5.29	0	0.86
4	15	11.03	5	2.81	10	8.12
5	20	21.38	10	15.48	10	19.51
6	40	40.82	10	8.15	15	7.01
7	40	31.57	10	8.28	20	19.04
8	100	101.08	15	18.02	20	21.52
9	100	120.67	20	20.12	20	14.08
10			20	12.97	20	13.87
11			20	10.87	30	26.65
12			30	30.45	30	27.54
13			30	32.09	30	23.47
14			40	33.96	40	45.39
15			40	40.90	50	43.39
16			50	42.32	100	96.27
17			100	130.27	100	107.63
18			100	119.99	100	106.53
SEP (v/v %)		**8.50**		**9.44**		**5.25**

Pred.:Prediction

CHAPTER 8

CONCLUSION

In this study, the aroma fingerprints of the extracted extra virgin olive oil samples obtained from different varieties and geographical regions and commercial extra virgin olive oil samples for the 2005-2006 and 2006-2007 harvest years were obtained by using an surface acoustic wave sensing electronic nose. The electronic nose was also used to detect adulterations of extra virgin olive oils with other monovarietal extra virgin olive oil samples and also with other edible oil samples such as sunflower, corn, soybean and hazelnut oils and to quantify the percentages of adulteration. PCA, Coomans' plot, PLS were conducted for analyses of the electronic nose data. At the end of this study:

- The aroma fingerprints of extracted extra virgin olive oils obtained from different varieties could be classified by PCA using electronic nose.
- Gemlik and Ayvalık extra virgin olive oil samples obtained from two different regions (İzmir and Edremit) were discriminated based on their aroma fingerprints using an electronic nose.
- Sensory evaluation of the Gemlik and Ayvalık olive oil samples obtained from two different regions (İzmir and Edremit) showed that the effect of geographical region on the organoleptic properties of olive oil samples could not be distinguished by the panelists.
- The acceptance test results represented that Gemlik olive oil samples obtained from Edremit was mostly liked by the panelists in 2005-2006 harvest year. Ayvalık olive oil was mostly preferred by the panelists in 2006-2007 harvest year.
- Commercial olive oil samples obtained from North and South of Aegean Region could be classified based on their aroma fingerprints by applying PCA.
- The adulteration of monovarietal olive oil samples could be determined by the electronic nose at higher than 10% adulteration level.

- The adulteration of extra virgin olive oil samples with other edible oils could be detected by the electronic nose at higher adulteration concentrations.
- As a conclusion, the electronic nose could be used in the oil industry for obtaining objective, low-cost and rapid sensory information based on the aroma fingerprints of the olive oils.

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APPENDIX A

Table A.1 The electronic Nose data for the extracted olive oils of 2005-2006 harvest Year

