

**EGE UNIVERSITY
GRADUATE SCHOOL OF APPLIED AND NATURAL SCIENCES**

(PhD THESIS)

**DIFFERENTIATION OF OLIVE OIL
PRODUCED IN DIFFERENT AREAS IN TURKEY
BASED ON MAJOR PARAMETERS
USING CLUSTERS/PCA
(PRINCIPAL COMPONENT ANALYSIS) METHODS**

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Zinar Pınar GÜMÜŞ tarafından Doktora Tezi olarak sunulan “**Differentiation of olive oil produced in different areas in Turkey based on major parameters using Clusters/PCA (principal component analysis) methods**” başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi’nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 04.02.2015 tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

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ÖZET

ANA PARAMETRELERE BAĞLI OLARAK TÜRKİYE'DE FARKLI BÖLGELERDE ÜRETİLEN ZEYTİNYAĞININ KÜMELEME/PCA (TEMEL BİLEŞEN ANALİZİ) METOTLARI KULLANILARAK AYRILMASI

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Doktora Tezi, Kimya Anabilim Dalı
Tez Danışmanı: Doç. Dr. Hasan ERTAŞ
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Bu tez çalışmasında, Türkiye'deki farklı bölgelerde üretilen zeytinyağlarının sterol kompozisyonu, yağ asidi metil esterleri, triasilgliseroller, ağır metalleri ve delta $^{13}\text{C}/^{12}\text{C}$ isotop oranları analizleri ile farklı ve bölgesel özelliklerini gösterilmiştir. Bu özellikler daha sonra farklı bölgelerden (Aydın, İzmir, Manisa, Bursa, Muğla, Edremit Körfezi) toplanmış zeytinyağları arasında temel bileşen analizi (PCA) ve kümeleme (Cluster) analizleri ile karşılaştırma yapılmıştır.

Bu tez ile bütün veriler ve analiz parametreleri derlenerek, farklı bölgelerden ve hatta yurt dışından ithal edilmiş olan zeytinyağını da ayırtetmek için kullanılabilecektir. Bu sonuçlar zeytinyağ üreticileri için yardımcı olacak ve tağşişle ilgili ticari endişeyi ortadan kaldıracak ve aynı zamanda belli bölgenin üretimi olan belli kalitede zeytinyağı talebini karşılamada kullanılabilecektir.

Anahtar Kelimeler: Zeytinyağı, coğrafik orijin, tağşiş, temel bileşen analizi, kümeleme analizi.

ABSTRACT**DIFFERENTIATION OF OLIVE OIL PRODUCED IN
DIFFERENT AREAS IN TURKEY BASED ON MAJOR
PARAMETERS USING CLUSTERS/PCA (PRINCIPAL
COMPONENT ANALYSIS) METHODS**

GÜMÜŞ, Zinar Pınar

PhD Thesis at Department of Chemistry

Supervisor: Assoc.Prof.Dr. Hasan ERTAŞ (Department of Chemistry)

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In this thesis, it was aimed to investigate the composition of olive oil samples collected from produced in different areas in Turkey namely; sterol compositions, fatty acid methyl esters, trace element composition, $^{13}\text{C}/^{12}\text{C}$ isotope ratios and triacylglycerols (TAG). These properties can be exploited to differentiate the olive oils obtained from Aydın, İzmir, Manisa, Bursa, Muğla, Edremit Körfezi between each other by using principal component analysis (PCA) and Cluster analysis.

Overall data of the above parameters was compiled to differentiate the olive oil from different areas, even though the olive oil samples imported from abroad. This method developed for differentiation is expected to be used for removing any hesitation for the adulteration in terms of trade concern and to meet consumer demands for high quality olive oil produced in particular geographical region.

Keywords: Olive oil, authentication, adulteration, principal component analysis, cluster analysis.

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LIST OF SYMBOLS

<u>Abbreviations</u>	<u>Explanation</u>
ECN42	Equivalent Carbon Number 42.
FAME	Fatty Acid Methyl Esters.
TAG	Triacylglycerols.
PCA	Principal Component Analysis.
HCA	Hyerarchical Cluster Analysis.
FID	Flame Ionisation Dedector.
HPLC	High Pressure Liquid Chromatography.
GC	Gas Chromatography.
RID	Refractive Index Dedector.
IRMS	Isotope Ratio Mass Spectroscopy
ICP-MS	Inductive Coupled Plasma Mass Spectroscopy.
A	Aydın Region
K	Edremit Bay Region
ML	Mugla Region
MS	Manisa Region
I	Izmir Region
B	Bursa Region

1. INTRODUCTION

1.1 General Knowledge about Olive Oil

Olive oil is a very important agricultural product of the Mediterranean region, especially, Spain, Italy, Greece, Tunisia, Syria, Turkey and Morocco. According to International Olive Oil Council (IOOC), Turkey produces 4.3 % of world's olive oil both in production and consumption (IOOC, 2014).

Recent popularity of olive oil could be attributed to its sensorial characteristics as well as its potential health benefits. These benefits have been related to its wellbalanced major and minor components. With increasing consumer demand for high quality olive oil, oil produced from olives of just one variety (monovarietal) or one geographical region have been appeared on the market. Therefore, it is becoming more important to characterize each monovarietal olive oil by its chemical and sensorial properties. Chemical composition of olive oils might also differ due to the influence of geographical, agronomic and technological factors (Aparicio and Luna 2002).

The first olive oil producer was uncivilized man who worked a stone mortar to press fallen olives and noticed that oil was dropped. Antique civilizations of the Middle East and the littoral regions of the Mediterranean basin have explained openly data farming of olive and production of oil.

Process techniques used in olive oil production has been developed with technological evolution over the centuries. After importance of olive oil was increased new progresses was developing and so mechanically extracting olive oil were implemented in the 1900s as a result of studies on percolation and centrifugation systems.

There are three extraction systems in use pressure, percolation and centrifugation, although centrifugation is by far the most common in olive mills all over the world.

To produce high-quality oil, the olives must be harvested without breaking the fruit skins, and fruit should be processed within 12 to 24 h of harvest. Fruit should be separated by quality, with each grade processed separately (Lipid Library, AOCS).

Recently, several studies have been conducted in order to emphasize the certification of the geographical origin of food products, since authenticity and quality issues can be often associated with a given geographical origin. The protected designation of origin (PDO) and Protected Geographical Indication (PGI) for agricultural products has been introduced with official European regulations, which allow the labelling of some products with the names of the geographical area of production. This designation guarantees that the quality of the product is apparently engaged to its geographical origin. (Ulberth and Buchgraber 2000, Babcock and Clemens 2004).

Chromatographic methods have been generally preferred in classification and adulteration studies. Although chromatographic methods supply high degree of precision, there is an increasing demand for rapid, inexpensive and effective techniques for determination of authenticity of olive oils.

To characterize each olive oil variety few series of chemical compounds or a univariate statistics is not adequate. Instead multivariate analysis techniques should be applied to a number of variables (chemical compounds and/or sensory descriptors). The multivariate data analysis enables the extraction of meaningful information from the large amount of data such as chemical and sensorial properties of olive oil (Aparicio and Luna 2002).

Multivariate data analysis can be used for both classification and regression issues. It is common to employ principal component analysis (PCA) which shows the relation between observations to classify olive oil with respect to variety or geographical origin. Olive oil varieties from the different geographical regions have been well classified by models based on principal component analysis (PCA) and hierarchical cluster analysis (HCA).

1.2 Types of Olive Oils

Edible oils are mainly vegetable oils which have been subjected to several processes to remove undesirable constituents. In order to make them suitable for human consumption, most edible oils are subjected to refining processes, such as neutralization, bleaching and deodorization. Among edible oils, only virgin olive oil can be consumed without refining. Edible oils are characterized by a wide range of physical and chemical properties, since their composition depends on the type of the oil.

Olive oil is the oil obtained only from the fruit of the olive tree (*Olea europaea* L.), to the exception of oils obtained using solvents or re-esterification processes and of any mixture with oils of other kinds. Virgin olive oils are the oils obtained from the fruit of the olive tree completely by mechanical or other physical means under conditions, particularly thermal conditions, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration.

According to the International Olive Oil Council and Turkish Food Codex, the designation and categorization of olive oils are explained below. Free acidity is expressed as % oleic acid (IOOC, 2014).

Extra virgin olive oil: not more than 0.8 g per 100 g.

Virgin olive oil: not more than 2 g per 100.

Ordinary virgin olive oil: not more than 3.3 g per 100 g.

Lampante virgin olive oil: virgin olive oil not fit for consumption as specified lampante virgin olive oil. It has free acidity of more than 3.3 g per 100 g and/or the organoleptic.

Refined olive oil is the olive oil obtained from virgin olive oils by refining methods. It has a free acidity of not more than 0.3 g per 100.

Olive oil is the oil consisting of a mix of refined olive oil and virgin olive oils fit for consumption. Olive oil has a free acidity of not more than 1 g per 100 g.

Olive-pomace oil is the oil obtained by treating olive pomace with solvents or other physical treatments, to the exclusion of oils obtained by re-esterification processes and of any mixture with oils of other kinds.

Olive pomace oil is the oil comprising the blend of refined olive pomace oil and virgin olive oils fit for consumption as they are. It has a free acidity of not more than 1 g per 100 g.

Olive Oil suitability for most uses depends on its quality and chemical composition. Several tests can be used to determine oil purity, such as the determination of triacylglycerols (TAGs), fatty acid methyl esters (FAMES), sterols and other constituents of the unsaponifiable fraction of oil.

1.3 Olive Oil Composition

Olive oil is a complex mixture consisting of two main groups of substances: (a) saponifiables, 98% of the chemical composition, for instance triacylglycerols (TAG), partial glycerides, free fatty acids and phosphatides; and (b) unsaponifiables, 2% of olive oil composition, for instance phytosterols, tocopherols, hydrocarbons, pigments, phenols, flavonoids or volatile compounds.

1.3.1 Triacylglycerols and fatty acids

Triacylglycerols include the trihydric alcohol glycerol esterified, nearly always, with long-chain fatty acids. When the two primary hydroxyl groups are esterified with different fatty acids, the resulting triacylglycerol can be asymmetric (AOCS Lipid Library).

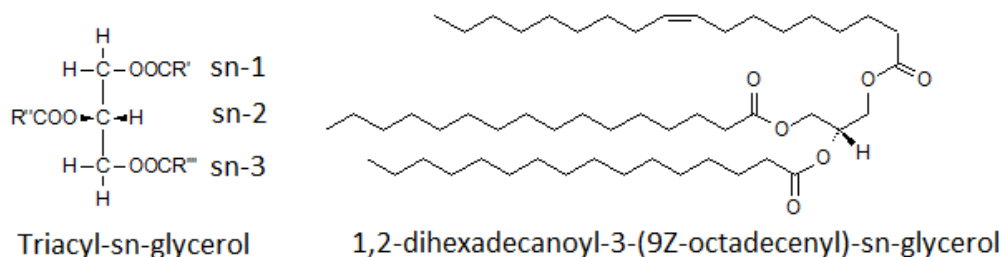


Figure 1.1. The Fischer projection of a triacyl-*sn*-glycerol (AOCS, Lipid Library).

While seed and fruit oils can contain an enormous range of different fatty acids especially C₁₈ unsaturated fatty acids is found in their chemical structure, especially oleate and linoleate are predominant. The TAG composition of olive oil is: OOO (40-59%), POO (12-20%), OOL (12.5-20%), POL (5.5-7%), SOO (3-7%) and smaller amounts of POP, POS, OLnL, LOL, OLnO, PLL, PLnO and LLL, LnLO, LnOP, PLP, SOP, EOO.

The fatty acid composition of olive oil is: myristic acid (C₁₄:0): ≤ 0.05%, palmitic acid (C₁₆:0): 7.5-20%, palmitoleic acid (C₁₆:1): 0.3-3.5%, heptadecanoic acid (C₁₇:0): ≤0.3%, stearic acid (C₁₈:0): 0.5-5%, oleic acid (C₁₈:1): 55-83%, linoleic acid (C₁₈:2): 3.5-21%, linolenic acid (C₁₈:3): 3.5-21%, eicosanoic acid (C₂₀:0): ≤0.6%, gadoleic acid (C₂₀:1): ≤0.4%, behenic acid (C₂₂:0): ≤0.2%, lignoceric acid (C₂₄:0): ≤0.2% (IOOC, 2003).

Saturated fatty acid components make up 10-40% of the total fatty acids in most natural lipids. The most present saturated fatty acids include 14, 16 and 18 carbon atoms. Thus, the fatty acid with 16 carbon atoms, hexadecanoic (palmitic) acid as it can seen in Figure 1.2.

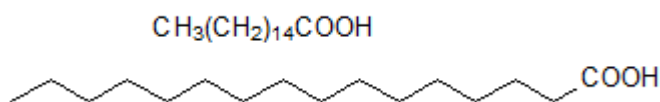


Figure 1.2. Structural formula of hexadecanoic (palmitic) acid (AOCS, Lipid Library).

Acetic or ethanoic acid (2:0) is of great importance in living tissues, as the biosynthetic precursor of fatty acids and innumerable other metabolites.

Propanoic acid (3:0) is important as the biosynthetic precursor of some amino acids. It is seldom found in esterified form in natural lipids.

Butanoic acid (4:0) comprises 3-4% by weight of the total fatty acids in cow's milk.

Hexanoic acid (6:0) comprises 1-2% of the total fatty acids in ruminant milk triacylglycerols. It is also found as a minor component of certain seed oils rich in medium-chain saturated fatty acids.

Medium-chain fatty acids, such as **octanoic** (8:0), **decanoic** (10:0) and **dodecanoic** (12:0), are found in esterified form in most milk fats, including those of non-ruminants, though usually as minor components. They are major components of such seed oils like coconut oil, palm kernel oil.

Myristic acid (14:0) is a pervasive component of lipids in most living organisms generally at levels of 1-2% only. However, it is more abundant in cow's milk fat, some fish oils and in those seed oils enriched in medium-chain fatty acids as. coconut and palm kernel oil.

Palmitic acid (16:0) is usually considered the most plentiful saturated fatty acid in nature. It is present in amounts that vary from 10 to 40% in seed oils like palm oil.

Stearic acid (18:0) is the second most abundant saturated fatty acid in nature and in vegeoils such as cocoa butter.

Eicosanoic acid (20:0) can be detected at low levels in most lipids of animals, and often in those of plants and microorganisms.

The most abundant monoenoic fatty acids in animal and plant tissues are 16 or 18 carbon atoms. The structural formula of the fatty acid with 18 carbon atoms is systematically named *cis*-9-octadecenoic (oleic) acid in Figure 1.3.

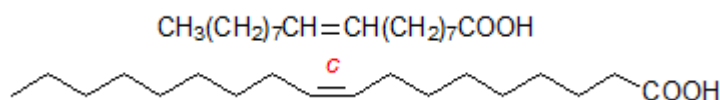


Figure 1.3. Structural formula of *cis*-9-octadecenoic (oleic) acid (AOCS, Lipid Library).

In the shorthand nomenclature, it is designated '18:1' (or 9-18:1 or 9c-18:1).

Triacylglycerols (or oils and fats) containing high proportions of monoenoic fatty acids are usually liquid at ambient temperature. Analogous fatty acids with *trans* double bonds are normally higher melting.

Isomers of Fatty Acids

Oleic acid (9*c*-18:1 or 18:1(*n*-9)) is by far the most abundant monoenic fatty acid in plant and animal tissues. Olive oil contains up to 78% of oleic acid.

9-*cis*-Hexadecenoic acid (palmitoleic acid, 9-16:1 or 16:1(*n*-7)) is a minor component of animal lipids, but it can be much more abundant in fish oils such as cod-liver oil.

3-*trans*-Hexadecenoic acid is an important constituent of the photosynthetic tissues (chloroplasts) of plants.

11-*cis*-Eicosenoic acid is a common if minor constituent of animal tissues and fish oils. It is also found in rapeseed oil and seed oils of related species.

Erucic acid (13-22:1) occurs naturally in fish oils and in small amounts in the phospholipids of animal tissues. Erucic acid is the major component (up to 66%) of the total fatty acids in rapeseed oil.

The lipids of all higher organisms contain appreciable quantities of polyunsaturated fatty acids ('PUFA') with methylene-interrupted double bonds, i.e. with two or more double bonds of the *cis*-configuration separated by a single methylene group.

Two principal families of polyunsaturated fatty acids occur in nature that are derived biosynthetically from **linoleic** (9-*cis*,12-*cis*-octadecadienoic) and **α -linolenic** (9-*cis*,12-*cis*,15-*cis*-octadecatrienoic) acids.

The (n-6) family of polyunsaturated fatty acids

Linoleic acid is the most abundant component of plant lipids, and of all the seed oils of commercial importance such as corn, sunflower and soybean oils usually contain over 50% of linoleate, and safflower oil contains up to 75%.

γ -Linolenic acid ('GLA' or 6-*cis*,9-*cis*,12-*cis*-octadecatrienoic acid or 18:3(*n*-6)) is usually found in a few seed oils, and those of evening primrose, blackcurrant have some commercial importance.

11-*cis*,14-*cis*-Eicosadienoic acid (20:2(*n*-6)) is a common minor component of animal tissues.

8-*cis*,11-*cis*,14-*cis*-Eicosatrienoic acid (dihomo- γ -linolenic acid or 20:3(*n*-6)) is the immediate precursor of arachidonic acid. However, it does not accumulate to a significant extent in animal tissue lipids, and is typically about 1-2% of the phospholipid fatty acids.

Arachidonic acid (5-*cis*,8-*cis*,11-*cis*,14-*cis*-eicosatetraenoic acid or 20:4(*n*-6)) is the most important metabolite of linoleic acid in animal tissues, both in quantitative and biological terms.

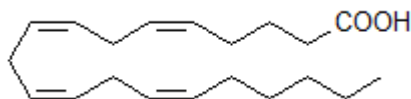


Figure 1.4. Structural formula of arachidonic acid (AOCS, Lipid Library).

4,7,10,13,16-Docosapentaenoic acid (22:5(*n*-6)) is usually a relatively minor component of animal lipids, but it is the main C₂₂ polyunsaturated fatty acid in the phospholipids of testes.

The (*n*-3) family of polyunsaturated fatty acids

α -Linolenic acid (9-*cis*,12-*cis*,15-*cis*-octadecatrienoic acid or 18:3(*n*-3)) is a major component of the leaves. It can amount to 65% of the total fatty acids of linseed oil. In contrast, soybean and rapeseed oils have up to 7% of linolenate.

11,14,17-Eicosatrienoic acid (20:3(*n*-3)) can usually be detected in the phospholipids of animal tissues but rarely at above 1% of the total. Higher concentrations may be found in fish oils.

Stearidonic acid (6,9,12,15-octadecatetraenoic or 18:4(*n*-3)) is occasionally found in plants as a minor component, and it occurs in algae and fish oils.

8,11,14,17-Eicosatetraenoic acid (20:4(*n*-3)) is found in most fish oils and as a minor component of animal phospholipids.

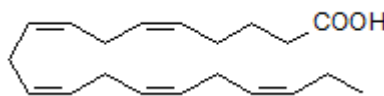


Figure 1.5. Structural formula of eicosapentaenoic acid (20:5(n:3)) (AOCS, Lipid Library).

5,8,11,14,17-Eicosapentaenoic acid ('EPA' or 20:5(*n*-3)) is one of the most important fatty acids of the (*n*-3) family. It occurs widely in algae and in fish oils, which are major commercial sources.

7,10,13,16,19-Docosapentaenoic acid (22:5(*n*-3)) is an important constituent of fish oils, and it is usually present in animal phospholipids at a level of 2 to 5%. It is known that it can be retro-converted to EPA although desaturation to DHA does not appear to occur.

4,7,10,13,16,19-Docosahexaenoic acid ('DHA' or 22:6(*n*-3)) is usually the end point of α -linolenic acid metabolism in animal tissues. It is a major component of fish oils.

The (n-6) family of polyunsaturated fatty acids

Oleate can be chain elongated and desaturated in animal tissues with **5,8,11-eicosatrienoic acid** (20:3(*n*-9) or 'Mead's acid') as the most important product. Other fatty acids of this family that may also be found at low levels include 18:2(*n*-9), 20:2(*n*-9) and 22:3(*n*-9).

1.3.2 Phenolic compounds

The phenolic compounds present in olive oil can be classified into a lipophilic group and a hydrophilic group. The total polyphenolic content of olive oil ranges from 50 to 1000 ppm. The major phenolic compounds in olive oil are gallic, caffeic, vanillic, *p*-coumaric, syringic, ferulic, homovanillic, *p*-hydroxybenzoic and protocatechuic acids, tyrosol and hydroxytyrosol.

The levels of total phenols and individual phenols in olive oil depend on agronomic factors, maturity of the olives, processing, packaging and storage. The

phenolic compounds present in olive oil are strong antioxidants and radical scavengers.

There are in good correlation of total polar phenol content with the stability of the olive oil. Phenolic compounds are more effective than tocopherols in increasing the stability of olive oil toward oxidation.

1.3.3 Phospholipids

Phospholipids are found in small quantities in freshly produced olive oils (40–135 mg/kg), being their concentration lower with oil aging. The most important phospholipids in olive oil are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine.

1.3.4 Tocopherols

Tocopherols and tocotrienols belong to the lipophilic group in olive oil. α -tocopherol is traditionally considered to be the major antioxidant of olive oil

1.3.5 Aliphatic alcohols

Aliphatic alcohols are compounds of linear structure. The main linear alcohols present in olive oil are docosanol (C22), tetracosanol (C24), hexacosanol (C26) and octacosanol (C28). Other alcohols with an odd atom number, present at trace levels, are tricosanol (C23), pentacosanol (C25) and heptacosanol (C27). Olive oil also contains two important diterpene alcohols: phytol and geranylgeraniol.

1.3.6 Wax esters

Wax esters occurring in vegetable oils are a group of compounds formed by esterification of high molecular mass alcohols with fatty acids. If the alcoholic group is a long chain aliphatic alcohol, it results in aliphatic waxes of 34–46 carbon atoms. Wax values in virgin olive oil, olive oil and refined olive oil are a maximum 250 and 350 mg/kg. Virgin olive oil has a higher content of C36 and C38 waxes than C40, C42, C44, and C46 waxes.

1.3.7 Hydrocarbons

The most important hydrocarbon found in both virgin and refined olive oils is squalene. This hydrocarbon is a precursor of sterols in vegetable oils. Other hydrocarbons have also been found in virgin olive oil.

1.3.8 Pigments

The main pigments present in edible oils are carotenoids. The main carotenoids present in olive oil are β -carotene and lutein. Other pigments also found in olive oils (10–30 mg/kg) are chlorophylls. Olive oil green colour is a consequence of the presence of these pigments.

1.3.9 Volatile and aromatic compounds

When Olive oil compared with other vegetable oils has a characteristic aroma and flavour. These sensory characteristics, together with nutritional aspects, are the main reasons for the increment of virgin olive oil consumption. A balanced flavour of green and fruity sensory characteristics of high quality olive oil has a profile of volatile compounds, mainly comprising aldehydes, esters, alcohols and ketones. Volatile components can be used to check the quality of olive oil, to detect an adulteration, to detect a possible off-flavour or to determine the variety of olive.

1.3.10 Phytosterols

Cholesterol (cholest-5-en-3 β -ol) is the most abundant member of a family of polycyclic compounds known as **sterols**. The basic sterol from which other sterol structures are defined is 5 α -cholestan-3 β -ol.

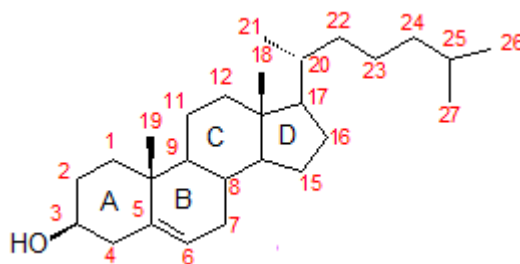


Figure 1.6. Structural formula of cholest-5-en-3 β -ol (AOCS, Lipid Library).

The phytosterols include campesterol, β -sitosterol, stigmasterol and Δ^5 -avenasterol, some of which are illustrated below in Figure 1.7.

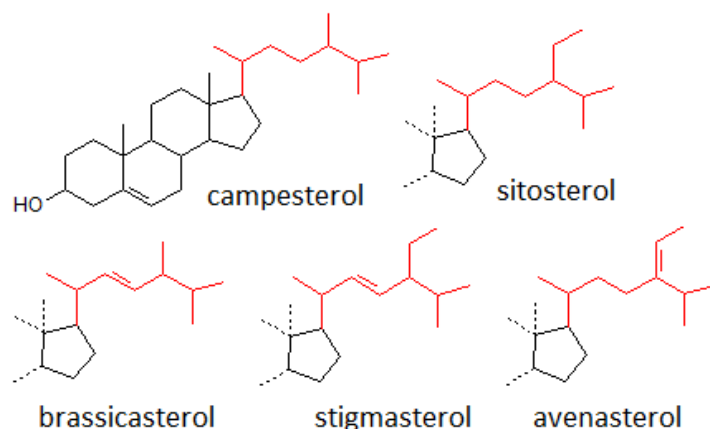


Figure 1.7 Structural formula of phytosterols (AOCS, Lipid Library).

Cholesterol is usually a minor component only of plant sterols. Phytosterols comprise a major proportion of the unsaponifiables in vegetable oils. Total sterol content in olive oil varies between 1000 and 2300 ppm.

Sterol composition and content of olive oil are influenced by cultivar, crop year, and degree of fruit ripeness, storage time of fruits before oil extraction and method of oil extraction. There are different types of sterols in vegetable oils: The main sterols in olive oil are β -sitosterol (75–90 %), Δ^5 -avenasterol (5–36 %) and campesterol (3 %). However, other sterols such as cholesterol, campestanol, stigmasterol, Δ^7 -campesterol, chlosterol, sitostanol, $\Delta^5,24$ -stigmastadienol, Δ^7 -stigmasterol and Δ^7 -avenasterol have been also found at trace levels. The main triterpene dialcohols are erythrodiol and uvaol. Their total content in olive oil ranged from 1 to 20 mg/g.

1.3.11 Proteins

Finally, the presence of proteins in vegetable oils has been described. The total protein content varies widely depending on the type of oil and its extraction method. On the other hand, it has been shown that there are significant differences in protein content in olive oils depending on the crop and the maturity of the fruit.

Oil suitability for most uses depends on its quality and chemical composition. Several tests can be used to determine oil purity, such as the determination of triacylglycerols, TAGs, sterols and other constituents of the unsaponifiable fraction of oil.

1.4 Authenticity of Olive Oil

Olive (*Olea europaea* L.) is one of the oldest agricultural tree crops worldwide and is an important source of oil with beneficial properties for human health. In comparison to commonly used vegetable oils, the cost of olive oil is higher. There is also the possibility of the addition of cheaper rates of olive oil to better graded ones for the economic reasons. Some analytical methods are using for determination of the adulteration which adding to low-level sunflower, canola, cotton or other seed oil to olive.

Food traceability implies the control of the entire chain of food production and marketing, allowing the food to be traced through every step of its production back to its origin. The verification of food traceability is necessary for the prevention of deliberate or accidental mislabeling, which is very important in the assurance of public health.

In the case of olive oils, the increase in the demand for high-quality olive oils has led to the appearance in the market of olive oils elaborated with specific characteristics. They include oils of certain regions possessing well-known characteristics, that is, olive oils with a denomination of origin, or with specific olive variety composition, that is, coupage or monovarietal olive oils. Olive oils obtained from one genetic variety of olive or from several different varieties are called monovarietal. Monovarietal olive oils have certain specific characteristics related to the olive variety from which they are detailed.

Regulation of olive oil can only go so far in resolving an issue of authenticity. The essential problem in authentication is setting parameters that accurately define the composition of pure, fresh oil. Any oil is a complex mixture of components. These include tri-, di-, and monoglycerides; free fatty acids; saturated, monounsaturated, polyunsaturated, and trans fatty acids; sterols; aliphatic and other alcohols; flavonoids; and a variety of other organic molecules.

For oils extracted from the same species of plant, regional variations in climate and soil conditions may affect the levels of some of these components. Protected designation of origin (PDO), protected geographical indication (PGI) and traditional speciality guaranteed (TSG) are geographical indications (GIs) defined in European Union Law to protect the names of regional foods. This law provides the protection of the reputation of the regional foods and prevention from the mean competition and misleading of consumers by virtual products which can be poor quality or different flavour.

A geographical indication is a name or sign that reflects the certain products in a specific geographical location or origin such as town, region or country. The labelling of VOOs with their geographical area of production is provided by European legislation. European Community (EC) introduce the indications to ensure that high quality parameters of olive oil are apparently engaged to its geographical origin (EC Regulation 2081, 1992).

1.4.1 Key components (Food quality)

The tests conducted can be loosely characterized into tests to determine the quality and authenticity of the oil and to detect the presence of the incorrect grade of olive oil. Free fatty acids, peroxide values, and ultraviolet (UV) determination are tests that ascertain the quality of the oil with respect to hydrolysis or oxidation of the oil.

The fatty acid profile, sterol profile, triglyceride analysis, and glyceryl 2-palmitate determination can be used to detect the presence of oils other than olive. Erthyrodiol, uvaol, wax, and stigmastadiene analysis are used to detect the presence of olive-pomace oil and/or refined oil in virgin olive oils.

Free fatty acids: The free fatty acid content, which is measured by total acidity, indicates quality, because acidity increases with hydrolytic activity, which could be due to poor quality, damaged olive fruit, or microbial activity.

Peroxide value: The oxidation of the oil is determined by the peroxide value, with a high value indicating a poor quality, older, or mistreated oil.

UV determination: The analysis of olive oils at certain UV wavelengths can be used to assess the oxidation of the oils and can also indicate the presence of a refined olive oil in virgin olive oil.

Fatty acids: Fatty acid composition is a useful indicator of purity or the presence of oils other than olive oil. High levels of trans fatty acids can indicate the presence of refined olive oil or other vegetable oils in a virgin olive oil.

Each type of oil usually has a fatty acid composition that is indicative of its plant source. So, although it may not be possible to confirm authenticity from fatty acid content alone, the presence of the wrong fatty acids can be a very good indication of adulteration.

Glycerol 2-palmitate: Additional information is gained by determining which fatty acids are located at the 2-position (middle) of the triglyceride. High levels of saturated fatty acids at the 2-position are indicative of adulteration with interesterified oil.

ECN42 (equivalent carbon number 42): The ECN42 is a test that measures the level of the triglyceride trilinolein and similar triglycerides. Trilinolein is a triglyceride that is abundant in oils such as sunflower, rapeseed, and grape seed but is virtually absent in olive oil. ECN 42 is a very useful parameter to determine for adulteration.

Sterols: The total sterol content and measurement of individual sterols (cholesterol, brassicasterol, campesterol, stigmasterol, delta-7 stigmasterol, and beta-sitosterol) gives an indication of authenticity.

Beta-sitosterol is present in particularly high levels relative to the other sterols in olive oil. High levels of brassicasterol indicate the presence of rapeseed oil, and high levels of delta-7-stigmasterol indicate the presence of sunflower oil.

Waxes and diols: Levels of waxes and diols, erythrodiol and uvaol, are much higher in solvent-extracted olive oil (olive-pomace oil or olive residue oil) than in other olive oils. Virgin olive oils, which are obtained by pressing the olive fruit, contain lower levels of these components than the cheaper olive-pomace oil.

Stigmastadienes: Some tests indicate whether the oil has been bleached during the refining process. Stigmastadienes are formed when the sterol components are exposed to the high temperatures of the refining process. Refining virgin olive oils is not permitted, so a relatively high level of stigmastadienes is a good indicator of the presence of cheaper refined oils.

Taste: Many would argue that the sensory characteristics of the oil are what matter most, but even the most sensitive palate cannot detect every case of adulteration. Taste testing does help indicate age, quality, and rancidity.

1.4.2 Three testing categories

The revised U.S. grade standards divide tests into three categories: mandatory, confirmatory, and optional. As might be expected, the mandatory tests will have to be performed on all lots of olive oil and olive-pomace oil. These tests will include flavor, odor, color, free fatty acid expressed as oleic acid, peroxide value, absorbency in UV, fatty acid composition, trans fatty acid content, desmethylsterol composition, total sterol, and stigmastadiene content.

There are many other tests one might wish to apply, such as pesticide residue testing and trace element content. In addition, there are many other oils to which one might wish to apply the methods about to be required for olive oil.

1.5 Literature Survey

A number of methods and limits were introduced by IOOC trade standard, into EC Regulation 2568/91 and into the Codex Alimentarius Standard for controlling product authenticity and quality. Among the established methods for the control of authenticity of olive oil, the determinations of fatty acids and triglycerides seem to be very useful.

Fatty acid analysis is usually performed by GC. The official bodies have established limits with regard to the content of fatty acids in olive oil. These limits are used for the discrimination between genuine olive oil and other vegetable oils.

The parameter ΔECN_{42} is a very useful and effective tool in the detection of the presence of the most common vegetable oils for the detection of adulteration of an olive oil with the following vegetable oils: sunflower, soybean, cotton, corn, walnut, sesame, safflower, canola and rapeseed. (Christopoulou et al., 2004)

Matos et al were investigated the usefulness of three chemical parameters which are tocopherols, sterols and fatty acids as a tool to discriminate three varietal olive oils in Portuguese. They are used statistical methods such as multivariate analysis of variance; principal components analysis (PCA) and cluster analysis were used to evaluate significant differences on the studied parameter. According to their results tocopherols, sterols and fatty acids olive oils show good nutritional characteristics. Each varietal olive oil characteristics contribute to the unique characteristics (Matos et al, 2007).

Plant sterols or phytosterol contents vary in different types of olive oil. Sterol profile was used for determining the adulteration. Even so, these characteristics are known to be affected by many factors agronomic climate, soil, water, geographic altitude, longitude, harvesting cultivar, ripeness, technological conservation of the fruit or of the oil, extraction systems, and processing refining, solvent extraction. The presence of olive–pomace oil in virgin oil can be detected from the levels of erythrodiol + uvaol. The diversity of these factors and their interrelationships make it very difficult to completely characterize the sterol profile and erythrodiol + uvaol content of a given product (Casas et al., 2004).

Fasciotti et al discuss several ways to assess the addition of soybean oil to olive oil. Mass spectra obtained by positive APCI-MS, after the direct injection of oil solutions allowed the identification of the main TAG ions present in these solutions and showed to be efficient to assess the addition of soybean oil to olive oil, through the detection of unexpected TAGs. They conclusive to classify olive oils as adulterated because certain TAGs (LLL, for example) do not occur in olive oils. They suggest that any investigation of olive oil adulteration by HPLC–APCI-MS because adulteration can be easily identified if unexpected TAGs are found in a suspected olive oil (Fasciotti et al, 2010).

A review of Abidi S.L. was investigated development and application of chromatographic analysis techniques for the isolation, purification, separation and

detection of the for plant sterols (Abidi et al., 2001). In recent years attention has been focused on authentication of the geographical origin of olive oil using in particular the profiles of volatile compounds sterols or free fatty acids, nuclear magnetic resonance fingerprinting and also isotope ratios and mineral content.

Isotope ratio analysis, $\Delta^{13}\text{C}/^{12}\text{C}$, was measured using elemental analyser isotopic ratio mass spectrometry (EA-IRMS) or gas chromatography/combustion/isotopic ratio mass spectrometry (GC/C-IRMS) in bulk olive oil or in some sub-components (individual fatty acids or aliphatic alcohol and sterols) has been shown to be useful for detecting the adulteration of olive oil with cheaper pomace olive oil or with other vegetable oils. (Camin et al, 2010)

The quantitative analysis of fatty acids, triglycerides or sterols can be useful for detection of olive oil adulteration. The HPLC-RID quantitative analysis of triglycerides is considered a potent method for detection of olive adulteration. Determination of sterols in olive oil or other vegetable oil is usually carried out by GC-FID (Khalid et al 2010).

Authentication covers many different aspects, including adulteration, mislabelling, and characterization and misleading origin. Several methods have been proposed for the monitoring of adulteration of virgin olive oils with other edible oils. Some oils normally added to VOO can be, either olive oils of lower quality (e.g. olive-pomace olive oil or virgin olive oil obtained by second centrifugation of the olives), or seed oils (e.g. corn, soybean, palm or sunflower oil, among others) (Pena et al, 2005).

A simple and sensitive method for the analysis of sterols, tocopherols and triterpenic dialcohols from the unsaponifiable fraction from oil samples in a single analytical run using liquid chromatography coupled to mass spectrometry was developed by Zarrouk et al. This methodology was suitable for the identification of 23 compounds belonging to different families present in olive oil and other kinds of oils (Zarrouk et al, 2009).

Trace elements analysis plays an important role for oil characterization and adulteration detection. The presence of metals in edible oils may be due to different factors: The metals can be incorporated into the oil from the soil or be

introduced during the manufacturing of the foodstuff. Therefore, it can be assumed that the trace elemental distribution in olive oils varies according to their origin and then it can be supposed that a statistical treatment on trace element data could allow a geographical characterization of different olive oils.

Inductively coupled plasma atomic emission (ICP-AES) and atomic absorption spectrometry (AAS) are the most commonly used techniques for the determination of metals in different samples. Fats and oils are particularly difficult to analyze for their trace metal contents since some of them are present at very low concentration levels. For this reason ICP-MS could be an interesting tool, not only because of its well-known high sensitivity, but also because it allows an easy multielement analysis for each single sampling of a complex matrix such as olive oil. The detection power of the technique was able to give good data for the determination of elements of interest in olive oil samples (Benincasa et al., 2007).

The volatile compounds were analysed by using headspace-mass spectrometer for the detection of adulterants in olive oil (Lorenzo et al., 2002)

In another study carried out by Zeiner et al., the elements analyzed for this screening were the following: Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Zn. Some are essential for human nutrition (Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Zn), for others effects on the oxidative stability of edible oils are reported (Cu, Fe, Mn, Ni, Zn) and some occur in oils due to environmental contamination (Cu, Pb). A microwave assisted decomposition of the olive oil in closed vessels using a mixture of nitric acid and hydrogen peroxide was applied for the sample preparation (Zeiner et al., 2005).

Authentication of virgin olive oils using PCA of TAG and fatty acid profile was reported that adulteration at the 20% levels could be clearly detected based on PCA plots of the liquid chromatographic data of TAG composition, and TAG profiles proved to be more discriminating than those of fatty acids (Lee et al., 2001 and Rezanka et al., 1999).

PCA and SIMCA have been used to characterize or classify 80 different olive oils according to their origin in Diaz et al study. According to their results PCA is used mainly to achieve a reduction of dimensionality, and to allow a

primary evaluation of category similarity. They were used the contents in the various triglycerides, or the various sterols, or both. Other variables usually measured, acidity, index of peroxide, colour, and fatty acids. Chemometric techniques have been defined as the utilization of mathematical and statistical methods for handling and interpreting chemical data (Diaz et al., 2005).

Fatty acid content can be used to the fingerprint each specific cultivar. Important aspects of fatty acids concern the geographical characterization of olive oils. Multivariate statistical analysis applied to fatty acids, triacylglycerols, sensory attributes, chemical components and also to minor compounds determined using ^1H NMR technique. Generally, the results obtained using different methodologies refer to specific conditions of cultivar, geographical area or year of harvesting and therefore the results requires further validations using, for instance, more cultivars, more geographical areas, more samples and more years (D'Imperio et al., 2007).

Study of Grigoriadou et al was explained SPE procedure was optimised for the subsequent extraction of squalene and α -tocopherol prior to HPLC of triacylglycerols (Grigoriadou et al, 2007). According to study of both SPE and SPME they was useful in the adulteration of virgin olive oil (Flores et al., 2006).

GC-MS was used to analysis of fatty acid methyl esters for control of contamination of olive oil like adding sunflower oil into olive oil as we seen in another study (Vazquez et al., 2003). A more recent study reports a discrimination study depending on five minor Tunisian olive cultivars by analyzing the sterol and phenol compositions (Manai-Djebali et al 2012). This study emphasis the importance of varieties to produce good quality oils with a chemical compositions. In addition, the VOO content of the oil samples were also studied to demonstrate the differences in phenols, tocopherol, sterols, triterpene dialcohols and triacylglycerols profiles may be explained by genetic factors. PCA and HCA analyses have shown a good discrimination between varieties according to phenol, triacylglycerol and sterol data.

According to a study has displayed that the effects of agronomic and climatic conditions variables on sterol profiles was ambiguous (Temime et al., 2008). The diversity of these factors and their interrelationships make it very

difficult to completely characterise the sterol profile of a given product. As well as rainfall, temperature and especially winter temperature is also important.

An overview of analytical methods like chromatographic and spectroscopic techniques for high quality food products with a clear geographical origin has been reported (Luykx et al., 2008). Nowadays, δC^{13} values are widely used as the indicators of authenticity for flavour, beverages, and foods. The variabilities observed in δC^{13} values of the major fatty acids because of plant type, geographic origin and year of harvest have been measured (Woodbury et al., 1998).

The authenticity of single seed vegeoils which utilise the C_3 photosynthetic pathway was investigated using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-SIRMS). Samples of authentic groundnut, palm, rapeseed and sunflower oils were derivatised to form fatty acid methyl esters (FAMES) and their carbon isotope ratios ($^{13}C/^{12}C$) determined (Kelly et al, 1997).

Longobardi and co-workers have carried out on samples of virgin olive oils (VOOs) coming from four different Greek Ionian islands and than they was analysed measurements of free acidity, peroxide content, spectrophotometric parameters, chlorophyll content, phenol, sterol, fatty acid and triacylglycerol composition. Their results have shown that the multidisciplinary approach that combines different types of analytical determinations improved the discrimination of geographical origin for Greek virgin olive oils. They have also noted that cultivar, besides geographical origin, has a strong effect of the physicochemical parameters of olive oils determined (Longobardi et al., 2012).

Thirty-nine single virgin olive oils from eight producer countries, but cultivated in the same orchard under identical agronomic and pedoclimatic conditions, were characterised by 64 volatile compounds quantified by HS-GC and applied linear discriminant analysis for check cluster the varietal virgin olive oils (Luna et al, 2006).

In a recent review, the literature of the application of exploratory and unsupervised/supervised chemometric methods on chromatographic data, using triacylglycerol composition for the characterization and authentication of several

foodstuffs such as olive oil, vegetable oils, animal fats, fish oils, milk and dairy products, cocoa and coffee have been reported (Bosque et al., 2012).

Clinical studies have demonstrated that dietary intake of phytosterols as a part of the normal diet, or as a supplement, may help reducing blood cholesterol levels through the inhibition of its absorption from the small intestine. Additionally, it has been suggested that sterols have antiinflammatory, antibacterial, antifungal, antiulcerative, antitumoral activities and antioxidant activity (Alves et al, 2005).

1.6 The Aim of the Thesis

Olive oil is an essential agricultural food product in Turkey. There are difficulties in controlling the quality and adulteration of this product. In order to overcome difficulties, the characterization of olive oil is a necessity for Turkey.

This thesis aims to construct a classification procedure and authenticity based on virgin olive oil samples both collected from different regions. For this purpose 49 samples collected from different regions (Marmara, North Aegean, Central Aegean and South Aegean) are evaluated by using chemical parameters. These regions are critical regions in Turkey in terms of olive oil production.

The aim of collecting samples from different areas is to establish a reliable data set representing for each region as much as possible. Then, the sample being in question can be more confidently classified and treated. It also gives an insight to which parameters are significant in assessment of olive oils for authentication purposes. By this means, possible qualitative differentiations at the regional level can be easily handled to find adulteration in olive oil. The chemical parameters include sterol composition, fatty acid methyl esters, triacylglycerols, elemental analysis, and isotope ratio of $\delta C^{13}/C^{12}$. After all the analyses were completed, principal component analysis (PCA) and cluster analysis have been applied to the data set for authentication and adulteration to show significant variables in overall data.

The findings of the study will provide a reliable way for the varietal authenticity of Turkish olive oil according to their sterol compositions,

triacylglycerols, fatty acid methyl esters, elemental analysis and isotope ratio of $\delta C^{13}/C^{12}$ profiles as the geographical indicators. Therefore, this systematic method and obtained data can be used in PDO or PGI labelling of Turkish olive oils.

1.7 Fundamentals of Analytical Methods

1.7.1 Chromatographic methods

The GC Instrument

The sample is injected into the inlet where it is volatilised and splitless or split proportion is carried onto the column by the carrier gas. The sample components are separated according to column properties. The separated sample components elute from the column into the detector where some physico-chemical parameter is detected and signal produced. The signal is then amplified and sent to the data system where the chromatogram is electronically constructed.

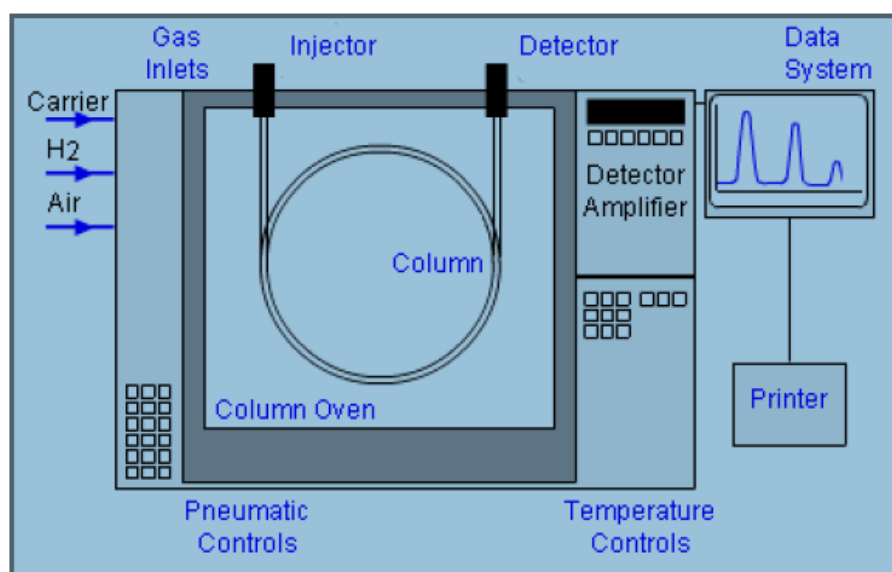


Figure 1.8. The GC instrument process (LC/GC's Chromacademy).

Gas chromatography uses a gaseous mobile phase to transport sample components through column which is containing stationary phase. There are several important detector characteristics. Dedector was chosen according to

physic-chemical properties of analyte because the detector responds the physico-chemical property of the analytes amplifies this response and generates an electronic signal for the data system for the produce a chromatogram.

A GC detector was been sensitivity (the signal output per unit concentration or mass of analyte), minimum detectability (S/N ratio) and can reliable linearity (for quantitative measurements).

Sensitivity: The detector sensitivity is equal to the output per unit concentration (for concentration sensitive detectors) or per unit mass (for mass flow sensitive detectors), of the analyte in the carrier gas.

Minimum detectability: This is the minimum concentration (or mass) of analyte that gives rise to a detector signal that can be clearly discerned from the noise. The term may also be seen written as Minimum DetecQuantity (MDQ) or Limit of Detection (LOD. A related term is the limit of quantitation (LOQ) which corresponds to the minimum analyte concentration that can be accurately and reproducibly detected for quantitative analysis.

Linear range: The instrument response curve shows an opposite behavior in relation to deviation from the linearity at high analyte concentration. In fact most detectors will deviate from linearity in this way and it is useful to establish this limit and range.

The flame ionisation detector (FID): This detector is the most widely used GC detector and was invented specifically for GC applications. It's high sensitivity and linear range for carbon-containing compounds make it very popular in organic analysis. A typical FID design is shown in Figure 1.9. The column effluent is mixed with hydrogen and a make-up gas (for capillary systems) before exiting via a small orifice (jet-tip) which is surrounded by a high flow of air. The Hydrogen is combustibile in air and can be lit via a remote glow plug.

The HPLC Instrument

HPLC was derived from classical column chromatography and has found an important place in analytical techniques. The major advancement in HPLC was found by the use of efficient separators used small particles high pumping pressures. The main components of an HPLC system are a high pressure pump, a column an injector system as well as detector.

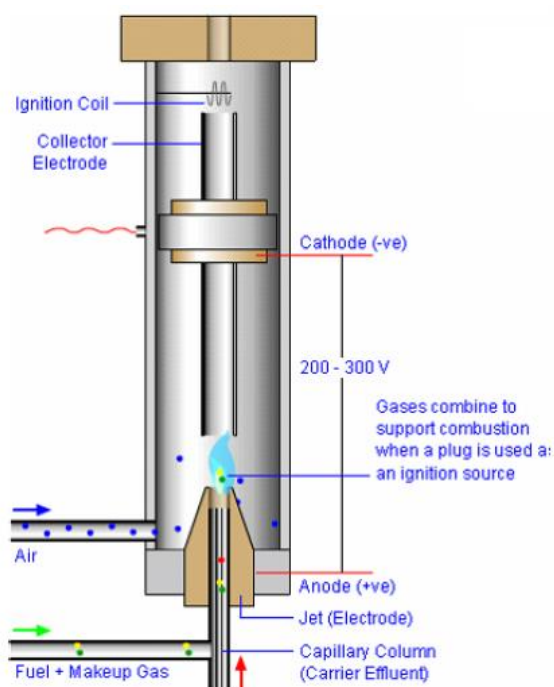


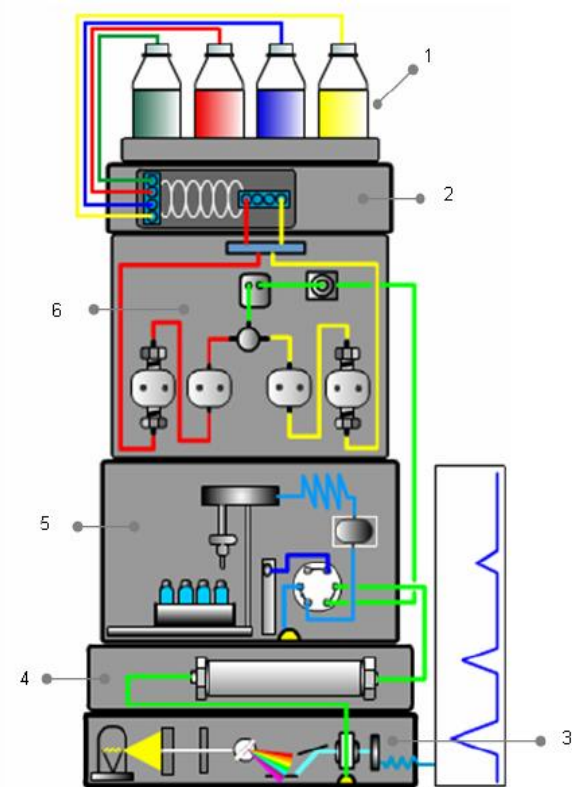
Figure 1.9. The overall view of FID detector showing the burning mechanisms occurred within the cell under flowing H gas. (LC/GC 's Chromacademy)

The system works as follows: eluent is filtered and pumped through a chromatographic column, the sample is loaded and injected onto the column and the effluent is monitored using a detector and recorded as peaks.

Columns are the main part of the chromatographic instruments. Heavy-wall glass, stainless steel and plastic are among materials that can withstand high pressures and are thus used construct HPLC columns. They must also be able to resist the chemical action of the mobile phase.

Usually a short guard column is placed in front of the analytical column. This serves to increase the life of the analytical column by removing particulate matter and contaminants from the solvents. Several detectors are coupled with HPLC system.