Sample Code	P1	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
M	18.5	206.5	464.0	375.5	35.5	77.0	34.5	0.0	0.0	15.5	71.5	0.0	0.0	0.0	0.0
M	19.5	164.5	469.0	593.0	7.5	53.0	32.0	0.0	0.0	0.0	78.0	0.0	0.0	0.0	10.5
M	9.5	161.5	485.0	586.5	17.5	49.0	35.0	0.0	0.0	8.5	70.0	0.0	9.0	0.0	12.5
M	23.5	126.0	400.5	522.5	7.5	47.5	32.0	0.0	9.5	0.0	79.0	0.0	18.5	20.5	0.0
M	25.0	151.5	381.0	449.0	0.0	30.0	32.0	0.0	0.0	12.5	50.5	0.0	0.0	32.5	0.0
M	22.5	153.5	397.0	443.5	0.0	23.5	23.5	0.0	0.0	12.5	17.0	0.0	8.0	36.0	13.5
M	17.0	113.5	346.0	499.0	0.0	34.0	35.5	0.0	0.0	27.0	15.0	0.0	22.0	31.0	25.5
M	15.0	128.0	333.5	448.0	0.0	27.0	24.0	0.0	0.0	0.0	19.5	0.0	22.0	32.0	16.0
M	12.5	123.0	358.5	495.0	0.0	29.0	36.5	0.0	0.0	0.0	27.5	0.0	19.0	33.0	21.0
E	8.0	73.0	620.0	199.0	0.0	22.0	36.0	97.0	8.5	51.0	32.5	0.0	0.0	0.0	0.0
E	10.0	69.5	604.5	289.5	0.0	0.0	38.5	81.0	8.0	66.0	0.0	0.0	0.0	0.0	0.0
E	0.0	50.0	544.0	267.5	0.0	0.0	21.0	84.5	8.5	25.5	0.0	0.0	0.0	0.0	0.0
E	0.0	40.5	543.5	226.0	48.5	0.0	48.5	49.0	8.0	30.0	23.0	0.0	0.0	0.0	10.5
E	0.0	33.0	576.5	246.5	0.0	0.0	35.0	74.5	18.5	16.0	33.0	0.0	0.0	0.0	0.0
E	0.0	34.0	533.5	229.5	0.0	0.0	43.0	72.5	7.5	17.5	21.0	0.0	0.0	0.0	0.0
E	0.0	57.5	475.5	273.0	55.0	0.0	40.5	0.0	0.0	57.0	23.5	0.0	10.0	0.0	0.0
E	10.5	56.0	558.0	328.5	0.0	8.0	43.0	25.0	13.5	59.5	14.5	0.0	0.0	0.0	0.0
E	9.5	52.5	520.0	333.0	11.0	10.0	39.5	19.5	0.0	46.0	32.0	0.0	0.0	0.0	0.0
G	0.0	84.5	203.0	312.5	63.0	40.0	23.0	0.0	8.5	9.5	269.5	0.0	11.5	0.0	0.0
G	0.0	65.0	162.5	123.0	87.5	18.0	16.0	0.0	0.0	0.0	249.0	0.0	29.0	0.0	0.0
G	0.0	61.5	151.5	122.5	78.0	0.0	11.5	0.0	0.0	8.5	185.5	0.0	27.0	10.0	13.0
G	7.5	8.5	339.0	8.5	129.5	0.0	32.0	0.0	0.0	18.0	248.5	0.0	0.0	0.0	0.0
G	0.0	8.5	331.0	12.0	105.0	0.0	35.0	0.0	19.0	0.0	240.5	0.0	0.0	0.0	0.0
G	9.0	0.0	324.5	11.0	51.5	0.0	19.5	40.0	9.5	12.0	230.0	0.0	0.0	0.0	0.0
G	0.0	41.5	403.5	126.0	109.0	0.0	43.0	0.0	17.0	46.5	0.0	0.0	0.0	0.0	17.5
G	22.0	46.5	421.5	137.0	116.0	0.0	43.5	0.0	0.0	7.5	178.0	0.0	0.0	0.0	0.0
G	17.5	46.5	435.5	147.5	58.5	0.0	37.5	53.0	0.0	9.5	176.5	0.0	0.0	0.0	9.0
A	0.0	117.0	158.5	328.5	60.5	20.5	19.0	0.0	9.0	21.0	297.5	0.0	0.0	0.0	0.0
A	0.0	65.5	134.0	101.0	48.5	0.0	21.0	0.0	9.5	0.0	260.0	0.0	0.0	0.0	0.0
A	0.0	69.0	154.0	135.5	78.5	0.0	21.5	0.0	8.0	11.5	273.0	0.0	0.0	0.0	0.0
A	0.0	52.0	135.5	137.0	66.5	0.0	21.5	0.0	10.5	9.0	297.5	0.0	0.0	0.0	0.0
A	0.0	50.5	126.0	98.5	64.5	0.0	17.0	0.0	20.0	7.5	276.0	0.0	0.0	0.0	0.0
A	0.0	47.0	133.0	139.5	65.5	0.0	22.0	0.0	10.0	0.0	293.5	0.0	0.0	0.0	0.0
A	0.0	85.5	173.0	281.0	57.5	20.0	21.0	0.0	19.0	0.0	287.5	0.0	0.0	0.0	0.0
A	9.0	50.5	192.5	202.0	75.0	0.0	27.0	0.0	10.0	9.5	302.0	0.0	0.0	0.0	0.0
A	12.0	37.0	186.0	219.0	25.0	0.0	27.5	29.0	0.0	0.0	275.5	0.0	0.0	0.0	0.0

P: Peak

(cont. on next page)

Table A.1 The electronic nose data for the extracted olive oils of 2005-2006 harvest year (cont.)