Refractive Index Detectors: The refractive index of a medium is the ratio of the speed of light in a vacuum to the speed in the medium. These detectors measure the change in refractive index in the eluent as the solute passes through the sample cell. This method of detection is less sensitive than UV detection although non-chromatographic compounds can be measured directly without derivitisation.



- 1) The mobile phase composition needs to be optimised in order to gain good separation,
- 2) Degassers are often used to remove air from the mobile phase –leading to better chromatographic baselines,
- 3) The detector conditions are chosen to give the best response to the analytes of interest and achieving good sensitivity,
- 4) The column dimensions and stationary phase chemistry are chosen and optimised to give separations of the quality required,
- 5) The autosampler introduces a plug of sample into the mobile phase flow which is then swept onto the column,
- 6) Dual reciprocating pumps are used to deliver the mobile phase at back pressures of up to 400 Bar. As steady stream of liquid delivered at constant flow rate is important.

Figure 1.10. HPLC System (LC/GC 's Chromacademy).

1.7.2 EA-IRMS instrument

Charged atoms and molecules are separated in a mass spectrometer on the basis of their mass-to-charge ratio (m/z): mass number (m) of an ion and total charge (z). The mass number is the sum of the total number of protons and neutrons in an atom, molecule or ion. The relative abundance of the ions is then determined by measuring the currents produced by the spatially separated ion beams.

The modern Nier-type mass spectrometer has three basic components an ion source, a mass analyser and an ion detector (collector) system. The solid or liquid sample is weighed into a small tin capsule and loaded into the Solid AutoSampler. When the AutoSampler is triggered, the sample drops into the combustion reactor that is held at 1020 - 1100°C depending on the elemental species of interest.

The sample and capsule melt in an atmosphere temporarily enriched with oxygen, where the tin promotes flash combustion. The combustion products are carried through an oxidation catalyst of CrO_3 by a constant flow of helium.

The oxidation products are then passed through a reduction reactor at 650°C containing copper granules, where oxides of nitrogen (NO , N_2O & N_2O_2) are reduced to N_2 and excess oxygen is removed. The resulting gas species (CO_2 , N_2 , SO_2 , H_2O) are then passed through a magnesium perchlorate filter to remove water. The remaining CO_2 , N_2 (if N was present in the original sample) and SO_2 (if S was present in the original sample) then pass through a short chromatographic column where they are time-separated. They then pass through a Thermal Conductivity Detector, and out of the vent on the top of the instrument.

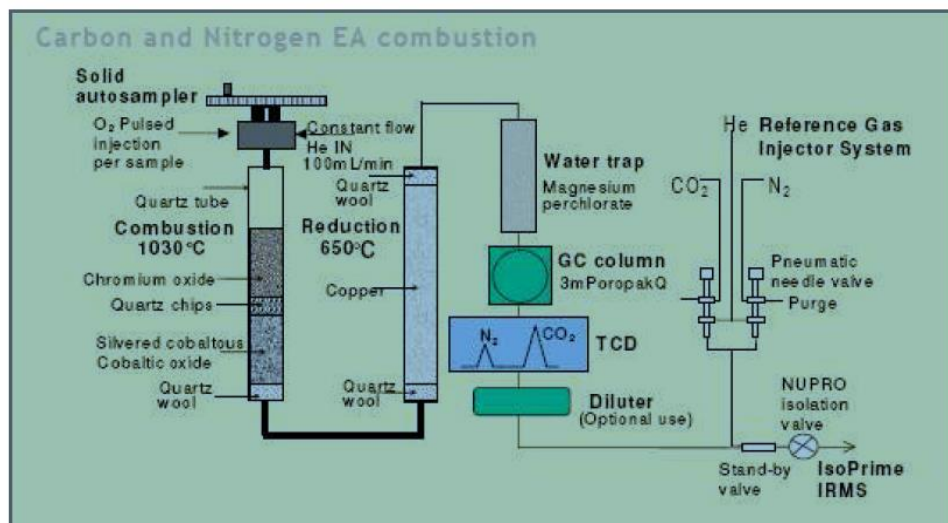


Figure 1.11. Carbon and Nitrogen EA Combustion (Euro Vector-Micromass)

When used with the IsoPrime, gas samples, typically H₂, N₂, CO, CO₂ and SO₂ are introduced into the source through a narrow capillary. The gas molecules then undergo ionisation, as a result of impact with a stream of electrons generated by a thorium coated tungsten filament 70 eV.

Excess energy transferred to the newly formed positively charged ion can cause bond dissociation (fragmentation or cracking). After ionisation of the sample gas, the positively charged ions are accelerated and collimated (ions moving in a parallel path) by a series of electrode 'lenses' to which variable potentials may be applied.

At this stage the ion beam is unresolved and enters the magnetic sector of the flight tube, which is designed to provide low-mass resolution of 1 atomic mass unit (amu) with an average resolution between any two peaks of 10% relative to the peak height.

The Field generated by the permanent or electromagnet deflects the ions into circular trajectories with radii proportional to the mass of the respective isotope. The resolved beams then continue along the flight tube to the collector assembly. The ion beam collides with the inside walls of a metal box (Faraday cup) and all secondary ion emission is suppressed.

An accurate measure of the charge deposited by the ion beam is obtained via an electron current that flows through resistors of 10^9 - $10^{12} \Omega$ depending on the relative intensity of the ion beams.

The ion beam defining slits are wider than the ion beams, resulting in the characteristic flat top peaks that are insensitive to drift, which may result from temperature variation affecting the geometry of the flight tube and electronic components used in mass selection. The overall result is stable, well-defined beams that can be utilised for precise measurement of the relative abundance of the isotopomers.

1.7.3 ICP MS instrument

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an analytical technique used for elemental determinations. ICP-MS has many advantages over other elemental analysis techniques. The ability to handle both simple and complex matrices with a minimum of matrix interferences due to the high-temperature of the ICP source, superior detection capability than ICP-AES with the same sample throughput and finally, the ability to obtain isotopic information.

An ICP-MS combines a high-temperature ICP (Inductively Coupled Plasma) source with a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. The ICP-MS instrument employs plasma as the ionization source and a mass spectrometer analyzer to detect the ions produced.

It can simultaneously measure most elements in the periodic and determine analyte concentration down to the sub nanogram-per-liter (ng/L) or part-per trillion (ppt) levels. It can perform qualitative, semi quantitative, and quantitative analysis, and since it employs a mass analyzer, it can also measure isotopic ratios. Figure 1.12 shows the main components of the ICP-MS system.

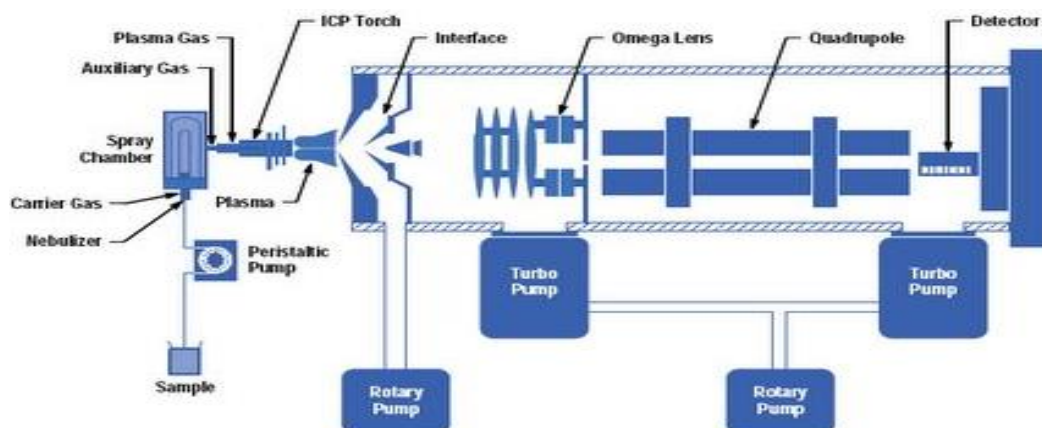


Figure 1.12. ICP-MS System (Agilent System)

The first step in analysis is the introduction of the sample. The most common method is the use of a nebulizer. This is a device which converts liquids into an aerosol, and that aerosol can then be swept into the plasma to create the ions. Nebulizers work best with simple liquid samples.

Many varieties of nebulizers have been coupled to ICP-MS, including pneumatic, cross-flow, Babington, ultrasonic, and desolvating types. The aerosol generated is often treated to limit it to only smallest droplets, commonly by means of a double pass or cyclonic spray chamber. Use of auto samplers makes this easier and faster.

The carrier gas (usually argon) is sent through the central channel and into the very hot plasma. The sample is then exposed to radio frequency which converts the gas into the plasma. The high temperature of the plasma is sufficient to cause a very large portion of the sample to form ions. This fraction of ionization can approach 100% for some elements but this is dependent on the ionization potential.

A fraction of the formed ions passes through a $\sim 1\text{mm}$ hole (sampler cone) and then a $\sim 0.4\text{mm}$ hole (skimmer cone). The purpose of which is to allow a vacuum that is required by the mass spectrometer. The first stage is usually based on a roughing pump, most commonly a standard rotary vane pump. This removes most of the gas and typically reaches a pressure of around 133 Pa by using turbomolecular pumps.

Before mass separation, a beam of positive ions has to be extracted from the plasma and focused into the mass-analyzer. It is important to separate the ions from UV photons, energetic neutrals and from any solid particles that may have been carried into the instrument from the ICP. Traditionally, ICP-MS instruments have used transmitting ion lens arrangements for this purpose the Einzel lens, the Barrel lens and Omega Lens can used in analysis.

Another approach is to use ion guides (quadrupoles, hexapoles, or octopoles) to guide the ions into mass analyzer along a path away from the trajectory of photons or neutral particles. The primary role of the ion lenses is to transfer and focus the ions efficiently into the mass filter. Recently, a compound or multi lens ion optic, for efficient ion focusing across, was introduced into the mass spectrometer in the sampling/scimming process. If these reach the detector, the background noise will increase, and detection limits will suffer. Off-axis “omega” lens on the instrument eliminates neutral species, ensuring very low backgrounds, enabling sub-ppt detection limits for most elements.

The Octopole Reaction System (ORS) features an off-axis reaction cell, which effectively removes spectral interferences in most complex sample matrices. The ORS-ICP-MS has been designed specifically to handle high matrices and elements suffering from significant Ar-based (plasma based) interferences such as Fe, Se, As etc. The ORS consists of an octopole ion guide, mounted off-axis to minimize random background levels, inside a cell that can be pressurized with a reaction gas (usually H₂ or He). Difficult polyatomic interferences such as Ar₂, ArCl and MAr are dissociated by collisions with the reaction gas within the cell, enabling otherwise interfering analytes to be determined.

Because the ORS employs simple reaction gases, side reactions that would create new, unpredictable interferences are eliminated, and the ORS can be operated in passive mode without a mass-filter. The plasma used in an ICP-MS is made by ionizing argon ($\text{Ar} \rightarrow \text{Ar}^+ + \text{e}^-$) gas. The energy required for this reaction is obtained by pulsing an electrical current in wires that surround the argon gas. After injecting the sample, the plasma's extreme temperature causes the sample to separate into individual atoms (atomization). Next, the plasma ionizes these atoms ($\text{M} \rightarrow \text{M}^+ + \text{e}^-$) so that they can be detected by the mass spectrometer.

An inductively coupled plasma (ICP) is sustained in a torch that consists of three concentric tubes, usually made of quartz. The end of this torch is placed inside an induction coil supplied with a radio-frequency electric current. A flow of argon gas (usually 14 to 18 L/min) is introduced between the two outermost tubes of the torch and an electrical spark is applied for a short time to introduce free electrons into the gas stream. These electrons interact with the radio-frequency magnetic field of the induction coil and are accelerated first in one direction, then 14 the other, as the field changes at high frequency (usually 27.12 million cycles per second).

The accelerated electrons collide with argon atoms, and sometimes a collision causes an argon atom to part with one of its electrons. The released electron is in turn accelerated by the rapidly-changing magnetic field. The process continues until the rate of release of new electrons in collisions is balanced by the rate of recombination of electrons with argon ions (atoms that have lost an electron). This produces a ‘fireball’ that consists mostly of argon atoms with a rather small fraction of free electrons and argon ions.

Making the plasma from argon has several advantages. First, argon is abundant and therefore cheaper than other noble gases. Argon also has a higher first ionization potential than all other elements except He, F, and Ne. Because of this high ionization energy, the reaction ($\text{Ar}^+ + \text{e}^- \rightarrow \text{Ar}$) is more energetically favorable than the reaction ($\text{M}^+ + \text{e}^- \rightarrow \text{M}$). This ensures that the sample remains ionized (as M^+) so that the mass spectrometer can detect it.

Argon can be purchased for use with the ICP-MS in either a refrigerated liquid or a gas form. However it is important to note that whichever form of argon purchased, it should have a guaranteed purity of 99.9% Argon at a minimum.

The ICP-MS allows determination of elements with atomic mass ranges 7 to 250. This encompasses Li to U. Some masses are prohibited such as 40 due to the abundance of argon in the sample. Other blocked regions may include mass 80 (due to the argon dimer), and mass 56 (due to ArO), the latter of which greatly hinders Fe analysis unless the instrumentation is fitted with a reaction chamber.

A typical ICP-MS will be able to detect in the region of ng/L to 10 or 100 mg/L or around 8 orders of magnitude of concentration units. ICP-MS and ICP-AES are powerful techniques because of regardless of the type of matrix, analysis of the samples most frequently encountered in food science can be considered in a number of discrete stages, namely sample collection and storage, preparation, pre-treatment, quantification, quality control and reporting.

1.7.4 Microwave digestion

Microwave oven was supplied to significant improvement in chemical reaction rates that are possible using microwave radiation. The aim of all digestion methods is to transfer the element(s) of interest into the final solution quantitatively and efficiently, preferably with total decomposition of the bulk matrix and removal of potentially interfering species.

As a rule, acid digestion procedures are employed for the determination of elements in solids subsequent to sampling and mechanical sample preparation in order to completely transfer the analytes into solution so that they can be introduced into the determination step in liquid form. A typical microwave acid digestion can be completed in a matter of minutes, whereas the same conventional hot plate digestion can take hours. Microwave digestion usually involves placing a sample in an acid solution and heating to high temperatures and pressures. These extreme conditions will dissolve most materials, but is potentially hazardous.

The goals of microwave digestion techniques are; complete solution of the elements, complete decomposition of the matrix, avoiding losses and contamination, reduction of handling and process times.

Liquids heat by two mechanism: dipole rotation and ionic conduction. Polar molecules will tend to align their dipole moments with the microwave electric field. Because the field is changing constantly, the molecules are rotated back and forth, which causes them collide with other nearby molecules. Ions in solution will tend to migrate in the presence of a microwave electric field. This migration causes the ions to collide with other molecules. Heat is generated when molecules or ions collide with nearby molecules or ions.

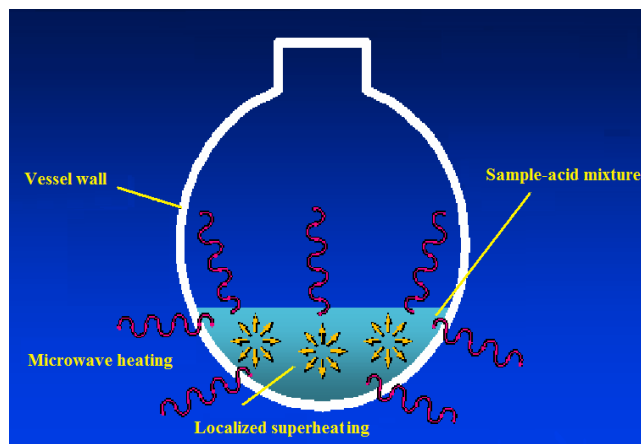


Figure 1.13. Heating Mechanism for Trace element.

Common to both methods is the temperature limitation as a consequence of the solution's boiling point and the risk of contaminants from the air. Volatile elements such as mercury may be lost during the digestion times of, typically 2-15 hours. The temperature limitation can be overcome by working with a closed pressure vessel. This rise in pressure results in a dramatic increase in the reaction kinetics allowing acid digestions to be carried out in a matter of hours or, if microwave heating is employed, in 20-40 minutes.

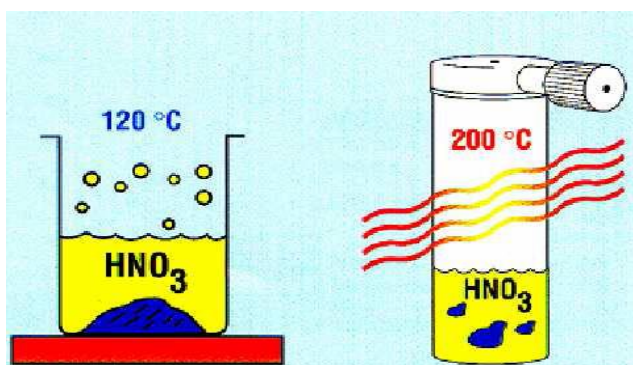


Figure 1.14. a) Hot plate, b) Closed vessel microwave digestion

The advantage of closed procedure in comparison with open digestion in a recycling device or with the traditional hot plate lies in the significantly higher working temperatures which can be achieved. In Figure 1.14, it was shown differences between open and closed digestion operating temperature. Closed digestion system was dramatically increase in the reaction kinetics, allowing digestions to be carried out in hours.

1.8 Chemometrics

The chemical discipline that uses mathematical and statistical methods, (a) to design or select optimal measurement procedures and experiments, and (b) to extract maximum amount of information when analysing multivariate (chemical) data. Multivariate data has traditionally been analyzed using one or two variables at a time. However, to discover the relationships among all samples and variables efficiently, we must process all of the data simultaneously. Chemometrics is the field of extracting information from multivariate chemical data using tools of statistics and mathematics. Chemometrics is typically used for one or more of three primary purposes:

- To explore patterns of association in data;
- To track properties of materials on a continuous basis; and
- To prepare and use multivariate classification models.

The algorithms in primary use in the field have demonstrated a significant capacity for analyzing and modeling a wide assortment of data types for an even more diverse set of applications.

1.8.1 Principle components analysis (PCA)

PCA is probably the most widespread multivariate statistical technique used in chemometrics, and because of the importance of multivariate measurements in chemistry. Principal components are linear combinations of the original variables calculated with the maximum variance criterion. Principal components are centered, uncorrelated, and ordered from largest to smallest variance. The goal of principal components analysis is to explain the maximum amount of variance with the fewest number of principal components.

The principal components are the linear combinations of the original variables that account for the variance in the data. The maximum number of components extracted always equals the number of variables. PCA is one of several multivariate methods that allow us to explore patterns in these data, similar to exploring patterns in psychometric data. Which compounds behave similarly? Which people belong to a similar group?

How can this behaviour be predicted from available information? In many cases the identities and amounts of unknowns may not be available in advance. The aims of PCA are to determine underlying information from multivariate raw data. The characteristics of each PC are usually the **scores** relating to the objects or samples and the **loadings** relating to the variables or measurements.

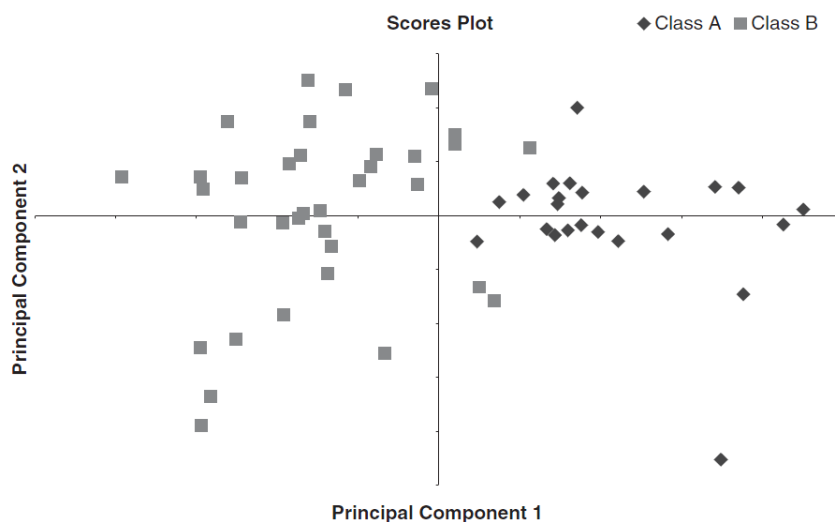


Figure 1.15. Principal Component scores plot for elemental composition of pots in two different groups (A and B)

1.8.2 Graphical representation of scores and loadings

Many modern multivariate statisticians think geometrically as much as numerically, and concepts such as PCs are often treated as much as objects in an imaginary space than mathematical entities. The algebra of multidimensional space is the same as that of multivariate statistics. However, now it is possible to obtain a large number of graphs rapidly using simple software. There are many ways of visualizing PCs and this section will illustrate some of the most common.

Case study I: The aim is to try to obtain the individual profiles of each compound in the chromatogram, and also their spectra. The raw data are a matrix whose *columns* relate to wavelengths and whose *rows* relate to elution time.

Case study II: The aims are to see which elements group together and also which physical constants are responsible for this grouping. Because all the

physical constants are on different scales, it is first necessary to standardize the data prior to performing PCA.

There are a number of important features of scores and loadings. It is important to recognise that the aim of PCA involves finding mathematical functions which contain certain properties which can then be related to chemical factors, and in themselves PCs are simply abstract mathematical entities.

1.8.3 Score plots

The score plot graphs the second principal component scores versus the first principal component scores. Scores plots can be used to answer many different questions about the relationship between objects and more examples. The PC plot for case study 1. Is the horizontal axis is the scores for the first PC, and the vertical axis for the second PC? This 'picture' can be interpreted as follows:

- The linear regions of the graph represent regions of the chromatogram where there are pure compounds, I and II.
- The curve portion represents a region of coelution.
- The closer to the origin, the lower the intensity.

How can these graphs help?

- The pure regions can inform us of the spectra of the pure compounds.
- The shape of the PC plot informs us of the amount of overlap and quality of chromatography.
- The number of bends in a PC plot can provide information about the number of different compounds in a complex multipeak cluster.

1.8.4 Loading plots

The loading plot provides information about the loadings of the first two principal components exactly the same principles apply in that the value of the loadings at one PC can be plotted against that at the other PC.

It is not, however, only the scores that are of interest but sometimes the loadings. Exactly the same principles apply in that the value of the loadings at one PC can be plotted against that at the other PC.

Loadings plots can be used to answer a lot of questions about the data, and are a very flexible facility available in almost all chemometrics software.

1.8.5 Scree plot and eigenvalues

Normally after PCA, the **size** of each component can be measured. This is often called an **eigenvalue**: the earlier (and more significant) the components, the larger their size. Using the size of eigenvalues we can try to estimate the number of significant components in the dataset. Scree plot where the smooth decrease of eigenvalues levels off.

The scree, or eigenvalue, plot provides one method for determining the number of principal components. The scree plot displays the component number versus the corresponding eigenvalue. The eigenvalues of the correlation matrix equal the variances of the principal components; therefore, choose the number of components based on the size of the eigenvalues.

1.8.6 Biplot graph

The biplot overlays the score and loading plots. Use the biplot to assess the data structure and loadings on one graph. The second principal component scores are plotted versus the first principal component scores. The loadings for these two principal components are plotted on the same graph. Note that if the scores are mean centered, the denominator equals the variance.

1.8.7 Cluster analysis

Cluster term was defined as distinct group of objects that are more similar to each other than to objects outside of the group. Cluster Analysis is a generic term for a wide range of numerical methods with the common goal of uncovering or discovering groups or clusters of observations that are homogeneous and separated from other groups.

Hierarchical classifications may be represented by a two dimensional diagram known as dendrogram, which illustrates the fusions made at each stage of the analysis. The dendrogram displays the groups formed by clustering of observations and their similarity levels. The cluster procedure classifies variables into groups when the groups are initially not known. The primary reason to cluster variables is to reduce the number of variables. Cluster observations and cluster variables use a hierarchical clustering procedure.

Cluster analysis divides data into groups (clusters) that are meaningful, useful, or both. If meaningful groups are the goal, then the clusters should capture the natural structure of the data. Cluster analysis has played an important role in a wide variety of fields. There have been many applications of cluster analysis to practical problems.

Exploratory data analysis such as PCA is used primarily to determine general relationships between data. Sometimes more complex questions need to be answered, such as, do the samples fall into groups?

Similarity:

The first step is to determine the similarity between objects. Four of the most popular ways of determining how similar objects are to each other are as follows.

1. **Correlation coefficient** between samples. A correlation coefficient of 1 implies that samples have identical characteristics, which all objects have with themselves. Some workers use the square or absolute value of a correlation coefficient, and it depends on the precise physical interpretation as to whether negative correlation coefficients simply similarity or dissimilarity.

2. **Euclidean distance:** The distance between two samples samples k and l is defined by

$$d_{kl} = \sqrt{\sum_{j=1}^J (x_{kj} - x_{lj})^2}$$

where there are j measurements and x_{ij} is the j th measurement on sample i .

The smaller is this value, the more similar are the samples, so this distance measure works in an opposite manner to the correlation coefficient and strictly is a dissimilarity measure. Although correlation coefficients vary between -1 and $+1$, this is not true for the Euclidean distance, which has no limit, although it is always positive. Sometimes the equation is presented in matrix format:

$$d_{kl} = \sqrt{(x_k - x_l) \cdot (x_k - x_l)'}$$

3. **Manhattan distance:** This is defined slightly differently to the Euclidean distance and is given by

$$d_{kl} = \sum_{j=1}^J |x_{kj} - x_{lj}|$$

Manhattan distance will always be greater than (or in exceptional cases equal to) the Euclidean distance.

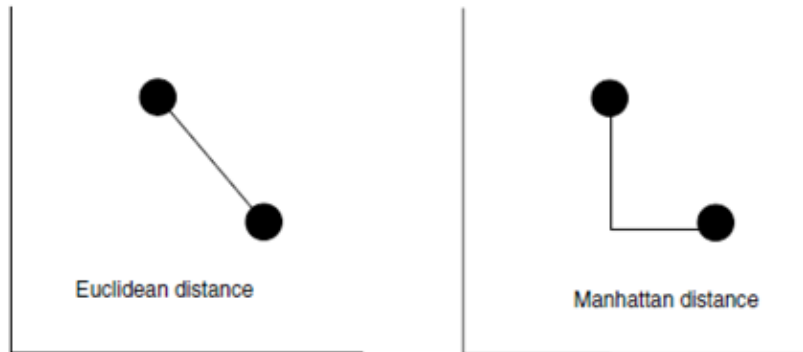


Figure 1.16. Euclidean and Manhattan distance

4. **Mahalanobis distance:** This method is popular with many chemometricians and, whilst superficially similar to the Euclidean distance, it takes into account that some variables may be correlated and so measure more or less the same properties.

The distance between objects k and l is best defined in matrix terms by

$$d_{kl} = \sqrt{(x_k - x_l) \cdot C^{-1} \cdot (x_k - x_l)'}$$

where C is the variance–covariance matrix of the variables, a matrix symmetric about the diagonal, whose elements represent the covariance between any two variables, of dimensions $J \times J$.

Linkage

The next step is to link the objects. The most common approach is called *agglomerative* clustering whereby single objects are gradually connected to each other in groups. Any similarity measure can be used in the first step, but for simplicity¹. From the raw data, find the two objects that are most similar (closest together). The main task is to recalculate the numerical similarity values between the new group and the remaining objects. The linkage method that you choose determines how the distance between two clusters is defined. At each amalgamation stage, the two closest clusters are joined. There are three principal ways of doing this.

a) **Nearest neighbor:** The similarity of the new group from all other groups is given by the highest similarity of either of the original objects to each other object.

b) **Furthest neighbor:** This is the opposite to nearest neighbour, and the *lowest* similarity is used. Note that the furthest neighbour method of linkage refers only to the calculation of similarity measures after new groups are formed, and the two groups (or objects) with highest similarity are always joined first.

c) **Average linkage:** The average similarity is used. There are, in fact, two different ways of doing this, according to the size of each group being joined together. Where they are of equal size (e.g. each consists of one object), both methods are equivalent. The two different ways are as follows.

- **Unweighted:** If group A consists of N_A objects and group B of N_B objects, the new similarity measure is given by

$$s_{AB} = (N_A s_A + N_B s_B) / (N_A + N_B)$$

- **Weighted:** The new similarity measure is given by

$$s_{AB} = (s_A + s_B) / 2$$

The terminology indicates that for the unweighted method, the new similarity measure takes into consideration the number of objects in a group, the conventional terminology possibly being the opposite to what is expected. For the first link, each method provides identical results.

There are numerous further linkage methods, but it would be rare that a chemist needs to use too many combinations of similarity and linkage methods, however, a good rule of thumb is to check the result of using a combination of approaches.

Ward: With Ward's linkage method, the distance between two clusters is the sum of squared deviations from points to centroids. The goal of Ward's linkage method is to minimize the within-cluster sum of squares. It tends to produce clusters with similar numbers of observations, but it is sensitive to outliers.

In Ward's linkage method, it is possible for the distance between two clusters to be larger than $d_{\max}$, the maximum value in the original distance matrix. If this occurs, the similarity will be negative.

Dendrograms

Often the result of hierarchical clustering is presented in the form of a dendrogram (sometimes called a 'tree diagram'). The objects are organised in a row, according to their similarities: the vertical axis represents the similarity measure at which each successive object joins a group. Using nearest neighbour linkage and correlation coefficients for similarities are shown in the Figure 1.17.

A good approach is to perform several different methods of cluster analysis and compare the results. If similar clusters are obtained, no matter which method is employed, we can rely on the results. Similarity level is measured along the vertical axis (you can also display the distance level), and the different observations are listed along the horizontal axis. The graph shows the way in which the clusters were formed— either by joining two individual observations, or pairing an individual observation with an existing cluster. As we can see in Figure 1.17, what similarity levels the clusters are formed, and the composition of the clusters of the final grouping of variables into clusters. The dendrogram displays the groups formed by clustering of variables, and their similarity levels.

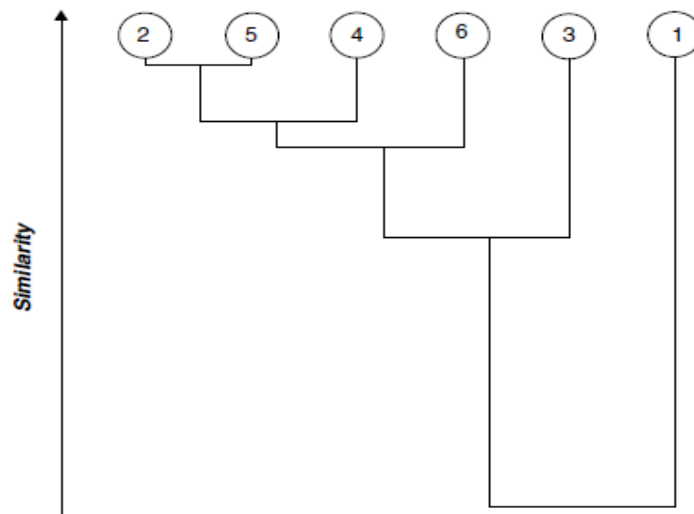


Figure 1.17. Dendrogram for cluster analysis example (Brereton, 2003).

2. EXPERIMENTAL

2.1 Oil Samples

Olive oils samples which belong to 49 different locations of Aegean and Marmara region of Turkey, and they were obtained from the different olive oil producer for 2010/2011 harvest year. 9 olive oils samples from Bursa (B), 11 samples from Edremit Bay (K), 8 samples from İzmir (I), 7 samples from Aydın (A), 6 samples from Muğla (ML) and 8 samples from Manisa (MS) could be seen in Table 2.1. The geographic regions are shown in a Turkey map in Figure 2.1. There were different variety of olive in Turkey correspondingly among these olive oil varieties Ayvalık, Gemlik were characteristic of Edremit Bay and Bursa respectively. Memecik and Uslu could be variety of Aydın and Muğla region. İzmir and Manisa has mixture of these varieties.



Figure 2.1. Geographic regions of olive produced in Turkey.

Table 2.1. Olive oil samples from north and south parts of Aegean Region and Marmara of Turkey belonging to 2010/2011 harvest years

Sample No	Geographic Origin	Sample Code	Sample No	Geographic Origin	Sample Code	Sample No	Geographic Origin	Sample Code
1	Altınoluk Adatepe	K1	17	Tilkiköy, saruhanlı	MS6	33	İsabeyli	A5
2	Altınoluk Doyran	K2	18	Salihli	MS7	34	Karacasu	A6
3	Altınoluk Avcılar	K3	19	Salihli	MS8	35	Bozdoğan	A7
4	Altınoluk Narlı	K4	20	Çakırlı Bursa	B1	36	Akkaya	ML1
5	Altınoluk Tahtakuşlar	K5	21	Çeltikçi	B2	37	Milas	ML2
6	Edremit Camcı	K6	22	Dutluca	B3	38	Fethiye	ML3
7	Edremit Çıkırıkçı	K7	23	Keramet	B4	39	Göcek	ML4
8	Edremit Dereli	K8	24	Orhangazi	B5	40	Milas	ML5
9	Edremit Güre Yassicalı	K9	25	Paşapınar	B6	41	Akkaya	ML6
10	Edremit Kızılçukur	K10	26	Sölöz	B7	42	Zeytinköy	I1
11	Edremit Sekveren Çamdibi	K11	27	Yeni Sölöz Bursa	B8	43	Selçuk	I2
12	Muradiye	MS1	28	Yeniköy	B9	44	Şirince	I3
13	Apak, Saruhanlı	MS2	29	Halaçlar, Çine	A1	45	Zeytinköy	I4
14	Muradiye	MS3	30	Sinekçiler, Nazilli	A2	46	Seferihisar	I5
15	Sarıçam	MS4	31	Saylar, Nazilli	A3	47	Tire	I6
16	Yağköy, Akhisar	MS5	32	Söke	A4	48	Bornova	I7
						49	Bornova	I8

2.2 Reagents

The reagents were used either analytical or chromatographic grade. Millipore ultrapure water (Type I) was used for all analysis. Potassium hydroxide, methanol, ethyl ether, anhydrous sodium sulphate, ethanol, acetone, hexane, ethyl ether, 2,2,4-trimethylpentane (isooctane), toluene, chloroform and α -cholestanol, 2,7-dichlorofluorescein, anhydrous pyridine were of analytical grade. BSTFA+TMCS (99:1, v/v) mixture was used as the derivatization agent for sterol analysis.

TLC plates coated with silica gel, without fluorescence indicator, thickness 0.25 mm were used. Carrier and Auxiliary gases: hydrogen, helium, air, chromatographic purity.

Nitric acid, Hydrochloric acid and standard solution of Fe, K, Ca, Na, Mg, Ag, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Tl, V, Zn were used in ICP studies. Li, Sc, Y, Ge standards were used as the internal standard mix. In IRMS measurements, the sample was weighed in standard tin capsules.

2.3 Apparatus

For sterol analysis; flask fitted with a reflux condenser having ground-glass joints, 500 ml separating funnel, 250 ml flasks, 100 μ l and 500 μ l microsyringes and rotary evaporator was used. The determination of sterol band on TLC plates (20 x 20 cm) was accomplished by using ultraviolet lamp having a wavelength of 366 or 254 nm.

In ICP analysis, Agilent 7500ce ICP-MS systems was used. The sample digestion was made by using CEM Mars 5 Microwave digestion system. Sterol and fatty acid methyl ester analysis were accomplished by using Thermo scientific GC-FID and Agilent 6890 GC-FID systems.

Agilent 1100 HPLC system with Refractive Index (RID) Agilent 1100 G1362A was used for triacylglycerols. EUROVECTOR Elemental Analysis (EA 8202) with Micromass Δ soprime (JB068) systems was used for isotop ratio analysis.

2.3 Procedures

2.3.1 Sterol analysis

Determination of sterol compositions method describes a procedure for determining the individual and total sterols content of fatty substances. α -cholestanol was used as a internal standard. The fatty substance was saponified with potassium hydroxide in ethanolic solution. The unsaponifiables are then extracted with ethyl ether (liquid-liquid extraction) (COI/T.20/Doc. No.10/ Rev.1 Determination of sterols composition and content by capillary column chromatography).

The sterol fraction is separated from the unsaponifiable extract by chromatography on TLC. The sterols recovered from the silica gel are transformed into trimethyl-silyl ethers with BSTFA+TMCS (99:1, v/v) and was analyzed by GC-FID systems.

Preparation of the unsaponifiables: Using the 500 μ l microsyringe introduce into flask a volume of 0.2% α -cholestanol solution in chloroform. Evaporate to dryness in a current of nitrogen and then weigh accurately 5 ± 0.0001 g into same flask.

Add 50 ml of 2 N ethanolic potassium hydroxide solution, fit the reflux condenser and heat to gentle boiling on a water bath until saponification takes place after saponification was formed added 50 ml of distilled water from the top of the condenser and cool the flask to room temperature. Transfer the contents of the flask into separating funnel add approximately three times with 80 ml of ethyl ether and then washing the ether solution with distilled water and finally was dried with anhydrous sodium sulphate, evaporate with rotary evaporator to dry, and then weigh after cooling in a desiccator.

Separation of the sterol fraction: Preparation of the basic plates: Immerse the silica gel plates completely in the 0.2 N ethanolic potassium hydroxide solution for 10 seconds, then allow to dry in a fume cupboard and finally place in a stove at 100 °C for one hour. Remove from the stove and keep in a desiccator.

Place the plate in the developing chamber prepared as specified in toluene-acetone mixture in the plate-developing chamber 95:5 (V/V). After removed the plate from the developing chamber and evaporate the solvent in a flow of hot air.

The plate was sprayed with the 2,7-dichlorofluorescein solution. After the plate is observed under ultraviolet light the sterol band and mark the limits of the band. On the silica gel in the marked area is scrape off with spatula. Added 10 ml of hot chloroform and residue wash with ethyl ether (10 ml each time), collecting the filtrate in the same flask attached to the funnel, evaporate the filtrate to dryness and then allow cooling in a desiccator prior to the weigh. The residue contained in the test tube consists of the sterol fraction.

Preparation of the trimethylsilyl ethers: The mixture of silylation reagent which is BSTFA+TMCS (99:1, v/v) and pyridine (1:1, v,v) was added in the ratio of 50 μ l for every milligram of sterols to the test tube containing the sterol fraction. After solution was completely dissolved then centrifuge for a few minutes. The clear solution was injected to gas chromatography system.

Gas chromatographic analysis: Instrument parameters of sterols as it can seen in Table 2.2 for sterol compositions of olive oils.

Table 2.2. Instrument parameters of GC-FID system for sterol.

Instrument	GC-FID System
Column	ZEBRON ZB-5ms Length: 30 m Internal Diameter: 0.25 mm Film thickness: 0.25 μ m
TemperatureProgramme	260 $\pm$ 5°C
Split ratio	1:50-1:100
Detector Oven temperature	290°C
Detector	FID
Carrier Gas	Hydrogen, 30 cm/sn.
Injection Volume	1 μ L

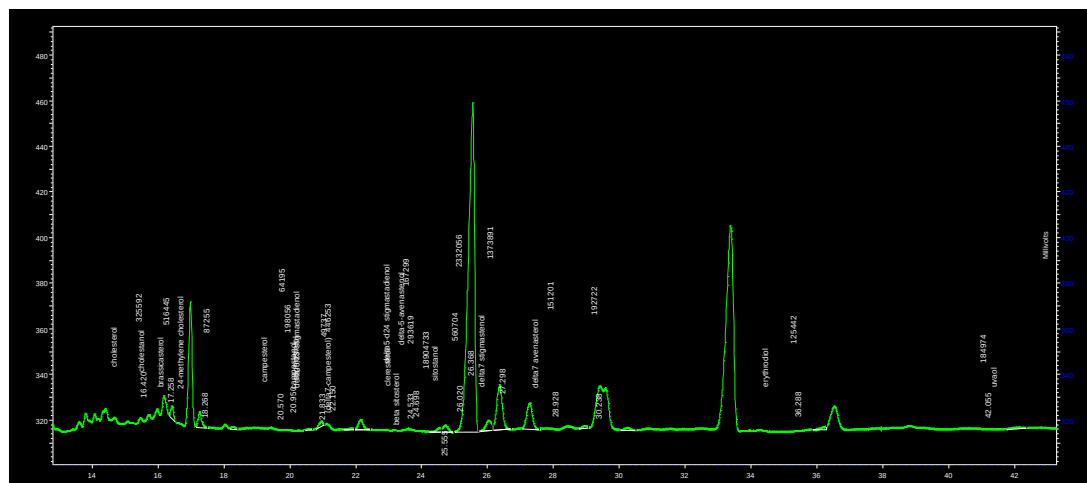


Figure 2.2. Sterol Chromatogram of olive oil.

While in Figure 2.2 are shown an example chromatogram for sterol of olive oil, in Table 2.3 was given Relative Retention Time of sterols according to betasitosterol.

Table 2.3. RRT of sterol in analytic methods

Peak	RRT	Peak	RRT
Cholesterol	0.65	$\Delta 5,23$ - stigmastadienol	0.94
α -Cholestanol	0.66	clerosterol	0.95
Brassicasterol	0.71	β -sistosterol	1.00
24- methylene cholesterol	0.80	sitostanol	1.01
campesterol	0.82	$\Delta 5$ -Avenasterol	1.02
campestanol	0.84	$\Delta 5,24$ -stigmastadienol	1.06
stigmasterol	0.86	$\Delta 7$ -stigmastenol	1.14
$\Delta 7$ - campesterol	0.91	$\Delta 7$ -avenasterol	1.19

Quantitative evaluation: Calculate the areas of the α -cholestanol and the sterol peaks using the manual peak integration. Any compound which is not included among those listed in 1 was ignored. Calculate the concentration of each individual sterol in mg/kg of fatty material as follows:

$$\text{sterol x} = \frac{A_x \cdot m_s \cdot 1000}{A_s \cdot m}$$

where:

A_x = peak area for sterol x, in square millimetres;

A_s = area of the α -cholestanol peak, in square millimetres;

m_s = mass of α -cholestanol added, in milligrams;

m = mass of the sample used for determination, in grams.

Expression of the results: Record individual sterol concentrations as mg/kg of fatty material and their sum as "total sterols"

Calculate the percentage of each individual sterol from the ratio of the relevant peak area to the total peak area for sterols.

$$\% \text{ of sterol x} = \frac{A_x}{\sum A} \cdot 1000$$

where:

A_x = peak area for x;

$\sum A$ = total peak area for sterols

2.3.2 Fatty acid methyl esters (FAME) analysis

The procedure includes the preparation of the fatty acid methyl esters from the fat, and analysis by capillary column gas chromatography. Identification of the fatty acids is made from their retention times. Quantification is carried out by determination of the ratio of the area under the relevant peak to the sum of the areas under all the peaks for the fatty acids.

Preparation of the methyl esters: Trans esterification was applied to oil for preparation of fatty acid methyl esters. This rapid method is applicable to olive oils. Methanolic potassium hydroxide was used trans-esterification for forming methyl esters in addition this methanolic potassium hydroxide act a reactive of saponification. After adding iso-octane this solution, it is mixed gently and than centrifuged. Upper phase was taken into vial for GC-FID analysis.

Gas chromatographic analysis: Table 2.4 are shown list of fatty acid methyl esters by using GC-FID. Trans fatty acids were listed on the too. Instrument parameters are shown in Table 2.5. (TS 4504 –TS EN ISO 15304).

Table 2.4. Fatty acid methyl esters in analytic methods.

No	Name	No	Name	No	Name
1	Caprylic Acid	13	Heptadecanoic Acid	25	Erucic Acid
2	Caproic Acid	14	Cis 10 Heptadecanoic Acid	26	Cis 11.14.17 Eicosatrienoic Acid
3	Capric Acid	15	Stearic Acid	27	Tricosanoic Acid
4	Undecanoic Acid	16	Oleic Acid	28	Arachidonic Acid
5	Lauric Acid	17	Linoleic Acid	29	Cis 13.16 Docosadienoic Acid
6	Tridecanoic Acid	18	Arachidic Acid	30	Lignoceric Acid
7	Myristic Acid	19	Gondoic Acid	31	Cis 5.8.11.14.17 Eicosapentaenoic Acid
8	Myristoleic Acid	20	Linolenic Acid	32	Nervonic Acid
9	Pentadecanoic Acid	21	Heneicosanoic Acid	33	Cis 4.7.10.13.16.19 Docosahexaenoic Acid
10	Cis 10 Pentadecanoic Acid	22	Cis 11,14 Eicosadienoic Acid	Trans Fatty Acids	
11	Palmitic Acid	23	Behenic Acid	1	Trans Oleic Acid
12	Palmiteloic Acid	24	Cis 8.11.14 Eicosatrienoic Acid	2	Trans Linoleic Acid
				3	Trans Linolenic Acid

Table 2.5. Instrument parameters of GC-FID system for fatty acid methyl esters

Instrument	GC-FID System
Column	SUPELCO SP TM-2560 Length: 100 m Internal diameter: 0.25 mm Film thickness: 0.20 µm
Oven Temperature Programme	Initial temp 140 °C, 4 °C/min to 240 °C, 5 min isothermal
Split ratio	1:100
Dedector temperature	260 °C
Dedector	FID
Carrier gas	Helium, 20 cm/sn.
Injection Volume	1 µL

Peak identification: The order in which the fatty acid methyl esters appear on the chromatogram is a direct function of their number of carbon atoms. The unsaturated esters are eluted after the corresponding saturated esters and their elution is a direct function of the number of double bonds. The *trans* fatty acid esters are eluted before the corresponding *cis* isomers.

The individual methyl esters are then identified by their retention times, which are compared with the retention times of the FAME mix reference standart solution. A specimen chromatograms are given in from Figure 2.3 to Figure 2.5. First chromatogram was shown all peaks.

Quantitative analysis: Calculate the areas of each peak using the electronic integrator. The percentage for each fatty acid is calculated from the ratio of the area under the corresponding peak to the sum of the areas under all the peaks using the formula:

$$100 \frac{A_x}{\sum A}$$

where:

A_x = peak area for fatty acid x and $\sum A$ = total of all the peak areas.

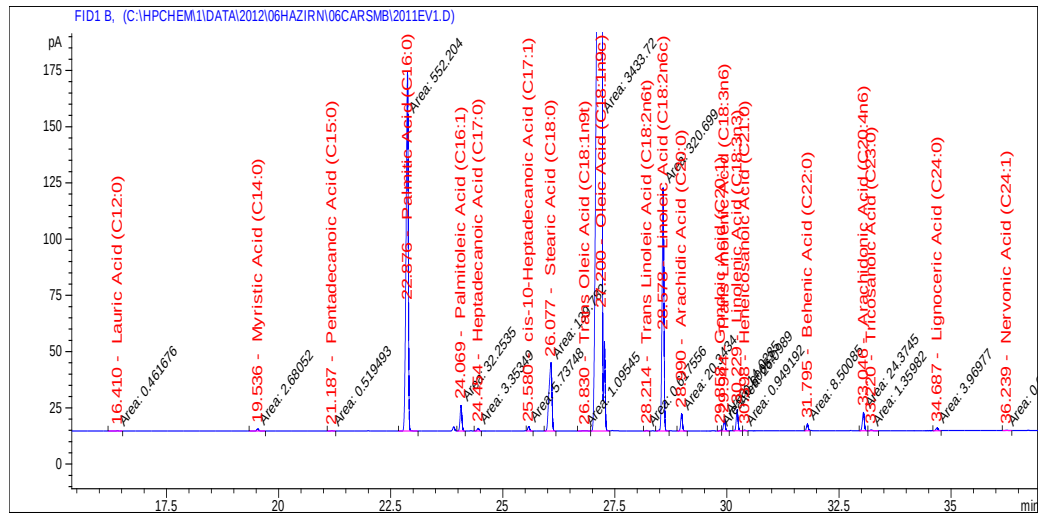


Figure 2.3. Fatty acid methyl esters chromatogram (1)

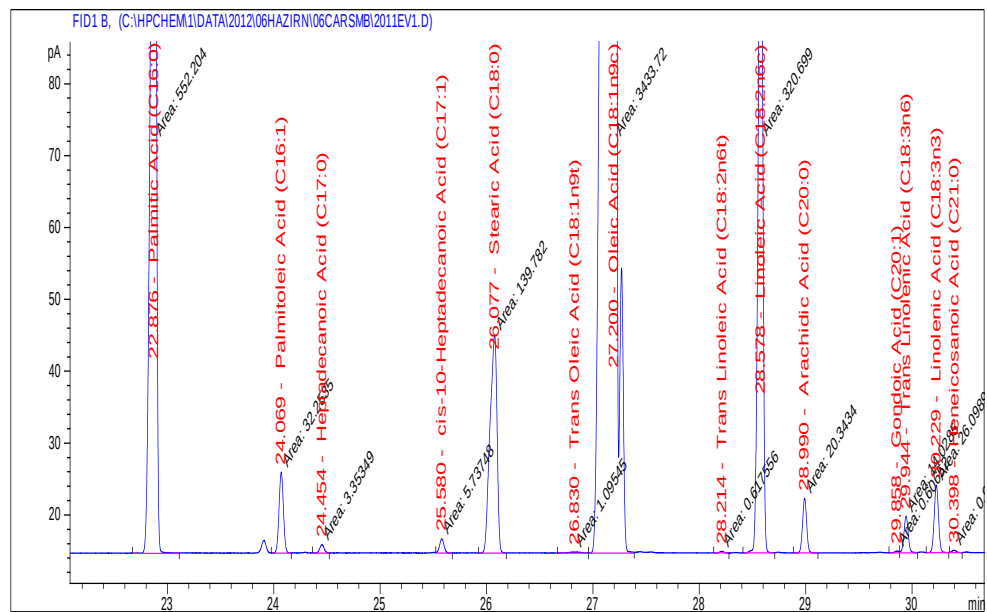


Figure 2.4. Fatty acid methyl esters chromatogram (2)

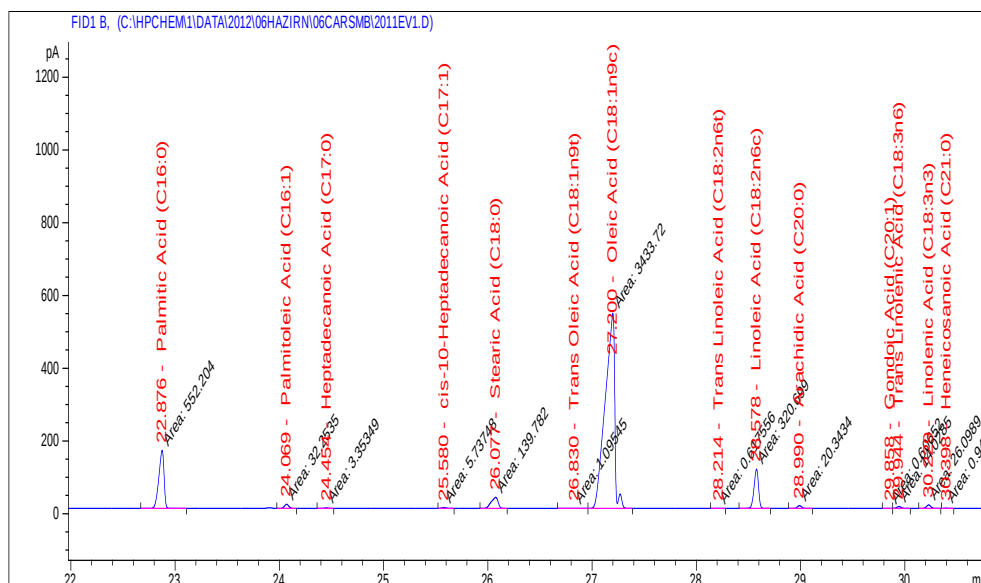


Figure 2.5. Fatty acid methyl esters chromatogram (3)

Determination of Triacylglycerols: Determination of the values of triacylglycerols (TAGs) obtained by HPLC. The method is applicable to the detection of the presence of small amounts of seed oils (rich in linoleic acid) in every class of olive oils (COI/T.20/Doc. No.20/ Rev.2 Determination of the difference between actual and theoretical content of triacylglycerols with ECN 42).

Preparation of the samples for HPLC analysis: A 5% solution of the sample to be analysed is prepared by weighing 0.5 ± 0.0001 g of the sample into a 10 ml graduated flask and making up to 10 ml with the solubilisation solvent (acetone).

Table 2.6. Fatty Acid MW and ECN number

Fatty acid (FA)	Abbreviation	Molecular weight (MW)	ECN
Palmitic acid	P	256.4	16
Palmitoleic acid	Po	254.4	14
Stearic acid	S	284.5	18
Oleic acid	O	282.5	16
Linoleic acid	L	280.4	14
Linolenic acid	Ln	278.4	12

2.3.3 Triacylglycerols

The triacylglycerols was listed below in order of expected elution in HPLC (normally only three peaks).

LLL

PoLL and the positional isomer LPoL

OLLn and the positional isomers OLnL and LnOL

PoPoL and the positional isomer PoLPo

PoOLn and the positional isomers OPoLn and OLnPo

PLLn and the positional isomers LLnP and LnPL

PoPoPo

SLnLn and the positional isomer LnSLn

PPoLn and the positional isomers PLnPo and PoPLn

Liquid chromatographic analysis: An example chromatogram for triacylglycerols of olive oil is shown in Figure 2.6 and Relative Retention Time of TAG according to OLL+LnPP is given in Table 2.8.

Table 2.7. Instrument parameters of HPLC system for TAG analysis.

Instrument	HPLC System
Column	PHENOMENEX Luna 5u C18 100A 250*4.60 mm
Dedector temperature	30 °C
Dedector	RID
Mobile Phase	Propionitrile
Flow Rate	1.5 ml/min
Injection Volume	10 µL

Table 2.8. RRT of TAG in analytic methods

Peak	RRT	Peak	RRT
LLL	0.63	SLL+PLO	1.11
OLLn+PoLL	0.65	PoOP+SPoL+SOLn+SPoPo	1.17
PLLn	0.71	PLP	1.23
OLL	0.79	OOO+PoPP	1.27
OOLn+PoPL	0.82	SOL	1.40
PLL+PoPoO	0.87	POO	1.43
POLn+PPoPo+PPoL	0.90	POP	1.64
OOL+LnPP	1.00	SOO	1.85
PoOO	1.06	POS+SLS	2.10

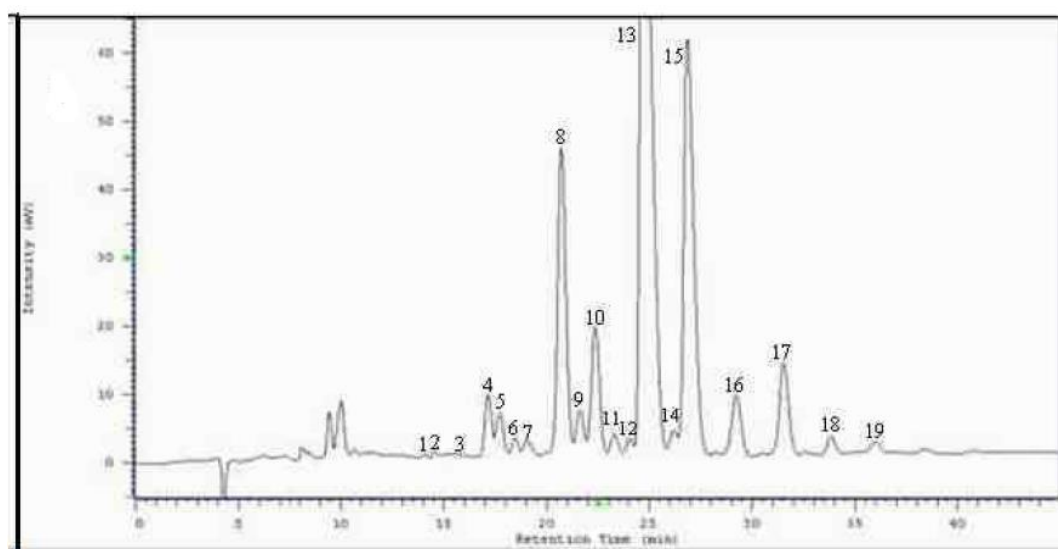


Figure 2.6. ECN42 chromatogram of olive oil

TAG profile with propionitrile: Main components of chromatographic peaks: **ECN42:** (1) LLL; (2) OLLn+PoLL; (3) PLLn; **ECN44:** (4) OLL; (5) OOLn+PoOL; (6) PLL+PoPoO; (7) POLn+PPoPo+PPoL; **ECN46:** (8) OOL+LnPP; (9) PoOO; (10) SLL+PLO; (11) PoOP+SPoL+SOLn+SPoPo; (12) PLP; **ECN48:** (13) OOO+PoPP; (14) SOL; (15) POO; (16) POP; **ECN50:** (17) SOO; (18) POS+SLS

2.3.4 Trace element analysis

Stock solutions of elements prepared in 5% of HNO₃ and 2% of HCl with deionized water. Standard solutions were prepared weekly by diluting stock solutions in 2% of nitric acid and 1% of HCl (EPA Methods 3051, AOAC 986.15A, TS EN 14332). Olive oils samples were collected from various locations with their known natural origin were analyzed as their trace metal content.

Sample preparation procedures: After weighting oil into Teflon PFA vessels to add HNO₃ to the sample and cap the vessel and so digestion procedure was applied. Cool for approximately 5 minutes and vent vessels and then transfer the samples into clean, acid washed volumetric flasks and dilute with ultrapure water and finally to transfer the samples into clean polyethylene bottles.

Table 2.9. Microwave digestion procedure

Heating Program: Ramp to Temperature						
Stage	Max. Power (W)	% Power	Ramp (min)	Pressure (psi)	Temperature (°C)	Hold (min)
1	600*	100	30:00	N.A	210	5:00

* Power should be adjusted up or down with respect to the number of vessels. General guidelines are as follows: 1-2 vessels (300 W), 3-6 vessels (600 W), 7 or more vessels (1200 W).

The optimized ICP-MS conditions are given in Table 2.10.

Table 2.10. Optimized conditions for ICP-MS system

Plasma conditions		Ion Lenses		Octopole Parametres	
RF Power	1500 W	Extract 1	-54.9 V	Octp RF	180 V
RF Matching	1.8 V	Extract 2	-120 V	OCTP Bias	-6 V
Sample Depth	8 mm	Omega Bias	-30 V	Reaction Cell	
Carrier Gas	0.91 L/min	Omega Lens	0.2 V	Reaction Mode	On
Make up Gas	0.17 L/min	Cell Entrance	-20 V	H ₂ Gas	0 mL/min
Nebulizer Pump	0.08 rps	QP focus	5 V	He Gas	1 mL/min
S/C temperature	2 °C	Cell Exit	-28 V	Ar Gas	15 mL/min

Isotopes of each element used in method validation studies and sample analysis are listed in Table 2.11.

Table 2.11 Used isotopes of analyzed elements

Metal	isotope	Metal	isotope	Metal	isotope
As	75	Cu	63	Ni	60
B	11	Fe	56	Pb	208
Ba	137	Hg	202	Sb	121
Ca	40	K	39	Se	82
Cd	111	Mg	24	Sn	118
Co	59	Mn	55	V	51
Cr	53	Na	23	Zn	66

2.3.5 Delta C13

150 µg olive oil was placed into tin capsules and injected onto the EA-IRMS. In the IRMS system, ultrapure helium (% 99.9995) was used carrier gas and carbondioxide (% 99.995) was used reference gas. Other parameters were shown in Table 2.12 (AOAC 984.23, AOAC 991.41 Methods (Official Methods of Analysis of the Association of Official Analytical Chemists 18th. Ed. 2005), TS EN 12140).

Table 2.12. Working conditions of IRMS system

Parameter		Parameter	
Carrier	100 kPa	Purge	80 mL/min
Front Reactor	1030 °C	Rear Reactor	650 °C
Oven	50 °C	Accelerating Voltage	3051.58 V
Extraction Voltage	73.25 % AV	Half Plate Differential	36.58 V
Z-Plates Voltage	- 35.68 V	Trap Current	361.6 µA
Electron Volts	90.37 eV	Ion Repeller Voltage	- 5.00 V
Magnet Current	3557.39 mA		

2.4 Data Analysis

Correlation tables of raw data were drawn for each analysis results and so relation between each variance were investigated according to their “r” values by using Microsoft Office Excel.

Data analysis was performed using multivariate statistical methods with MINITAB 15 Statistical Software. All analyses were statistically analysed. PCA and HCA models were built to analyze the influence of the geographical origin. Results of PCA are visualized by scores, loadings and biplot and scree plots. Score plots were constructed to observe principal groupings among observations. To determine the variables responsible from the groupings among observations loading plots were used. Scree plot was given proportional % of eigenvalues and cumulative percentage of components.

Cluster observations dendrogram of geographical regions were shown grouping between geographical origins and cluster variables were established relation among the variances.

3. RESULTS AND DISCUSSION

In this thesis, 2010/2011 olive season is scheduled depending on the climatic conditions and geographical changes occurred in the characteristic of olive oil have been studied scientifically. For this purpose, 49 samples which are deemed to be pure and–extracted from olives by known manufacturers were collected. These samples were collected from different regions including Marmara, North Aegean, Central Aegean and South Aegean regions. Since, these regions are quite critical in respect to oil production at which they accounted almost for 80.5% of total of Turkey annual capacity overall. Minimum 6 maximum 12 samples were collected from each region.

The aim of collecting samples from different areas is to establish a reliable data set representing for each region as much as possible. Then, the sample being in question can be more confidently classified and treated. This study also gives an insight to which parameters are significant in assessment of olive oils to put forth the possible qualitative differentiations at the regional level easily and to find adulteration in olive oil.

Sterol compositions, FAME, TAG $\delta^{13}\text{C}/^{12}\text{C}$ isotope ratio and trace elements and analyses were performed in GC, HPLC, ICP-MS and IRMS systems and the obtained results were evaluated using MINITAB 15 Statistical Software.

3.1 Analysis of Sterol Compositions

3.1.1 Column chart of sterol compositions

Sterol compositions are the indicators of the authentication and purity of olive oil. Upon addition of any seed oil into olive oil samples, some of the parameters will be found higher than standard value range. Particularly, cholesterol, stigmasterol, campesterol, Δ^7 -stigmasterol and betasitosterol composition give a clear knowledge about purity and adulteration while some minor sterol results indicate the geographical origin and adulteration. Percentage of Cholesterol levels within each sample was utilised to judge on authenticity of sample and regional properties in Figure 3.1.

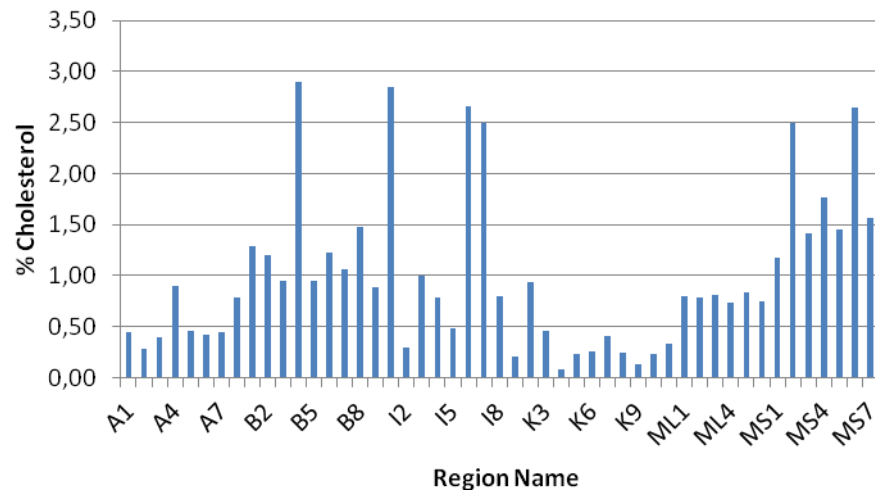


Figure 3.1. Column Chart illustration of relative percentage of cholesterol levels in different samples with coded regions.

Although the relative percentage level of betasitosterol level has significantly contributed to identify the adulteration and quality purposes. In this case, according to the data presented in Figure 3.2, there is no sufficiently enough variation was seen among the samples from different regions to aid unequivocally for the determination of the samples whether adultareted or not.

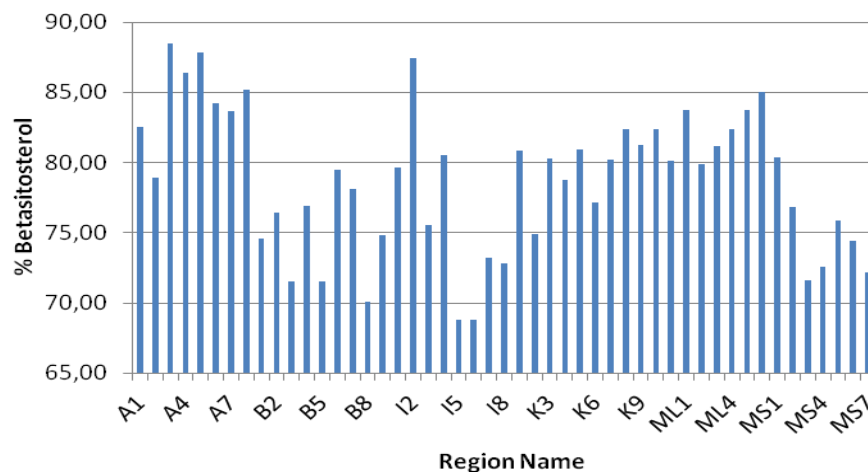


Figure 3.2. Column Chart illustration of relative percentage of betasitosterol levels in different samples with coded regions.

Although the most abundant sterol in olive oil (illustrated as relative percentage in Figure 3.2), betasitosterol, is not sufficient alone to identify authentication, it was seen that D5-avenastanol is sufficient both in regional grouping and to identify adulteration in the column chart.

ML and A regions's olive oil have included D5-avenastanol in proportion as maximum 4% while adulterated oil as abbreviated A2 has contained 0.28 % according to the data presented in Figure 3.3

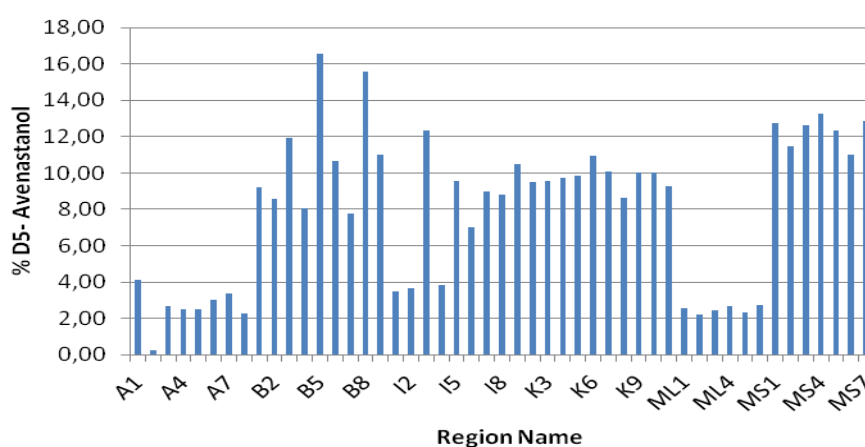


Figure 3.3. Column Chart illustration of relative percentage of D5-Avenastanol levels in different samples with coded regions.

Variation of campestanol level generally varied under 1 % regions of A, I, K, ML and MS olive oils as it can be seen in Figure 3.4. Campestanol percent of A2 was been 6.86%. Campestanol can be exposed to adulteration and can performed authentication according the column chart illustration.

In Figure 3.5, it was shown that percentage of D7-Avenasterol levels have given the differences according to each region. ML and MS (under 0.50%) have similar ratio like B and K (between 0.50-1.50%) regions olive oil while adulterated oil has contain 2.42% D7-Avenasterol.

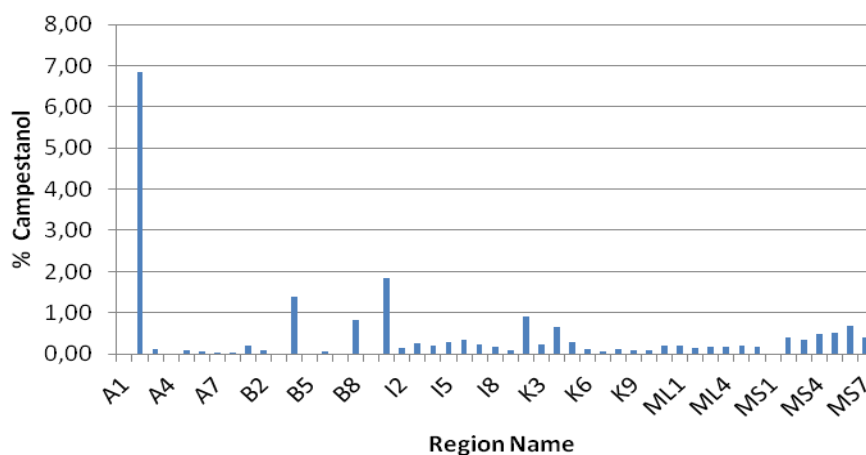


Figure 3.4. Column Chart illustration of relative percentage of campestanol levels in different samples with coded regions.

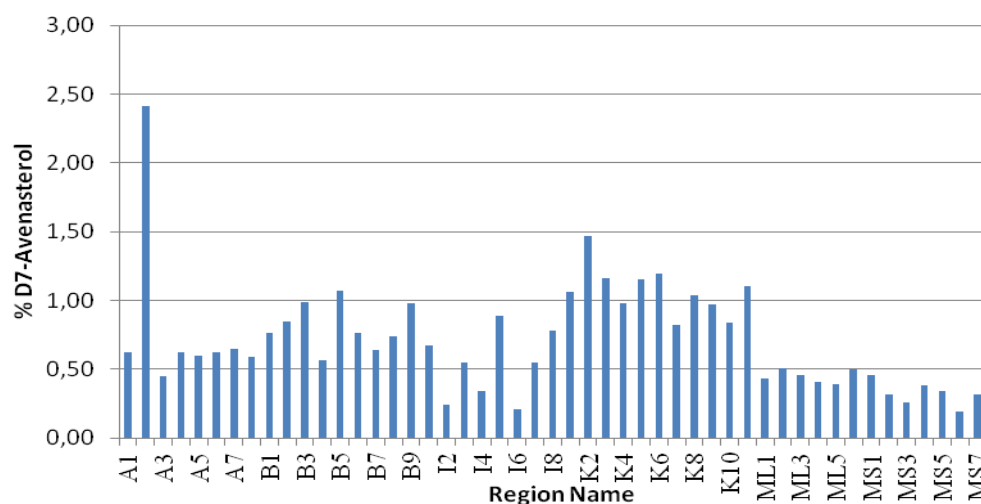


Figure 3.5. Column Chart illustration of relative percentage of D7-avenasterol levels in different samples with coded regions.

D7-Stigmastenol can be used for adulteration because all region's level of D7-Stigmastenol are similar but adulterated oil has got significantly high percentage level of D7-Stigmastenol (4.32%) than others as it can be seen in Figure 3.6.

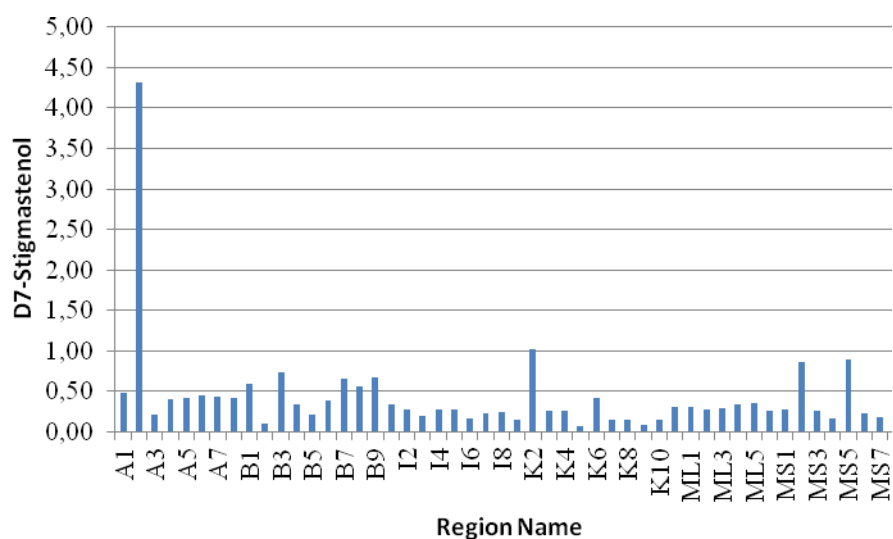


Figure 3.6. Column Chart illustration of relative percentage of D7-stigmastenol levels in different samples with coded regions.

3.1.2 Correlation tables of sterol compositions of raw data

When we were investigated correlation of relationship between each percentage of sterol composition data in Table 3.1 shows that there was no meaningful relationship between each variance of sterol compositions. The highest correlation was among of cholesterol and campestanol but only their “r” value was equal 0.671 that was not enough linear correlation between percentage of cholesterol and campestanol.

Table 3.1. Correlation of relationship between each sterol composition data.

%percentage	CHOLESTEROL	24-METILEN CHOLESTEROL	CAMPESTEROL	CAMPESTANOL	STIGMASTEROL	D5-23 STIGMASTADIANOL	CLEROSTEROL	BETASITOSTEROL	D5- AVENASTENOL	D5-D24 STIGMASTADIONOL	D7 STIGMASTENOL	D7- AVENASTEROL
CHOLESTEROL	1,000											
24-METILEN CHOLESTEROL	-0,319	1,000										
CAMPESTEROL	-0,371	-0,034	1,000									
CAMPESTANOL	0,617	-0,163	-0,279	1,000								
STIGMASTEROL	-0,085	-0,064	0,337	-0,447	1,000							
D5-23 STIGMASTADIANOL	0,258	0,002	0,132	0,077	0,160	1,000						
CLEROSTEROL	0,371	0,098	-0,640	0,087	-0,008	-0,047	1,000					
BETASITOSTEROL	-0,508	0,341	0,603	-0,281	0,150	-0,064	-0,290	1,000				
D5-AVENASTENOL	0,214	-0,169	-0,675	0,111	-0,343	-0,141	0,277	-0,738	1,000			
D5-D24 STIGMASTADIONOL	0,540	-0,457	-0,264	0,192	-0,108	0,158	0,031	-0,747	0,334	1,000		
D7 STIGMASTENOL	0,149	0,083	-0,161	0,128	0,191	-0,057	0,218	-0,115	0,039	-0,031	1,000	
D7-AVENASTEROL	-0,052	0,082	-0,192	0,010	-0,066	0,034	0,059	-0,074	0,274	-0,022	0,380	1,000

Figure 3.7 and 3.8 were plotted percentage value of sterols compositions as an example for showing pairwise relation between components and parameters were selected from correlation according to “r” values of among these variances.

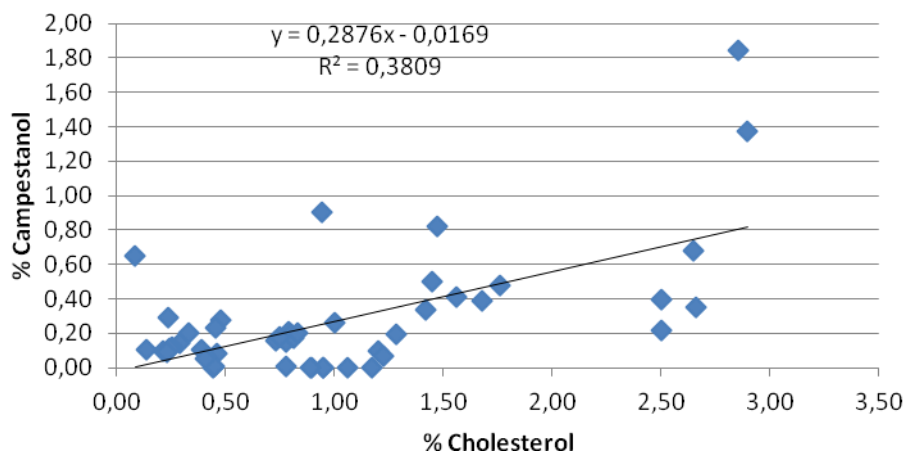


Figure 3.7. Correlation graph between percentage value of cholesterol vs campestanol

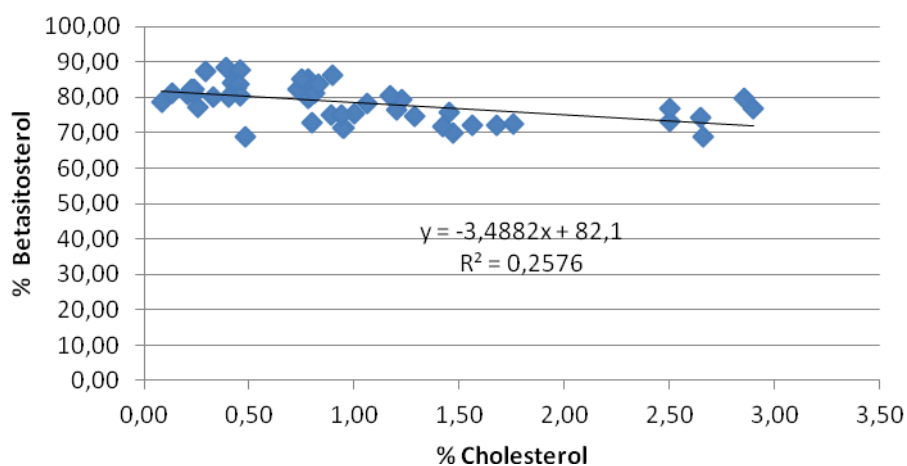


Figure 3.8. Correlation graph between percentage value of cholesterol vs betasitosterol.

3D scatterplot was drawn to show relation between components for authentication. This variances were selected from loading score that variances have high scores and so correlation of trinity was investigated. As it can be seen from 3D scatter plot sterol compositions have not been used for authentication.

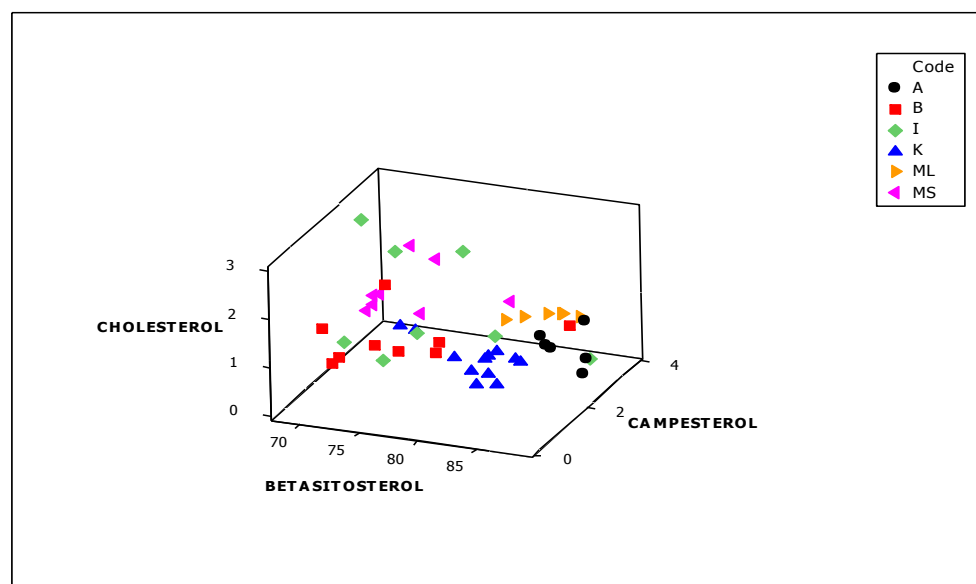


Figure 3.9. 3D scatterplot of cholesterol vs campesterol vs betasitosterol for geographical region.

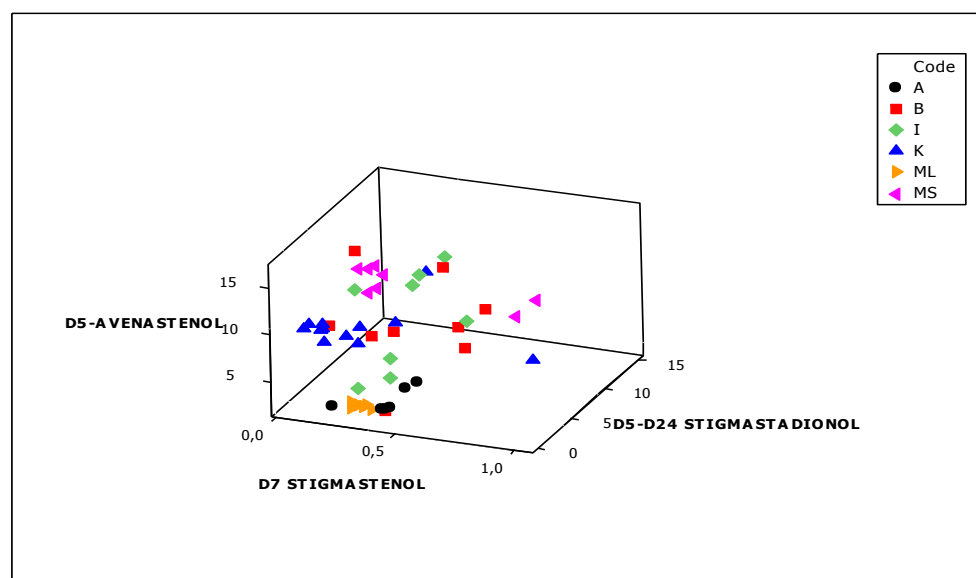


Figure 3.10. 3D scatterplot of d5-avenastenol vs d5-d24 stigmastadionol vs d7 stigmastenol for geographical region.

3.1.3 PCA and HCA for sterol compositions

A previous study was shown that cultivar, besides geographical origin, has a strong effect of the physicochemical parameters of olive oils determined. Thus, it would be appropriate to select a single cultivar and study the effect of geographical origin (Longobardi et al., 2012).

According to the score plot graph shown in Figure 3.11, Bursa İzmir, and Manisa regions were differentiated from other regions clearly with some exceptions such as I2 and MS1 was not differentiated. In addition a good clustering was shown for Aydın, Mugla and Edremit Bay regions as a result of score plot graph provided.

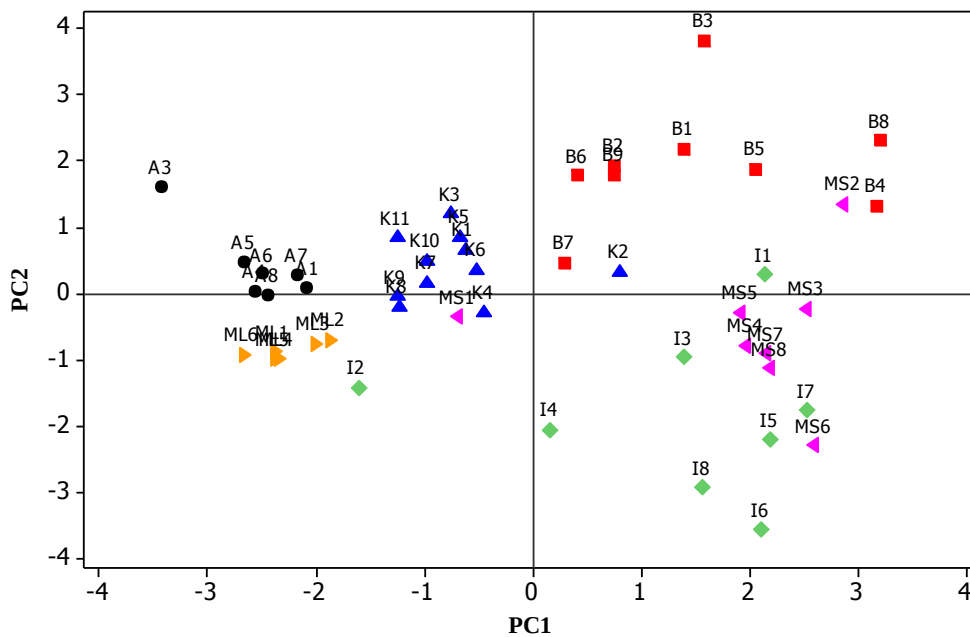


Figure 3.11. PCA Score plot of sterols for geographical regions.

Given the priority regarding to the score plot graph, while B, MS and I region were separated with the basis of PC1 from others, the effect of PC2 was paramount on K, ML and A regions. The effect of PC1 and PC2 on the regions of A, ML, K and MS olive oils are accumulated while the regions of I and B were

distributed. While PC1 has a role to split of MS, PC2 was effected on the grouping of MS.

Bursa as abbreviated B region was splitted and clustered with positive impact both PC1 and PC2. İzmir regions was separated from B under the influence of PC1 as it can seen in Figure 3.11.

When the PCA was made, the results of the modelling power analysis show that β -sitosterol, Δ -5-avenasterol, clerosterol, Δ -7-avenasterol Δ -7-stigmasterol and 24-methylene cholesterol are the more important variables to characterize the olive oils, according to the loading plot in Figure 3.12.

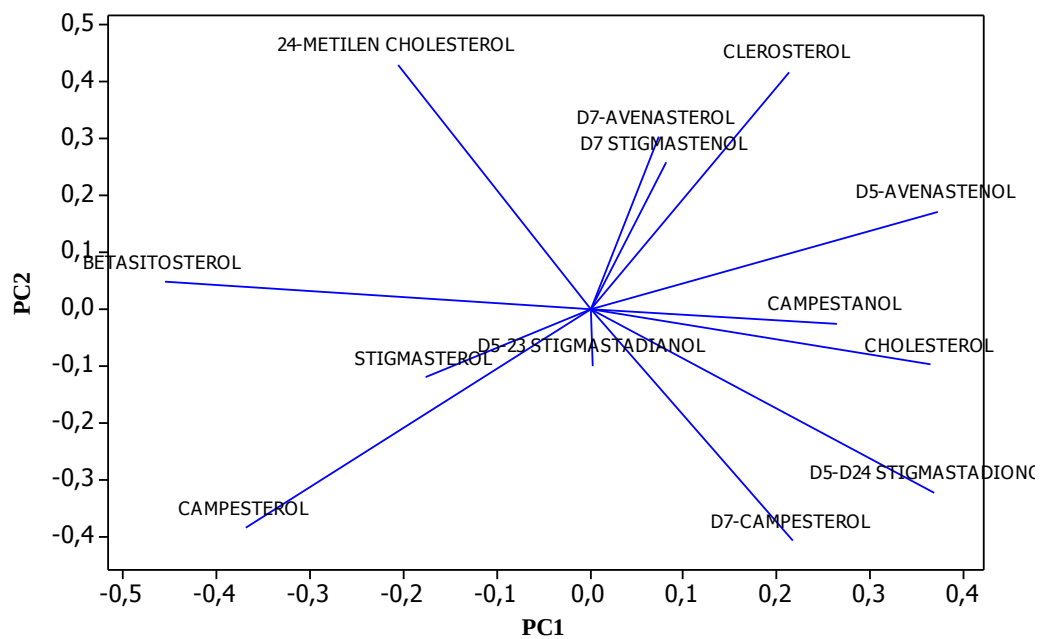


Figure 3.12. PCA Loading plot of sterols for geographical regions.

Clerosterol, Δ -7-avenasterol Δ -7-stigmasterol was been the more important variable to characterize the Bursa olive oils and cholesterol, Δ -7-campesterol, Δ -5-24 stigmasterol was the more important variable to characterize the İzmir and Manisa olive oils as it is shown in the loading plot of Figure. 3.12.

Biplot graph in Figure 3.13 shows that the more important variables to characterize the olive oil for each region in order of campesterol for ML, betasitosterol for A, 24-methylene cholesterol for K, delta 7 sterols for B, d7 campesterol, d5-d24 stigmasitadienol, cholesterol ve campestanol for MS and I.

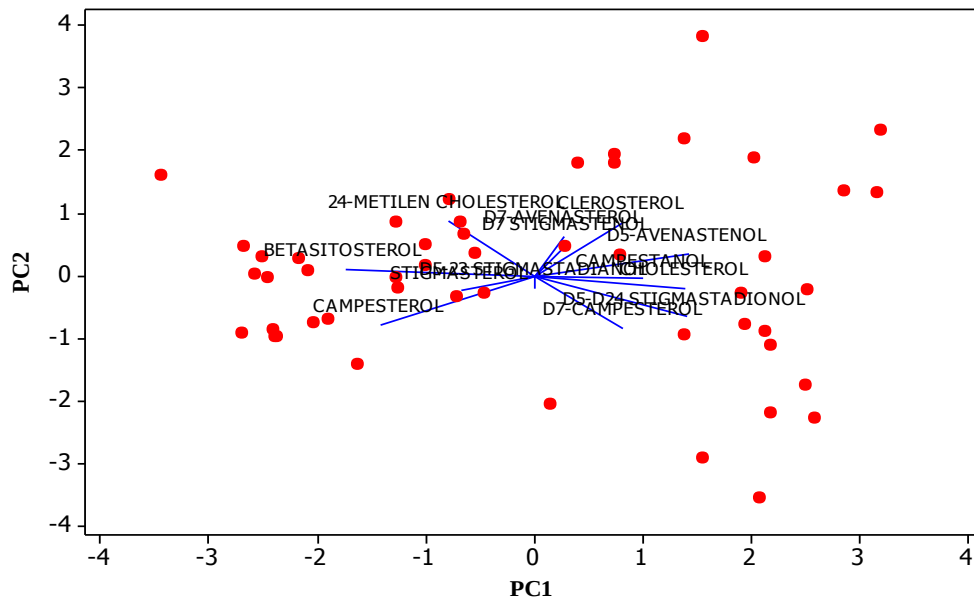


Figure 3.13. PCA Biplot of sterols for geographical regions.

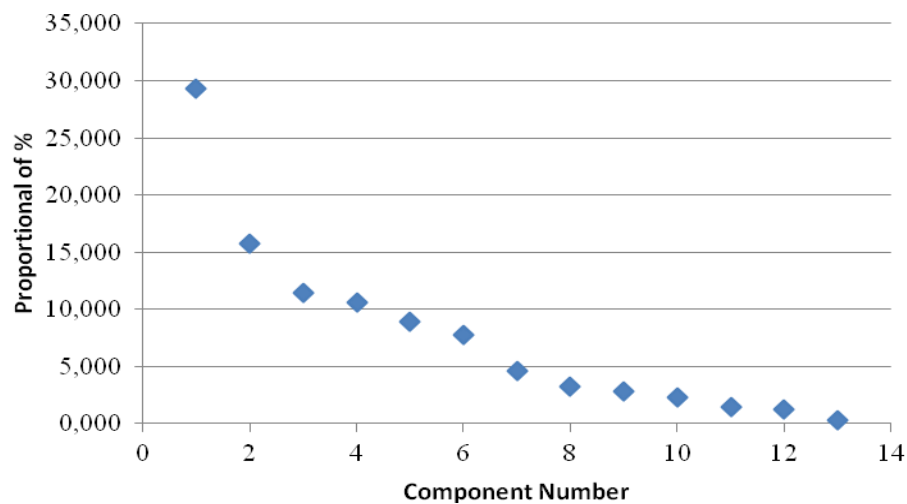


Figure 3.14. Proportional % eigen values vs component number for sterol variances.

Percent eigen value, proportion of % and cumulative percentage was shown in Figure 3.14 and Table 3.2. According to cumulative percentage of parametres was effected on the parting of regions.

Table 3.2. Percent eigen values of sterols.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	3.82	29.37	29.37
2	2.05	15.79	45.16
3	1.48	11.40	56.57
4	1.38	10.60	67.16
5	1.16	8.94	76.10
6	1.01	7.80	83.90
7	0.61	4.66	88.56
8	0.42	3.22	91.78
9	0.37	2.87	94.65
10	0.30	2.32	96.97
11	0.19	1.46	98.43
12	0.16	1.24	99.68
13	0.04	0.32	100.00

The data were also evaluated by cluster analysis. These data were also auto-scaled using the ward linkage method, and the Euclidean distances. Standardize variables were used to calculate sample interpoint distances and similarities using sterol results were shown in Figure 3.15. With respect to this dendrogram, ward method has done regional grouping. In other words, the oils from regions with more similar properties were clustered under the same groups. The results correlated well with other results from the PCA.

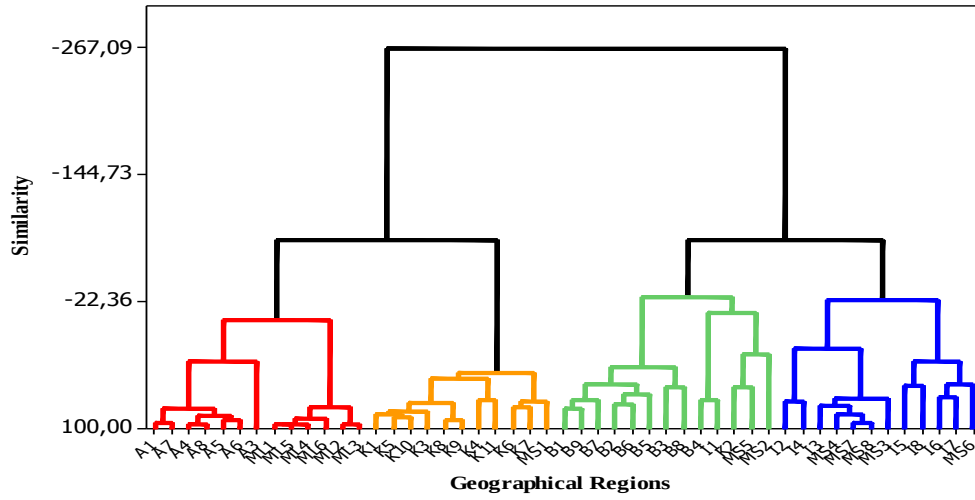


Figure 3.15. Cluster observations dendrogram of sterols according to the ward linkage, Euclidean distance with standardized variables for geographical regions.

Table 3.3. Cluster observations and similarity of geographical regions according to dendrogram of sterols.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	13	AYDIN (A) and MUĞLA (ML)
ORANGE	11	EDREMIT BAY (K)
GREEN	13	BURSA (B) and some MS and I region
BLUE	12	MANİSA (MS) and IZMİR (I)

When we was interpreted to final grouping of observations into clusters according to Figure 3.15 and Table 3.3, the orange cluster has the higher similarity level.

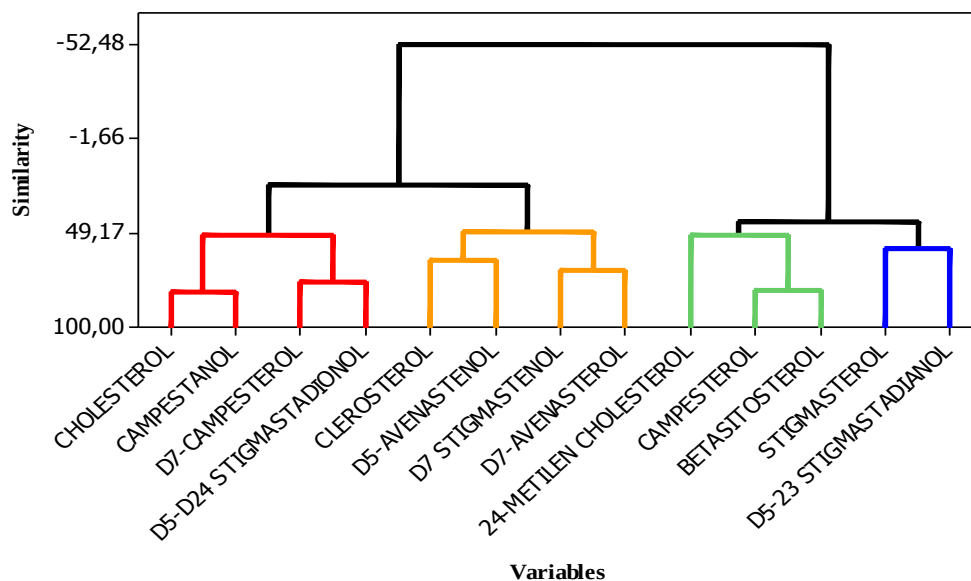


Figure 3.16. Cluster variables dendrogram of sterols according to the ward linkage, correlation coefficient distance for variables.

For the sterol composition of data, the final partition contains four clusters classified by similarities in Table 3.4. The most important variables for differentiating fresh oils according to degree of ripening were D7-campesterol/b-sitosterol, uvaol/stigmasterol, clerosterol/D5-avenasterol and sitostanol/uvaol, while stored oils were differentiated by campestanol/stigmasterol, erythrodiol, stigmasterol/D7-campesterol, D5-avenasterol, 24-methylene-cholesterol/beta-sitosterol and 24-methylene-cholesterol. Results demonstrated that sterols and triterpene diols can be used as indicators of variety and degree of ripening among virgin olive oils (Lukic et al., 2013).

Table 3.4. Cluster variables and similarity of geographical regions according to dendrogram of sterols.

CLUSTER	VARIABLES	SIMILARITY
RED	4	Cholesterol, Campestanol, Δ 7-Campesterol, Δ 5- Δ 24 - Stigmastadionol
ORANGE	4	Clerosterol, Δ 7-avenasterol Δ 7-stigmastanol, Δ 5-Avenastanol
GREEN	3	24-methylene cholesterol, campesterol, betasitosterol
BLUE	2	Stigmastanol, Δ 5- Δ 23 - Stigmastadionol

According to sterol dendrograms, it was found that Aydın (A) and Muğla (ML) olive oils are very close to each other, and their properties are in the same group by effect of cholesterol, campestanol, D7-campestanol, D5-24-stigmastadionol as it was seen in Figure 3.16.

24-methylene cholesterol, campesterol and betasitosterol have to role on the separation and grouping of Bursa from other geographical region according to cluster variable dendrogram of sterol compositions.

Manisa and Izmir were splitted and clustered by impact of stigmasterol and D5-23 stigmastadianol additionally other sterols were played role on the parting of region of Edremit bay is abbreviated K.

Beta-sitosterol, d5-avenasterol and campesterol were the most representative sterols. Cholesterol, stigmasterol, clerosterol and d7-stigmastenol were also found in all samples and sterols compositions were achieved for authenticity of PDO olive oils and the characterization of monovarietal olive oils.(Alves et al., 2005). Most of sterols compounds are significantly affected by the geographical origin (Temime et al, 2008) at the same time sterol compositions of olive oil were an indicator for adulteration (Khalid et al, 2010).

3.1.4 PCA and HCA of sterol compositions for North-South

According to PCA score plot as shown in Figure 3.17, PC2 was major variance for the separated north region olive oils from south region olive oils.

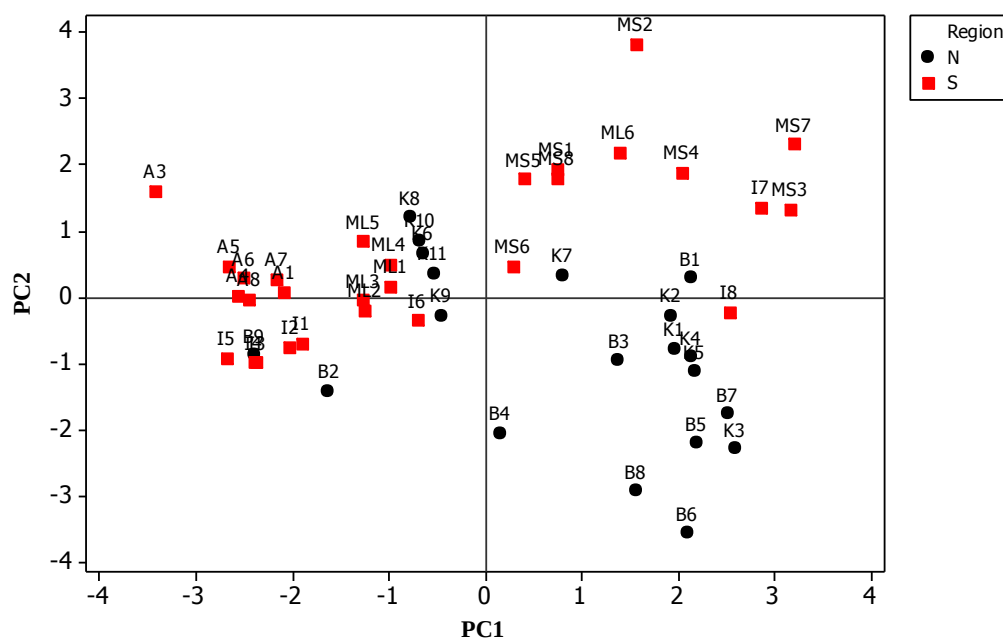


Figure 3.17. PCA score plot of sterol for North-South.

PC1 was divided both north and south region olive oils and so regions have distributed with its effect.

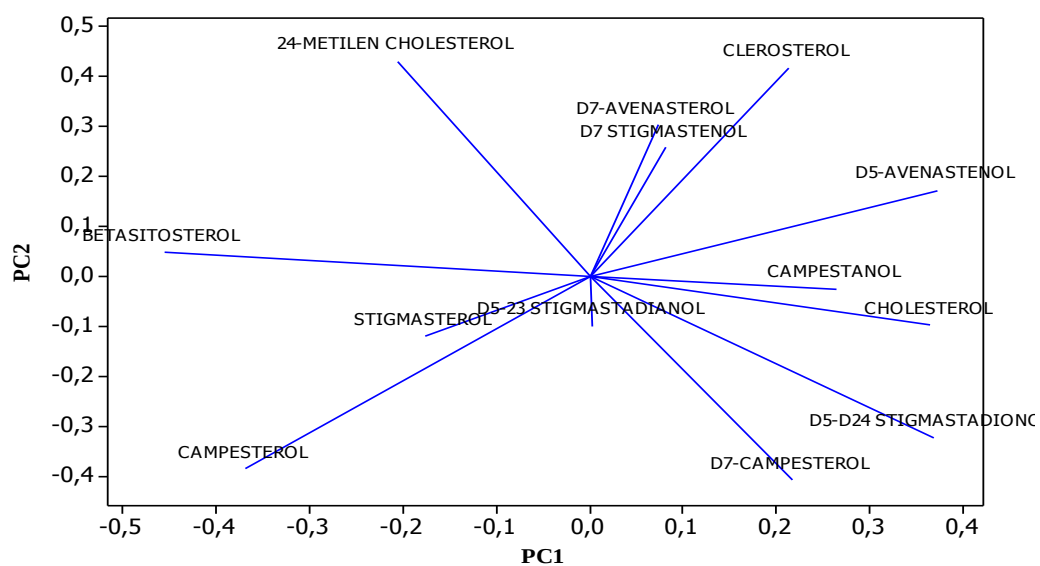


Figure 3.18. PCA Loading plot of sterol for North-South.