Sample Code	P1	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
D	39.0	0.0	98.0	0.0	85.0	0.0	41.5	0.0	0.0	49.0	185.5	0.0	0.0	0.0	0.0
D	54.0	0.0	93.5	0.0	65.0	0.0	33.0	0.0	0.0	26.5	198.5	0.0	0.0	0.0	0.0
D	52.5	0.0	82.5	0.0	86.0	0.0	0.0	0.0	0.0	10.5	217.5	0.0	0.0	0.0	0.0
D	48.0	0.0	61.5	0.0	70.5	0.0	61.5	0.0	0.0	8.5	202.0	0.0	0.0	0.0	0.0
D	0.0	83.5	651.5	245.5	36.0	0.0	36.0	0.0	0.0	0.0	184.0	0.0	0.0	0.0	0.0
D	0.0	75.0	661.5	268.5	26.5	0.0	25.5	0.0	0.0	0.0	184.0	0.0	0.0	0.0	0.0
D	0.0	81.5	656.0	293.5	29.0	0.0	32.5	0.0	0.0	27.5	177.0	0.0	0.0	0.0	0.0
D	0.0	81.0	642.5	277.0	24.5	0.0	29.0	0.0	0.0	0.0	162.0	0.0	0.0	0.0	0.0
D	0.0	68.5	683.0	277.5	27.0	0.0	36.5	0.0	0.0	20.5	175.5	0.0	0.0	0.0	0.0
N	0.0	217.5	728.5	66.0	0.0	0.0	0.0	72.0	73.0	22.0	45.5	24.5	0.0	0.0	0.0
N	0.0	246.0	725.5	157.0	0.0	0.0	21.5	65.0	66.5	19.0	34.0	93.5	0.0	0.0	0.0
N	0.0	221.0	677.0	155.5	0.0	0.0	7.5	72.0	53.5	8.0	67.5	45.5	0.0	0.0	0.0
N	0.0	229.0	710.0	168.0	0.0	0.0	8.5	65.5	56.0	0.0	10.5	58.5	0.0	0.0	0.0
N	0.0	193.0	536.5	122.0	0.0	0.0	0.0	44.5	95.5	0.0	17.0	77.5	0.0	0.0	0.0
N	0.0	176.5	529.0	121.5	0.0	0.0	0.0	46.5	101.5	11.5	58.5	78.5	0.0	0.0	0.0
N	0.0	180.5	541.0	116.0	0.0	0.0	0.0	48.0	102.5	0.0	16.0	56.5	0.0	0.0	0.0
N	0.0	193.5	539.5	125.0	0.0	0.0	0.0	46.0	108.0	0.0	32.0	52.5	0.0	0.0	0.0
N	0.0	201.0	552.0	122.0	0.0	0.0	0.0	48.5	105.5	9.0	35.0	66.5	0.0	0.0	0.0
GE	0.0	220.0	243.0	576.0	27.5	41.0	32.5	22.0	0.0	0.0	161.5	0.0	8.5	0.0	12.0
GE	17.0	188.5	247.0	340.0	45.0	0.0	25.0	34.0	0.0	10.5	193.0	0.0	20.0	7.5	25.0
GE	16.5	167.0	245.0	312.5	26.5	0.0	23.5	29.5	0.0	8.5	154.5	0.0	9.0	8.5	13.0
GE	0.0	119.5	290.5	385.0	9.5	0.0	24.5	27.5	0.0	26.0	156.5	0.0	11.0	9.5	18.5
GE	0.0	130.5	271.5	396.5	49.0	0.0	25.5	0.0	8.0	13.0	160.5	0.0	18.5	0.0	0.0
GE	8.5	123.0	278.5	386.5	0.0	8.0	20.0	51.0	0.0	21.5	148.0	0.0	24.0	8.5	11.0
GE	0.0	146.5	247.5	365.5	29.5	7.5	10.0	33.0	8.0	0.0	157.0	0.0	19.0	0.0	26.5
GE	0.0	121.5	240.0	407.0	25.5	7.5	24.0	19.0	0.0	0.0	154.5	0.0	10.0	0.0	14.0
GE	0.0	116.0	252.0	382.0	26.0	0.0	20.5	20.5	8.5	12.0	180.0	0.0	9.5	8.0	21.0
AE	10.0	300.5	254.0	511.0	12.5	8.5	19.5	0.0	0.0	0.0	110.5	0.0	17.5	34.0	28.0
AE	8.5	288.0	253.5	604.0	11.0	9.0	8.5	0.0	0.0	0.0	110.0	0.0	7.5	34.5	39.5
AE	0.0	267.5	245.0	432.5	21.0	0.0	7.5	0.0	0.0	7.5	106.5	0.0	24.0	39.0	44.0
AE	0.0	391.5	304.0	860.0	0.0	52.5	22.0	0.0	9.5	7.5	124.0	0.0	18.0	29.5	31.0
AE	18.0	481.5	285.5	787.5	0.0	43.0	35.5	0.0	19.5	0.0	121.0	0.0	7.5	0.0	34.5
AE	0.0	488.0	269.0	758.5	0.0	46.0	41.0	0.0	11.0	0.0	116.5	0.0	17.0	9.0	25.5
AE	0.0	482.5	286.5	786.5	0.0	49.5	36.0	0.0	18.5	0.0	120.0	0.0	18.5	0.0	13.0
AE	11.5	523.5	297.5	786.0	0.0	50.0	39.5	0.0	9.5	0.0	110.5	0.0	7.5	0.0	0.0
AE	11.5	459.0	278.5	781.5	0.0	54.0	40.0	0.0	20.5	0.0	109.5	0.0	9.0	0.0	8.0