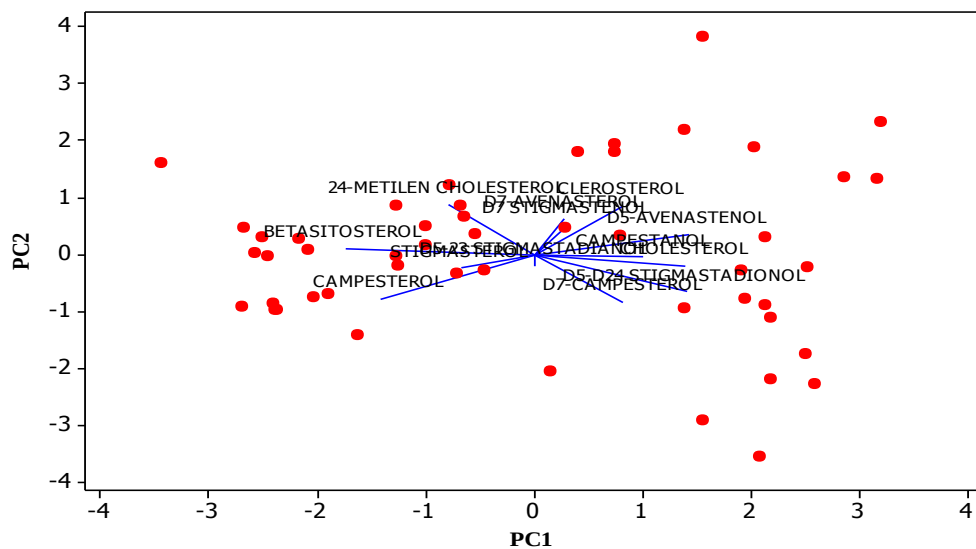


Figure 3.19. PCA biplot of sterol for North-South.

β -sitosterol, Δ^5 -avenasterol, clerosterol, Δ^7 -avenasterol Δ^7 -stigmastenol and 24-methylene cholesterol are the most important variables to characterize the “north” olive oils, according to the biplot in Figure 3.19. In contrast, campesterol, Δ^7 -stigmastenol, cholesterol, Δ^7 -campesterol, Δ^5 -24 stigmastadienol are the most important variables to characterize the “South” olive oils.

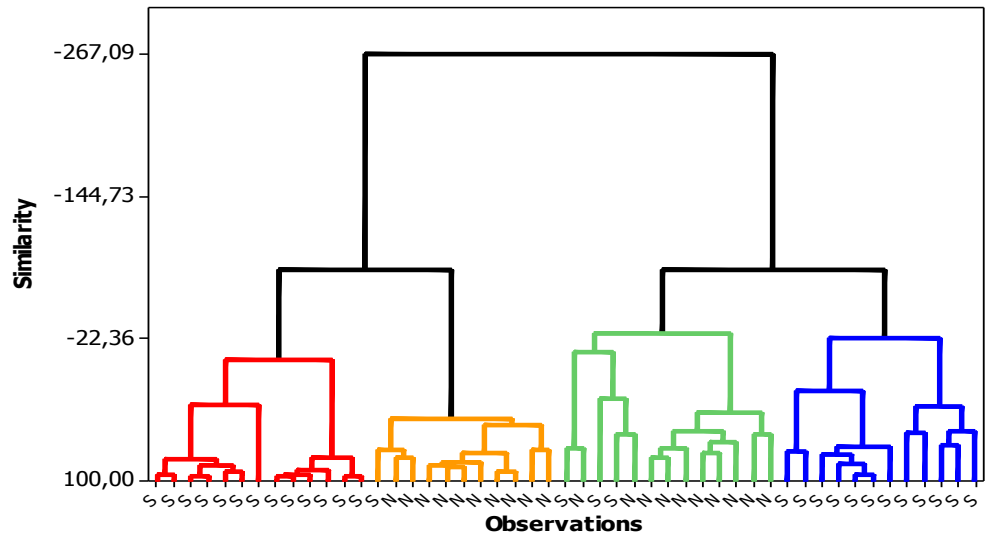


Figure 3.20. Cluster observations dendrogram of sterol for North-South according to the ward linkage, Euclidean distance with standardize variables for geographical regions.

Cluster observations dendrogram which was drawn with ward method was provide good clustering except some regions according to sterol variances as it can be seen in Figure 3.20. In spite of this, ward method was not clearly divided in two group as North and South.

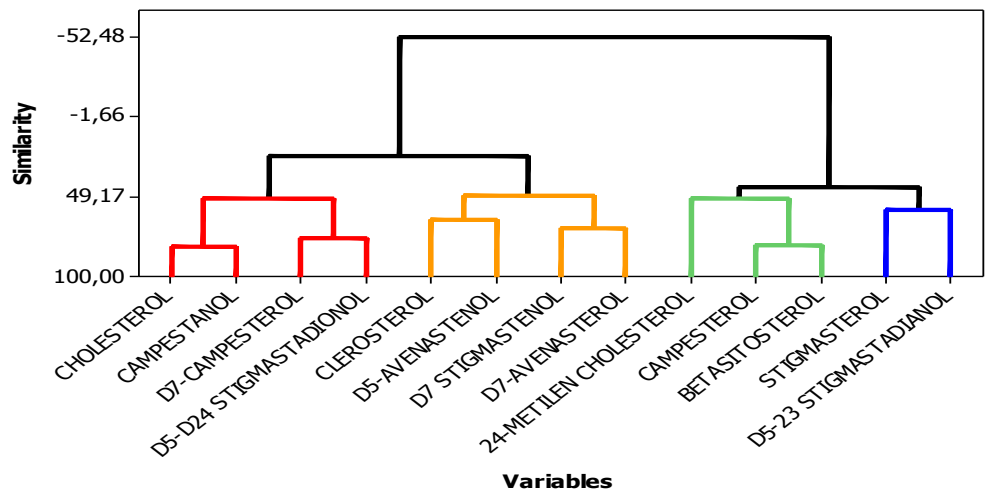


Figure 3.21. Cluster variables dendrogram of sterol of North-South according to the ward linkage, correlation coefficient distance for variables.

When all regions are divide into two groups as north and south, it can be that PC2 is the separative variance. Also HCA results are supply this.

3.1.5 PCA and HCA of sterol compositions for adulteration

The score plot graph shown in Figure 3.22, introduces the closeness of oils having similar properties and the exclusion of the adulterated oil (A2).

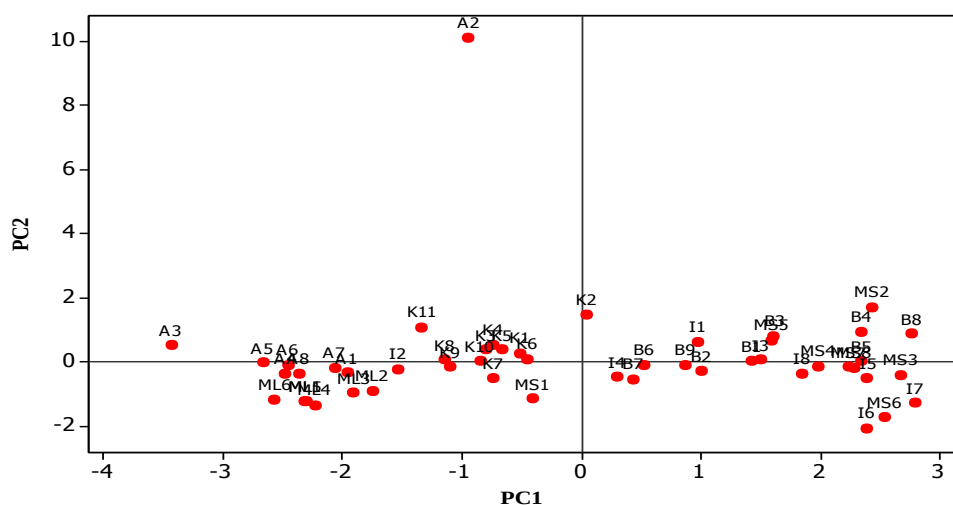


Figure 3.22. Score plot of sterols for adulteration.

In Figure 3.22, it cannot be seen differences in the regions since the generated oil have different properties. Because of this other regions were migrated on the zero line according to PC2 with effect of betasitosterol.

According to loading plot in Figure 3.23, sterol variables have shown similar properties. Campestanol, Δ^7 -stigmastenol, Δ^7 -avenasterol, Δ^7 -sterol, 24-methylene cholesterol are the most important variables to discriminate authentication in the olive oils.

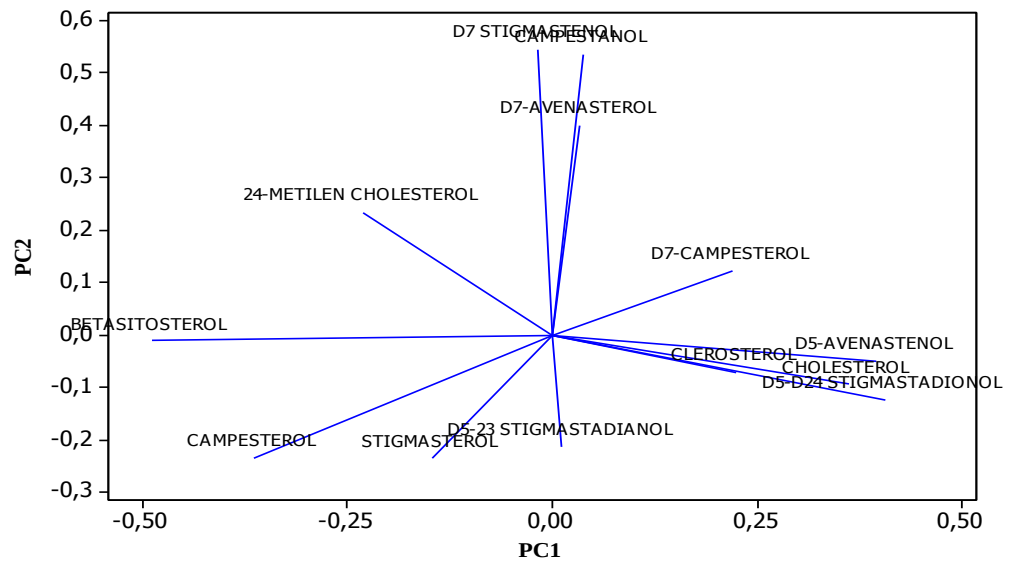


Figure 3.23. Loading plot of sterols for adulteration.

Biplot has shown relationship between samples and sterol parameters in Figure 3.24. Groups were formed effect of these parameters on the region. Cholesterol, $\Delta 7$ -stigmastenol, $\Delta 7$ -avenasterol results are given knowledge about purity and authentication and grouping according to geographical region.

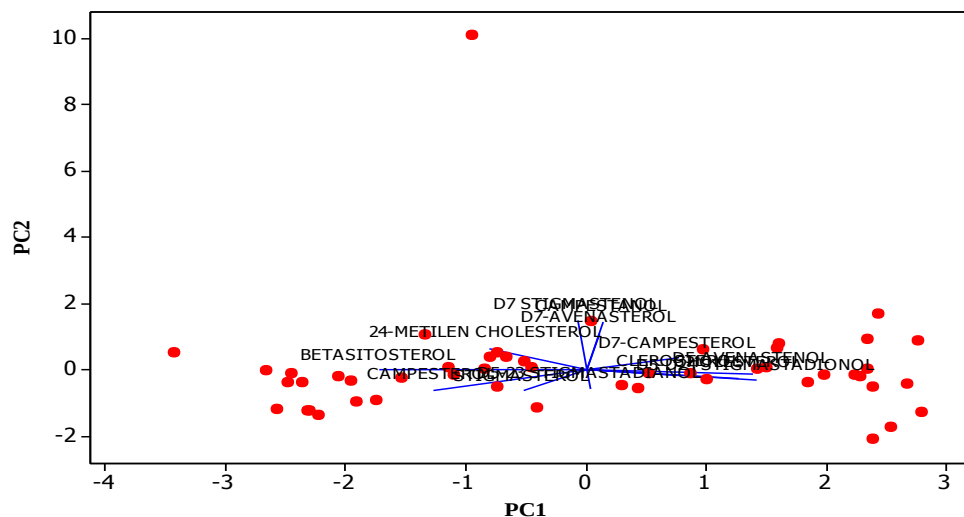


Figure 3.24. Biplot of sterols for adulteration.

According to the scree plot graph, PC1 has the highest eigen value. So, the column chart was drawn for the cholesterol parameter and it was seen that cholesterol isn't sufficient alone to identify authentication. And also, betasitosterol which is considered as fundamental for olive oil isn't sufficient alone to identify adulteration too.

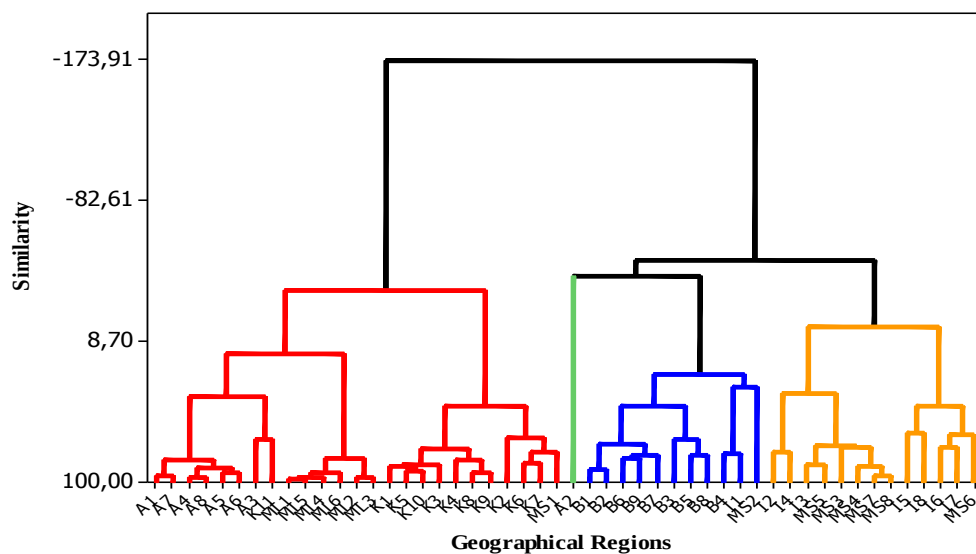


Figure 3.25. Cluster observations dendrogram of sterol for adulteration according to the ward linkage, Euclidean distance with standardize variables for geographical regions.

Cluster observations dendrogram of ward methods was been successful for the separate adulterated olive oil however ward method's dendrogram was executed neither cluster for each region variances nor spare of adulterated olive oil.

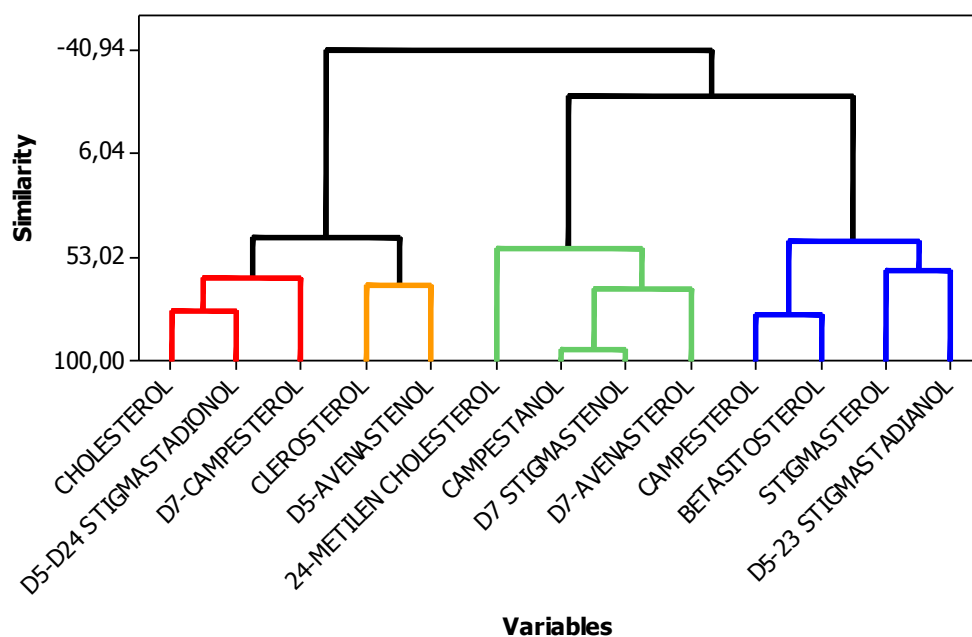


Figure 3.26. Cluster variables dendrogram of sterol for adulteration according to the ward linkage, correlation coefficient distance for variables.

Sterol compositions can be used to determine for adulteration of olive oil as we can openly seen score plot and dendrogram drawn by ward method.

3.2 Results of Fatty Acid Methyl Esters (FAMES)

3.2.1 Column chart of FAMES compositions

Fatty acid methyl esters are explained authenticity of olive oil. Linoleic acid value is sufficient to understand addition of seed oil because linoleic acid value is so high in seed oil. Palmitic acid, Linolenic acid, Behenic acid, Oleic acid parametres are support to find to adulteration in olive oil.

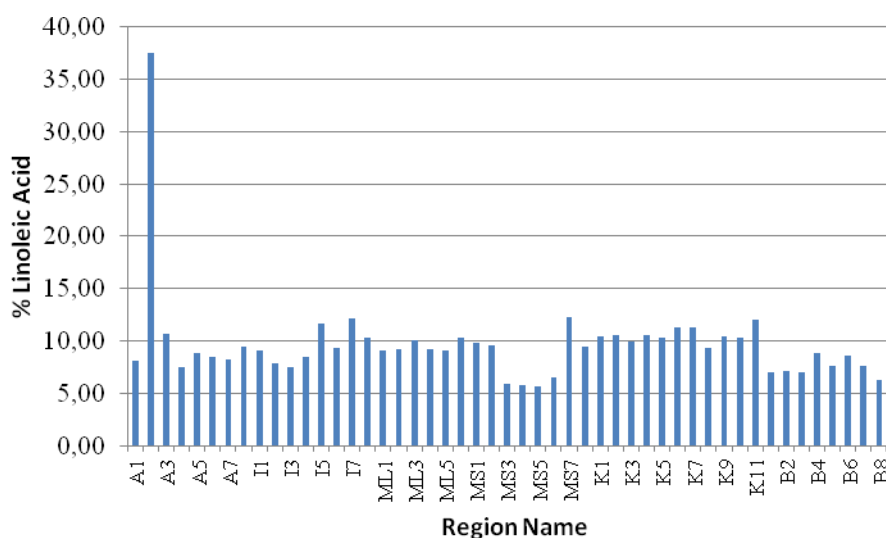


Figure 3.27. Column Chart illustration of relative percentage of Linoleic Acid levels in different samples with coded regions.

When we looked the demonstration of relative percentage of linoleic acid in Figure 3.27, linoleic acid level has role identification of adulteration. Although linoleic acid generally varied 5.00-10.00 % approximately all, linoleic acid level of A2 has uprate 35.00 %.

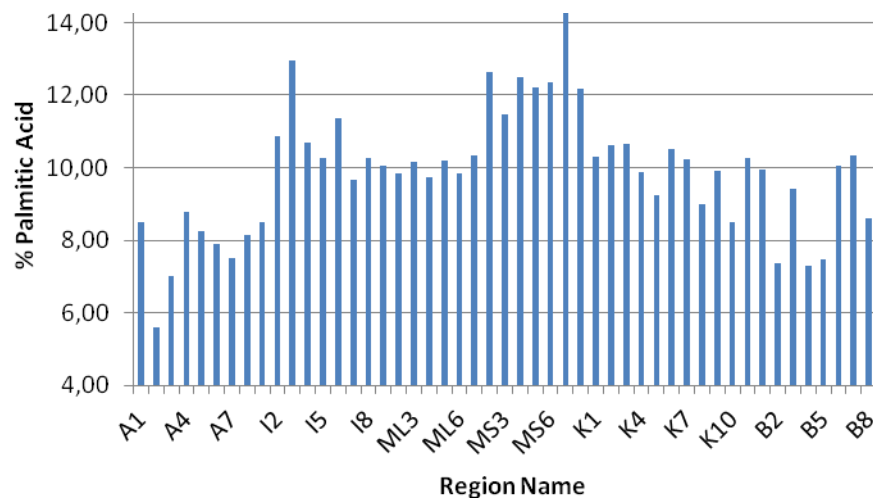


Figure 3.28. Column Chart illustration of relative percentage of Palmitic Acid levels in different samples with coded regions.

According to column chart illustration of relative percentage of palmitic acid and linolenic acid were shown in Figure 3.28 and 3.29, all regions have usually similar level of fatty acid methyl esters. Adulterated oil was shown differences from others olive oils but there was no certain distinction.

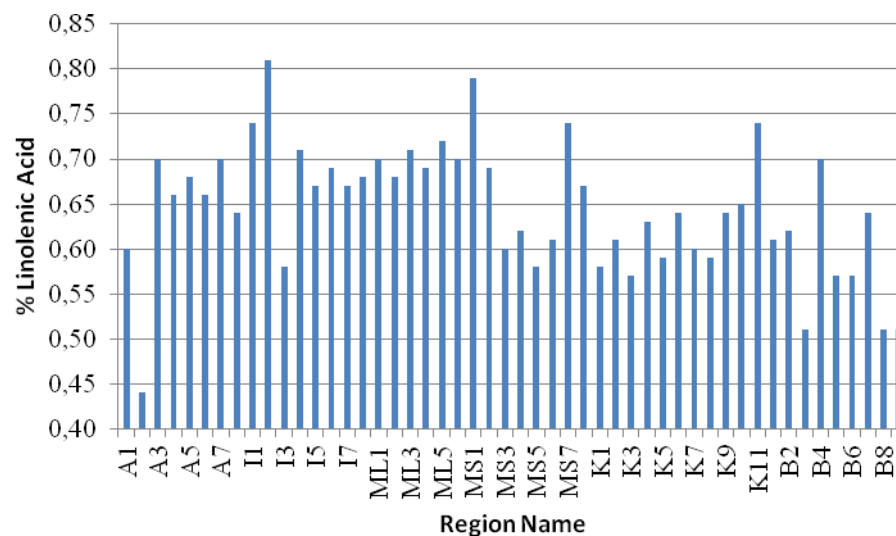


Figure 3.29. Column Chart illustration of relative percentage of Linolenic Acid levels in different samples with coded regions.

Oleic acid acts as separative variance for adulteration as it can be seen in Figure 3.30. Oleic acid, palmitic acid and linolenic acid have no impression on the authentication. The monounsaturated fatty acids have great importance because of their nutritional implication and their effect on oxidative stability of oils (Aparicio, et al. 1999). As the most abundant fatty acid in olive oil, the amount of oleic acid varied remarkably between the olive oil samples.

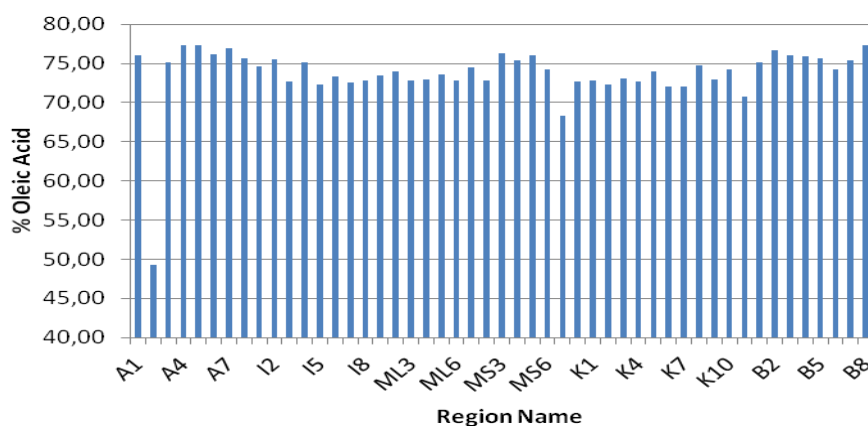


Figure 3.30. Column Chart illustration of relative percentage of Oleic Acid levels in different samples with coded regions.

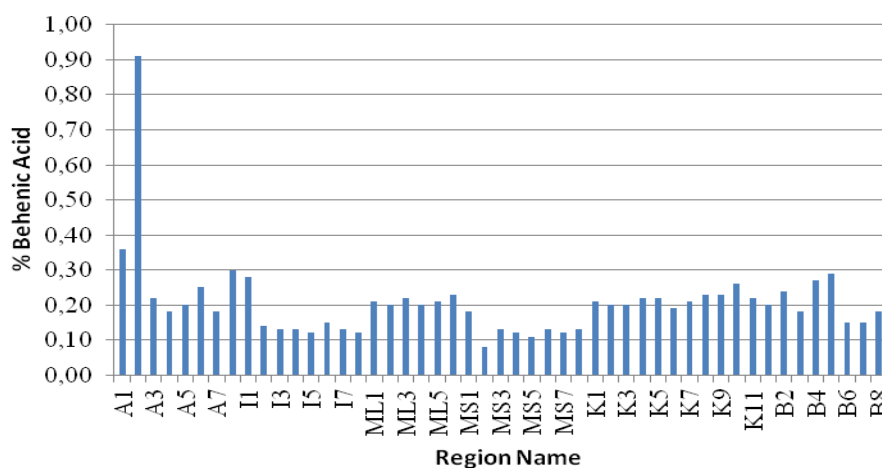


Figure 3.31. Column Chart illustration of relative percentage of Behenic Acid levels in different samples with coded regions.

According to column graphic in Figure 3.31, behenic acid has effective for adulteration and authentication. While proportion of Behenic acid is similar for K, B, ML and A regions, I and MS have generally varied same level. Behenic acid level of A2 was 0.90 %.

3.2.2 Correlation tables of FAMES data

When we were examined correlation of relationship between each percentage fatty acid methyl esters composition data in Table 3.5, there was relationship between some variance of percentage fatty acid methyl esters compositions.

The highest correlation was among of arachidic acid and behenic acid and so their “r” value was equal 0.888 that was not good linear correlation between percentage of arachidic acid and behenic acid because some data was shown distribution therefore linear connection was been between some data.

As it was seen in Table 3.5 some relation was shown inverse serial correlation such as arachidic acid, gondoic acid, heneicosanoic acid between palmitic acid data.

Table 3.5. Correlation of relationship between each fatty acid methyl esters data.

%percentage	PALMITC	PALMITOLC	HEPTADECANIC	STEAIC	OLEC	LINOLEC	ARACHIDC	GONDIC	LINOLENC	HENEICOSONC	BEHENC	ARACHIDONC	LIGNOCERC	ERVONC
PALMITIC	1,000													
PALMITOLEIC	0,672	1,000												
HEPTADECANOIK ASIT	0,260	0,294	1,000											
STEARIC	-0,670	-0,336	0,128	1,000										
OLEIC	-0,560	-0,131	-0,238	0,595	1,000									
LINOLEIC	0,013	-0,249	-0,029	-0,413	-0,769	1,000								
ARACHIDIC	-0,791	-0,575	-0,108	0,634	0,319	-0,002	1,000							
GONDOIC	-0,787	-0,642	-0,369	0,264	0,142	0,358	0,706	1,000						
LINOLENIC	0,061	-0,126	-0,516	-0,496	-0,289	0,423	-0,127	0,263	1,000					
HENEICOSONOIC	-0,399	-0,297	-0,249	0,144	0,064	0,131	0,579	0,338	0,098	1,000				
BEHENIC	-0,723	-0,612	-0,261	0,418	0,208	0,132	0,888	0,732	-0,043	0,697	1,000			
ARACHIDONIC	-0,398	-0,223	-0,125	0,383	0,424	-0,416	0,604	0,252	-0,189	0,443	0,602	1,000		
LIGNOCERIC	-0,543	-0,332	-0,129	0,344	0,141	0,057	0,790	0,497	-0,027	0,817	0,806	0,622	1,000	
NERVONIC	-0,338	-0,318	-0,243	0,082	0,120	0,016	0,573	0,267	0,110	0,731	0,608	0,573	0,740	1,000

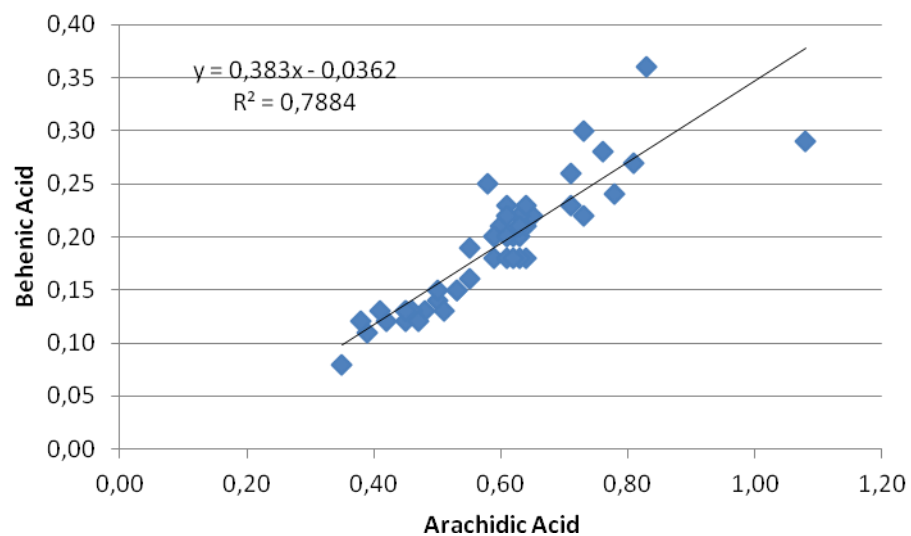


Figure 3.32. Correlation graph between percentage value of arachidic acid vs behenic acid.

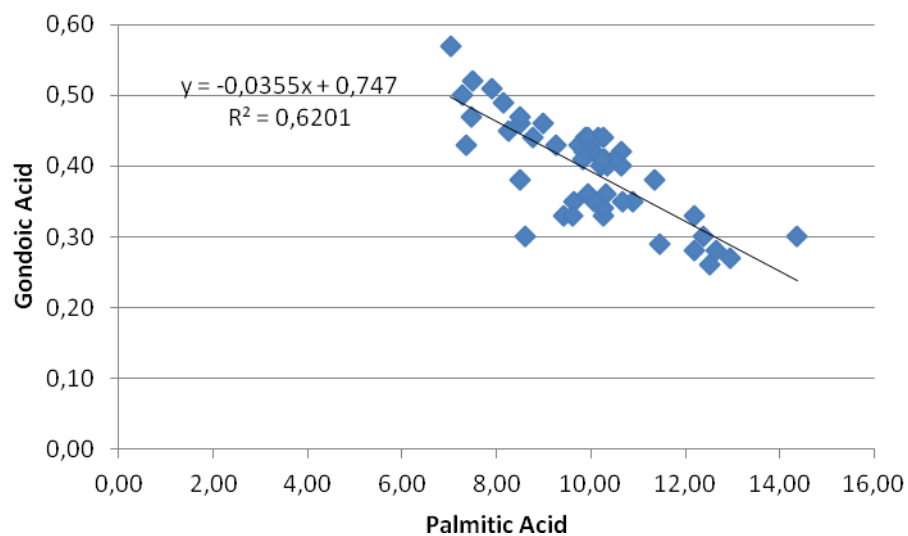


Figure 3.33. Correlation graph between percentage value of gondoic acid vs palmitic acid.

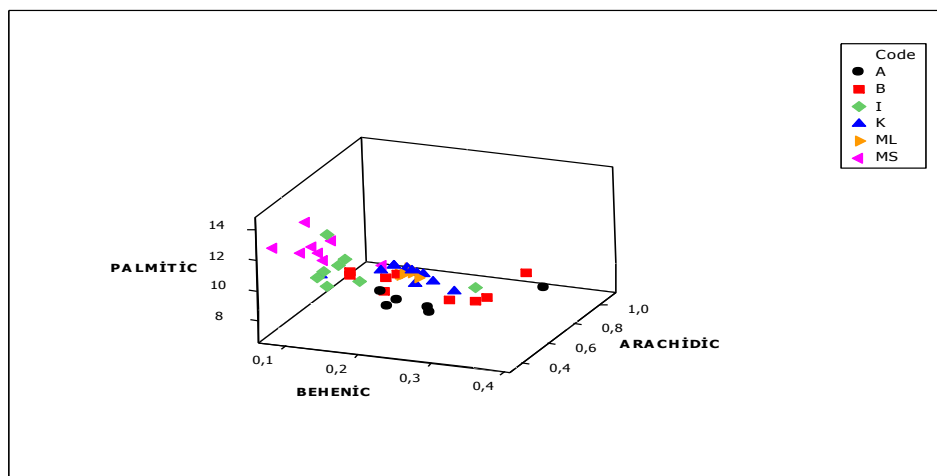


Figure 3.34. 3D Scatterplot of palmitic vs arachidic vs behenic acids for geographical region.

3D scatterplot was drawn to show relation between components for authentication. This variances were selected from loading score that variances have high scores and so correlation of trinity was investigated. As it can be seen in Figure 3.34 and 3.35, 3D scatter plot fatty acid methyl esters compositions have not been used for authentication.

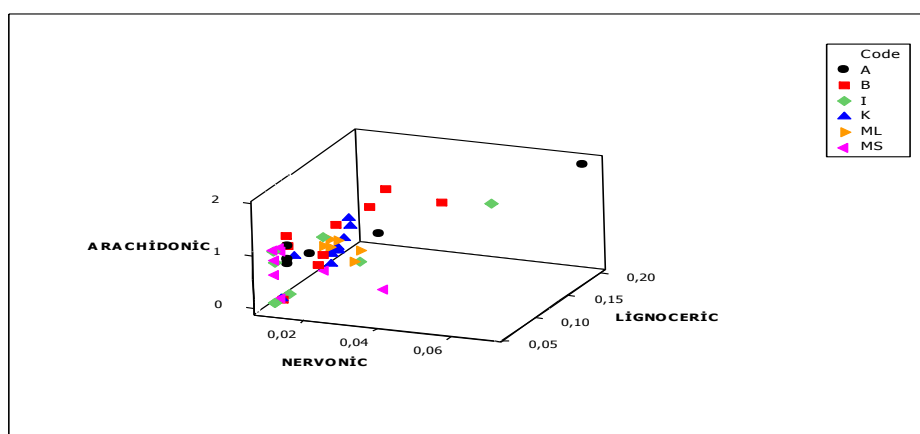


Figure 3.35. 3D Scatterplot of arachidonic vs lignoceric vs nervonic acids for geographical region.

3.2.3 PCA and HCA for FAME compositions

FAME analyses results were evaluated by using PCA and HCA. So it was seen that oils from Bursa differentiates clearly from other regions and it has a distribution in itself according to score plot in Figure 3.36. Also olive oils from Edremit Bay are clustered by differentiating from other regions according to fatty acids composition as we can see dendrogram of cluster observations in Figures 3.40 and 3.41.

While olive oils of Bursa was separated with PC2 from others, PC2 was active role on K and also PC1 are efficient on the clustering of Edremit Bay (K) therefore olive oils from K region have shown different properties. K and ML regions were aggregated while others were distributed. Both PC1 and PC2 are effected Izmir (I) region although Muğla (ML), Manisa (MS) regions were separated PC1. Aydın (A) was shown dispersed as it can plotted in PCA score plot graph in Figure 3.36.

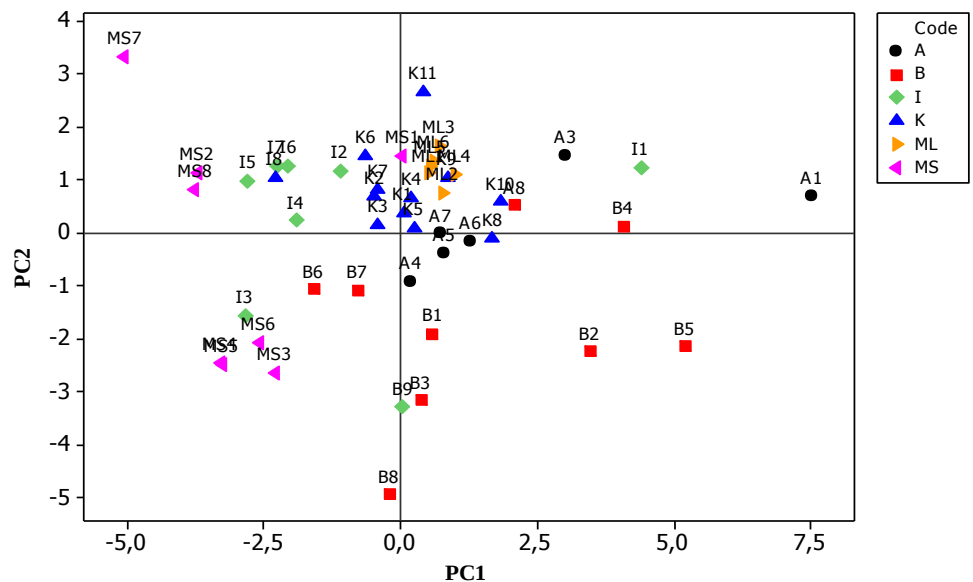


Figure 3.36. PCA Score plot of fame for geographical regions.

As PCA score plot of FAME, olive oils of K and ML were clustered in spite of B, MS, I and A were showed distribution.

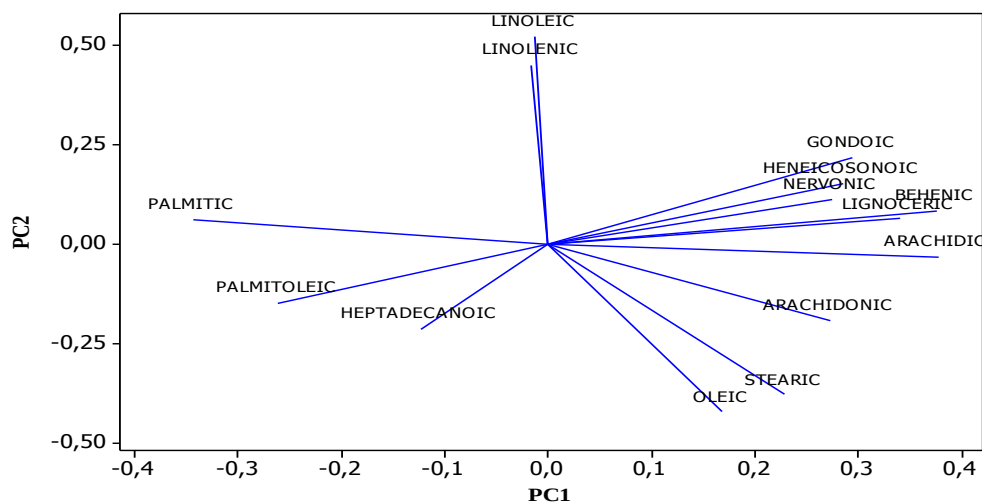


Figure 3.37. PCA Loading plot of fame for geographical regions.

According to loading plot in Figure 3.37, palmitic acid, palmitoleic acid and heptadecanoic acid play more effective role for parting MN and I region than others. K and ML regions were under the influence of linoleic acid and linolenic acid. Gondoic acid, heneicosonoic acid, nervonic acid, behenic acid, lignoceric acid and arachidic acid become effective on the other regions.

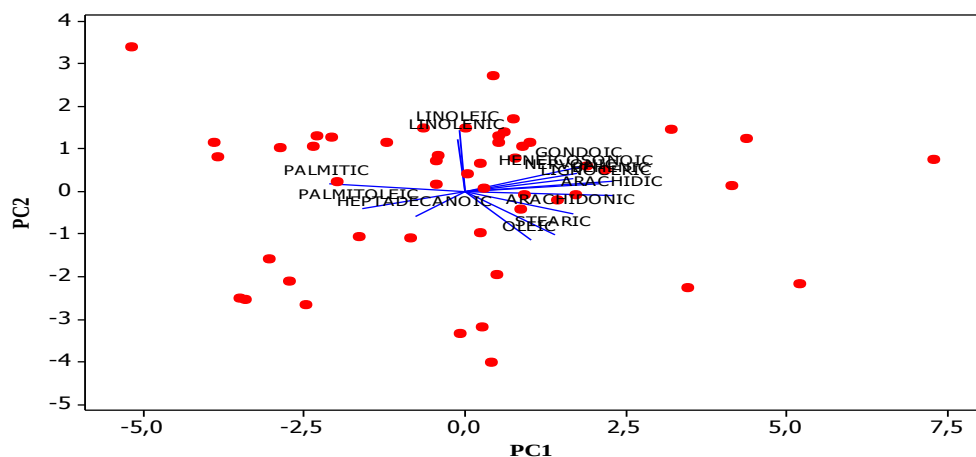


Figure 3.38. PCA Biplot of fame for geographical regions.

Even though the percent existence of oleic acid have a similar range in olive oil, oleic acid and stearic acid have played active role on the separation olive oils of Bursa (B) region.

Palmitic acid, palmitoleic acid and heptadecanoic acid play more effective role for parting I region. K region was under the influence of linoleic acid and linolenic acid as we can seen graph of biplot in Figure 3.38. A previous study on Sicilian olive oils suggested that although the effect of the cultivar is predominant in the olive oil classification based on the fatty acid composition, a minor but well defined geographic effect is also present (D'Imperio, et al. 2007).

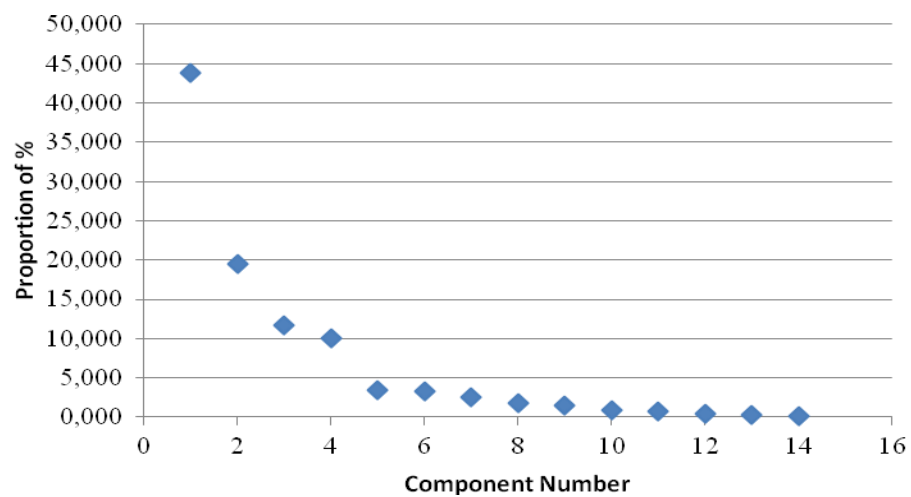


Figure 3.39. Proportional % eigen values vs component number for fame variances.

Percent eigen values were shown in Figure 3.39 and Table 3.6. According to cumulative percentage, first six parametres was effected on the parting of regions. PC1 has 43,76 % proportion that means PC1 was important variance for clustering of regions.

Q			Qubit
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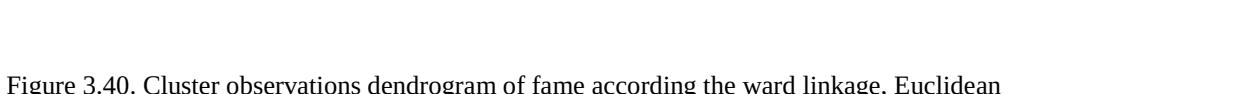


Table 3.7. Cluster observations and similarity of geographical regions according to dendrogram of fatty acids.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	28	AYDIN (A), MUĞLA (ML), EDREMIT BAY (K)
GREEN	21	BURSA (B), MANISA(MS) and IZMIR (I)

We could easily observe that the olive oils from the same location are clustered together and distinguish the presence of two main clusters of olive oil samples which are K and ML according to ward linkage dendrogram in Figure 3.40. To classify the olive oils according to regions ward linkage methods was provided not so good cluster observations as regard to fatty acid methyl esters.

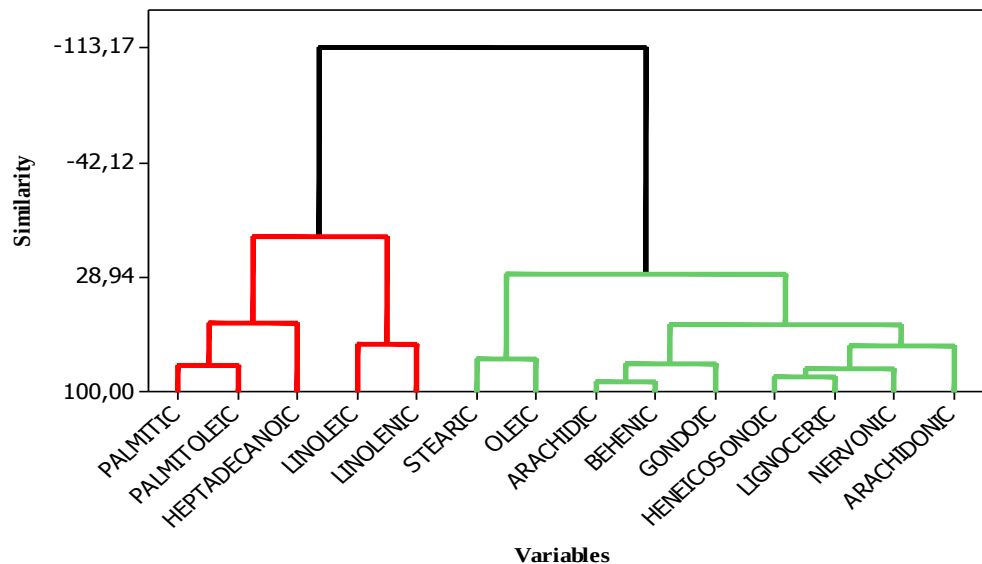


Figure 3.41 Cluster variables dendrogram of fatty acids according to the ward linkage, correlation coefficient distance for variables.

Table 3.8. Cluster variables and similarity of geographical regions according to dendrogram of fatty acids.

CLUSTER	VARIABLES	SIMILARITY
RED	5	Palmitic, Palmitoleic, Heptadecanoic, Linoleic, Linolenic Acid
GREEN	9	Stearic, Oleic, Arachidic Acid, Behenic, Gondoic, Heneicosonoic, Lignoceric, Nervonic, Arachidonic Acid

Cluster variables dendrogram of fame was shown linoleic acid and linolenic acid were effected on the geographical region of ML and K.

When PCA and HCA results of FAME was investigated which have not dominant effect on the separation of regions.

3.2.4 PCA and HCA of FAME compositions for North-South

Splitting the regions as north and south was not provide a specific grouping according to score plot in Figure 3.43. So it was not be possible to cluster the regions according to fatty acids.

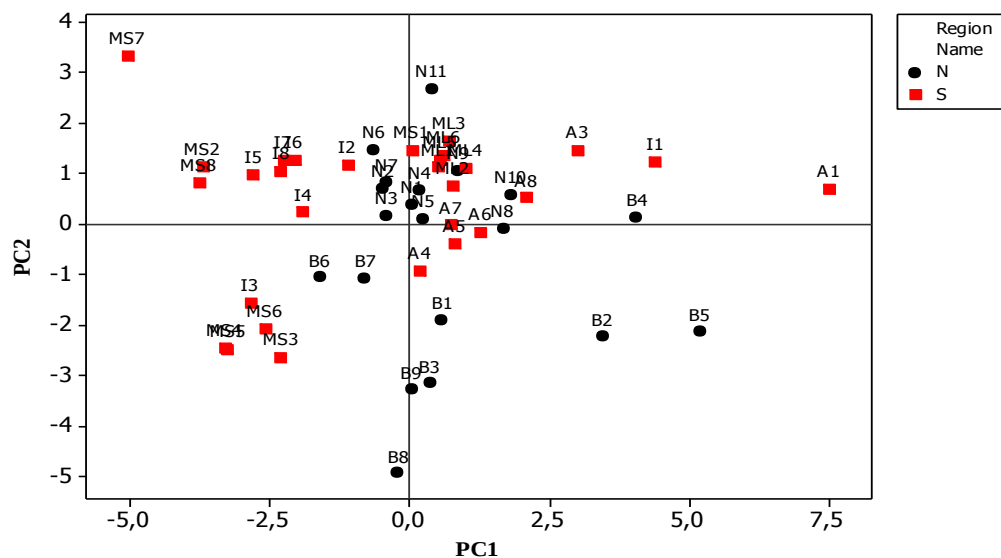


Figure 3.42. PCA score plot of FAME for North-South.

According to score plot of fatty acid methyl esters as we seen in Figure 3.42 regions were not divided clearly in two groups as a north and south.

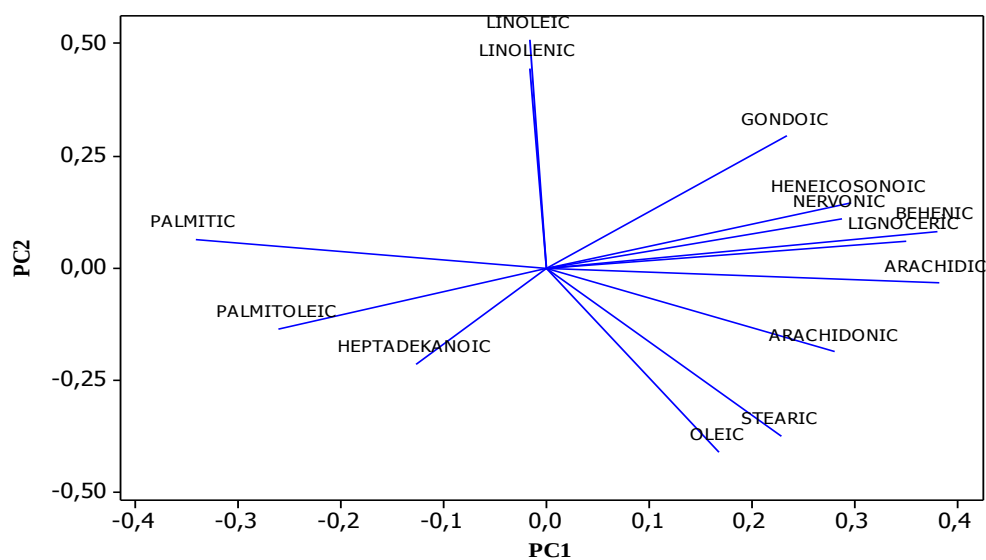


Figure 3.43. PCA Loading plot of FAME for North-South.

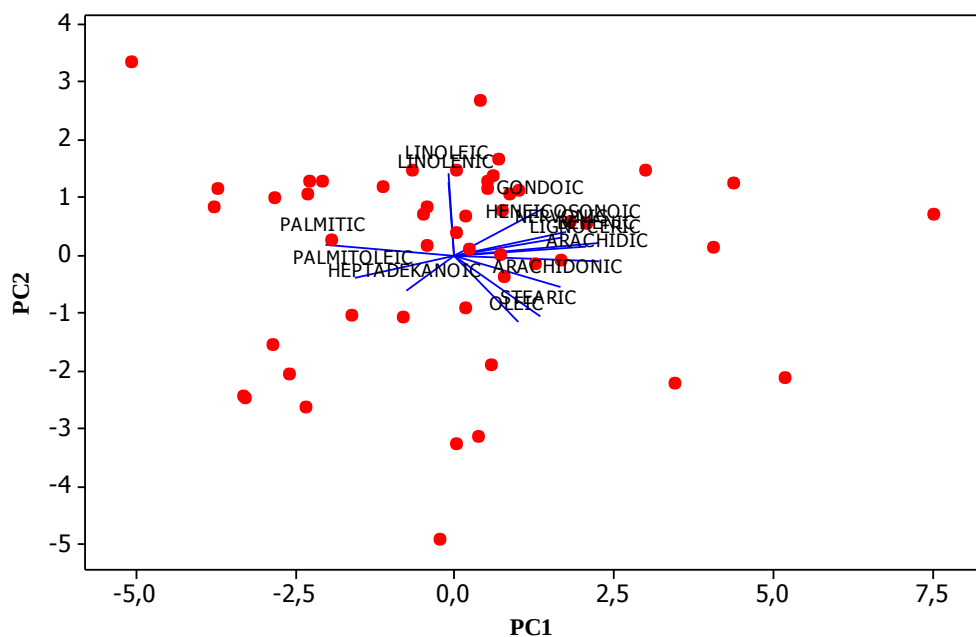


Figure 3.44. PCA biplot of FAME for North-South.

As we can see biplot in Figure 3.44, fatty acid variances were not formed groups. Linoleic and linolenic acid are the most important variances according to loading plot in Figure 3.43.

According to cluster observations dendrogram plotted using ward linkage methods in Figure 3.45, there were not a clear differentiation as North and South.

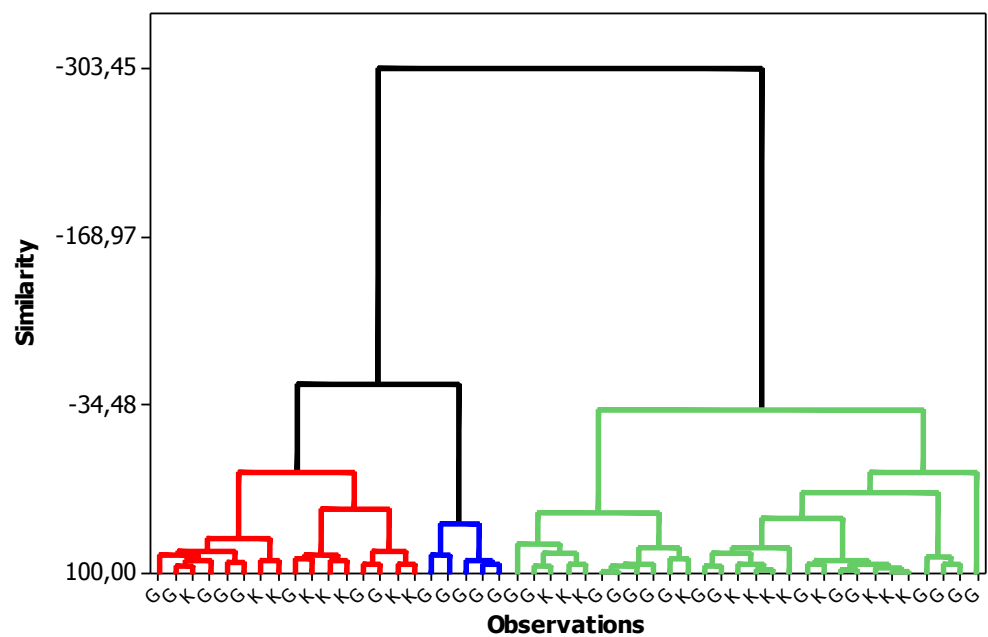


Figure 3.45. Cluster observations dendrogram of FAME for North-South according the ward linkage, Euclidean distance with standardize variables for geographical regions.

There was no distinct differences between dendograms which were drawn single and ward method. Regions were shown distribution and not indicated expressive clustered as north and south.

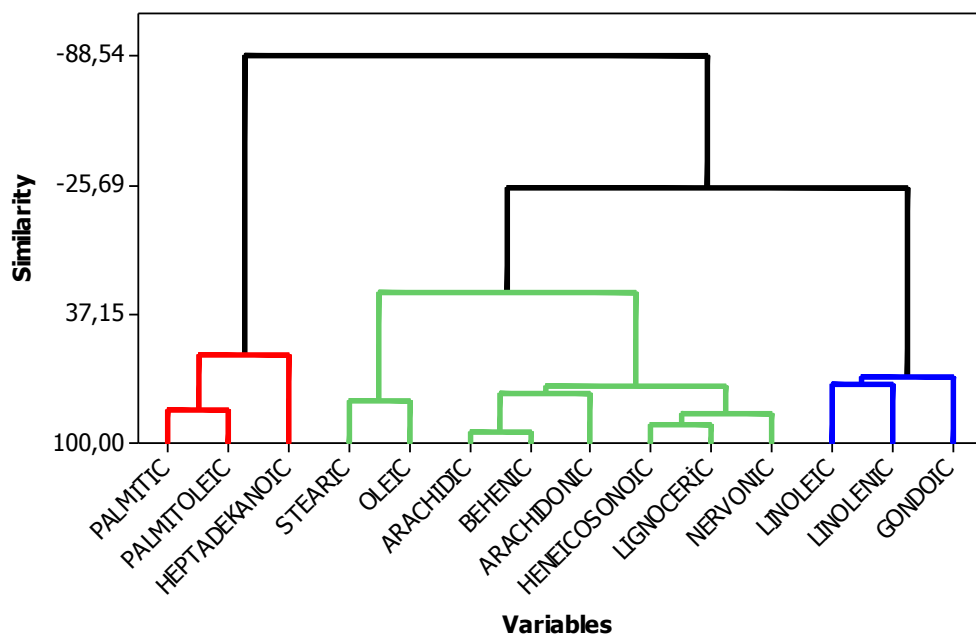


Figure 3.46. Cluster variables dendrogram of FAME of North-South according to the ward linkage, correlation coefficient distance for variables.

3.2.5 PCA and HCA of FAME compositions for adulteration

While the most abundant fatty acid in olive oil is oleic acid but in seed oils, linoleic acid is the most present. And so, the increase in the quantity of linoleic acid indicates the addition of seed oil to olive oil.

As we can see in Figure 3.48 and Figure 3.49, loading plot and biplot graphs obtained by PCA analysis of fatty acids clearly shows that the main component to identify adulteration is linoleic acid. Thus, linoleic acid is the most effective parameter to identify adulteration.

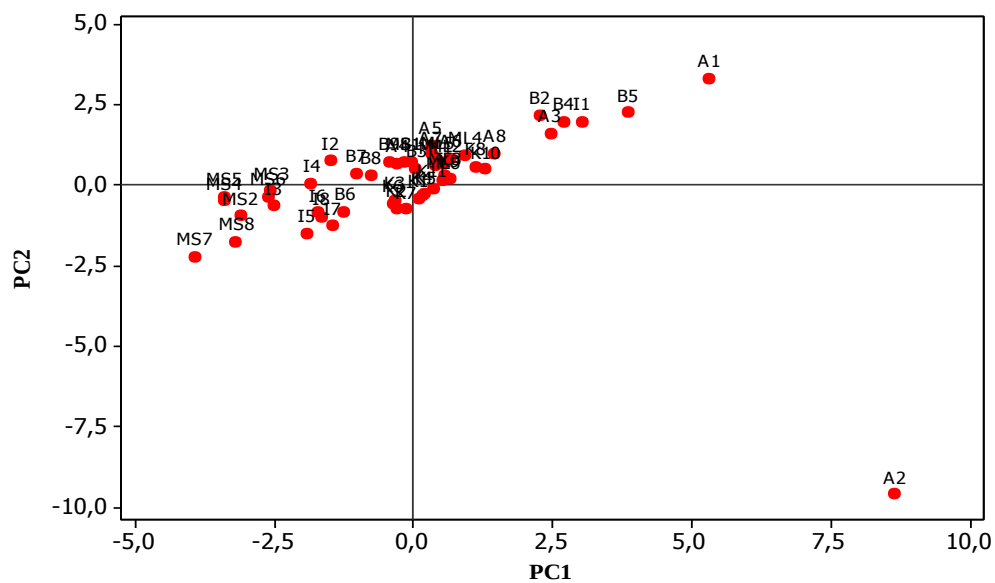


Figure 3.47. PCA scores plots of fatty acid methyl esters for authentication.

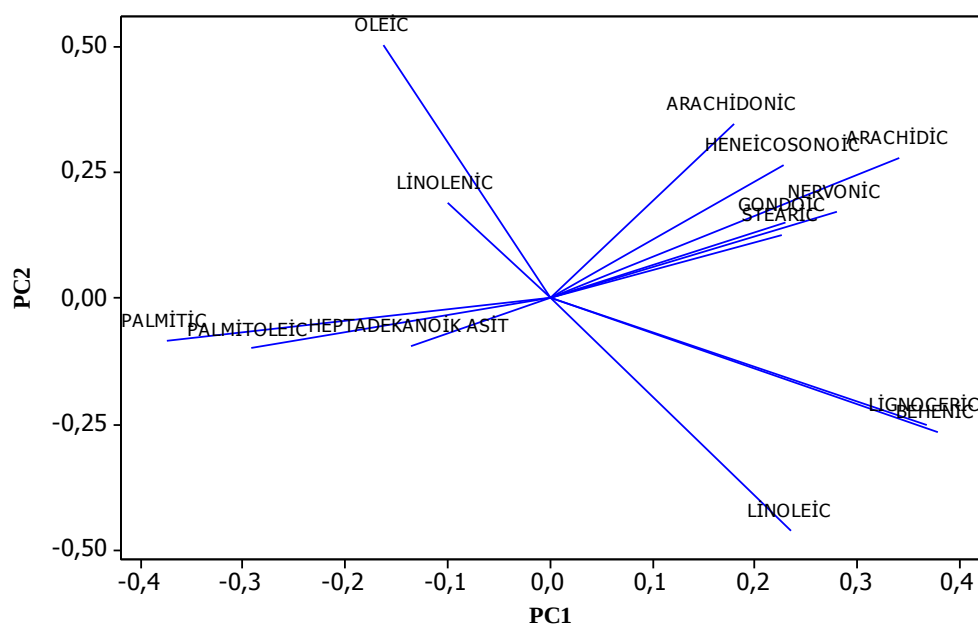


Figure 3.48. PCA loading plots of fatty acid methyl esters for adulteration.

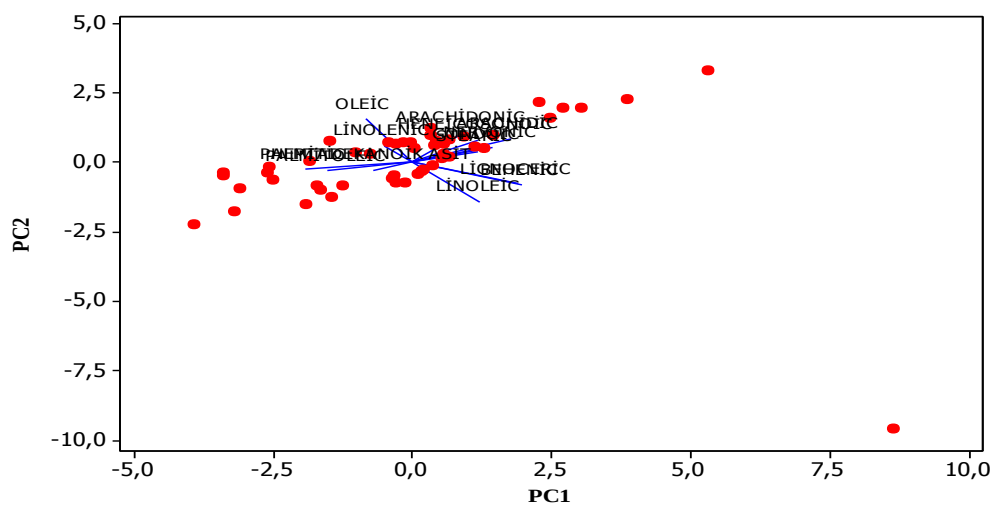


Figure 3.49. PCA biplots of fatty acid methyl esters for adulteration.

According to loading plot and biplot shown in Figure 3.48 and Figure 3.49, linoleic acid is the most important parameter for adulteration.

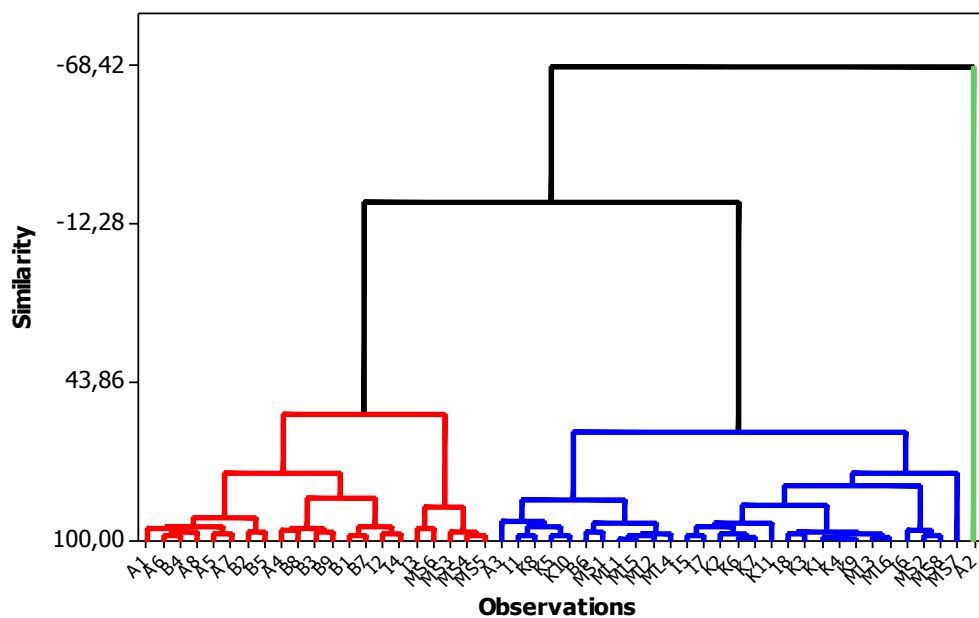


Figure 3.50. Cluster observations dendrogram of FAME for adulteration according to the ward linkage, Euclidean distance with standardize variables for geographical regions.

In the dendrograms which are drawn using fatty acid methyl ester compositions, the adulterated oil was remained outside. However a complete separation has not been provided for regional grouping. So, fatty acid methyl ester compositions are more effective for adulteration rather than for regional grouping as it can be seen in Figure 3.50.

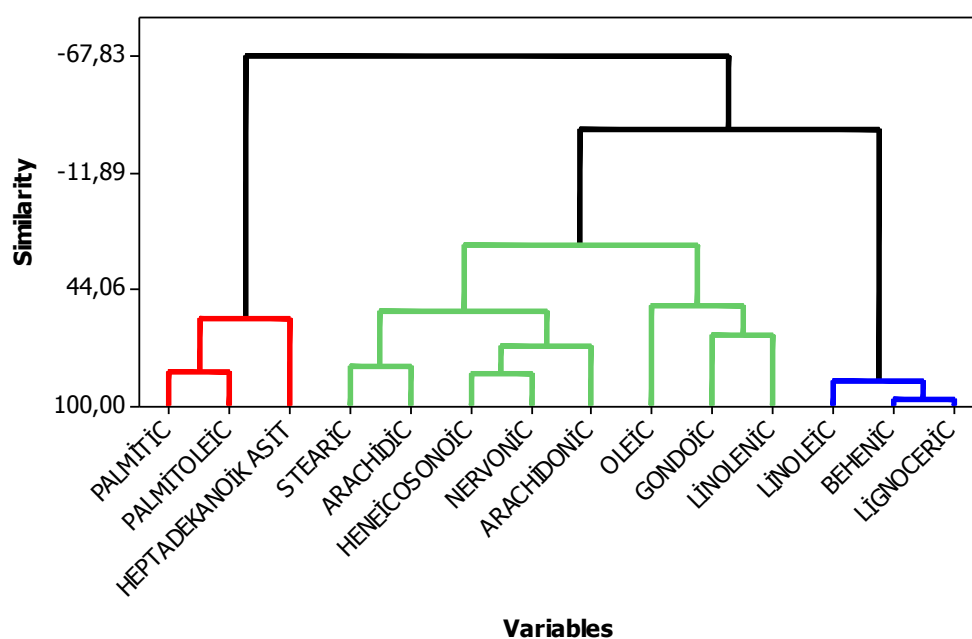


Figure 3.51. Cluster variables dendrogram of FAME for adulteration according to the ward linkage, correlation coefficient distance for variables.

Fatty acid methyl esters can not be use for authentication while FAME can easily except adulterated oil.

3.3 Results of Triacylglycerols (TAGs)

3.3.1 Column chart of TAGs compositions

The names of triacylglycerols analyzed by HPLC are listed below in Table 3.9.

Table 3.9. Abbreviations and equivalent carbon number (ECN) of fatty acid.

Fatty acids	Abbreviation	ECN
Palmitic Acid	P	16
Palmitoleic Acid	Po	14
Stearic Acid	S	18
Oleic Acid	O	16
Linoleic acid	L	14
Linolenic Acid	Ln	12

Trilinolein (LLL) is the fundamental component to identify authentication. Column chart in Figure 3.52 also supports this. As we can expected LLL is the major component for determination of adulteration. Equivalent carbon number of LLL is 42 that means three linoleic acid react with glycerol to form triacylglycerols and so ratio of LLL was increased.

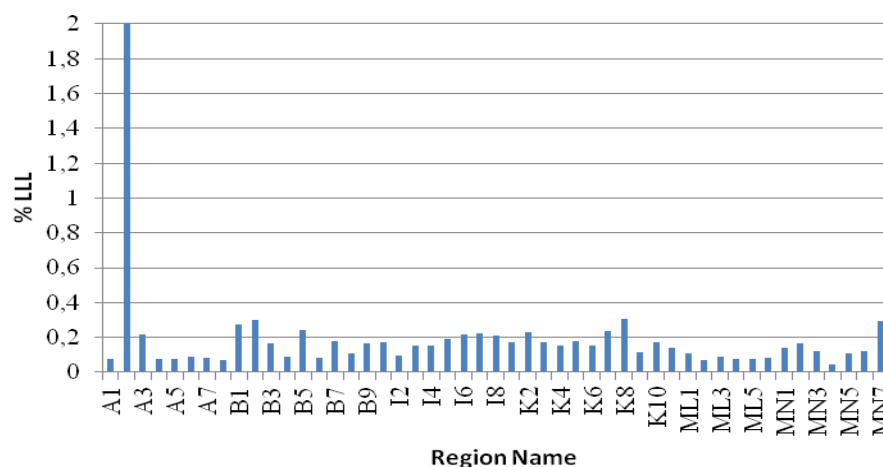


Figure 3.52. Column Chart illustration of relative percentage of LLL levels in different samples with coded regions.

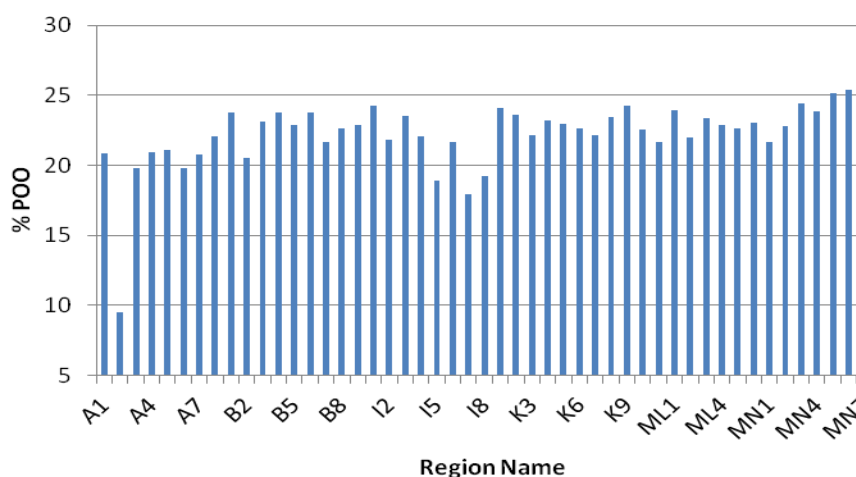


Figure 3.53. Column Chart illustration of relative percentage of POO levels in different samples with coded regions.

According to column chart illustrations of relative percentage of POP, SOO and OOO+PoPP shown in Figure 3.53, Figure 3.54 and Figure 3.55, level of all regions have generally varied similar level. Adulterated oil was shown differences from others even so POP, SOO and OOO+PoPP can not be used to determine for adulteration. These triacylglycerols have no impression on the authentication.

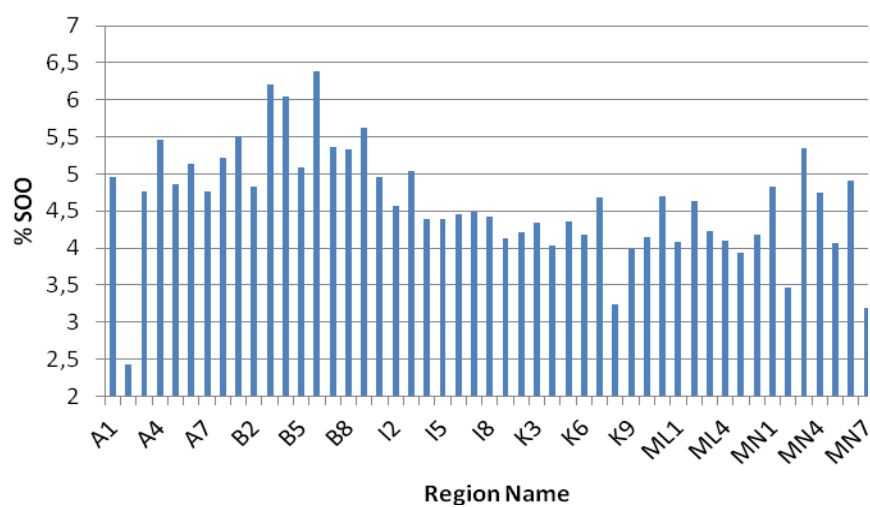


Figure 3.54. Column Chart illustration of relative percentage of SOO levels in different samples with coded regions.

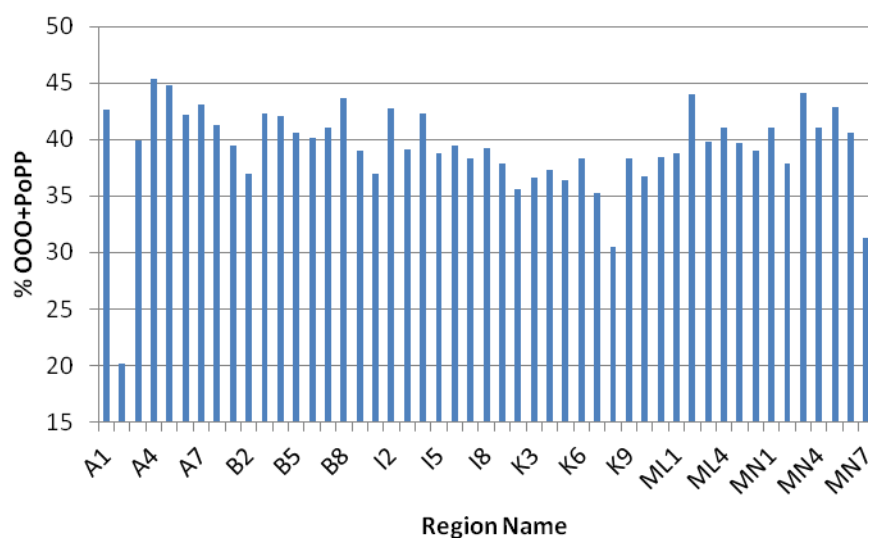


Figure 3.55. Column Chart illustration of relative percentage of OOO+PoPP levels in different samples with coded regions.

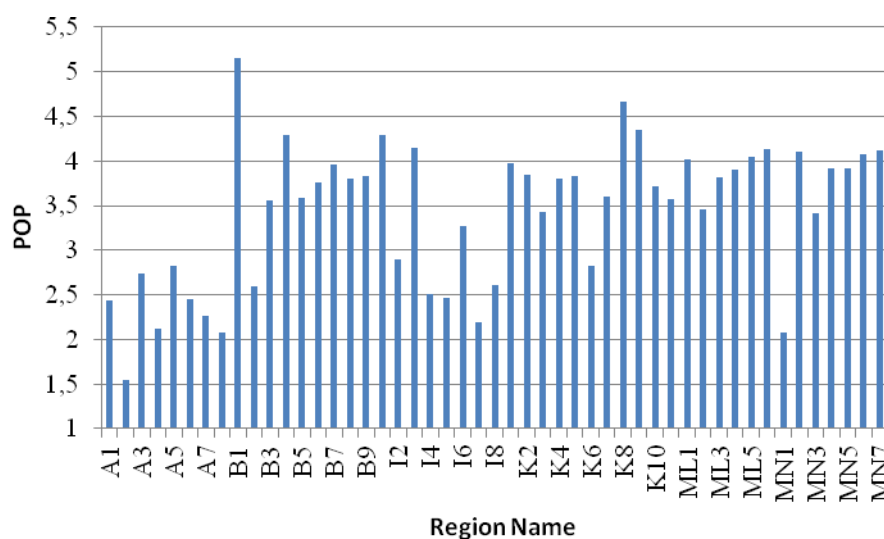


Figure 3.56. Column Chart illustration of relative percentage of POP levels in different samples with coded regions.

When we investigated column charts of OOO+PoPP, SOL, PLL+PoPoO, OLL in Figures from 3.57 to 3.60, we recognized that these triacylglycerols give knowledge to detect adulteration.

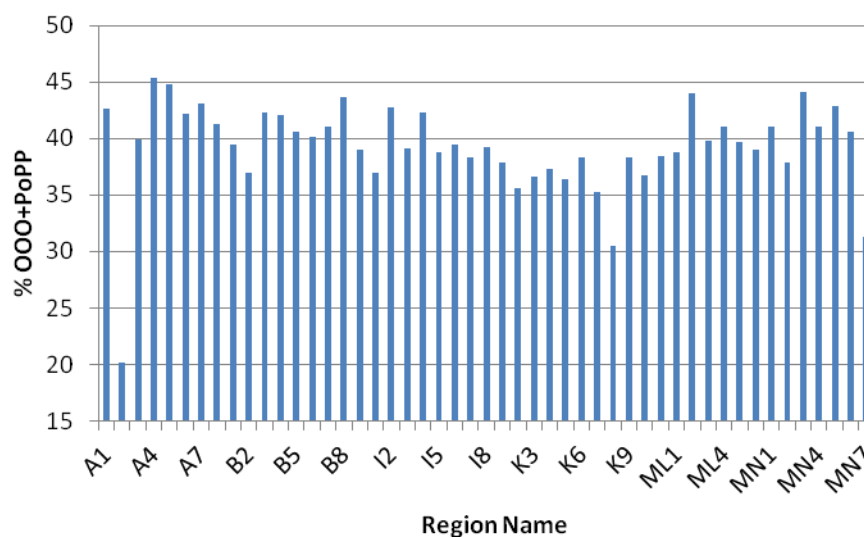


Figure 3.57. Column Chart illustration of relative percentage of OOO+PoPP levels in different samples with coded regions.

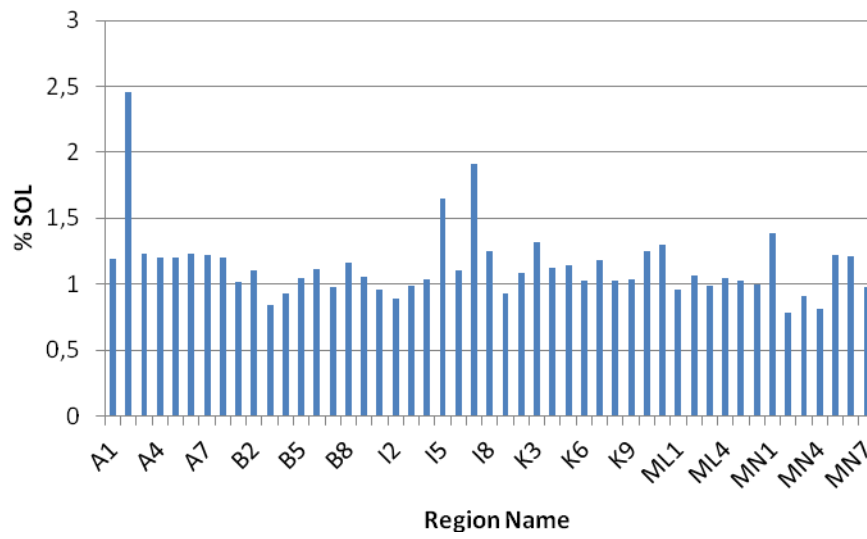


Figure 3.58. Column Chart illustration of relative percentage of SOL levels in different samples with coded regions.

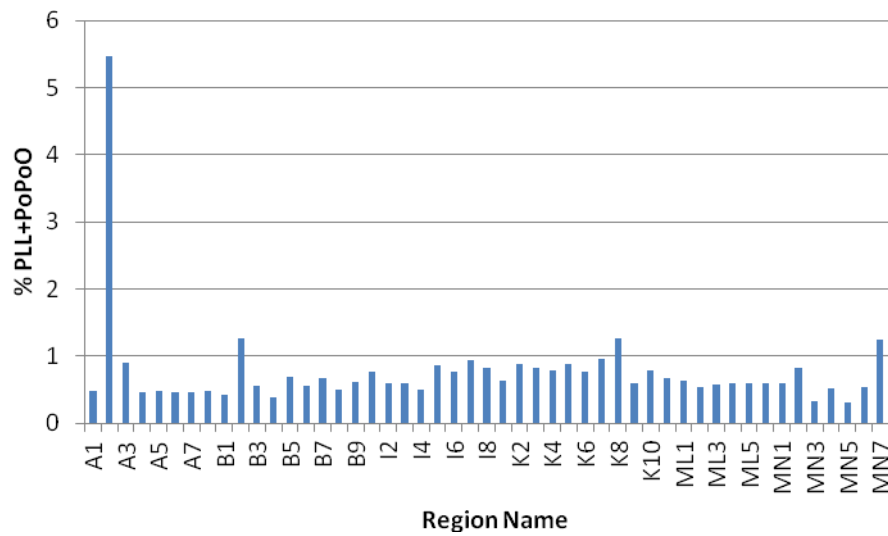


Figure 3.59. Column Chart illustration of relative percentage of PLL+PoPoO levels in different samples with coded regions.

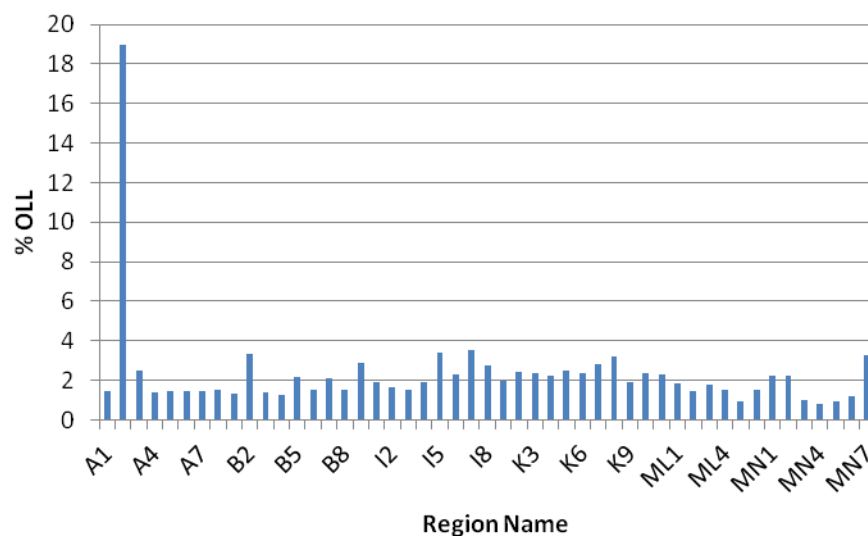


Figure 3.60. Column Chart illustration of relative percentage of OLL levels in different samples with coded regions.

Consequently we can say analysis of triacylglycerols are so important for adulteration. When we calculate the equivalent carbon number of them, we noticed that only ECN42 has no role of determination on adding seed oil to olive oil. ECN44 (OLL, PLL, PoPoO), ECN46 (PoPP) and ECN48 (SOL, OOO) have an impact factor for adulteration.

3.3.2 Correlation tables of triacylglycerols data

When we were examined correlation of relationship between each percentage triacylglycerols data in Table 3.10 shows that there was relationship between some variance of triacylglycerols compositions.

The highest correlation was among of PoOO and PoOP+SPoL+SOLn+SPoPo and so their “r” value was equal 0.875 that was not good linear correlation between them because some data was shown distribution therefore linear connection was been between some data.

As it was seen in Table 3.10 some relation was shown inverse serial correlation such as PLL+PoPoO, OLLn+PoLL, SLL+PLO between OOO+PoPP data of triacylglycerols.

Table 3.10. Correlation of relationship between each triacylglycerols data.

% percentage	LLL	OLLn+PoLL	PLLn	OLL	OOLn+PoOL	PLL+PoPoO	POLnPoPoPoPPoL	OOL+LnPP	PoOO	SLL+PLO	PoOPSPoLSOLnSPoPo	PLP	OOOPoPP	SOL	POO	POP	SOO	SOS+SLS
LLL	1,000																	
OLLn+PoLL	0,858	1,000																
PLLn	0,540	0,389	1,000															
OLL	0,766	0,794	0,341	1,000														
OOLn+PoOL	0,131	0,287	0,134	0,162	1,000													
PLL+PoPoO	0,778	0,788	0,344	0,868	0,248	1,000												
POLn+PoPoPo+PPoL	0,114	0,285	-0,024	0,087	0,254	0,349	1,000											
OOL+LnPP	0,418	0,550	0,206	0,781	0,198	0,666	-0,023	1,000										
PoOO	0,032	0,008	-0,070	0,348	0,040	-0,158	0,506	-0,652	1,000									
SLL+PLO	0,548	0,644	0,189	0,711	0,114	0,831	0,303	0,683	0,243	1,000								
PoOP+SPoL+SOLn+SPoPo	0,029	0,048	-0,163	0,232	-0,155	-0,055	0,666	-0,463	0,875	-0,046	1,000							
PLP	0,225	0,288	0,352	0,260	0,214	0,298	0,239	0,235	0,134	0,297	0,162	1,000						
OOO+PoPP	-0,703	-0,716	-0,238	0,702	-0,057	-0,830	-0,334	-0,462	0,030	-0,829	-0,147	-0,322	1,000					
SOL	0,132	0,126	0,261	0,427	-0,065	0,172	-0,288	0,550	0,452	0,153	-0,386	0,323	0,002	1,000				
POO	0,133	-0,203	-0,246	0,448	-0,209	-0,252	0,160	-0,712	0,568	-0,122	0,458	-0,102	-0,144	0,661	1,000			
POP	0,163	0,060	-0,073	0,160	-0,212	0,039	0,161	-0,477	0,477	0,051	0,430	-0,079	-0,408	0,562	0,767	1,000		
SOO	0,243	-0,419	0,004	0,371	-0,320	-0,484	-0,370	-0,462	0,057	-0,688	-0,085	-0,343	0,573	0,001	0,047	0,172	1,000	
SOS+SLS	0,087	-0,135	0,268	0,294	-0,339	-0,186	-0,087	-0,556	0,409	-0,376	0,208	-0,160	0,061	0,334	0,363	0,392	0,586	1,000

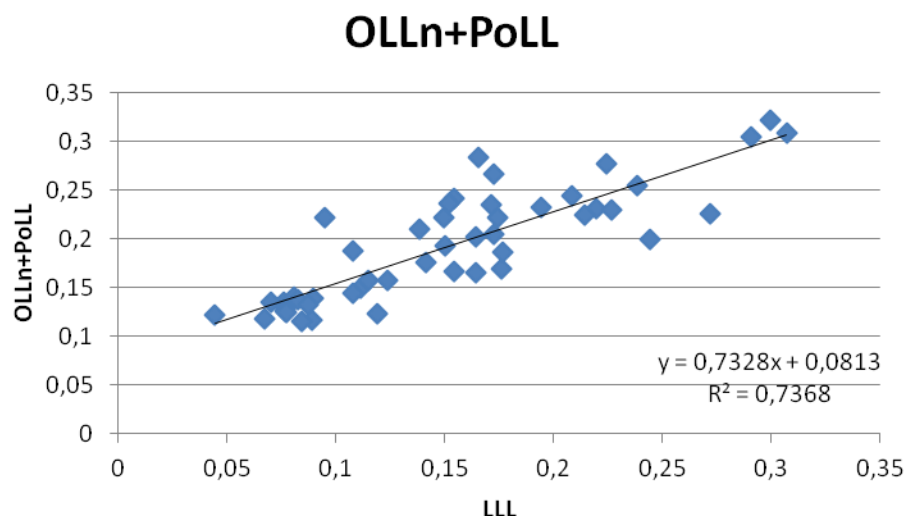


Figure 3.61. Correlation graph between percentage value of LLL vs OLLn+PoLL.

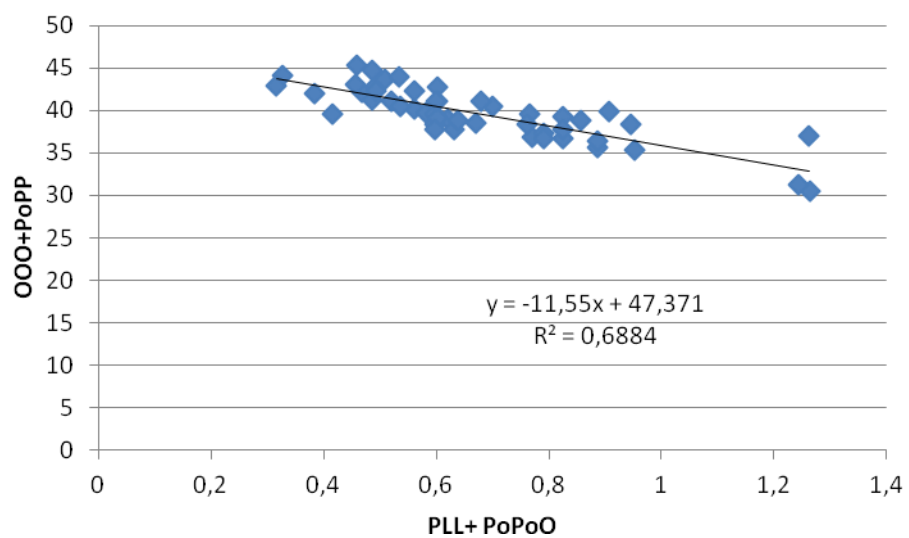


Figure 3.62. Correlation graph between percentage value of PLL+PoPoO vs OOO+PoPP.

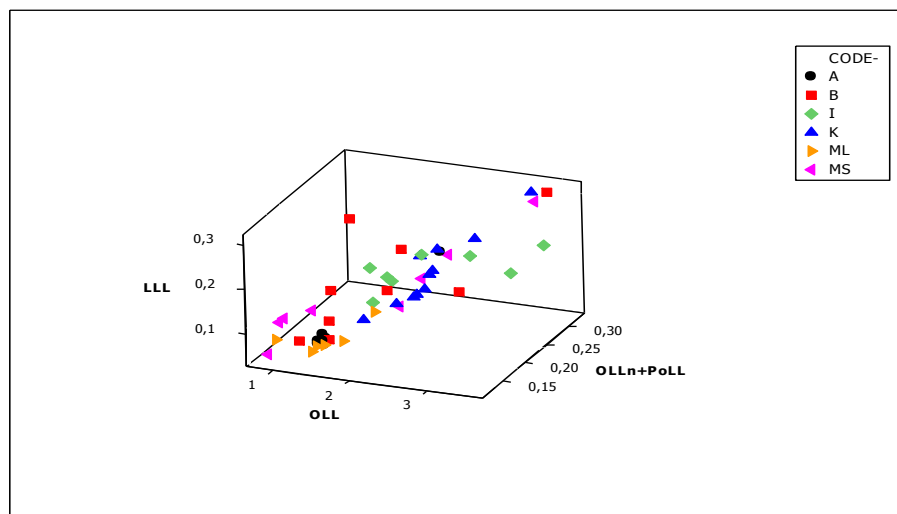


Figure 3.63. 3D Scatterplot of LLL vs OLLn+PoLL vs OLL for geographical region.

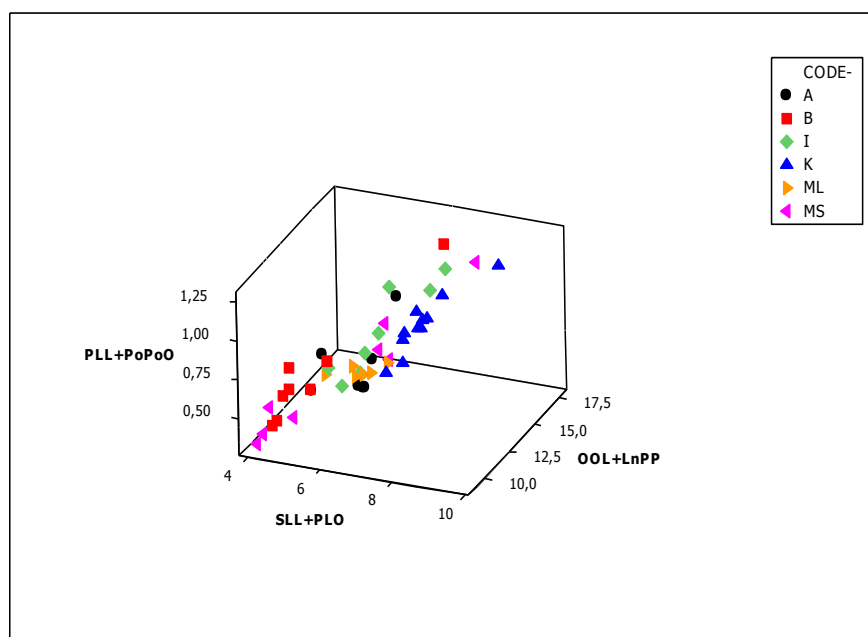


Figure 3.64. 3D Scatterplot of PLL+PoPoO vs OOL+LnPP vs SLL+PLO for geographical region.

3D scatterplot was drawn to show relation between components for authentication. This variances were selected from loading score that variances have high scores and so correlation of trinity was investigated. As it can be seen from Figure 3.63 and 3.64, 3D scatter plots of TAG compositions have not been used for authentication.

3.3.3 PCA and HCA of TAG compositions

K (Edremit Bay) and Izmir (I) were separated from other region with effect of PC1 as we have seen in Figure 3.66. Aydın (A) region was clustered under impressed both PC1 and PC2. PC1 and PC2 have a positive effect on the Mugla (ML) and Aydın (A) region's clustering.

Olive oil of B2 region was effected variance of PC1 and so it was became distant from other olive oil of same region.

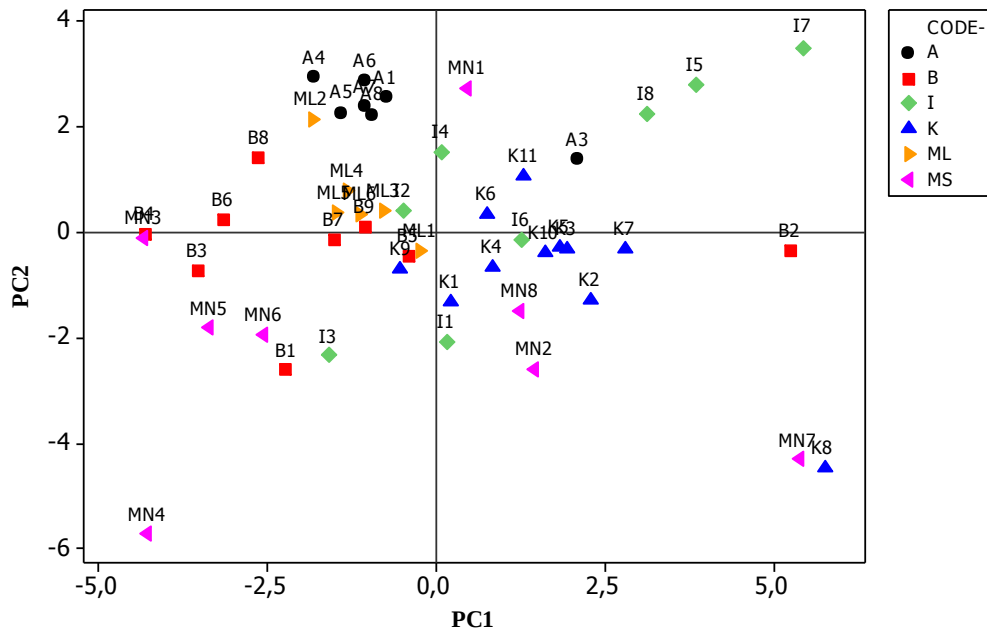


Figure 3.65. PCA score plot of TAG for geographical region.

In Figure 3.65 Score plot of TAG shows that I and MS regions have neither cluster nor formal dispersion. Olive oil of Aydın region was shown both clustering and splitting from others.

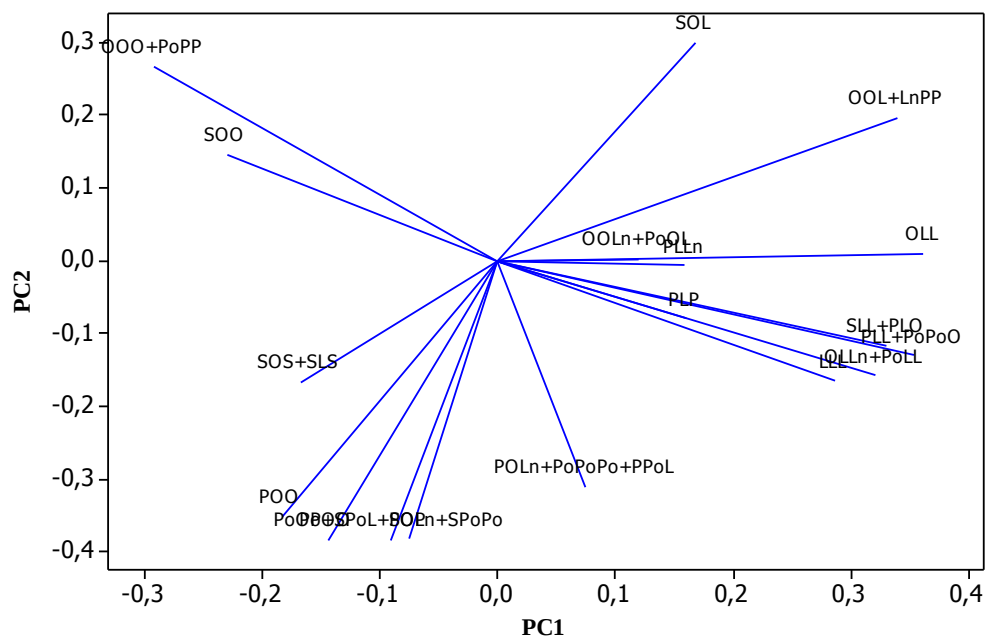


Figure 3.66. PCA loading plot of TAG for geographical region.

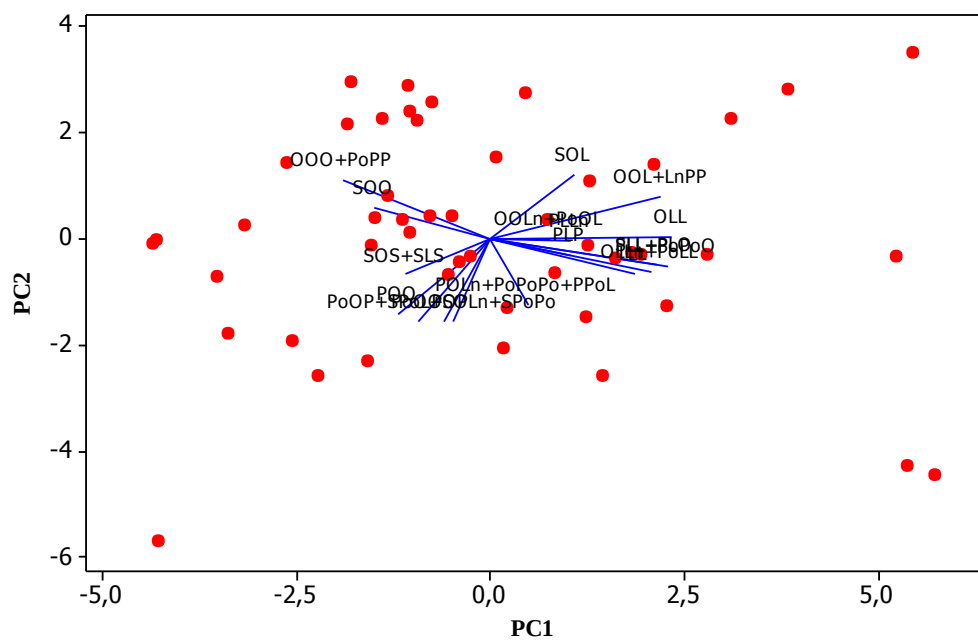


Figure 3.67. PCA biplot of TAG for geographical region.

OOl+LnPP was most effective component on the İzmir while OOLn+PoOL, PLP and PLLn on K region. ML region has influenced from OOO+PoPP and SOO variance as we can see in Figure 3.66.

In Figure 3.68 and Table 3.11, percent eigen value first six are shown. According to cumulative percentage first six parameters was effected on the parting of regions.

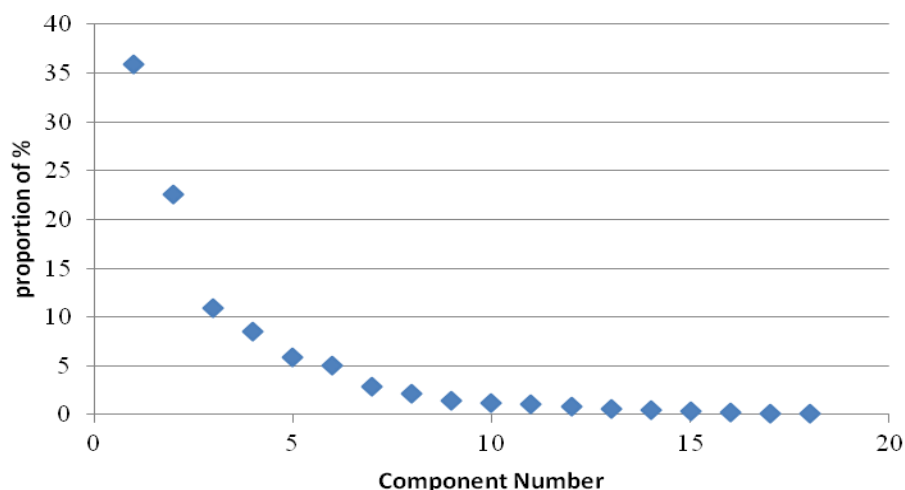


Figure 3.68. Proportional % eigen values vs component number for TAG variances.

In spite of PC1 has 35,96 % proportion that means PC1 was important variance but there is no powerful effect on the clustering.

Results of PCA and HCA analyses show a good discrimination between varieties according to phenol, triacylglycerol and sterol data. These components seem to be an effective tool to discriminate between the varieties. The presence of a high 1,2,3-triolelylglycerol (OOO) level in olive oil constitutes a favorable authenticity indicator. Triacylglycerol composition can also be used as a measurement of the quality and purity of vegeoils (Manai-Djebali et al., 2012).