P: Peak

Table A.2 The electronic nose data for the extracted olive oils of 2006-2007 harvest year

Sample Code	P1	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
M	23.5	103.0	421.0	0.0	124.5	45.0	0.0	87.0	23.0	110.0	111.0	0.0	0.0	0.0
M	45.0	103.5	419.5	0.0	120.5	39.0	19.5	91.5	23.0	87.5	0.0	0.0	0.0	0.0
M	42.5	86.5	398.5	0.0	103.0	36.5	21.5	81.5	23.0	81.5	0.0	0.0	0.0	0.0
M	30.5	116.5	499.0	0.0	113.5	45.0	0.0	112.0	0.0	110.0	0.0	0.0	0.0	0.0
M	32.5	116.5	510.5	0.0	102.5	34.0	18.0	76.0	0.0	90.0	0.0	0.0	0.0	0.0
M	33.5	108.0	471.0	0.0	105.5	33.0	0.0	88.5	13.0	139.0	0.0	0.0	0.0	0.0
M	34.5	31.5	108.0	0.0	44.5	0.0	0.0	117.0	27.5	27.5	0.0	142.0	0.0	0.0
M	36.0	31.5	99.5	0.0	46.5	0.0	0.0	114.0	32.0	69.0	0.0	165.0	0.0	0.0
M	36.0	34.5	115.0	0.0	48.5	0.0	0.0	110.0	25.0	59.5	0.0	163.0	0.0	0.0
E	32.0	24.5	321.0	0.0	43.5	21.5	0.0	98.5	21.5	40.5	20.0	33.5	0.0	0.0
E	33.0	17.0	305.5	0.0	43.0	0.0	0.0	93.5	27.5	25.0	0.0	43.0	0.0	0.0
E	29.5	20.0	316.0	0.0	42.0	0.0	0.0	84.5	16.5	10.0	0.0	24.5	0.0	0.0
E	41.5	0.0	292.0	0.0	28.5	25.0	0.0	62.5	17.0	84.5	826.0	61.5	0.0	0.0
E	38.5	0.0	289.5	0.0	30.5	8.5	0.0	53.0	12.0	53.0	200.0	58.5	0.0	0.0
E	36.5	10.5	284.5	0.0	34.0	0.0	0.0	52.5	20.5	16.0	129.5	56.0	0.0	0.0
E	25.0	17.0	302.5	0.0	34.5	0.0	9.0	75.0	8.5	8.0	44.5	97.5	0.0	0.0
E	29.5	0.0	331.5	0.0	31.5	0.0	10.5	37.5	0.0	27.5	22.0	52.5	0.0	0.0
E	29.0	8.0	316.0	0.0	38.0	0.0	0.0	50.5	0.0	25.5	148.0	55.0	0.0	0.0
E	20.0	0.0	134.0	0.0	20.5	21.5	0.0	57.0	23.0	78.5	0.0	0.0	0.0	0.0
E	27.0	0.0	152.5	0.0	26.0	26.0	0.0	73.5	23.0	78.5	0.0	0.0	0.0	0.0
E	28.0	0.0	145.0	0.0	24.0	23.0	0.0	56.0	16.0	59.0	0.0	0.0	0.0	0.0
G	43.5	187.5	610.0	0.0	141.5	20.5	8.5	152.5	23.5	71.5	150.5	0.0	0.0	0.0
G	46.0	192.0	623.5	0.0	140.5	9.0	0.0	158.5	32.5	32.5	167.5	0.0	0.0	0.0
G	36.5	206.5	671.0	0.0	155.5	0.0	7.5	155.5	0.0	45.0	157.5	0.0	0.0	0.0
G	43.5	177.0	650.5	0.0	96.0	0.0	17.0	140.5	19.0	52.0	167.5	0.0	0.0	0.0
G	52.5	178.0	604.5	0.0	104.5	0.0	16.5	138.0	0.0	45.5	171.0	0.0	0.0	0.0
G	49.5	179.0	631.5	0.0	97.5	0.0	7.5	140.5	0.0	46.5	161.5	0.0	0.0	0.0
G	18.0	52.0	378.5	0.0	45.0	0.0	0.0	56.0	0.0	74.5	130.5	0.0	0.0	0.0
G	20.0	51.5	372.5	0.0	45.0	0.0	0.0	56.5	0.0	69.0	131.5	0.0	0.0	0.0
G	8.0	54.0	382.0	0.0	42.5	0.0	0.0	55.0	8.0	48.0	135.0	0.0	0.0	0.0