Table 3.11. Percent eigen values of TAG.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	6,472	35,955	35,956
2	4,060	22,557	58,512
3	1,961	10,897	69,409
4	1,542	8,565	77,974
5	1,048	5,822	83,796
6	0,913	5,073	88,869
7	0,518	2,878	91,747
8	0,378	2,102	93,849
9	0,259	1,439	95,288
10	0,209	1,163	96,451
11	0,183	1,019	97,470
12	0,142	0,787	98,257
13	0,101	0,558	98,816
14	0,083	0,463	99,279
15	0,063	0,352	99,631
16	0,036	0,200	99,831
17	0,020	0,111	99,942
18	0,010	0,058	100,000

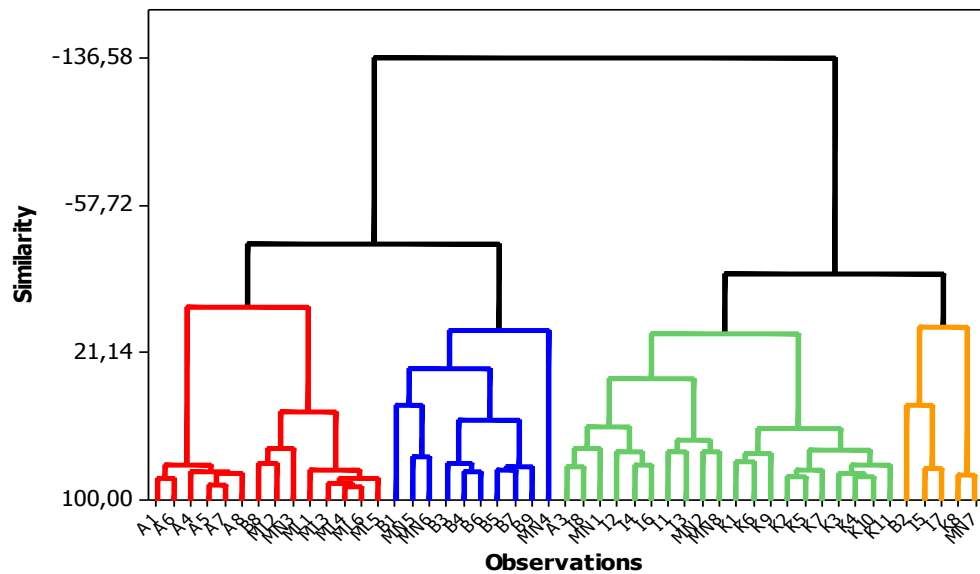


Figure 3.69. Cluster observations dendrogram of TAG according to the ward linkage, Euclidean distance with standardize variables for geographical regions.

Table 3.12. Cluster observations and similarity of geographical regions according to dendrogram of TAG.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	14	AYDIN (A) and MUĞLA (ML)
ORANGE	5	B2, I5, I7, K8, MN7
GREEN	20	EDREMIT BAY (K) and some region of MS and I
BLUE	10	BURSA (B) and some regions of MS.

The data were also evaluated by cluster analysis. These data were also auto-scaled using the ward linkage method as shown in Figure 3.69. There was three cluster for each metod. Aydın (A), Edremit bay (K) region and Muğla (ML) were clustered as regards ward linkage metod.

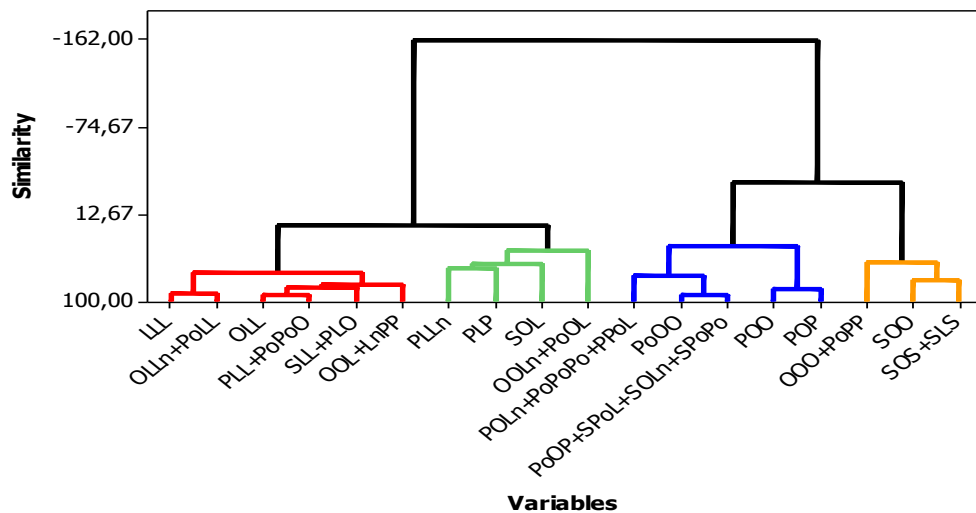


Figure 3.70. Cluster variables dendrogram of TAG according to the ward linkage, correlation coefficient distance for variables.

Table 3.13 Cluster variables and similarity of geographical regions according to dendrogram of TAG.

CLUSTER	VARIABLES	SIMILARITY
RED	6	LLL, OLLn+PoLL, OLL, PLL+PoPoO, SLL+PLO, OOL+LnPP
ORANGE	3	OOO+PoPP, SOO, SOS+SLP
GREEN	4	PLLn, PLP, SOL, OOLn+PoOL,
BLUE	5	POLn+PoPoPo+PpoL, PoLL, PoOP+SPoL+SOLn+SPoPo, POO,POP

TAGs have not important role for authentication as we can seen all plots and dendrogram of TAG.

Fatty acid or triglyceride composition data have been previously used for the determination of the geographical origin of olive oils They concluded that both parameters may be successfully used for the differentiation of geographical origin, cultivar and stage of maturity when combined with chemometric analysis. Zeiner et al. (2005) determined trace element content has been successfully used for the determination of the geographical origin of olive oils. Benincasa et al. (2007) used ICP-MS to determine 18 elements in olive oils from four different regions in Italy (Calabria, Apulia, Umbria and Abruzzo). Their results showed that application of chemometrics methods to trace element data achieved satisfactory differentiation of olive oils according to geographical origin.

3.3.4 PCA and HCA of TAG compositions for North-South

As it can be seen in Figure 3.71, the regions were dispersed as north and south according to PCA analyses of TAG analyses results.

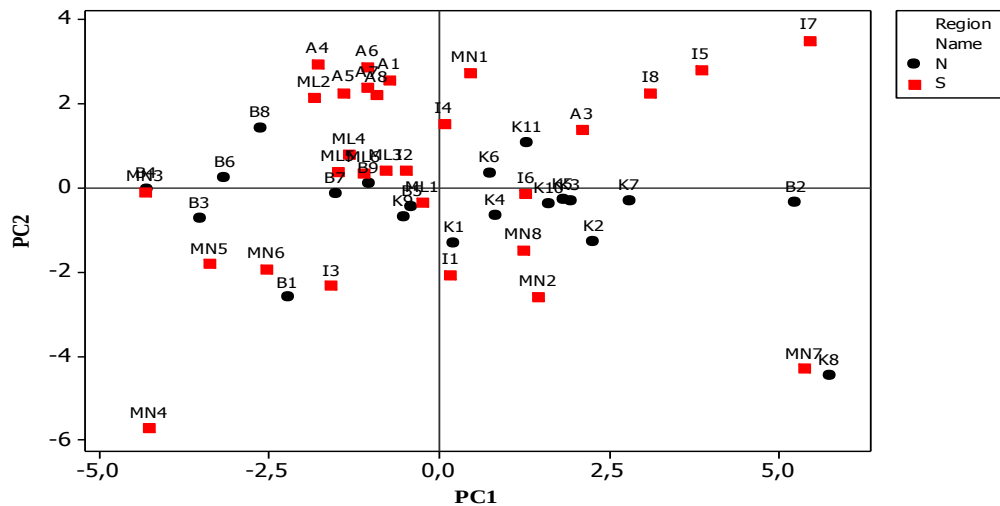


Figure 3.71. PCA score plot of TAG for North-South.

While PC2 has effected two region, impression of PC1 was not noticed on the regions.

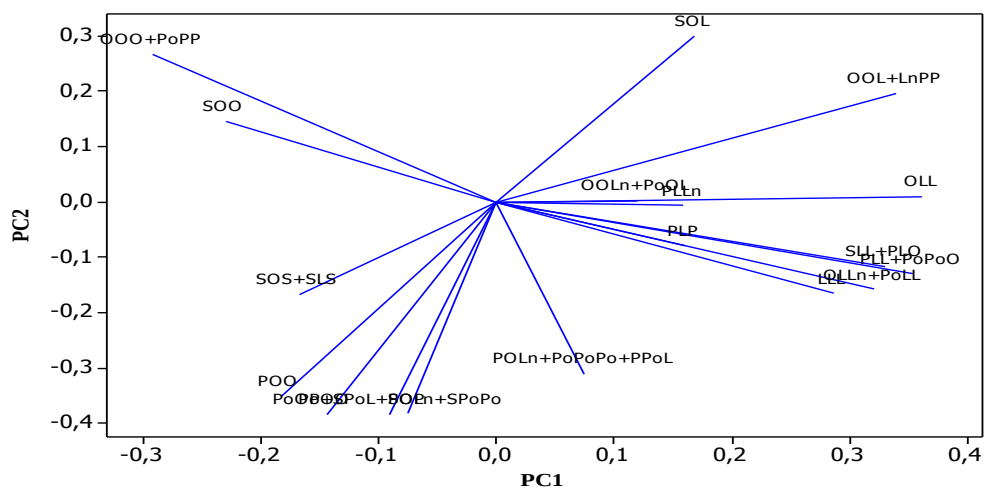


Figure 3.72. PCA loading plot of TAG for North-South.

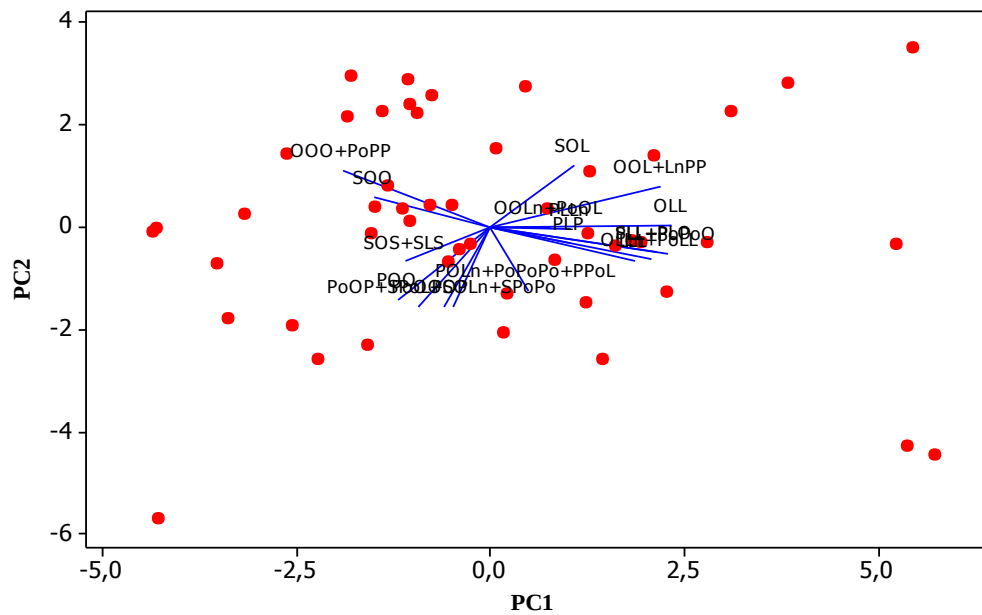


Figure 3.73. PCA biplot of TAG for North-South.

We have found that OOO+PoPP, SOL, OOL+LnPP AND SOO have the most important variable for the differentiation of the region of south according to loading and biplot graph in Figure 3.72 and Figure 3.73.

According to ward linkage methods dendograms in Figure 3.74, it was not possible to see clustering clearly in north and south olive oils.

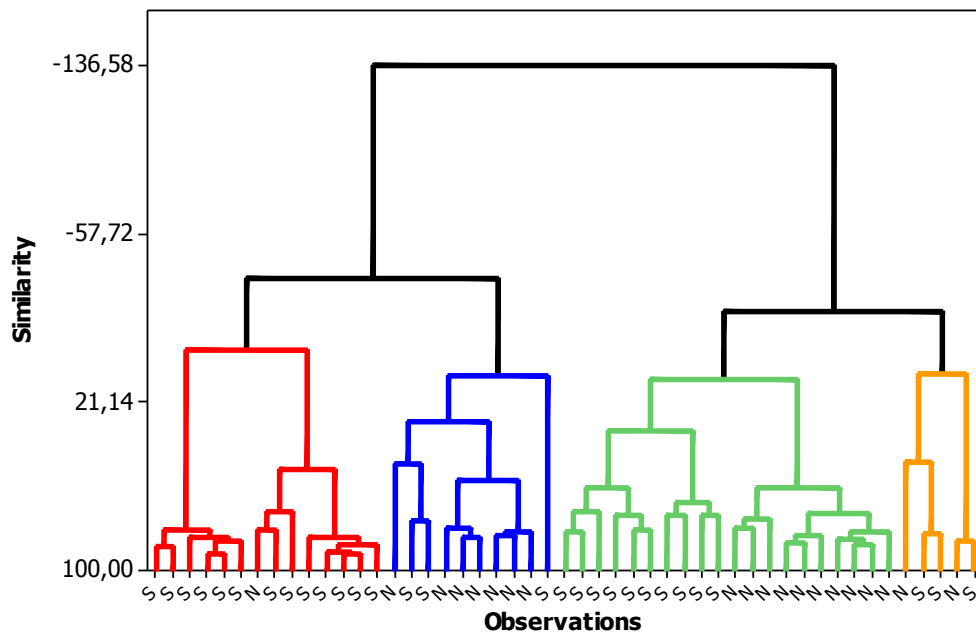


Figure 3.74. Cluster observations dendrogram of TAG for North-South according to the ward linkage, Euclidean distance with standardize variables for geographical regions.

Hyerarchical cluster analysis with single and ward method were supplied score plot graph. Some south and north regions are clustered but it was not clearly distinction.

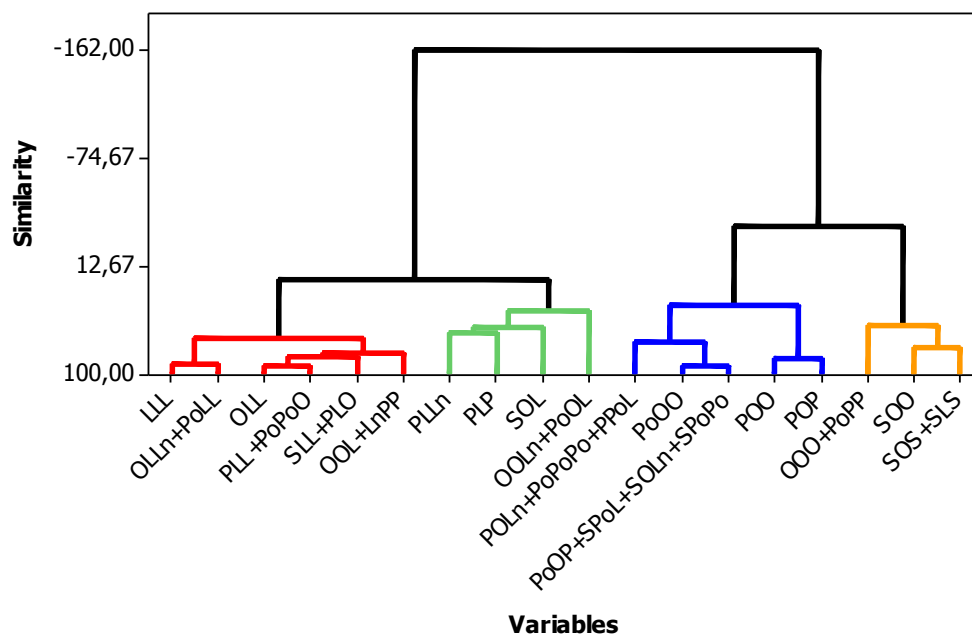


Figure 3.75. Cluster variables dendrogram of TAG of North-South according to the ward linkage, correlation coefficient distance for variables.

3.3.5 PCA and HCA of TAG compositions for adulteration

LLL is explained adulteration and purity of olive oil and LLL is singly sufficient for adulteration.

Values obtained from fatty acid methyl ester analysis and values obtained from HPLC triacylglycerol (TAG) analysis were placed onto a table. The results have shown that LLL ratio is the most effective component to identify adulteration. According to dendograms obtained with cluster analyses, regions were grouped and the olive oil with seed oil addition was excluded.

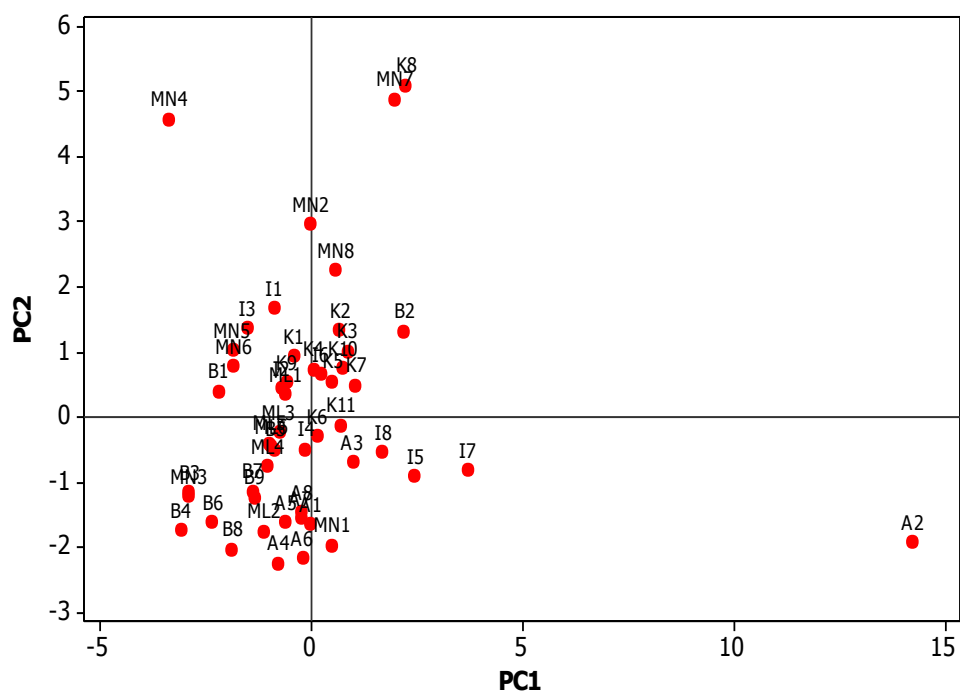


Figure 3.76. PCA score plot of TAG for adulteration.

According to score and loading graphs in Figure 3.76 and Figure 3.77, they could be said that trilinolein (LLL) is the fundamental component to identify authentication.

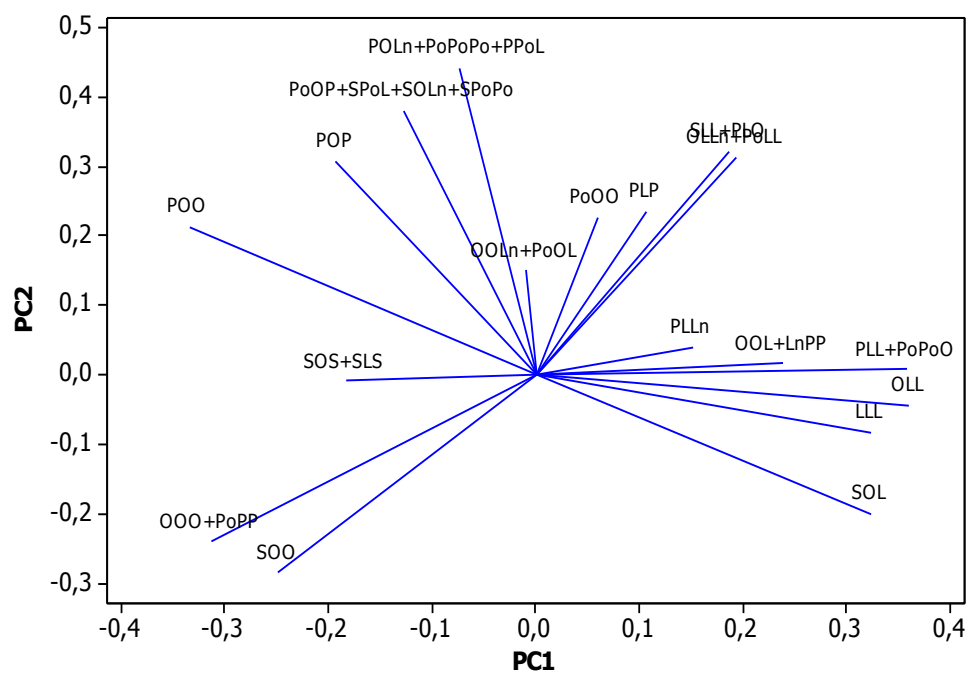


Figure 3.77. PCA loading plot of TAG for adulteration.

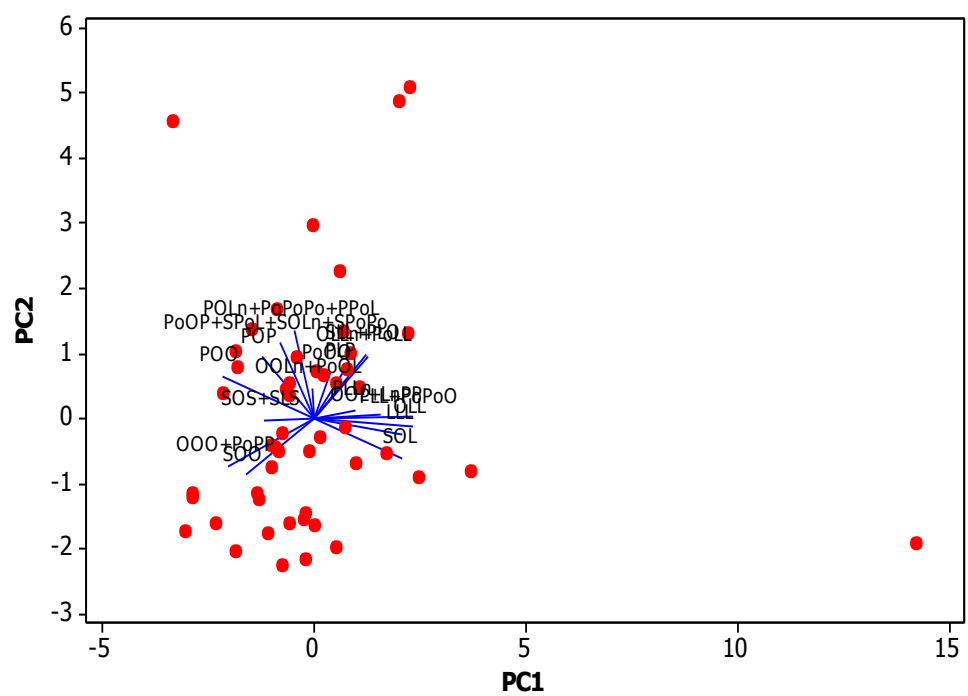


Figure 3.78. PCA biplot of TAG for adulteration.

Impressure of LLL (ECN42) variance was shown in Figure 3.77 and Figure 3.78 for authentication effect of OLL ve PLL+PoPoO (ECN44) were investigated as a result of this ECN44 was important variance to find adding seed oil into olive oil too.

According to the dendrogram plotted using ward method in Figure 3.79, the adulterated oil (A2) had been excluded clearly.

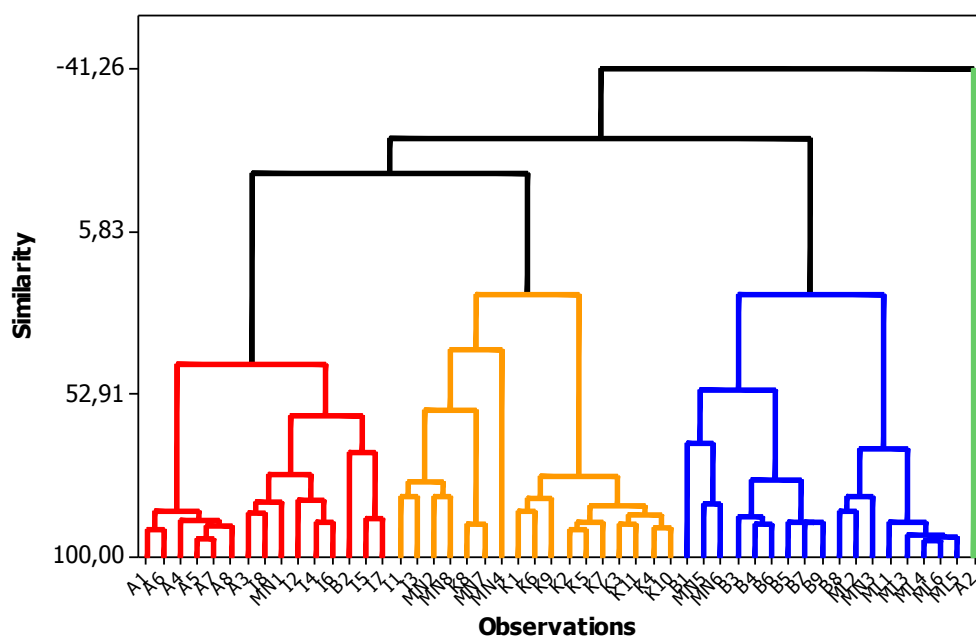


Figure 3.79. Cluster observations dendrogram of TAG for adulteration according to the ward linkage, Euclidean distance with standardize variables for geographical regions.

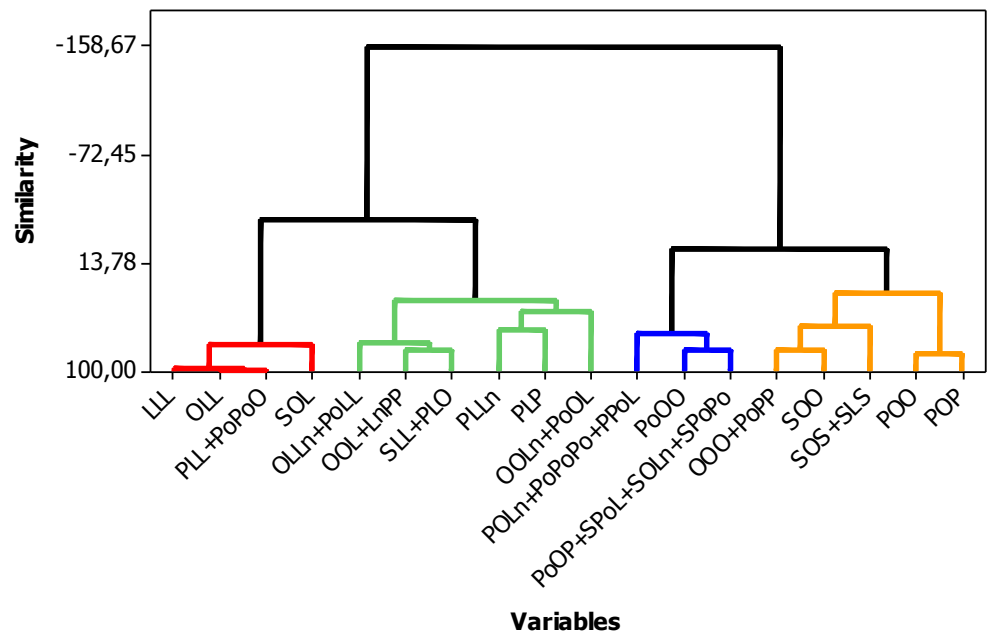


Figure 3.80. Cluster variables dendrogram of TAG for adulteration according to the ward linkage, correlation coefficient distance for variables

3.4 Results of Trace Elements

3.4.1 Column chart of trace elements data

According to column charts that were drawn for Fe56 and Zn results obtained with heavy metal analysis, it was seen that they are more effective for regional grouping individually in Figure 3.81 and 3.82. It means that Fe56 and Zn are important for determination of authenticity.

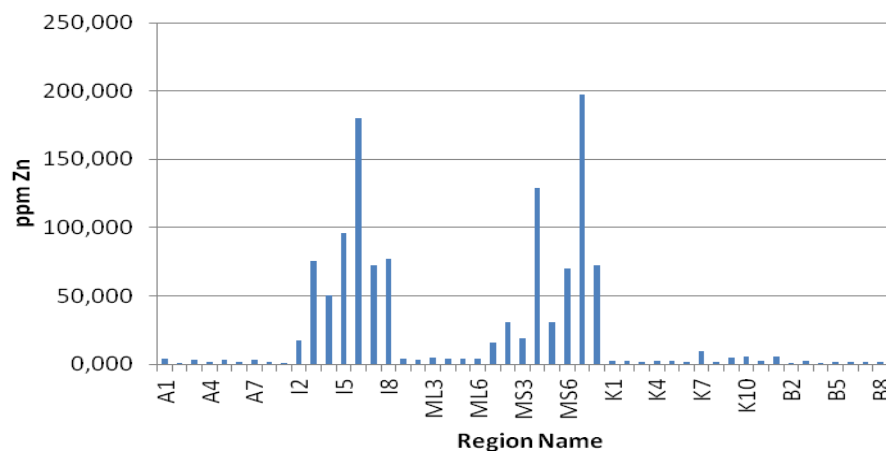


Figure 3.81. Column Chart illustration of concentration of Zn levels in different samples with coded regions.

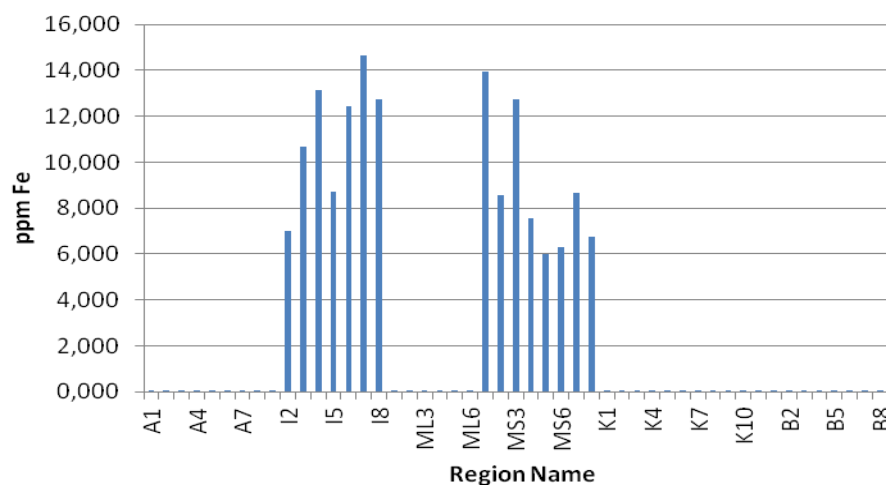


Figure 3.82. Column Chart illustration of concentration of Fe levels in different samples with coded regions.

3.4.2 Correlation tables of trace elements data

When we were analyzed correlation of relation between each concentration of trace elements data in Table 3.14 shows that there was correlation between some variance of triacylglycerols compositions.

The highest correlation was among of Ba and Pb and so their “r” value was equal 0.856 that was not good linear correlation between them because some data was shown distribution therefore linear connection was been between some data.

As it was seen in Table 3.14 some relations were shown inverse serial correlation for example between Mg and Fe, As and Fe, As and Zn data of triacylglycerols.

When we investigated correlation tables of sterol compositions, fatty acid methyl esters, triacylglycerols and trace elements there was no meaningful correlation between each data therefore column chart of adjudicated variances was plotted to explain authentication and adulteration by this way sequestered effect of variances was seen on the classification of geographical regions and adding seed oil to olive oil with drawing column chart.

Table 3.14. Correlation of relationship between each trace elements data.

	<i>V</i>	<i>Cr</i>	<i>Mn</i>	<i>Co</i>	<i>Ni</i>	<i>Cu</i>	<i>Zn</i>	<i>As</i>	<i>Ba</i>	<i>Pb208</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Ca</i>	<i>Fe56</i>
V	1,000														
Cr	0,288	1,000													
Mn	-0,151	0,128	1,000												
Co	0,189	-0,069	-0,037	1,000											
Ni	0,593	0,059	-0,078	0,176	1,000										
Cu	-0,295	0,049	0,565	0,164	-0,109	1,000									
Zn	-0,491	0,581	0,273	-0,183	-0,352	0,429	1,000								
As	0,674	0,049	-0,118	0,126	0,491	-0,319	-0,536	1,000							
Ba	0,022	0,104	0,459	0,597	0,155	0,409	0,186	-0,052	1,000						
Pb208	-0,070	0,240	0,574	0,559	0,076	0,492	0,378	-0,128	0,856	1,000					
Na	0,468	0,269	0,061	0,098	0,294	-0,300	-0,162	0,452	0,181	0,089	1,000				
Mg	0,822	0,025	-0,110	0,106	0,603	-0,329	-0,660	0,745	-0,023	-0,175	0,505	1,000			
K	0,169	0,145	0,402	0,121	0,029	0,022	0,100	0,034	0,559	0,421	0,243	0,073	1,000		
Ca	-0,350	0,289	0,347	-0,125	0,009	0,467	0,679	-0,449	0,265	0,346	-0,137	-0,454	0,081	1,000	
Fe56	-0,680	0,069	0,311	-0,099	-0,443	0,456	0,693	-0,683	0,133	0,288	-0,510	-0,836	-0,072	0,680	1,000

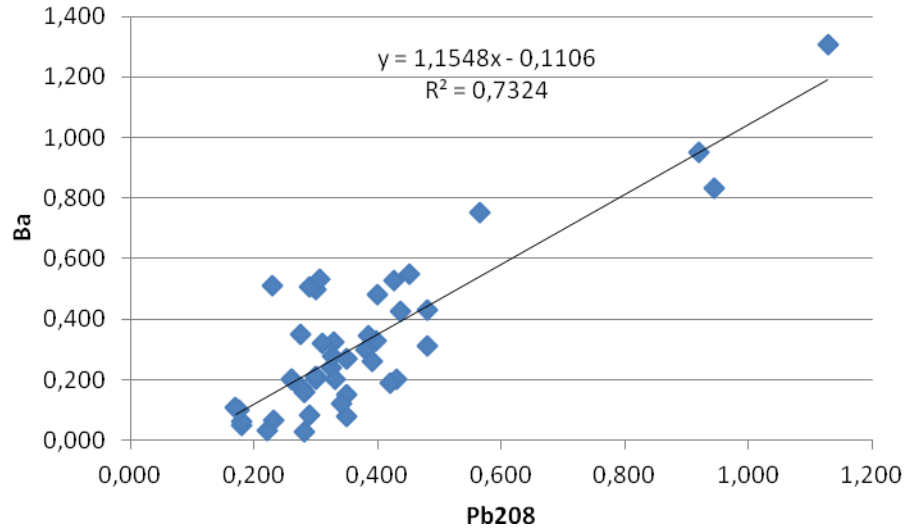


Figure 3.83. Correlation graph between percentage value of LLL vs OLLn+PoLL.

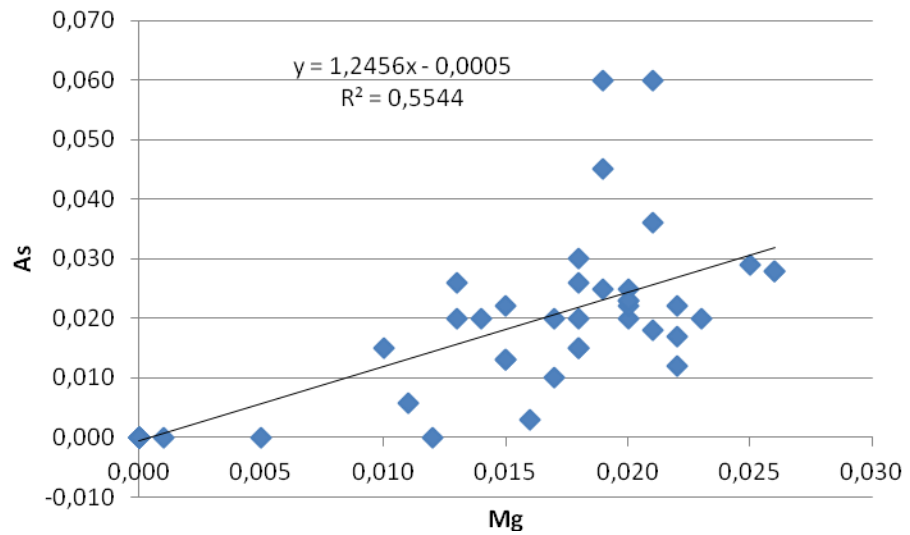


Figure 3.84. Correlation graph between percentage value of LLL vs OLLn+PoLL.

3D scatterplot was drawn to show relation between components for authentication. This variances were selected from loading score that variances have high scores and so correlation of trinity was investigated. As it can be seen from 3D scatter plots in Figure 3.85 to Figure 3.87, trace elements compositions can be used for authentication especially Manisa and İzmir.

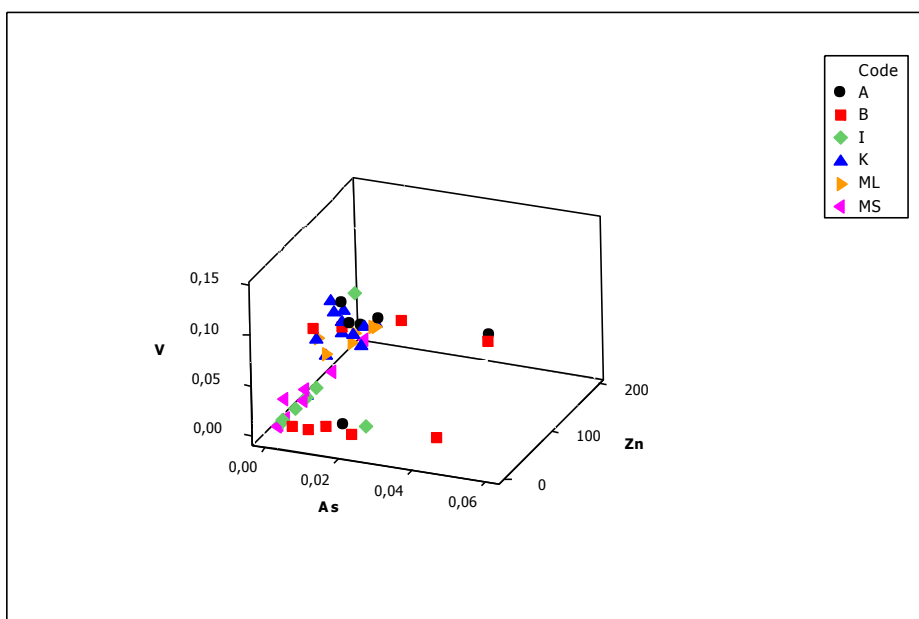


Figure 3.85. 3D Scatterplot of V vs Zn vs As for geographical region

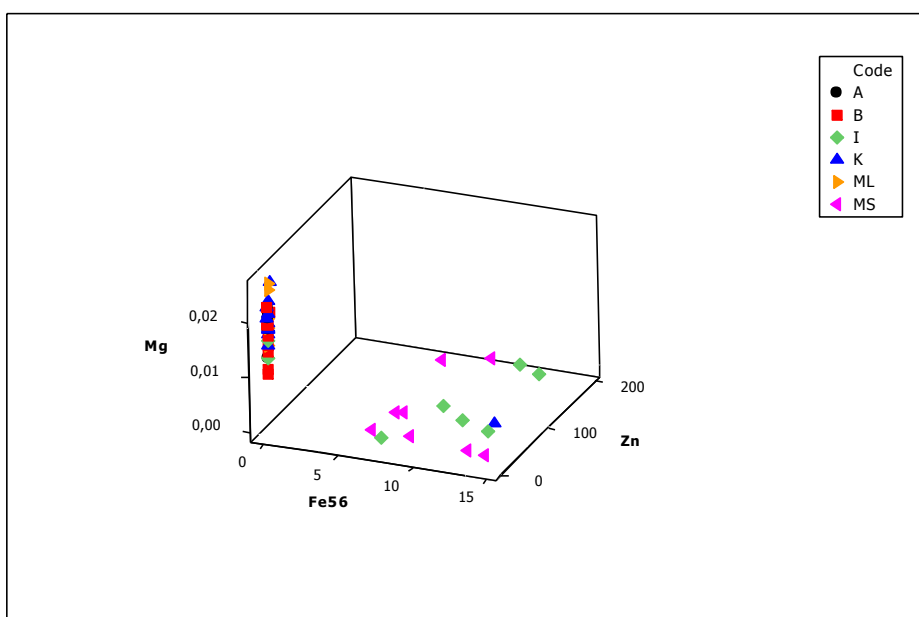


Figure 3.86. 3D Scatterplot of Mg vs Zn vs Fe for geographical region

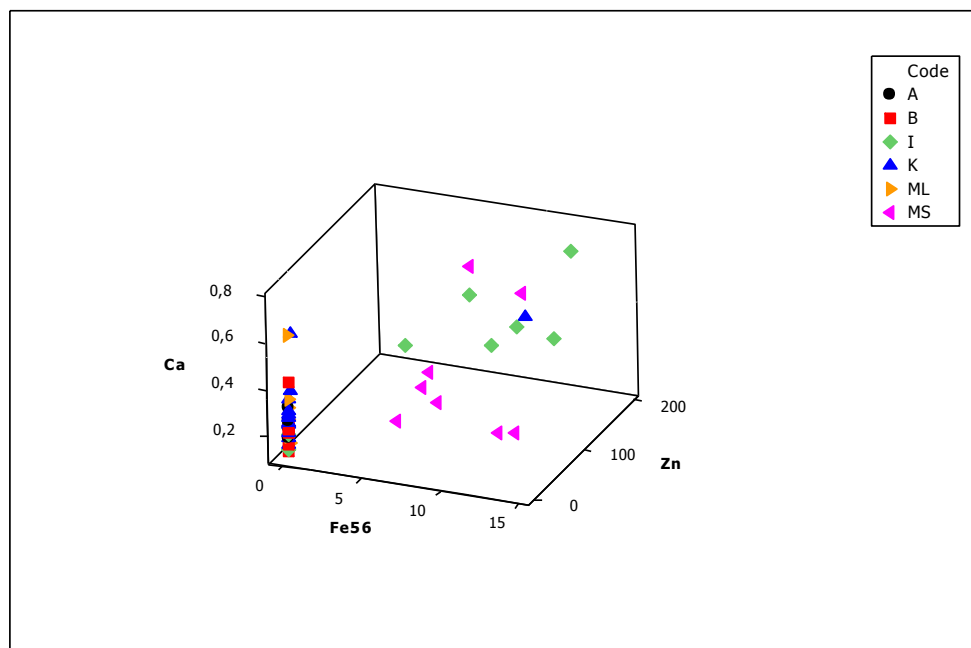


Figure 3.87. 3D Scatterplot of Ca vs Zn vs Fe for geographical region

The geographical traceability of virgin olive oil can be controlled by chemical species that are linked to the production area. Trace elements are among these species. The hypothesis is that the transfer of elements from the soil to the oil is subjected to minor variations and therefore this chemical information can be used for geographical traceability. The analysis of elements by ICP-MS might be a good technique for performing the backward traceability of olive oils. The coincidence in the selection of the elements for classifying soils olive oils in accordance with the geographical provenance indicates that some elements present in the soils of the orchards might also be detected in the olives and olive oils from the olive trees planted there, though at very lower concentrations (Beltran et al. 2015).

3.4.3 PCA and HCA for trace elements

According to PCA and HCA performed, İzmir and Manisa had been formed a group, the other regions had been formed another group. Score plot in Figure 3.88 was supported this result.

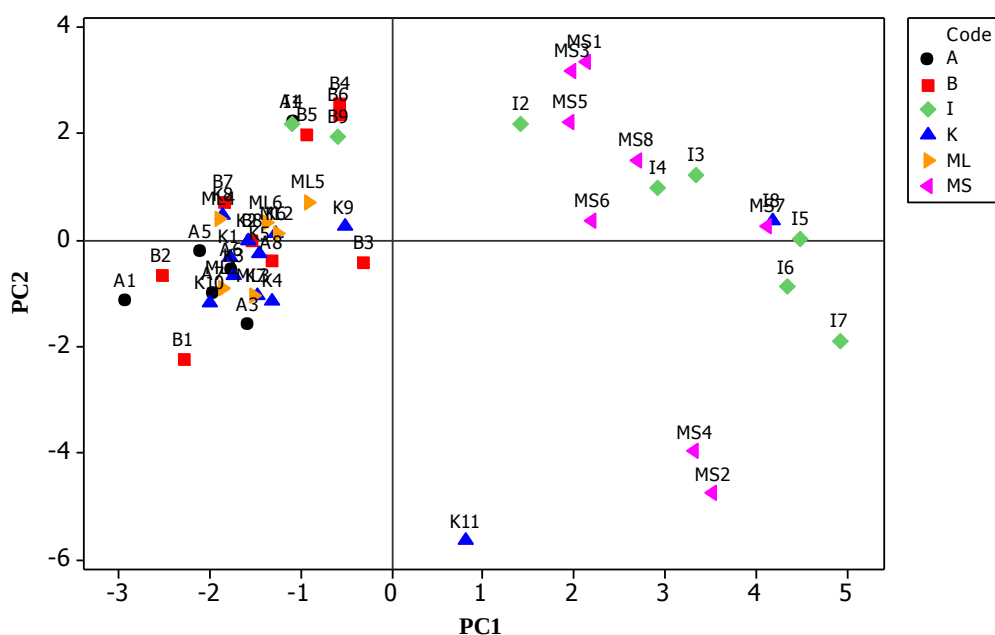


Figure 3.88. PCA score plot of trace elements for each region.

PC1 are most effective variety for the separate MS and I than others. PC2 was come into play important role on others regions especially K and ML region's olive oil are clustered.

K11 was separated from others olive oils of same region. When we looked maps of Turkey region of K11 was present on the piedmont of Kaz on the other hand Manisa region has effected by mountain of Spil. İzmir and Manisa were very close neighbour city therefore content of trace elements of İzmir region was shown similarity with Manisa especially on the variances of Fe and Zn.

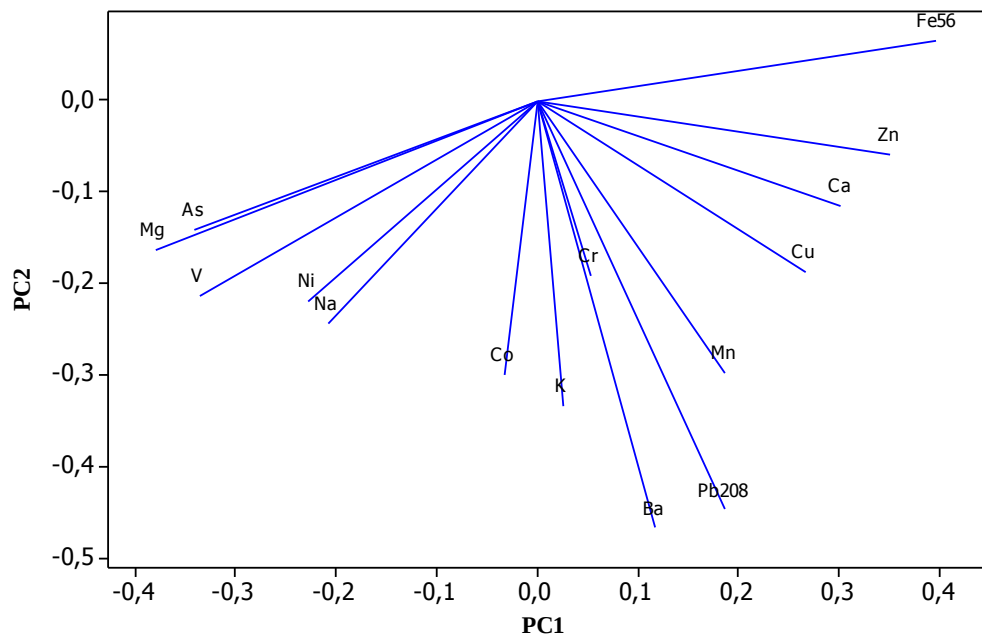


Figure 3.89. PCA loading plot of trace elements for each region.

According to score plot graph MS and I regions are part from others regions nevertheless loading plot graph showed that MS are more affected from Mn and Pb208 while I region are impressed Cu and Ca trace elements.

Na, Mg and As are effected “Muğla” region’ olive oil. Zn and Fe56 are most important parametres for “İzmir” and “Manisa” according to biplot graph in Figure 3.90. However, the existence of Ca and Cu trace elements might apparently affect on distribution of biplots constructed between first and second components. Figure 3.90. when it, further ,was investigated the Loading plots, it can be seen that this distribution, particularly the accumulation of data for regions ML, A B and K were affected by the trace elements levels such as Mg, V, As Ni and Na levels within the olive oil seen loading plot clearly in Figure 3.89.

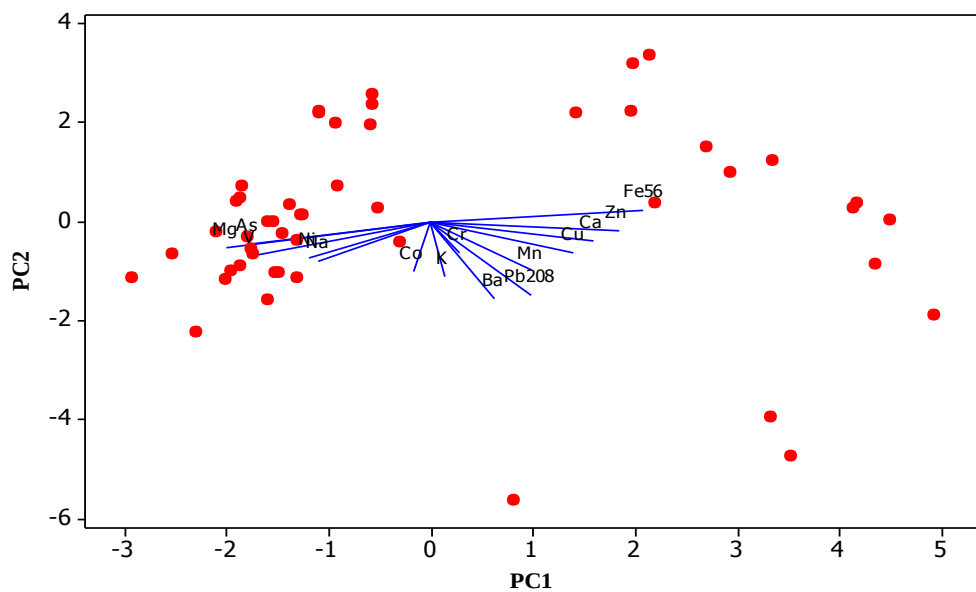


Figure 3.90. PCA biplot of trace elements for each region.

Proportion of percent and cumulative percentage of eigen values were shown in Table 3.15.

Table 3.15. Percent eigen values of trace elements.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	5,250	34,998	34,998
2	3,322	22,149	57,147
3	1,660	11,068	68,215
4	1,089	7,260	75,476
5	1,007	6,712	82,187
6	0,695	4,634	86,821
7	0,574	3,824	90,645
8	0,355	2,368	93,013
9	0,289	1,924	94,937
10	0,274	1,828	96,765
11	0,167	1,110	97,875
12	0,129	0,860	98,735
13	0,092	0,611	99,346
14	0,069	0,459	99,805
15	0,029	0,195	100,000

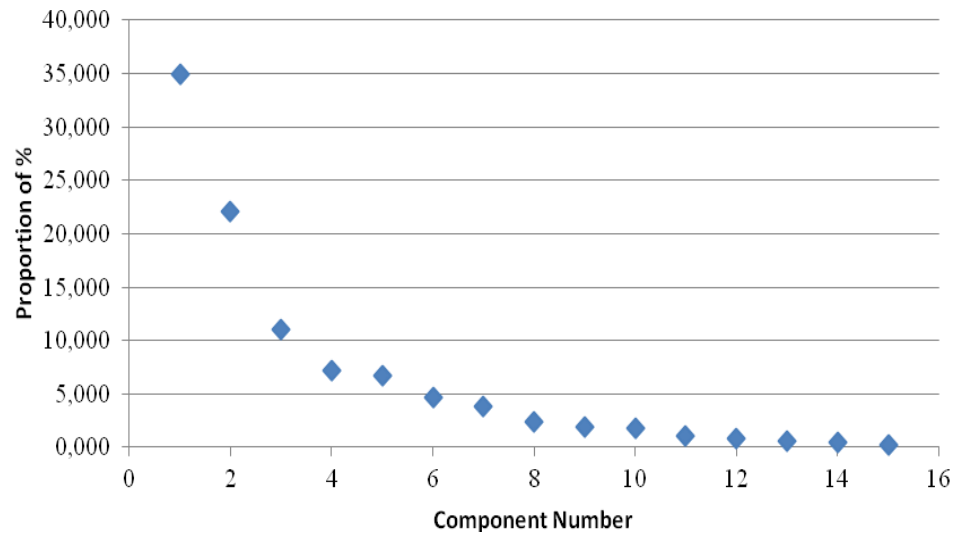


Figure 3.91 Proportional % eigen values vs component number for trace elements variances.

When we make analysis about trace elements results we noticed MS and I region olive oils have different properties than others and MS and I are grouped according to PCA regard as impacts of Fe56, Zn and Ca was clearly shown in score plots of principal components analysis.

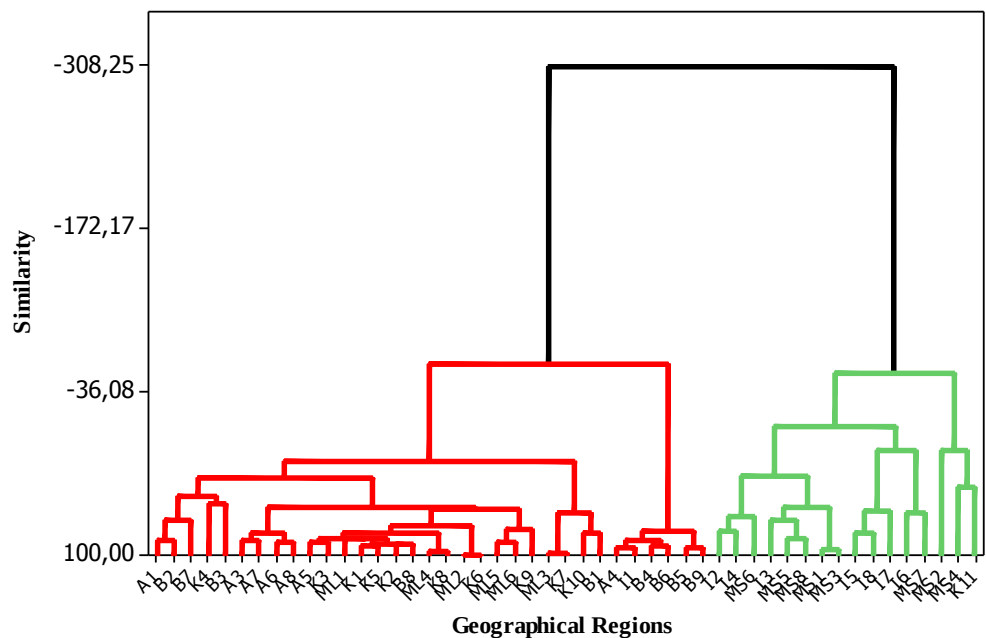


Figure 3.92. Cluster observations dendrogram of trace elements according to the ward linkage, Euclidean distance with standardize variables for geographical regions.

Manisa (ML) and İzmir (I) region's olive oils are separated from others region according to ward linkage method heavy metal dendrograms in Figure 3.92.

Table 3.16. Cluster observations and similarity of geographical regions according to dendrogram of trace elements.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	23	Rest of other regions.
GREEN	16	MANISA (MS) and IZMIR (I)

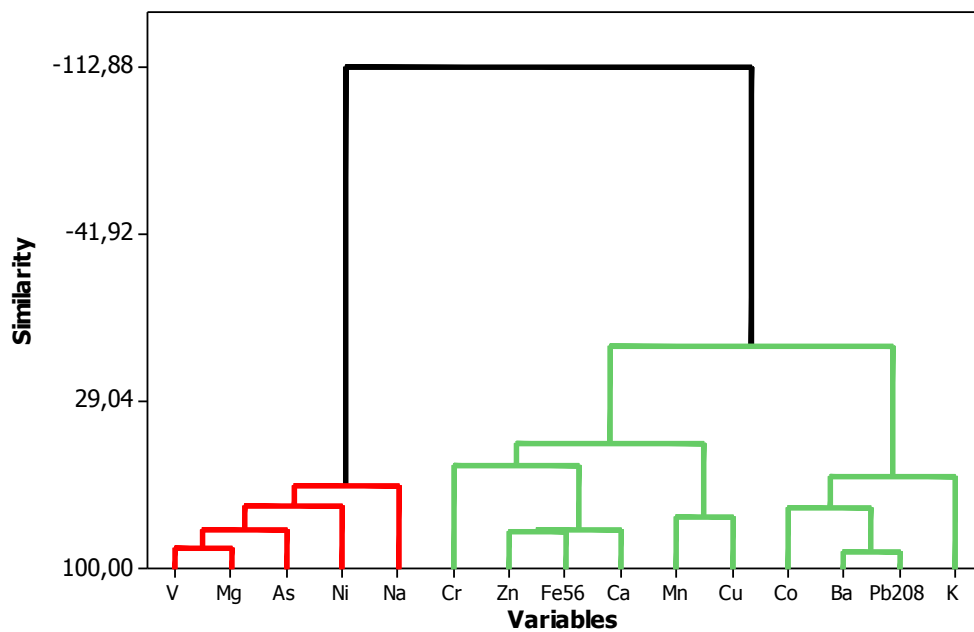


Figure 3.93. Cluster variables dendrogram of trace elements according to the ward linkage, correlation coefficient distance for variables.

Table 3.17. Cluster variations and similarity of geographical regions according to dendrogram of trace elements.

CLUSTER	VARIATIONS	SIMILARITY
RED	10	Cr, Fe, Zn, Ca, Mn, Cu, Co, Ba, Pb, K
GREEN	5	V, Mg, As, Ni, Na

Trace elements analysis was given very important knowledge for geographical distribution.

3.4.4 PCA and HCA for trace elements without Fe and Zn

Since both Fe and Zn make grouping by themselves, PCA analysis had been repeated after removing those metals and so olive oils from Manisa and İzmir had been concentrated on one side in Figure 3.94 according to PC1.

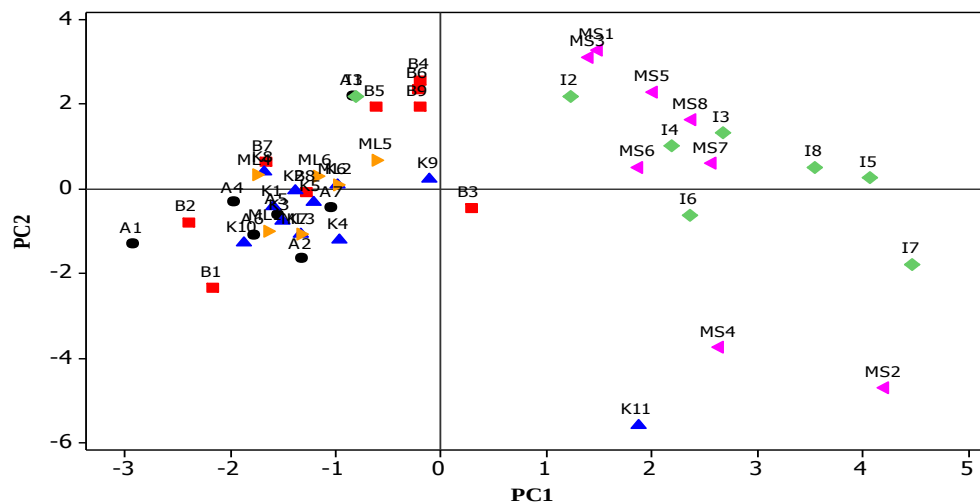


Figure 3.94. PCA score plot of trace elements without Fe-Zn for each region.

When we compared with score plots of all trace elements and without Fe56 and Zn there was not a significant differentiation between them. After remove Fe56 and Zn we can see effect of other trace elements openly. Ca and Cu were dominant variance, Mn was followed them for I and MS region ML, K, B, A's

region olive oils were impressed Na, Ni, Mg, As and V as it can be seen loading plot in Figure 3.95.

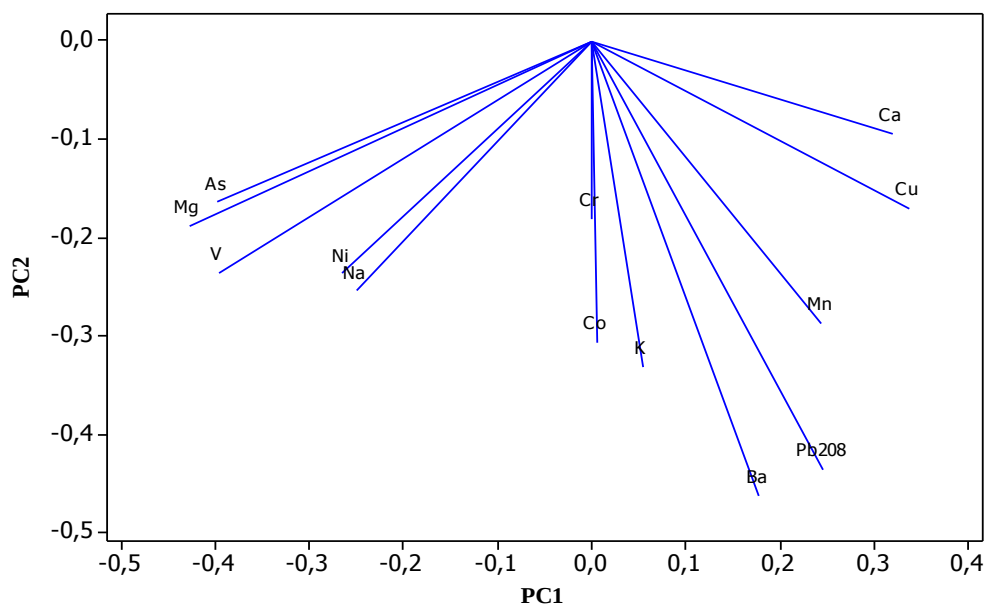


Figure 3.95. PCA loading plot of trace elements without Fe-Zn.

Ca and Cu's effect was clearly seen in biplot graph in Figure 3.96 after removing Zn and Fe56 metals.

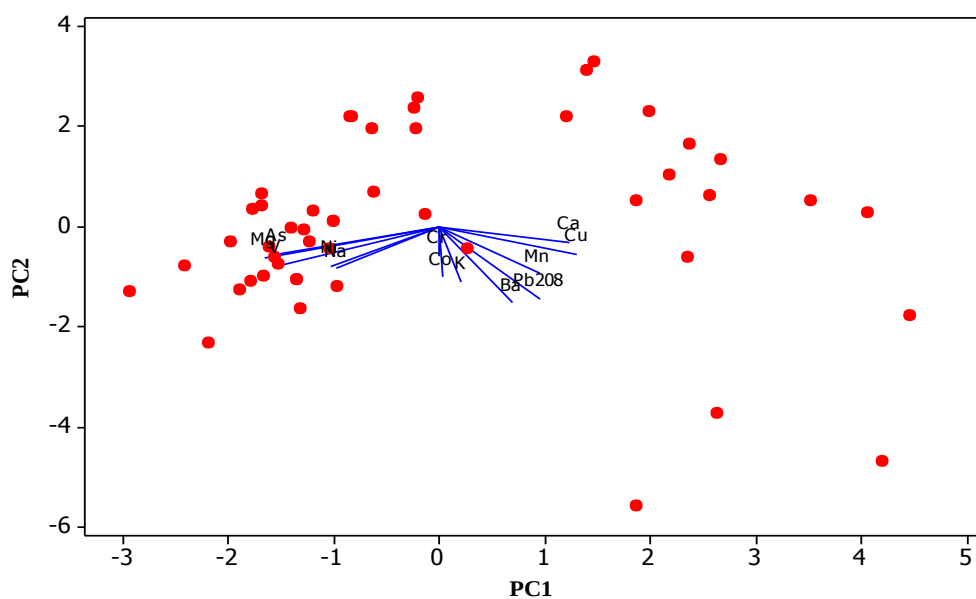


Figure 3.96. PCA biplot of trace elements without Fe-Zn.

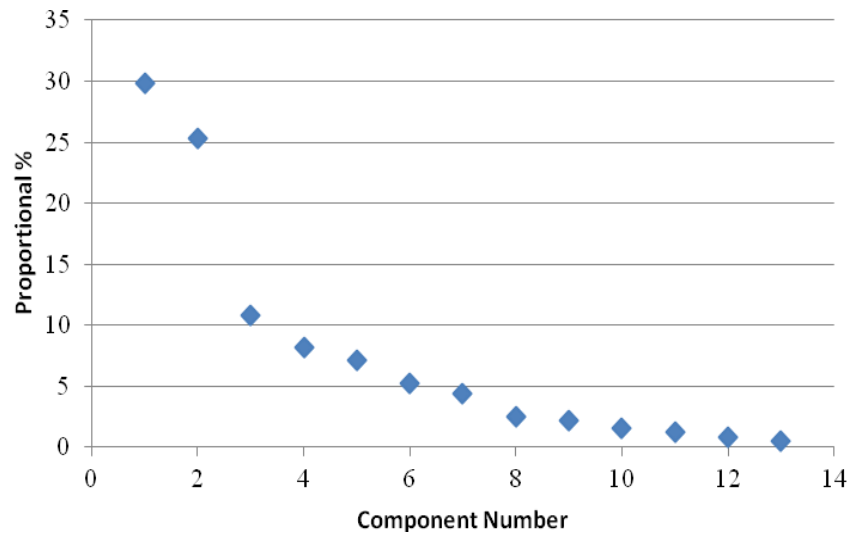


Figure 3.97. Proportional % eigen values vs component number for trace elements without Fe-Zn.

Removing of Fe56 and Zn trace elements from parameters causes evident changing of proportion of % eigen value were on the PC1 and PC2. PC1 of proportion % was decreased whereas PC2 of proportion % was increased as it can be shown in Table 3.18.

Table 3.18. Percent eigen values of trace elements without Fe-Zn.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	3,885	29,885	29,885
2	3,298	25,369	55,254
3	1,408	10,833	66,086
4	1,071	8,238	74,324
5	0,928	7,137	81,462
6	0,686	5,278	86,740
7	0,573	4,407	91,147
8	0,325	2,500	93,647
9	0,282	2,170	95,817
10	0,201	1,546	97,364
11	0,163	1,251	98,614
12	0,107	0,820	99,434
13	0,074	0,566	100,000

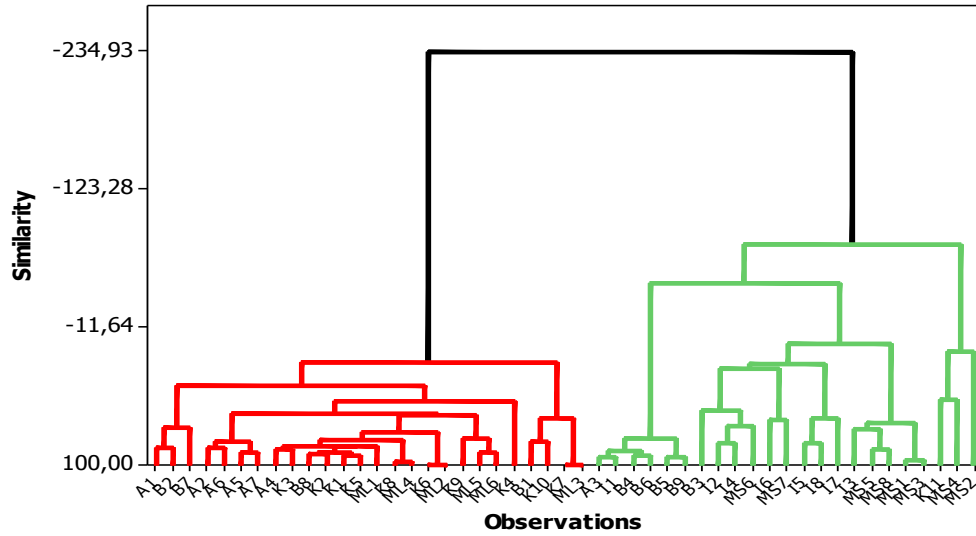


Figure 3.98. Cluster observations dendrogram of trace elements without Fe and Zn according the ward linkage, Euclidean distance with standardize variables for geographical regions.

When Zn and Fe56 metals were removed, the ward method cluster analysis dendrograms are not made regional cluster as we can seen in Figure 3.98.

Table 3.19. Cluster observations and similarity of geographical regions according to dendrogram of trace elements without Fe and Zn.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	23	Rest of other regions.
GREEN	16	MANISA (MS), IZMIR (I) and 5 regions from BURSA (B).

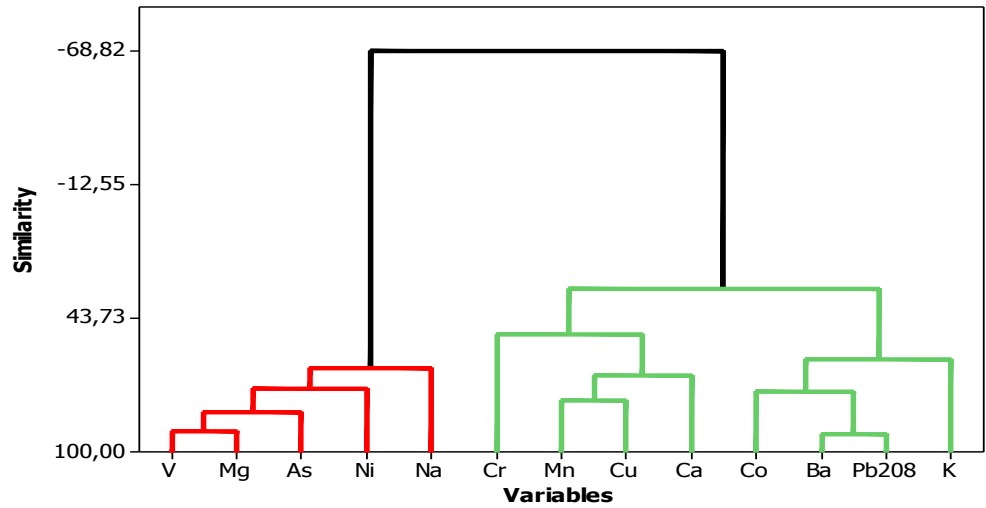


Figure 3.99. Cluster variables dendrogram of trace elements without Fe and Zn according to the ward linkage, correlation coefficient distance for variables.

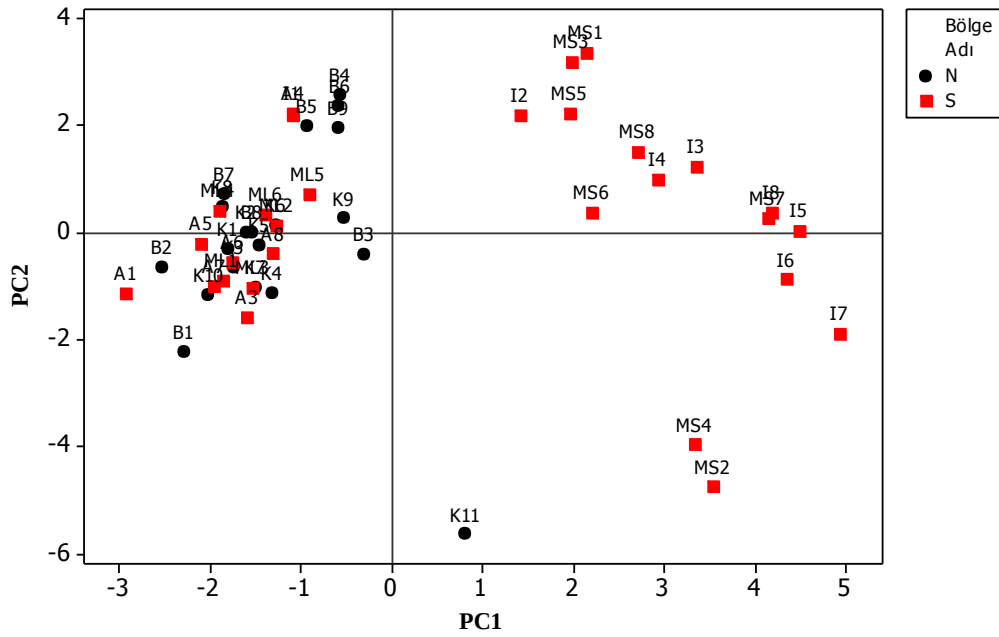
Fe and Zn were played important role for regional clustering of regions.

Table 3.20. Cluster variables and similarity of geographical regions according to dendrogram of trace elements without Fe and Zn.

CLUSTER	VARIABLES	SIMILARITY
RED	8	Cr, Ca, Mn, Cu, Co, Ba, Pb,K
GREEN	5	V, Mg, As, Ni, Na

3.4.5. PCA and HCA of trace elements for North-South

When olive oils are divided two groups as north and south there was not seen clear cluster between two regions. As expected some south olive oils are separated from others as we can seen in Figure 3.100.



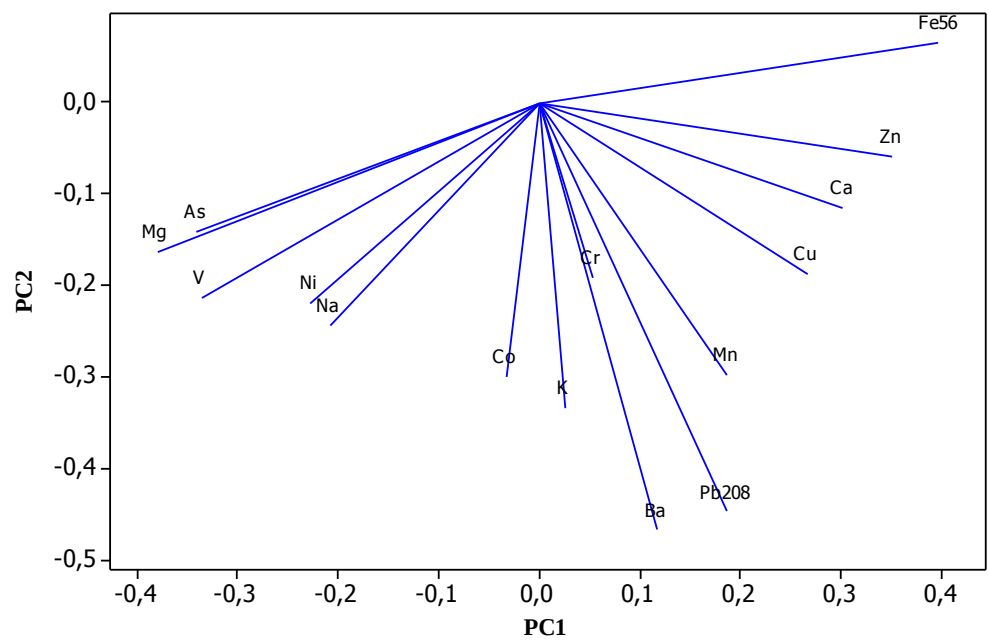


Figure 3.101. PCA Loading plot of heavy metal for North-South.

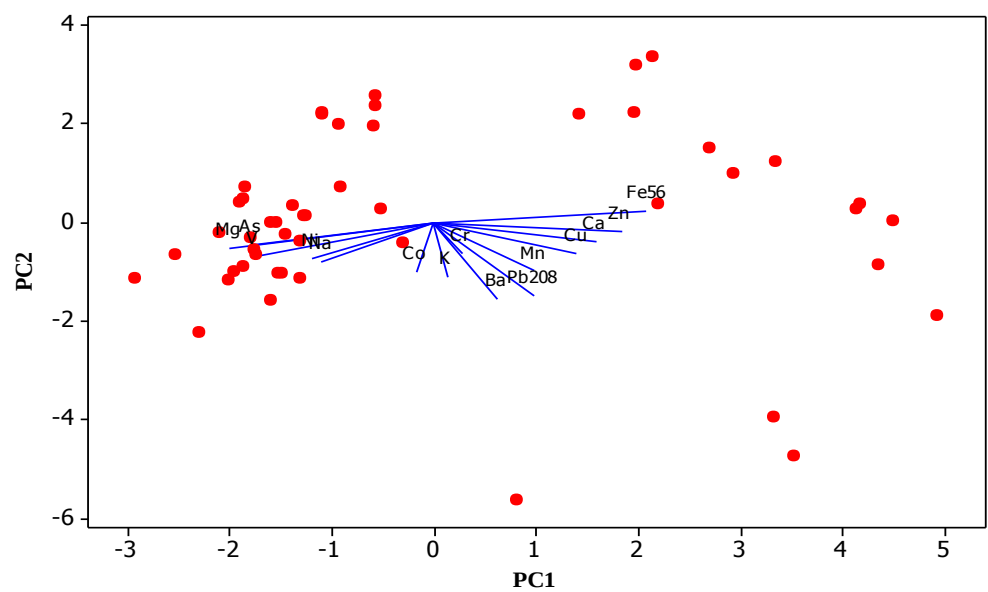


Figure 3.102. PCA biplot of of heavy metal for North-South.

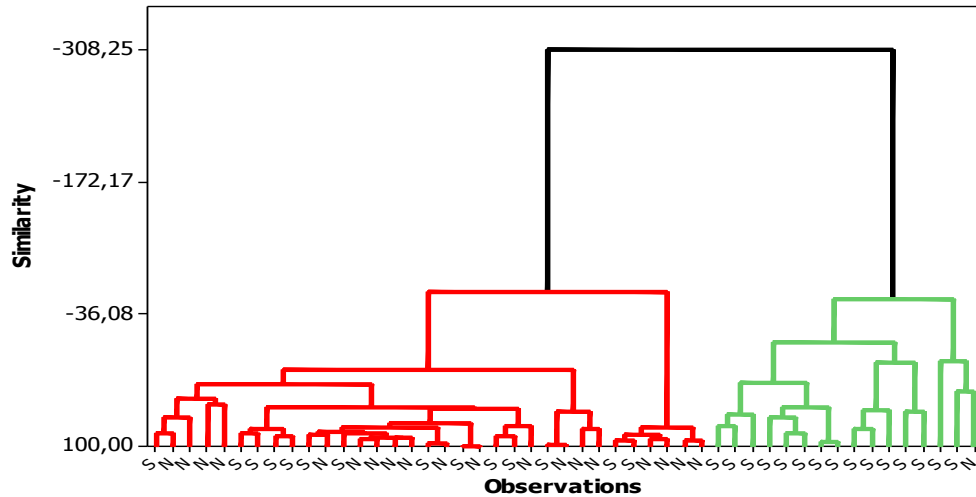


Figure 3.103. Cluster observations dendrogram of TAG for North-South according to the ward linkage, Euclidean distance with standardized variables for geographical regions.

When we divide regions into two groups as north and south, cluster observations dendrograms of the ward linkage method are not shown grouping into 2 regions clearly as we can see in Figure 3.103 although HCA was clustered some south and north variances.

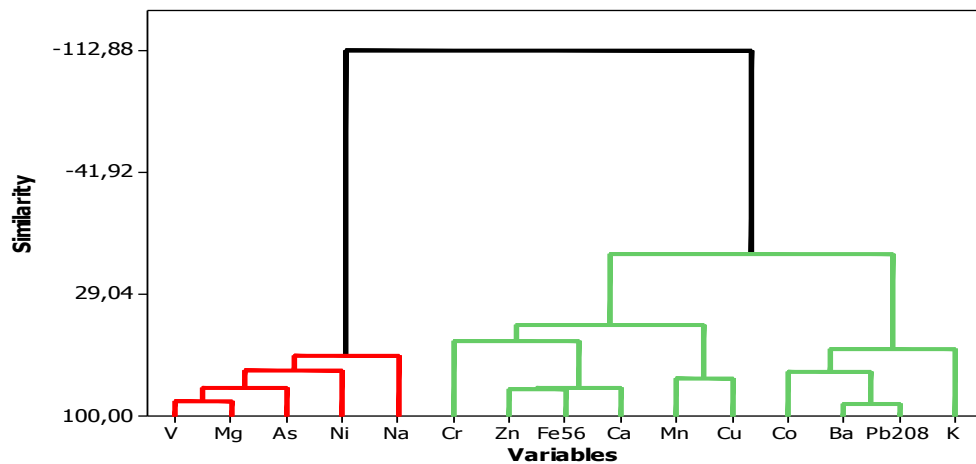


Figure 3.104. Cluster variables dendrogram of TAG of North-South according to the ward linkage, correlation coefficient distance for variables.

15 regions' olive oils of the south region were separated from all others according to the score plot and dendrograms.

3.4.6 PCA and HCA for trace elements for adulteration

In the regional grouping according to heavy metal analysis, İzmir and Manisa was remain on a side, the other regions was remain on the other side. It was occurred such a differentiation for grouping but this is not sufficient for authentication according to score plot graph in Figure 3.105.

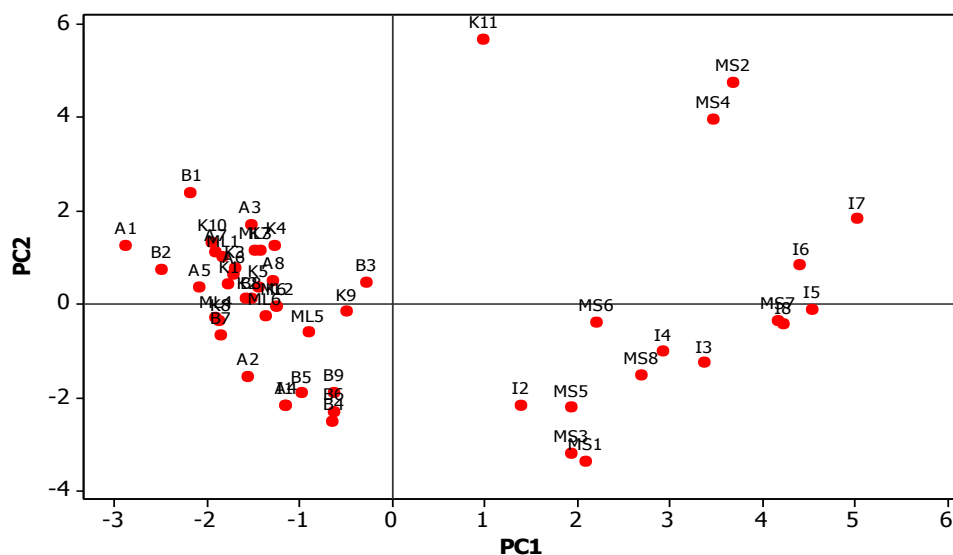


Figure 3.105. PCA score plot of trace elements for adulteration.

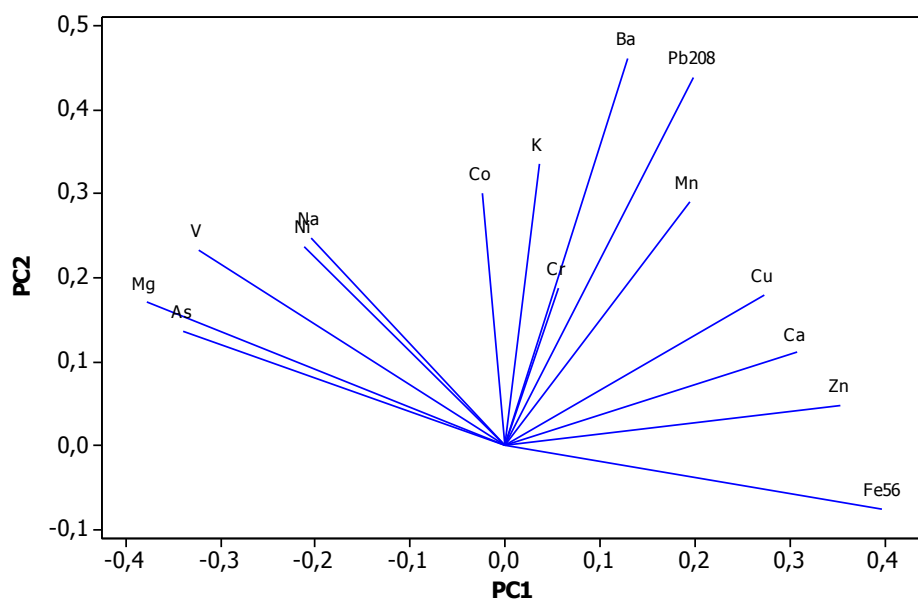


Figure 3.106. PCA loading plot of trace elements for adulteration.

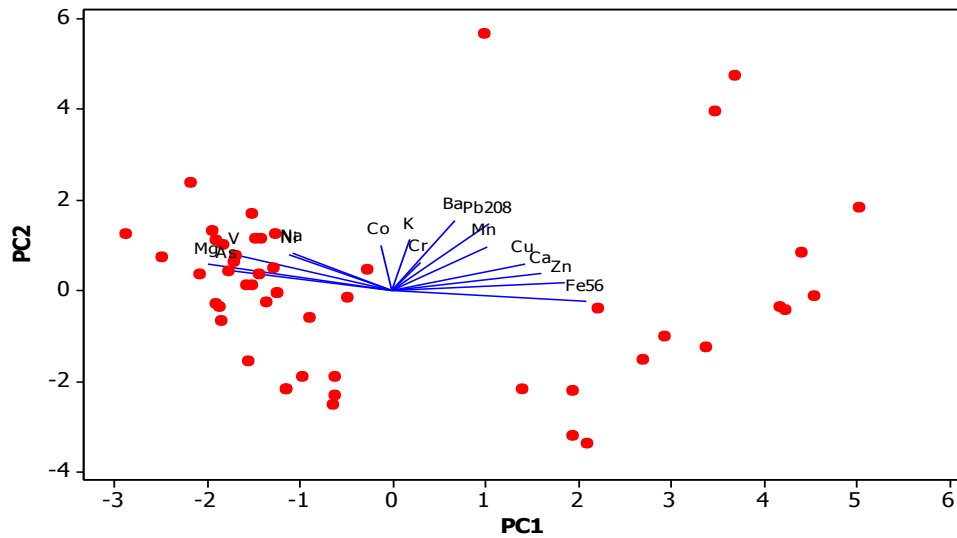


Figure 3.107. PCA biplot of trace elements for adulteration.

Zn and Fe56 are most important parameters for İzmir and Manisa region according to loading plot and biplot graph in Figure 3.106 and 3.107.

As a result of the PCA, it was seen that heavy metal analysis is not selective for adulteration. However according to cluster dendrogram, it was seen that heavy metal analysis is better for grouping by regions in Figure 3.108. Thus, heavy metal analysis can guide us to determine the region of an olive oil which is unknown according to dendrogram.

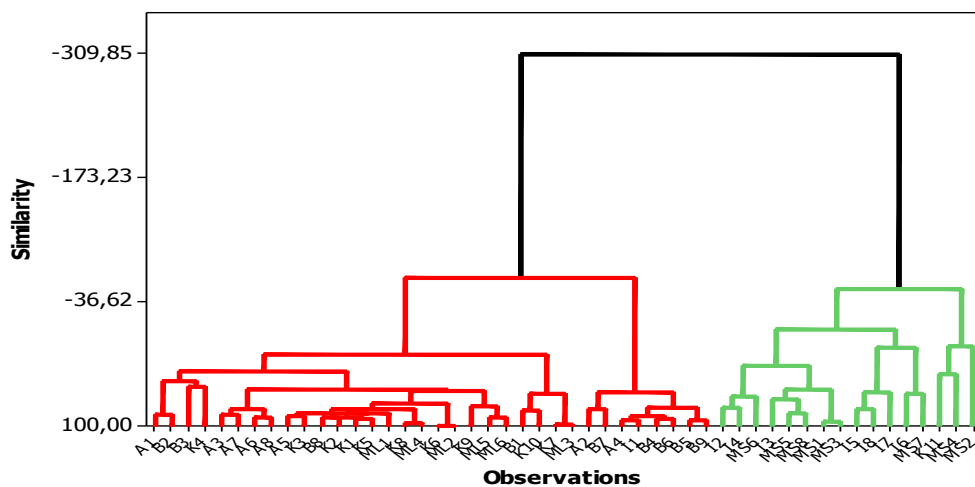


Figure 3.108. Cluster observations dendrogram of trace elements for adulteration according the ward linkage, Euclidean distance with standardize variables for geographical regions.

Heavy metal analysis can guide us to determine the region of an olive oil which is unknown from Manisa (MS) and İzmir (I) according to PCA where into Muğla (ML) was clustered by HCA analysis.

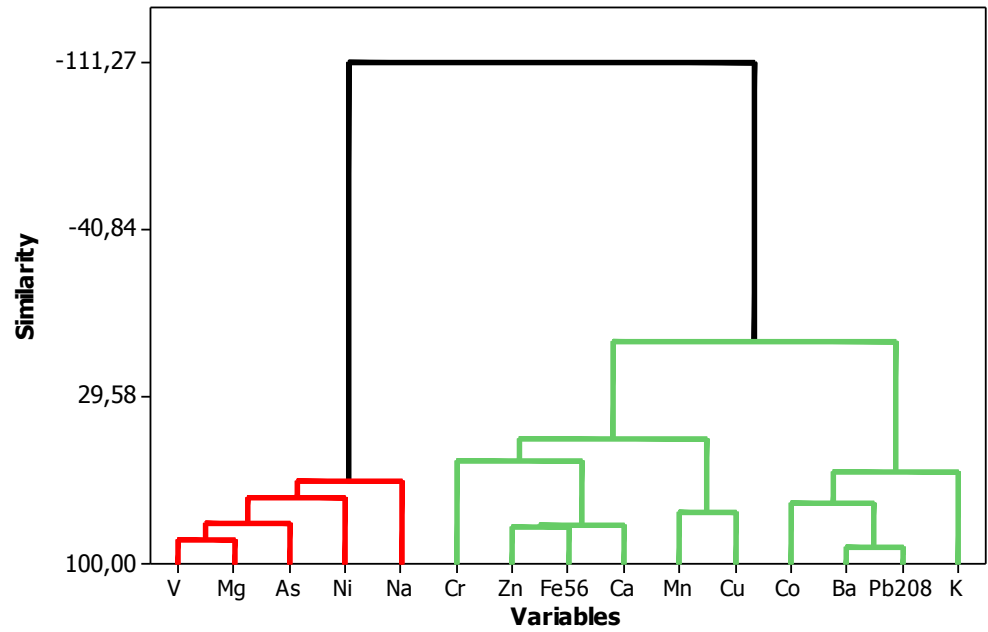


Figure 3.109. Cluster variables dendrogram of trace elements for adulteration according to the ward linkage, correlation coefficient distance for variables.

3.5 Delta C13 ($\delta^{13}\text{C}/^{12}\text{C}$ isotope ratio)

For isotope ratio $\delta^{13}\text{C}/^{12}\text{C}$ analysis, oils were analyzed as bulk oil in IRMS system. The resulting isotope ratios were shown in the Figure 3.110. $\delta^{13}\text{C}/^{12}\text{C}$ isotope ratio analysis is very effective for geographical regional grouping but it isn't effective to identify authentication alone. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ isotope ratios in an effort to classify olive oil samples according to geographical origin (Karabagias et al., 2013).

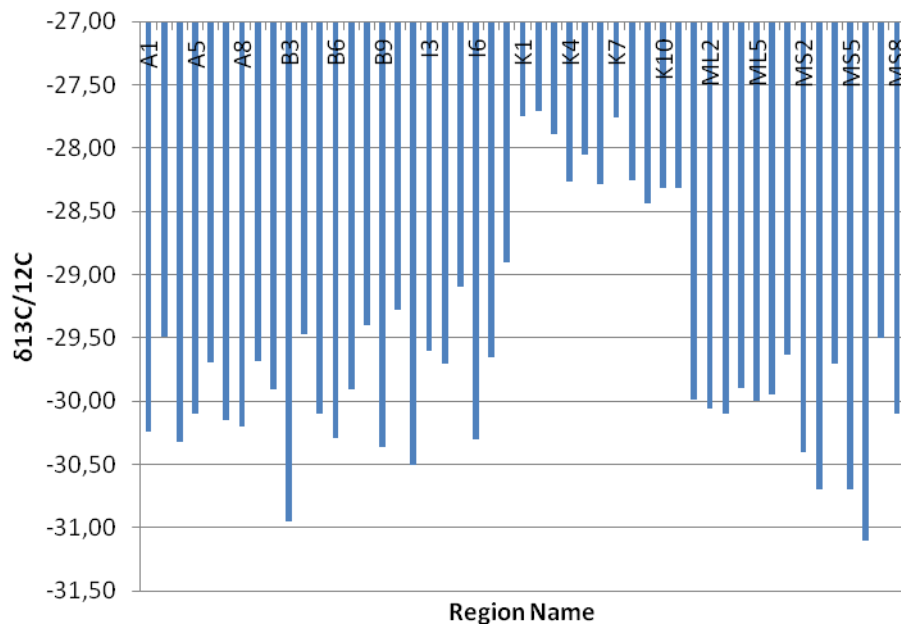


Figure 3.110. Column Chart illustration of Delta C13 levels in different samples with coded regions.

As it can be seen in Figure 3.110, Edremit Bay' olive oils are different from other region oils. Delta C13 most important variance for Edremit Bay's olive oils. We can determine adulteration in groups and regional grouping according to Delta C13 parametres.

MN, ML, I, B regions are shown similar values. K region have higher values than others. When the investigation of Aydın olive oils adulterated oil has been higher Delta C13 value from other Aydın olive oils. Delta C13 was not singly use for determination of adulteration but was so effected for authentication of Edremit Bay (K) region's olive oil.

3.6 $\delta^{13}\text{C}/^{12}\text{C}$ Isotope Ratio with Other Variables

$\delta^{13}\text{C}/^{12}\text{C}$ isotope ratios can be exploited for authentication of olive oil. Indeed, isotope fractionations of carbon both biotic and abiotic factors, producing isotope patterns with ability of characterising geographic origin and meteorological conditions as a consequence of $\delta^{13}\text{C}/^{12}\text{C}$ ratio was correlated with variances of fatty acid methyl esters, sterol compositions, triacylglycerols and trace elements. These parameters, were chosen judiciously for this correlation, since obtained biplot and loading plots from PCA and plotted column charts have illustrated significant influence on the differentiation of olive oil samples studied. Isotope analysis is a powerful tool for tracing the geographical origin and identifying the provenance of agro-products. (Zhao et al., 2014) Isotope ratios of carbon and oxygen can be used for authentication and traceability of olive oil.

3.6.1 Correlation of $\delta^{13}\text{C}/^{12}\text{C}$ isotope ratio with sterols percentage levels

After all possible productive combinations between $\delta^{13}\text{C}/^{12}\text{C}$ isotope ratio with Sterols percentage levels were investigated, then, the percentage value of each parameters such as 24-methylene cholesterol, D5-23-stigmastadianol, D5-24-stigmastadianol, D7-avenasterol, cholesterol, clerosterol, D5-avenasterol were chosen in order to establish to the link between $\delta^{13}\text{C}$ ratio and sterol values.

In the previous section as it can be seen in Figure 3.110, $\delta^{13}\text{C}/^{12}\text{C}$ ratios has influence on olive oils produced in the Edremit bay as abbreviated K for classification as geographically.

Angerosa et al. (1999) applied IRMS to 42 olive oil samples from Italy, Greece, Morocco, Spain, Tunisia and Turkey to determine their geographical origin. Values for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ determined were similar to those of the present study. The authors concluded that olive oils could be differentiated according to climatic areas and that the introduction of $\delta^{18}\text{O}$ values of sterols and aliphatic alcohols as opposed to bulk olive oil $\delta^{18}\text{O}$ values was a substantially more useful index. $^{13}\text{C}/^{12}\text{C}$ isotopic profiles could provide information about the geographical origin of samples. Their results can be essential tools for a registered trademark, such as the Denomination of Protected Origin (DOP) and Indication of Protected Geographical Area (IGP).

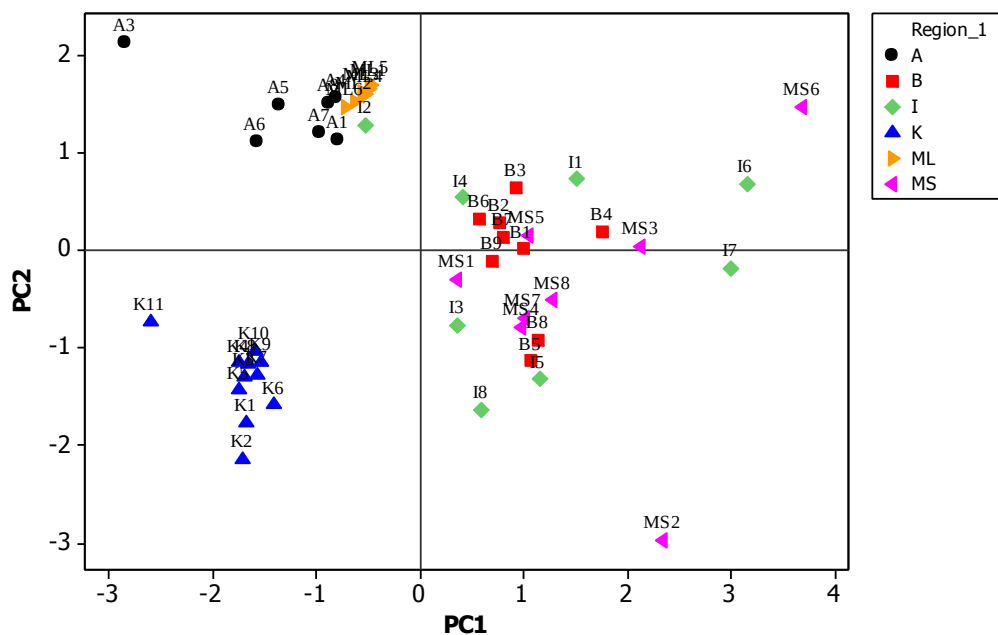


Figure 3.111. Score plot of $\delta^{13}\text{C}$ and some variances of sterols compositions.

According to the PCA model of data, K was classified and aggregated on the basis of the score plots it was seen in Figure 3.111.

I, MS and B was clustered on the right side of PC1 despite this effect of PC2 was shown on the grouping these regions especially B was under the influence of PC2.

Both A and ML regions were grouped from each other with the aid of PC1 and PC2. It is interesting to note that, the variance of ML region has been very close to each other while variances of Aydın region showed more openly distributed cluster at which in this way it was then split from ML. The effect of C13 correlated with sterol composition, it can be expected, the region of K was clearly separated than the all other regions..

When it was compared the score plots of whole sterol parameters and the score plots of relation of sterol parameters with $\delta^{13}\text{C}/^{12}\text{C}$ isotope ratios (Figure 3.111) were provided to present a more effective grouping formed between regions in this way. It can be concluded that the use deltaC13 data with sterol percentage parameter data for clustering was found to be better than that of sterol composition data alone.

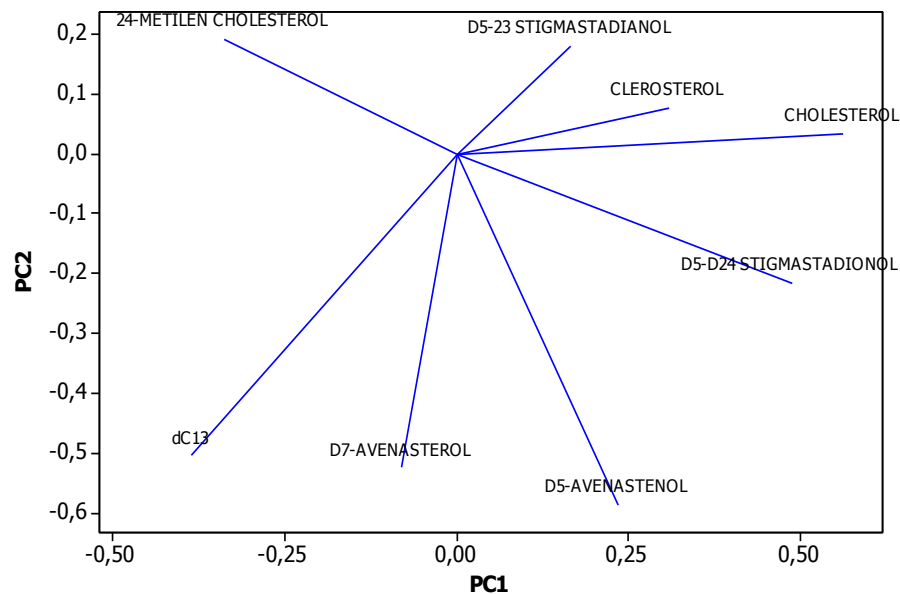


Figure 3.112. Loading plot of $\delta^{13}\text{C}$ and some variances of sterols compositions.

As it was seen loading plot Figure 3.112 clerosterol, cholesterol and D5-D24-stigmastadional have impressed on the grouping of B, I and MS while $\delta^{13}\text{C}$ have effect on the K (Edremit Bay) olive oils.

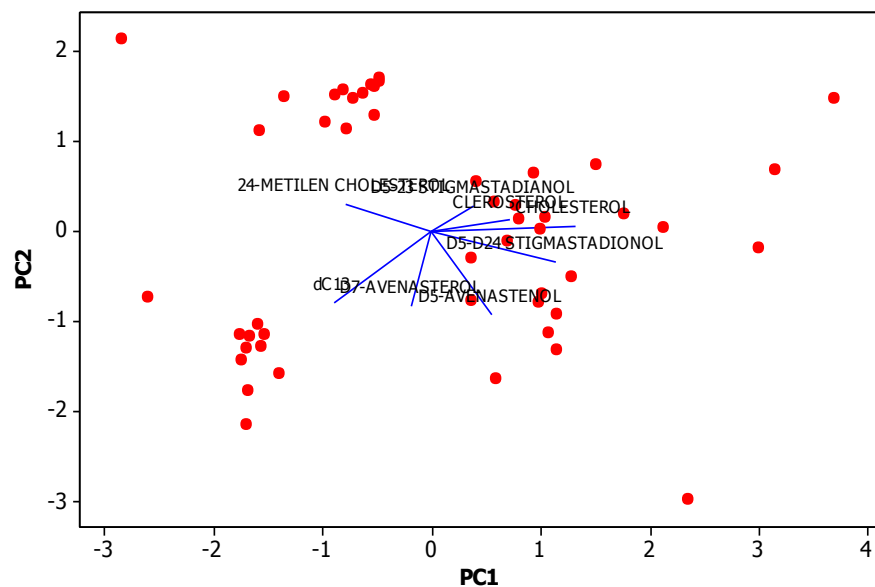


Figure 3.113. Biplot of $\delta^{13}\text{C}$ and some variances of sterols compositions.

24 methylene cholesterol has role of splitting of regions A and ML it was openly seen in biplot graph in Figure 3.113.