P: Peak

(cont. on next page)

Table A.2 The electronic nose data for the extracted olive oils of 2006-2007 harvest year (cont.)

Sample Code	P1	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
A	42.5	174.0	216.5	0.0	71.5	55.5	0.0	65.0	36.5	14.0	23.0	23.0	0.0	0.0
A	42.5	165.5	213.0	0.0	71.5	43.0	0.0	62.5	37.0	13.5	17.0	31.0	0.0	0.0
A	41.0	192.5	206.5	0.0	77.0	20.5	0.0	43.0	32.0	18.0	43.0	11.0	0.0	0.0
A	51.5	55.0	150.5	0.0	0.0	0.0	0.0	63.0	37.0	20.0	0.0	151.0	0.0	0.0
A	52.5	55.5	113.0	0.0	9.0	0.0	0.0	60.5	32.0	12.0	33.0	122.5	0.0	0.0
A	61.0	57.5	101.0	0.0	7.5	0.0	0.0	57.0	30.5	0.0	14.0	365.0	0.0	0.0
D	0.0	175.0	471.5	0.0	143.5	36.5	0.0	67.5	34.0	10.5	184.0	0.0	0.0	0.0
D	31.5	192.5	468.5	0.0	127.0	32.5	15.5	74.0	30.5	28.5	181.0	0.0	0.0	0.0
D	34.5	184.5	454.5	0.0	113.5	30.5	19.0	74.0	29.0	22.5	167.5	0.0	0.0	0.0
D	43.5	184.5	510.5	0.0	142.0	23.5	21.0	53.5	25.0	14.5	171.5	0.0	0.0	0.0
D	36.5	193.0	509.5	0.0	133.5	22.0	18.0	53.0	20.5	25.0	174.0	0.0	0.0	0.0
D	37.0	177.5	495.5	0.0	137.5	22.5	18.5	51.0	22.5	27.5	167.0	0.0	0.0	0.0
N	53.5	368.5	717.5	0.0	190.5	31.0	31.0	33.0	8.0	28.0	37.5	19.0	0.0	0.0
N	48.0	336.0	672.0	0.0	177.0	14.0	28.0	40.0	18.0	0.0	23.5	17.0	0.0	0.0
N	56.5	386.0	677.5	0.0	172.5	32.0	31.0	37.0	9.0	16.0	29.0	0.0	0.0	0.0
N	18.0	75.0	572.5	0.0	101.0	10.5	8.5	64.5	31.0	36.0	21.5	0.0	0.0	0.0
N	22.5	79.5	626.5	0.0	110.0	0.0	0.0	55.0	24.5	10.0	25.0	0.0	0.0	0.0
N	19.5	56.5	476.0	0.0	59.0	0.0	0.0	70.0	11.5	20.0	34.0	0.0	0.0	0.0
GE	22.5	136.5	375.0	0.0	163.0	28.0	0.0	126.5	22.0	26.5	0.0	0.0	0.0	0.0
GE	32.5	133.0	365.5	0.0	142.0	24.0	0.0	56.5	14.5	18.5	0.0	0.0	0.0	0.0
GE	23.5	131.5	373.5	0.0	149.0	19.5	0.0	109.5	29.5	39.5	0.0	0.0	0.0	0.0
GE	29.5	126.5	380.0	0.0	90.0	9.0	0.0	109.5	29.0	21.0	0.0	0.0	0.0	0.0
GE	37.5	115.0	376.0	0.0	100.0	0.0	0.0	103.5	33.5	24.0	0.0	0.0	0.0	0.0
GE	34.0	103.5	344.5	0.0	92.5	0.0	0.0	95.0	37.0	30.0	0.0	0.0	0.0	0.0
GE	35.5	105.0	433.0	0.0	36.5	0.0	0.0	60.5	0.0	31.0	0.0	0.0	0.0	0.0
GE	40.5	112.0	425.5	0.0	35.5	0.0	0.0	62.5	8.0	27.0	0.0	0.0	0.0	0.0
GE	44.0	118.0	428.0	0.0	33.0	0.0	0.0	43.5	7.5	12.5	0.0	0.0	0.0	0.0
AE	0.0	81.0	177.0	9.5	51.5	50.5	0.0	41.5	26.5	0.0	33.0	0.0	0.0	0.0
AE	27.0	115.0	226.0	10.5	63.0	45.5	0.0	68.5	48.5	31.5	67.5	0.0	0.0	0.0
AE	21.5	103.0	216.5	0.0	68.0	35.0	0.0	62.0	47.0	26.5	49.5	10.0	0.0	0.0
AE	0.0	332.0	189.0	0.0	95.0	39.0	0.0	40.0	20.5	26.0	79.0	0.0	40.5	0.0
AE	0.0	325.0	185.0	0.0	94.0	35.0	0.0	36.0	18.0	19.5	77.0	0.0	41.0	0.0
AE	0.0	350.0	186.5	0.0	93.5	28.5	0.0	30.5	17.5	24.5	76.0	0.0	35.0	0.0
AE	18.0	314.5	199.5	0.0	123.0	27.0	0.0	41.0	18.0	31.0	75.0	0.0	0.0	0.0
AE	0.0	259.0	193.0	0.0	104.0	12.0	0.0	36.5	18.5	36.0	70.0	0.0	0.0	0.0
AE	8.5	251.0	192.5	0.0	107.5	22.0	0.0	31.0	18.5	0.0	77.0	0.0	0.0	0.0

P: Peak

Table A.3: The electronic nose data for the commercial extracted olive oils of 2005-2006 harvest year

Sample Codes	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
Ez	64.792	34.708	100.875	520.333	230.125	87.250	32.208	0.000	42.708	9.708	180.250	22.167	50.375	11.125	174.167	179.167	50.750	0.000
Ez-Org	42.500	26.917	88.500	512.417	408.375	45.375	53.625	0.000	28.833	20.208	234.667	14.000	51.458	15.542	195.292	195.042	38.083	0.000
KucKuy1	1.417	2.083	54.917	354.667	122.333	70.583	13.958	0.000	34.250	21.875	275.042	19.042	37.458	21.875	191.375	246.833	39.458	0.000
KucKuy2	52.083	32.500	106.625	450.208	190.375	72.292	34.625	0.000	37.250	11.583	170.542	8.458	33.250	23.458	146.042	0.000	41.750	0.000
Hav	4.042	8.292	57.000	272.167	65.625	105.292	2.375	0.000	40.083	19.250	298.792	9.958	39.958	23.042	177.750	0.000	25.833	0.000
Altol	61.750	35.625	105.250	504.583	308.208	63.417	46.625	0.000	23.375	4.792	171.417	14.708	38.667	11.875	174.875	137.292	36.958	0.000
AltolSulbas	11.542	0.625	0.000	476.250	0.000	50.458	0.000	0.000	45.375	29.875	257.875	10.333	30.958	29.750	129.167	0.000	57.625	43.625
Edr	59.917	35.000	87.292	494.458	298.583	75.875	41.042	0.000	27.333	15.125	182.125	16.292	47.435	9.542	173.375	196.583	30.417	0.000
Bur	48.958	40.208	61.083	497.250	186.500	86.708	27.391	0.000	37.000	9.167	246.125	15.917	39.708	30.625	212.417	182.292	62.958	0.000
Gom	44.708	37.833	53.208	512.583	178.833	89.000	19.417	0.000	35.667	12.083	244.458	15.292	52.750	18.625	231.500	217.417	47.958	0.000
Ayv	57.480	37.440	66.440	454.840	168.760	90.400	31.320	0.000	42.080	19.320	227.080	14.800	53.640	20.440	154.880	184.680	59.280	0.000
Altova	40.500	29.667	77.583	427.792	126.167	74.000	23.042	0.000	46.583	20.042	204.125	7.333	49.792	29.875	129.667	0.000	49.458	0.000
Zey	31.917	12.792	92.500	495.833	192.667	50.875	34.292	0.000	40.042	6.000	280.083	17.500	38.125	17.083	134.083	225.375	52.958	0.000
Akh	31.958	3.208	68.750	373.917	56.250	81.917	2.125	31.125	49.167	15.792	182.750	11.333	50.917	20.333	141.167	179.125	34.542	0.000
Men	19.750	0.000	70.375	519.875	47.500	89.500	7.750	0.000	43.417	21.542	310.083	18.958	58.042	18.375	191.042	244.458	63.167	0.000
Tep	22.083	25.348	45.042	750.625	140.000	70.583	2.208	27.750	45.667	5.625	45.667	5.250	37.000	22.417	116.667	126.083	40.375	0.000
Bay	8.417	4.875	77.708	533.042	99.208	91.333	0.625	1.458	92.250	70.958	30.292	18.625	40.667	22.292	167.958	161.250	41.333	0.000
Sel	5.875	59.167	78.875	218.333	26.708	130.750	8.292	126.333	85.667	15.375	231.417	27.125	40.958	12.625	154.750	240.917	59.125	145.875
Ayd	49.111	28.000	103.556	477.806	88.028	93.750	6.278	8.583	68.500	9.583	140.083	20.917	41.417	23.556	149.778	0.000	54.639	0.000
Ort	30.583	9.125	69.708	454.417	63.958	120.750	11.208	32.750	55.708	23.583	221.917	29.042	60.167	30.083	172.583	251.583	78.875	0.000
Koc	20.600	4.760	71.040	531.800	54.520	135.200	1.880	3.400	65.880	27.200	167.200	26.240	48.040	25.080	187.960	269.320	53.840	0.000
Mil	0.000	0.000	50.208	411.250	64.458	79.917	1.292	0.000	73.083	21.958	33.708	11.875	43.458	24.333	167.292	156.542	39.958	0.000

Table A.4 The electronic nose data for the commercial extracted olive oils of 2006-2007 harvest year

Sample code	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
Ez	53.960	22.320	130.160	409.800	391.800	75.440	88.560	0.000	25.320	11.240	66.160	35.680	74.560	11.360	240.440	277.120	66.320	0.000
KucKuy	50.800	29.400	107.560	405.120	126.200	120.000	61.880	0.000	51.160	50.320	152.160	40.880	107.360	20.440	295.960	351.960	123.360	0.000
Altol	63.292	0.000	82.292	401.208	166.208	61.917	51.167	0.000	42.583	26.750	127.875	25.458	73.542	5.833	185.958	226.083	57.333	0.000
Edr	88.125	8.125	47.833	455.917	134.750	69.500	13.250	0.000	22.417	20.500	124.000	19.250	47.625	10.292	162.167	226.375	64.750	0.000
Hav	56.786	37.964	53.571	648.179	96.536	85.464	0.000	14.286	52.714	10.679	152.571	51.929	33.750	195.321	106.714	224.429	61.286	0.000
Bur	66.042	46.208	74.667	479.292	111.000	63.250	44.583	0.000	43.542	52.625	128.708	37.417	29.000	23.292	154.500	259.087	64.000	0.000
Gom	76.333	9.000	80.042	385.500	258.417	36.167	38.792	0.000	31.792	12.083	162.917	9.417	38.333	11.000	96.667	224.167	78.750	0.000
Ayv	89.964	40.321	98.179	499.821	201.429	54.643	0.000	22.107	47.667	9.679	138.000	39.143	23.179	133.214	81.429	155.250	36.464	0.000
Altova	51.040	28.880	86.160	476.880	130.040	94.600	76.760	0.000	42.520	27.520	37.920	51.080	71.200	20.160	281.320	381.200	83.240	0.000
Zey	46.417	2.625	92.375	403.583	200.375	62.208	32.083	0.000	26.917	15.333	123.417	19.417	39.917	13.167	164.000	238.125	95.500	0.000
Tep	39.958	39.583	75.042	897.208	119.625	219.833	0.000	0.000	31.292	10.417	8.542	56.208	34.500	210.375	101.792	127.875	52.542	0.000
Bay	35.750	31.042	0.000	354.458	14.417	127.739	37.458	0.000	59.708	47.000	34.792	58.917	98.042	42.458	218.958	263.625	114.208	91.833
Ode	31.480	0.000	69.480	495.920	39.840	179.000	0.000	0.000	55.208	8.640	49.480	56.760	23.680	197.240	71.880	165.600	46.880	0.000
Tire	34.500	21.958	93.125	819.250	71.000	244.125	0.708	0.000	38.125	16.833	16.500	24.875	50.458	17.208	228.208	210.917	62.458	0.000
Sel	44.375	34.625	50.708	857.375	78.667	158.375	10.958	0.000	42.292	28.667	30.750	24.833	87.625	15.667	191.583	192.458	64.625	0.000
Kus	46.400	23.120	82.280	627.480	67.160	186.040	0.000	0.000	108.840	5.560	20.760	39.120	21.760	152.000	84.680	90.520	42.800	0.000
Ger	31.360	28.360	72.320	946.680	108.480	148.360	9.880	0.000	44.000	10.720	31.960	24.480	46.280	51.880	194.520	202.240	55.200	0.000
Ayd	21.375	4.083	66.458	591.250	73.458	260.000	0.000	0.000	38.833	14.333	19.875	13.833	26.208	18.583	122.417	137.417	80.042	0.000
Ort	62.292	44.542	64.833	857.250	110.250	242.458	4.625	0.000	24.167	26.375	52.417	36.167	88.833	42.000	218.125	258.000	96.625	0.000
Kosk	34.630	1.889	68.741	519.000	40.667	196.852	0.000	2.074	85.185	16.000	48.444	56.481	38.259	172.444	153.778	226.077	70.370	0.000
Dal	24.542	2.542	73.583	525.708	53.000	188.833	0.000	0.000	66.917	7.708	25.000	34.667	32.125	116.042	141.500	155.292	46.417	0.000
Koc	35.875	12.792	100.333	550.583	76.000	285.417	0.000	0.000	57.500	6.250	11.208	40.333	49.083	126.042	125.292	150.667	53.250	0.000
Erb	38.880	3.400	39.280	257.920	19.920	325.458	16.360	0.000	229.840	53.920	89.080	26.160	49.560	30.840	238.120	261.080	88.560	75.520
Cine	30.269	2.808	9.962	264.808	4.269	258.923	0.000	0.000	48.269	9.346	10.154	12.885	33.923	29.423	164.038	161.000	72.000	79.462
Mil	37.778	22.444	93.370	614.444	70.667	190.593	0.000	0.000	61.148	10.074	10.222	31.926	31.037	146.667	116.037	168.593	56.815	0.000
Karbur	82.920	19.640	78.640	345.200	39.000	74.960	20.760	0.000	86.600	18.400	34.560	16.840	38.880	8.680	196.400	168.080	68.400	0.000