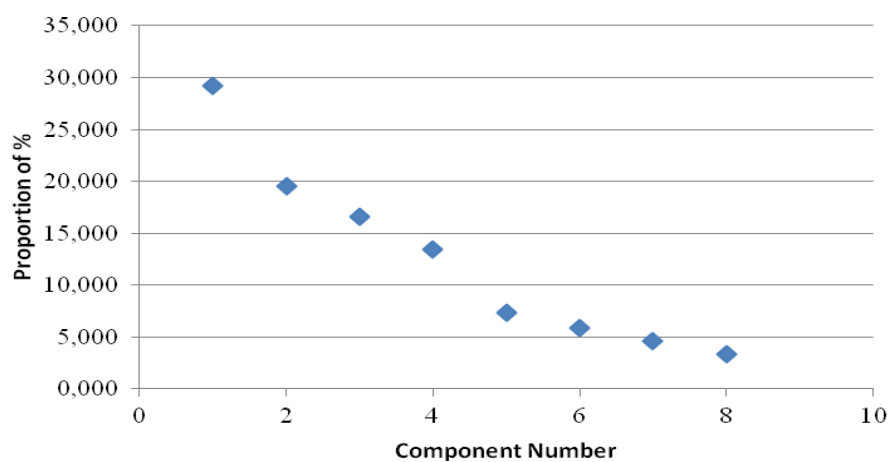


Figure 3.114. Proportional % eigen values vs component number for $\delta^{13}\text{C}$ and some variances of sterols compositions.

Table 3.21. Percent eigen values of $\delta^{13}\text{C}$ and some variances of sterols compositions.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	2,335	29,189	29,189
2	1,567	19,583	48,772
3	1,329	16,611	65,383
4	1,074	13,425	78,808
5	0,590	7,374	86,182
6	0,469	5,863	92,045
7	0,368	4,595	96,640
8	0,269	3,360	100,000

According to eigen values of $\delta^{13}\text{C}$ and some variances of sterols compositions eigen values of PC1 and PC2 have very high values it was seen in Figure 3.114 and Table 3.21.

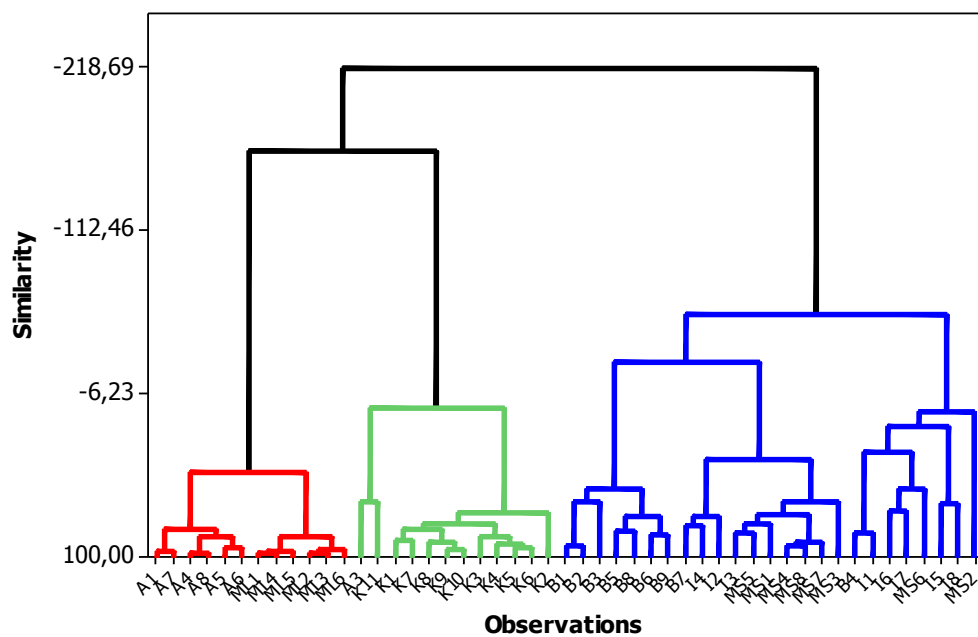


Figure 3.115. Cluster observations dendrogram of $\delta^{13}\text{C}$ and some variances of sterols compositions according the ward linkage, Euclidean distance with standardize variables for geographical regions.

Table 3.22. Cluster observations and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and sterols.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	13	Aydın (A) and MUGLA (ML)
GREEN	11	EDREMIT BAY (K)
BLUE	25	BURSA (B), MANISA (MS) AND İZMİR (I)

First of all we were contrasted single and ward linkage dendograms, K, ML and MS was much better clustered when ward linkage metod was used.

Olive oils from ML was openly separated others region according to both single and ward method linkage dendrograms.

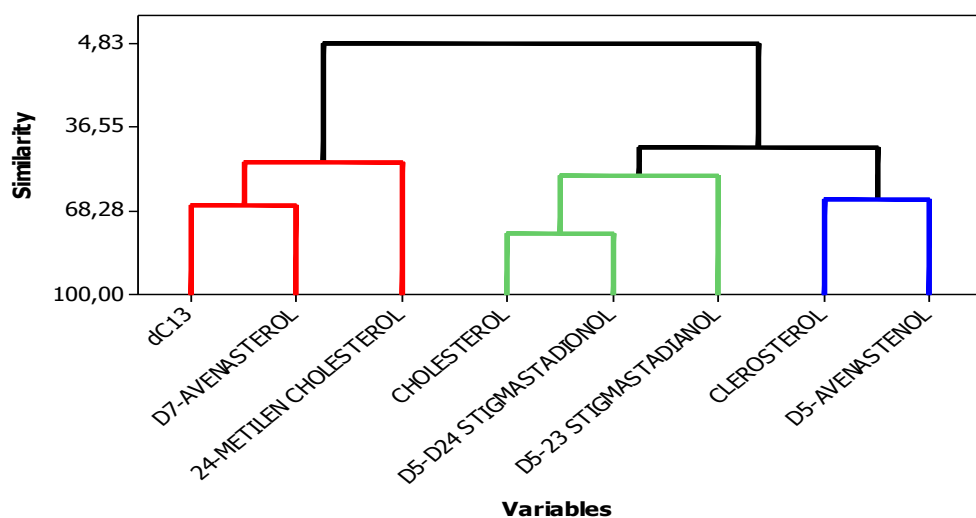


Figure 3.116. Cluster variables dendrogram of $\delta^{13}\text{C}$ and some variances of sterols compositions according the ward linkage, correlation coefficient distance for variables.

Table 3.23. Cluster variables and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and sterols.

CLUSTER	VARIABLES	SIMILARITY
RED	3	$\delta^{13}\text{C}$, $\Delta 7$ -avenasterol, 24-methylene cholesterol.
GREEN	3	Cholesterol, $\Delta 5$ - $\Delta 24$ – Stigmastadionol, $\Delta 5$ - $\Delta 23$ – Stigmastadionol.
BLUE	2	Clerosterol, $\Delta 5$ -Avenastanol

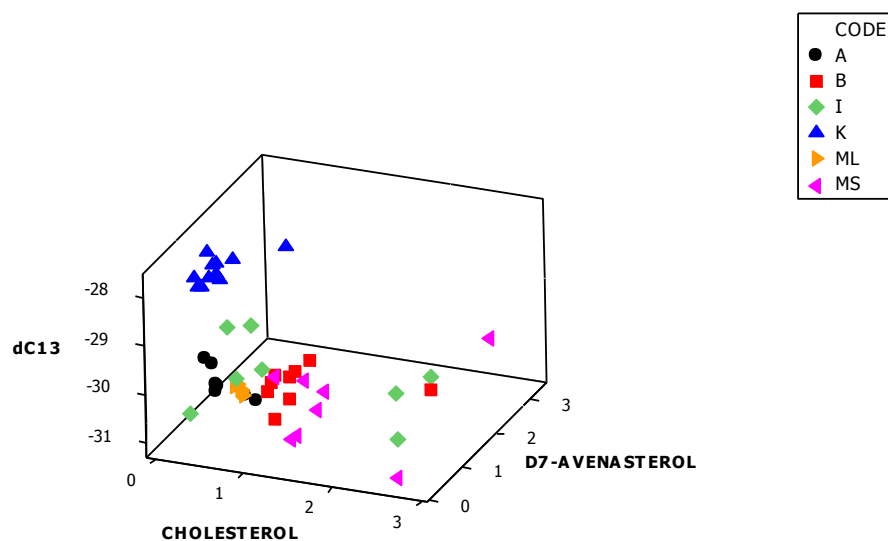


Figure 3.117.3D-Scatterplot of $\delta^{13}\text{C}/^{12}\text{C}$ vs d7-avenasterol vs cholesterol for geographical region.

When we was run cluster obsevation with ward linkage Edremit Bay (K) was formed near group like olive oils of ML region.

3.6.2 $\delta^{13}\text{C}/^{12}\text{C}$ with FAMEs

After all relationship creative between percentage of fatty acid methyl esters and $\delta^{13}\text{C}$ sisotope ratio were utilized, and so, in preference to lignoceric acid, palmitoleic acid, stearic acid, arachidonic acid and nervonic acid were selected for the connection with $\delta^{13}\text{C}$.

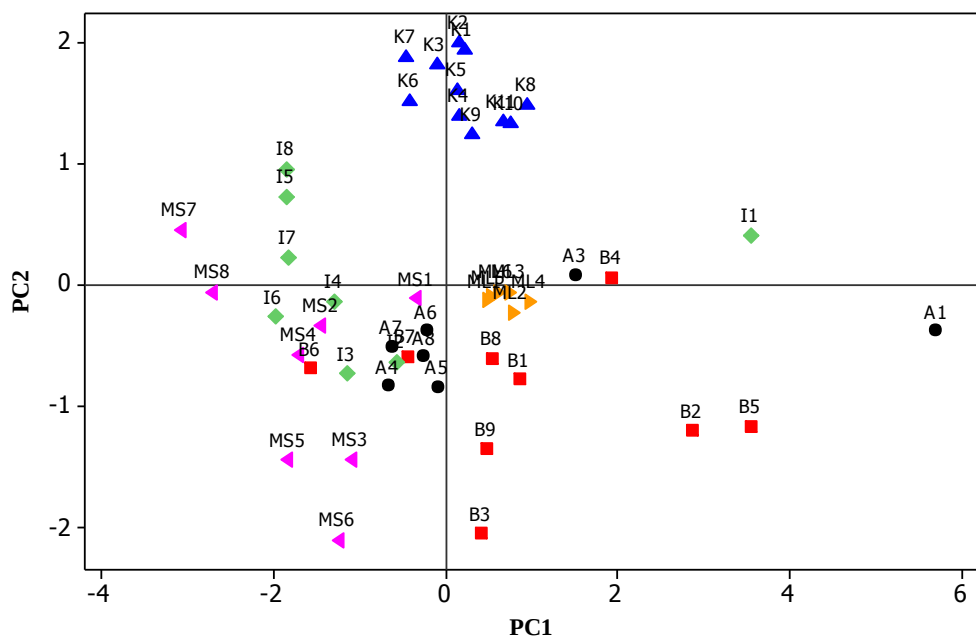


Figure 3.118. Score plot of $\delta^{13}\text{C}$ and some variances of FAME compositions.

As it was illustrated score plot of variances of fatty acid methyl esters have not very good effect for authentication when we was utilized relationship between some fatty acid methyl esters and $\delta^{13}\text{C}$ isotope ratio values by using PCA region of K was transparently splitted from others in Figure 3.118. In addition of this result B was shown better dispersion according to all fatty acid methyl esters score plots that means only fatty acid methyl esters have more important role for classification of B region.

Olive oils of ML region was separated with effect of PC1 and clustered with PC2 at the same time as I and MS were splitted with PC1. Both PC1 and PC2 have role on the region of B. It can demonstrated in score plots B, I and MS were shown distribution.

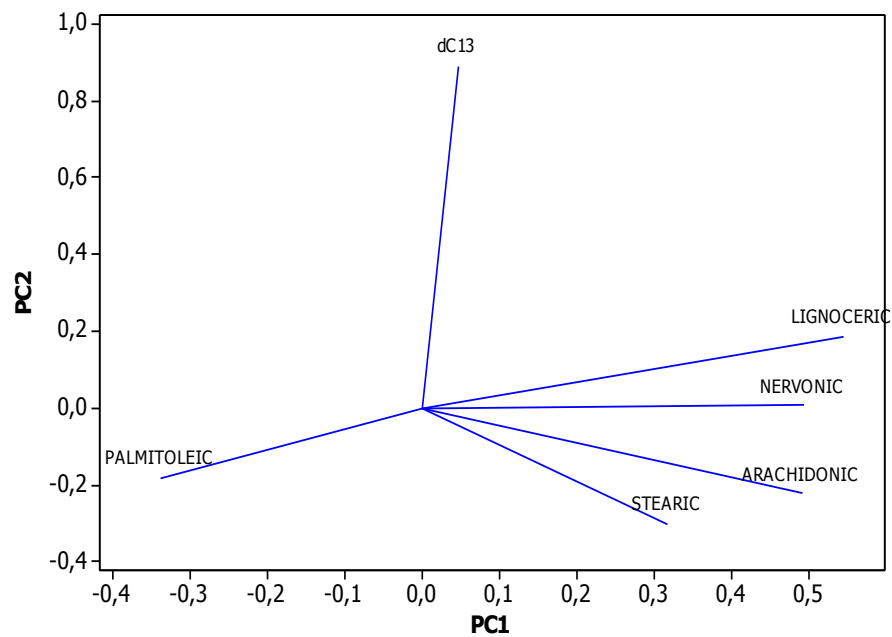


Figure 3.119. Loading plot of $\delta^{13}\text{C}$ and some variances of FAME compositions.

The most important variance was been $\delta^{13}\text{C}$ isotope ratio for authentication of EdremitBay (K) it was shown clearly in loading plot graph in Figure 3.119 and biplot graph in Figure 3.120.

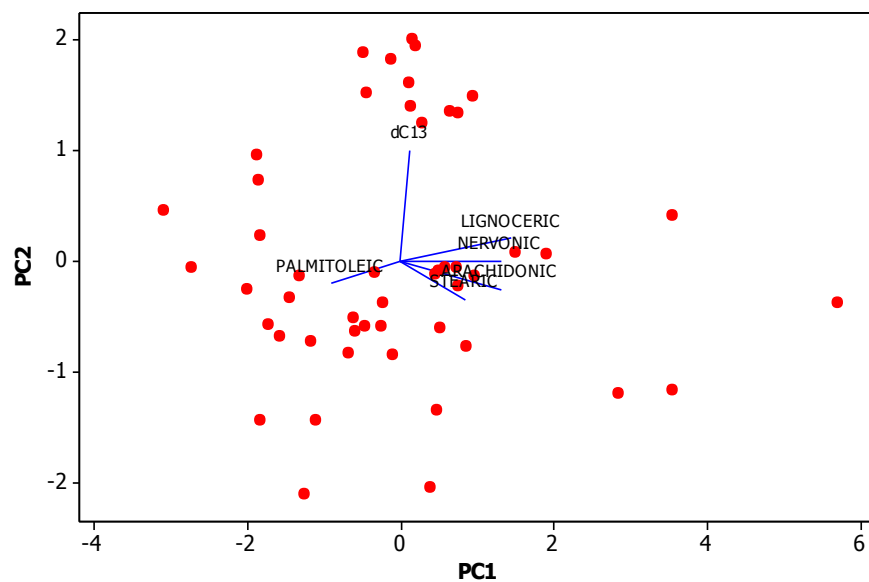


Figure 3.120. Biplot of $\delta^{13}\text{C}$ and some variances of FAME compositions.

Palmitoleic acid was effected variance on regions of I and MS and lignoceric acid, nervonic acid, arachidonic acid and stearic acid were impressed on the other regions for authentication.

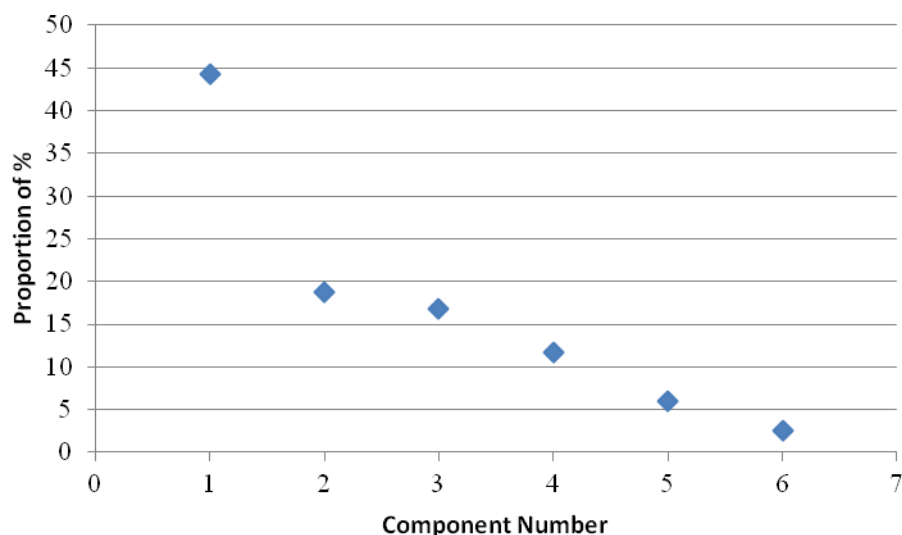


Figure 3.121. Proportional % eigen values vs component number for $\delta^{13}\text{C}$ and some variances of FAME compositions.

According to eigen value of $\delta^{13}\text{C}$ and some variances of fatty acids methyl esters compositions of first variance has very high value in Figure 3.121.

Table 3.24. Percent eigen values of $\delta^{13}\text{C}$ and some variances of FAME compositions.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	2,656	44,270	44,270
2	1,122	18,701	62,971
3	1,010	16,836	79,807
4	0,706	11,762	91,569
5	0,357	5,953	97,522
6	0,149	2,478	100,000

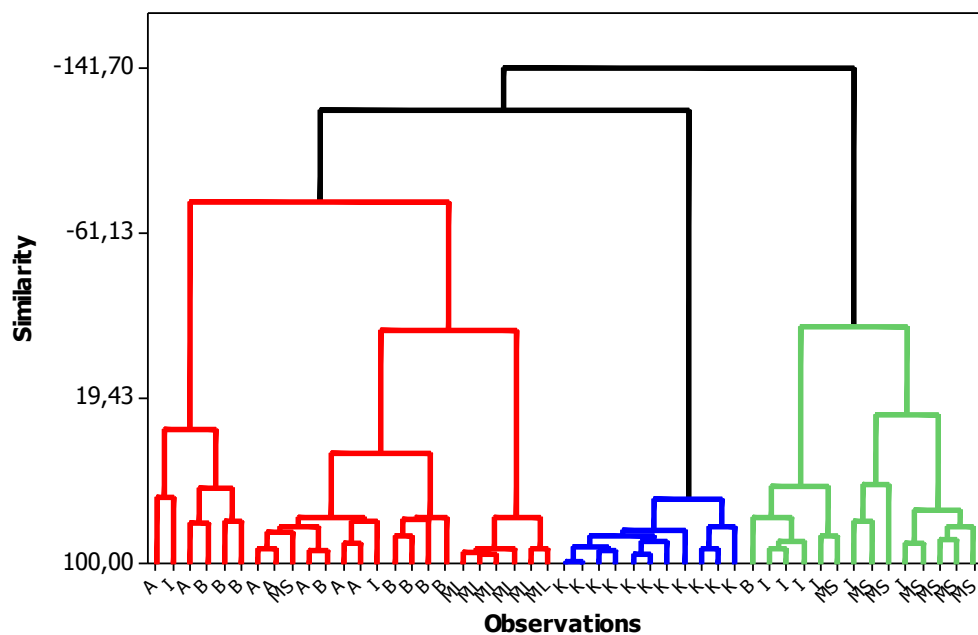


Figure 3.122. Cluster observations dendrogram of $\delta^{13}\text{C}$ and some variances of FAME compositions according the ward linkage, Euclidean distance with standardize variables for geographical regions.

When we were investigated ward linkage dendrograms, K and ML were much better grouped when ward linkage metod was used. Moreover clustering of region of ML and K were much better with effect of $\delta^{13}\text{C}$.

Table 3.25. Cluster observations and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and FAME.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	13	MUGLA (ML), Aydın (A) and BURSA (B)
GREEN	11	MANISA (MS) AND IZMIR (I)
BLUE	25	EDREMIT BAY (K)

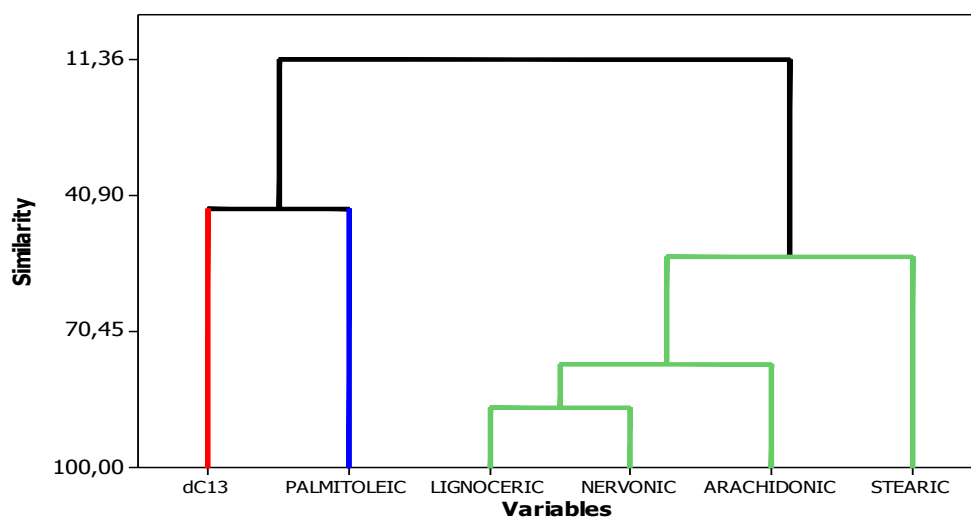


Figure 3.123. Cluster variables dendrogram of $\delta^{13}\text{C}$ and some variances of FAME compositions according the ward linkage, correlation coefficient distance for variables.

Table 3.26. Cluster variables and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and FAME.

CLUSTER	VARIABLES	SIMILARITY
RED	1	$\delta^{13}\text{C}$
GREEN	4	Lignoceric, Nervonic, Arachidonic, Stearic Acid.
BLUE	1	Palmitoleic Acid.

3D Scatterplot of dC13 vs LIGNOCERIC vs PALMITOLEIC

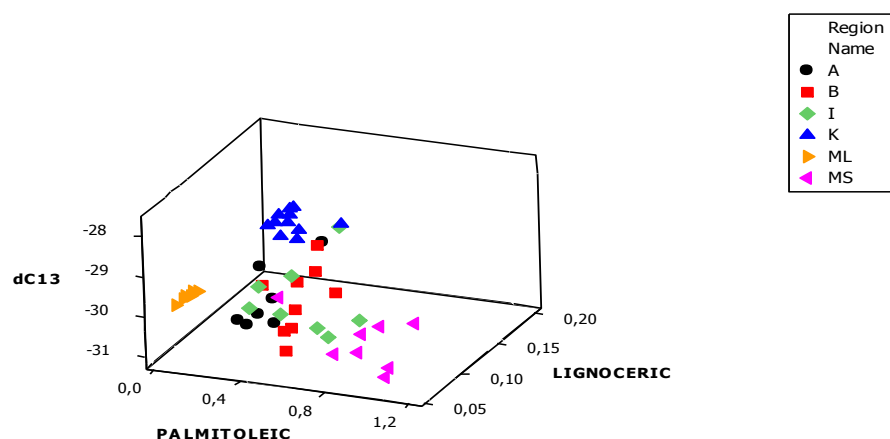


Figure 3.124. 3D-Scatterplot of $\delta^{13}\text{C}/^{12}\text{C}$ vs lignoceric vs palmitoleic for geographical region.

As it can be seen, the effect of fatty acid methyl esters which are selected according to loading score, and $\delta^{13}\text{C}/^{12}\text{C}$ on the authentication of Edremit Bay region in Figure 3.124.

3.6.3 $\delta^{13}\text{C}/^{12}\text{C}$ with TAGs

After all correlation between percentage of triacylglycerols and $\delta^{13}\text{C}$ isotope ratio values were analysed and so LLL, OLLn+PoLL, PLLn, OLL, OOLn+PoOL, PLL+PoPoO, SLL+PLO were selected for the connection with $\delta^{13}\text{C}$.

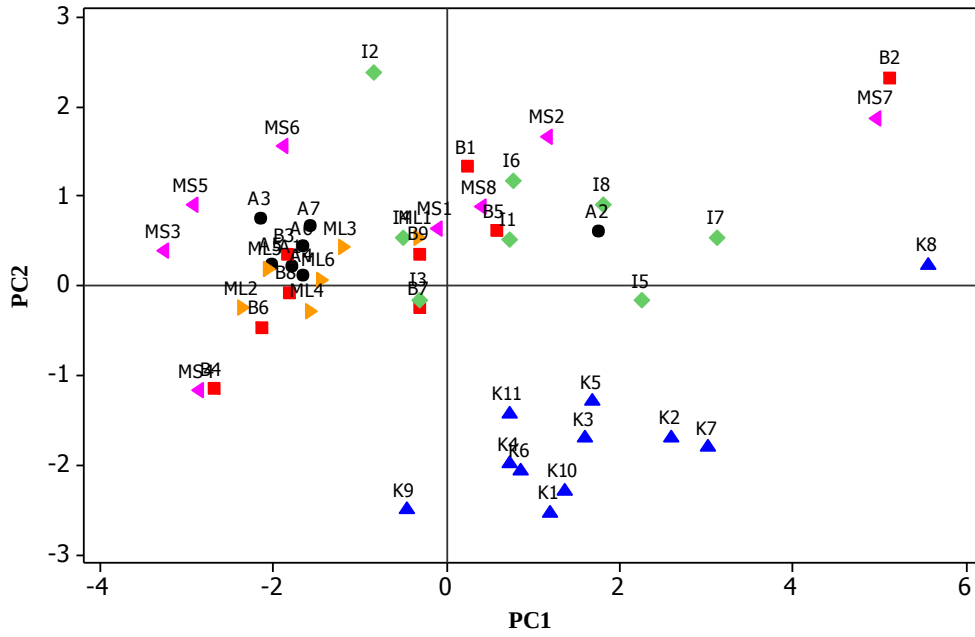


Figure 3.125. Score plot of $\delta^{13}\text{C}$ and some variances of TAG compositions.

K (Edremit Bay) was separated from other region with effect of PC1 as we have seen in Figure 3.125. Aydın (A) region was clustered under impressed both PC1 and PC2 as it was seen in score plot of $\delta^{13}\text{C}$ and some variances of TAG compositions. When we was investigated all regions of site of K were shown dispersion by effect of PC1 even so olive oils of Aydın (A) region were league together.

As anticipated region of K was separated and clustered under the influence of $\delta^{13}\text{C}$. Variances of OOLn+PoOL and PLLn were most effective component on the İzmir (I) as we can seen in Figure 3.126.

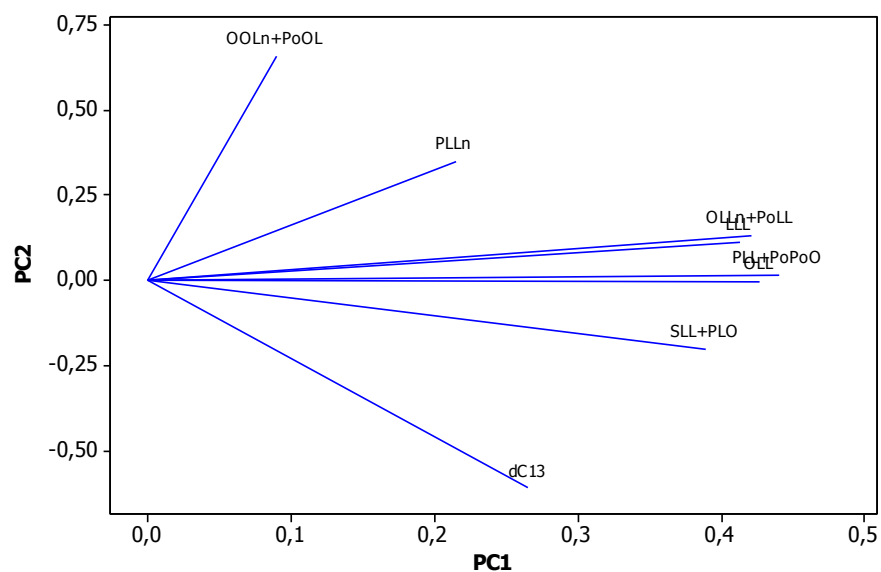


Figure 3.126. Loading plot of $\delta^{13}\text{C}$ and some variances of TAG compositions.

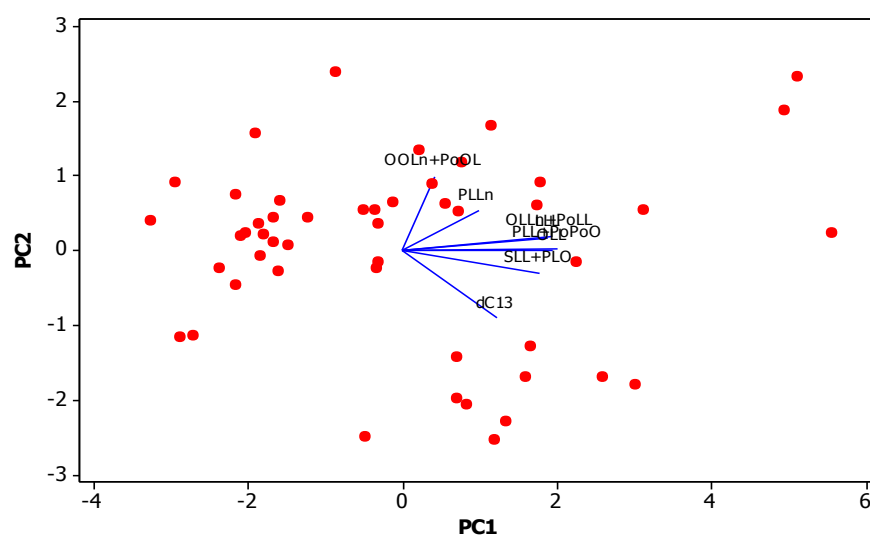


Figure 3.127. Biplot of $\delta^{13}\text{C}$ and some variances of TAG compositions.

According to eigen values $\delta^{13}\text{C}$ and some variances of triacylglycerols of, first variance has very high value in Figure 3.128.

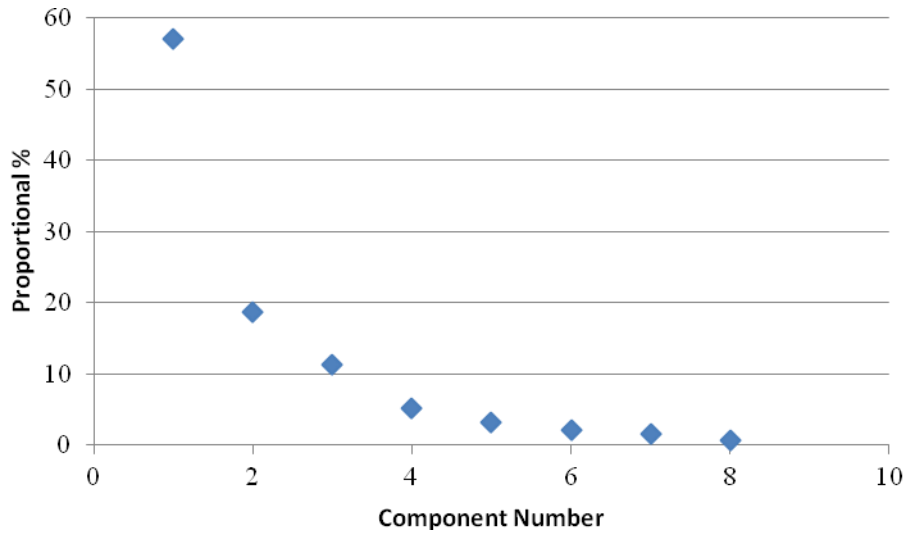


Figure 3.128. Proportional % eigen values vs component number for $\delta^{13}\text{C}$ and some variances of TAG compositions.

Table 3.27. Percent eigen values of $\delta^{13}\text{C}$ and some variances of TAG compositions.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	4,569	57,107	57,107
2	1,493	18,668	75,775
3	0,910	11,371	87,146
4	0,421	5,267	92,413
5	0,253	3,165	95,578
6	0,166	2,080	97,657
7	0,126	1,580	99,237
8	0,061	0,763	100,000

The data were also evaluated by cluster analysis. These data were also auto-scaled using the ward linkage method as shown in Figure 3.129. Ward method has not done regional grouping except K. In other words, there was only one cluster for linkage method. Edremit bay (K) region was clustered and grouped as regards ward linkage method.

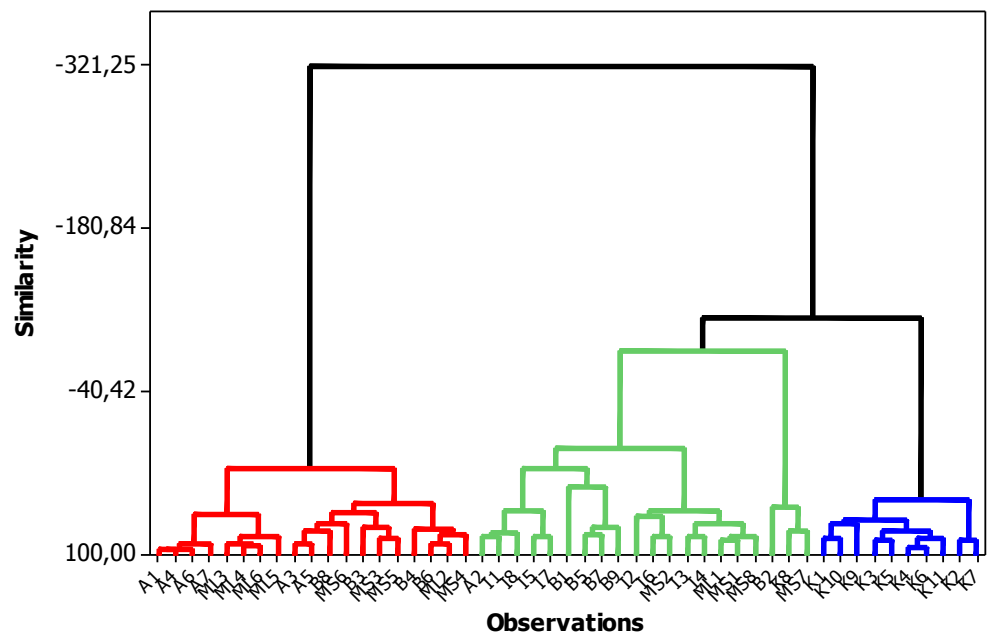


Figure 3.129. Cluster observations dendrogram of $\delta^{13}\text{C}$ and some variances of TAG compositions according the ward linkage, Euclidean distance with standardize variables for geographical regions.

Table 3.28. Cluster observations and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and TAG.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	19	MUGLA (ML), AYDIN (A) and some regions of BURSA (B)
GREEN	20	MANISA (MS), IZMIR (I) and and some regions of BURSA (B)
BLUE	10	EDREMIT BAY (K)

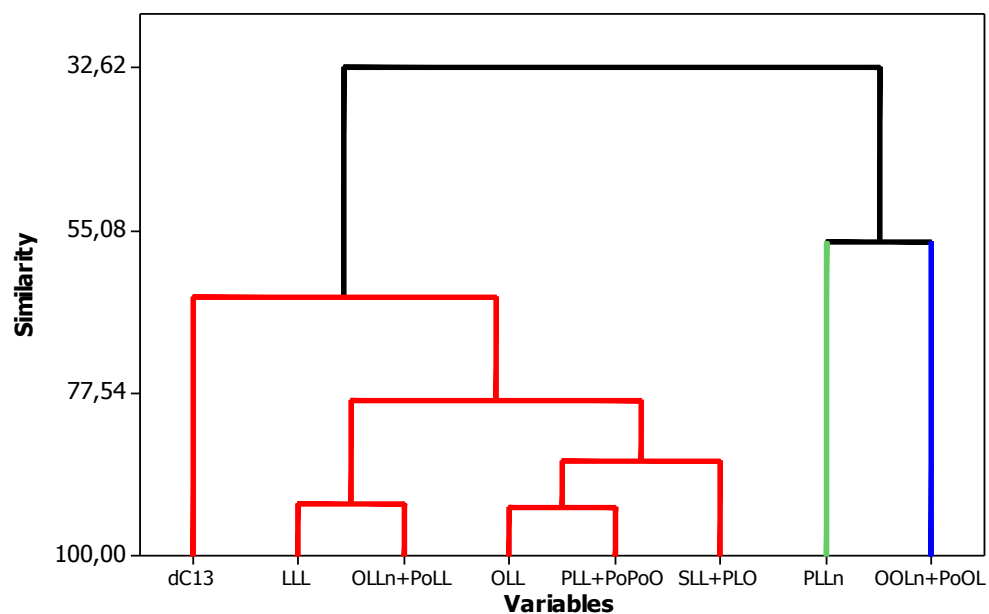


Figure 3.130. Cluster variables dendrogram of $\delta^{13}\text{C}$ and some variances of TAG compositions according the ward linkage, correlation coefficient distance for variables.

Table 3.29. Cluster variables and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and TAG.

CLUSTER	VARIABLES	SIMILARITY
RED	6	$\delta^{13}\text{C}$, LLL, OLLn+PoLL, OLL, PLL+PoPoO, SLL+PLO
GREEN	1	PLLn
BLUE	1	OOLn+PoOL

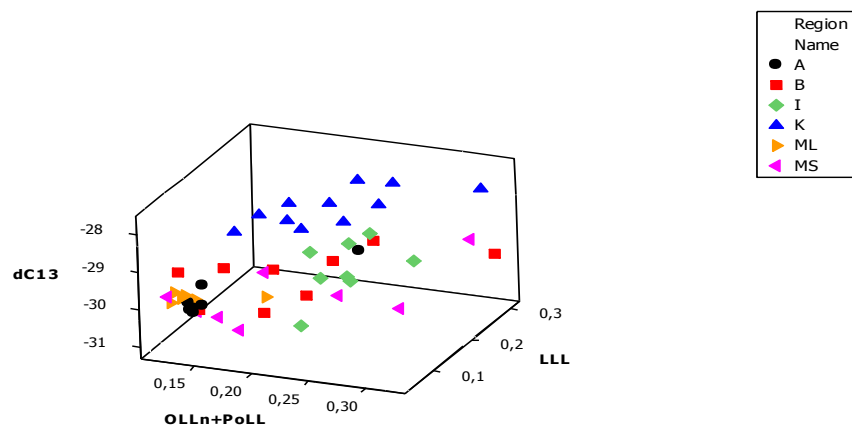


Figure 3.131. 3D-Scatterplot of $\delta^{13}\text{C}/^{12}\text{C}$ vs LLL vs OLLn+PoLL for geographical region.

3.6.4 $\delta^{13}\text{C}/^{12}\text{C}$ with trace elements

After all connection between concentrations of trace elements and $\delta^{13}\text{C}$ isotope ratio values were analysed, copper (Cu), zinc (Zn), iron (Fe) and calcium (Ca) were selected for the link with $\delta^{13}\text{C}$.

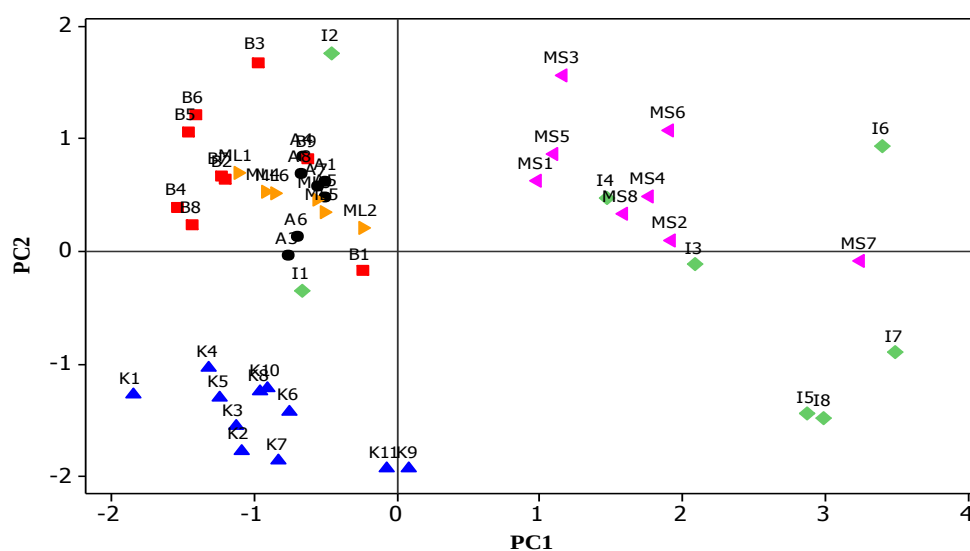


Figure 3.132. Score plot of $\delta^{13}\text{C}$ and some variances of trace elements compositions.

When we reduce the number of variances to five parametres for authentication we were realized they will haven to form class for some regions.

I and MS was splitted with PC1 from rest of others but PC1 was not supplied clustering on I and MS. K was seperated from B, A and ML under influence of PC2 as it can seen in Figure 3.132.

I and MS regions have neither cluster nor dispersion. Olive oils of K region was shown both clustering and splitting from others.

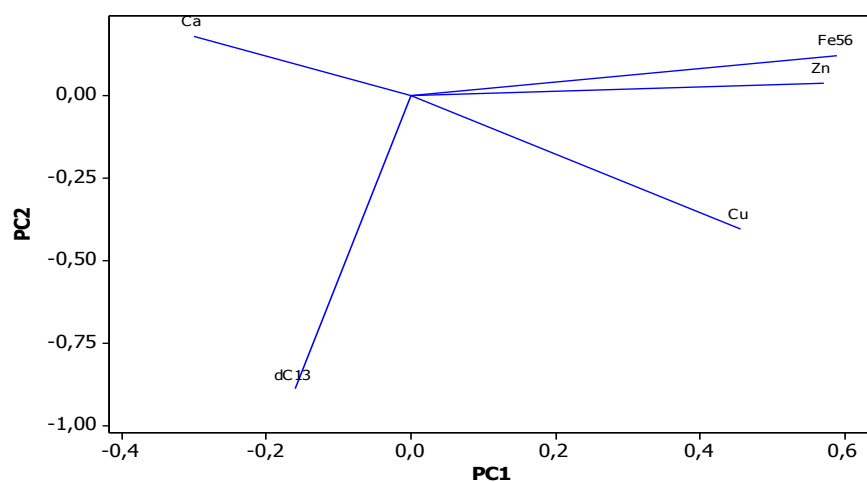


Figure 3.133. Loading plot of $\delta^{13}\text{C}$ and some variances of trace elements compositions.

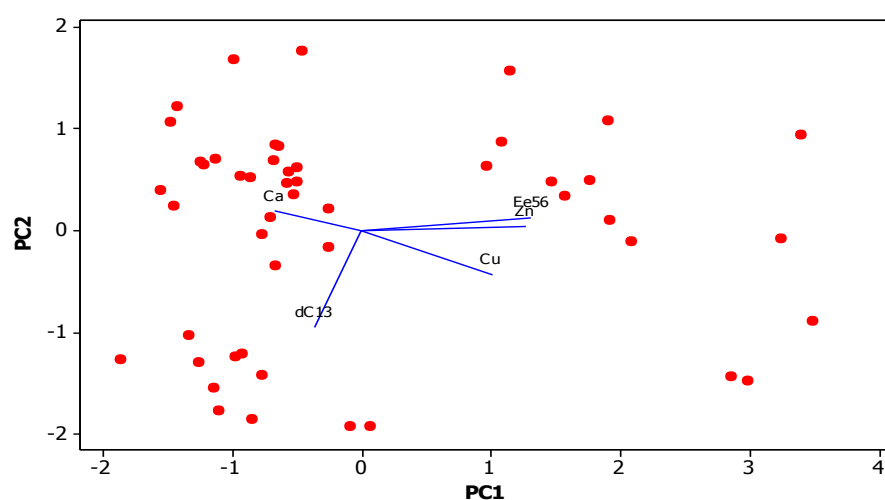


Figure 3.134. Biplot of $\delta^{13}\text{C}$ and some variances of trace elements compositions.

$\delta^{13}\text{C}$ was been most effective component on the Edremit bay (K) while Ca on Bursa (B) region and Muğla (ML). Izmir (I) and Manisa (MS) region has influenced from Fe56 and Zn variance as we can seen in loading plot graph in Figure 3.133 and biplot graph in Figure 3.134.

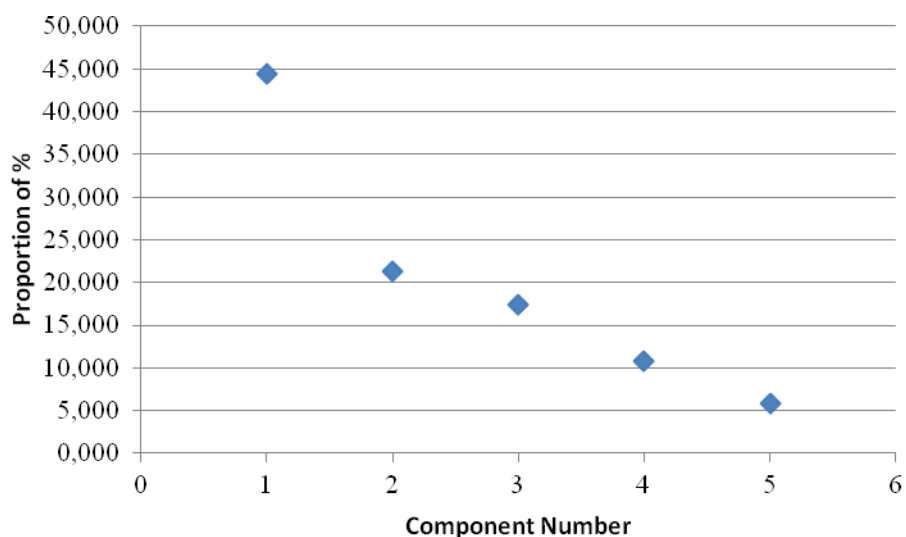


Figure 3.135. Proportional % eigen values vs component number for $\delta^{13}\text{C}$ and some variances of heavy metal compositions.

Table 3.30. Percent eigen values of $\delta^{13}\text{C}$ and some variances of heavy metal compositions.

Component Number	Eigen Value	Proportion of %	Cumulative Percentage
1	2,219	44,377	44,377
2	1,069	21,381	65,758
3	0,874	17,477	83,235
4	0,543	10,867	94,102
5	0,295	5,898	100,000

According to eigen values of $\delta^{13}\text{C}$ and some variances of trace elements of first variance has very high value in Figure 3.135.

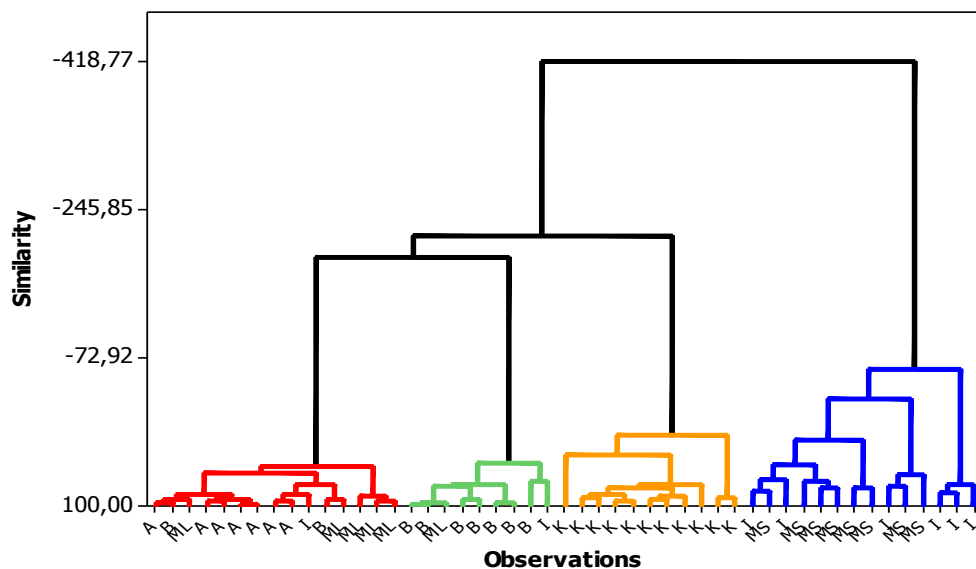


Figure 3.136. Cluster observations dendrogram of $\delta^{13}\text{C}$ and some variances of heavy metal compositions according the ward linkage, Euclidean distance with standardize variables for geographical regions.

Data were also auto-scaled using the ward linkage method by using cluster observations as shown in Figure 3.136. Edremit bay (K) was grouped as regards ward linkage, euclidean distance with standardize variables.

Table 3.31. Cluster observations and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and trace elements.

CLUSTER	OBSERVATIONS	SIMILARITY
RED	16	AYDIN (A) and MUĞLA (ML)
ORANGE	11	EDREMIT BAY (K)
GREEN	13	BURSA (B)
BLUE	12	MANİSA (MS) and IZMIR (I)

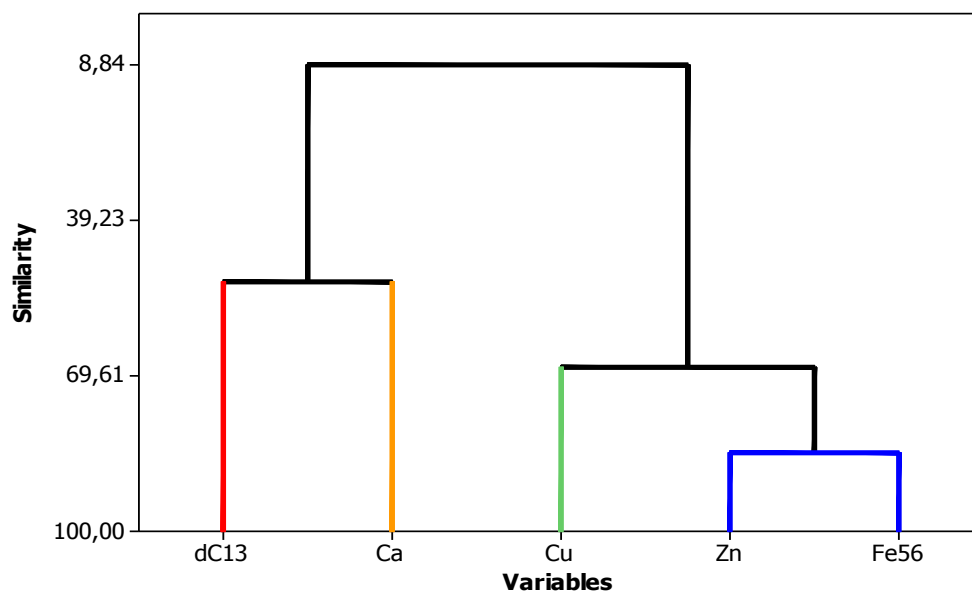


Figure 3.137. Cluster variables dendrogram of $\delta^{13}\text{C}$ and some variances of heavy metal compositions according the ward linkage, correlation coefficient distance for variables.

Table 3.32. Cluster variables and similarity of geographical regions according to dendrogram of $\delta^{13}\text{C}$ and trace elements.

CLUSTER	VARIABLES	SIMILARITY
RED	1	$\delta^{13}\text{C}$
ORANGE	1	Ca
GREEN	1	Cu
BLUE	2	Zn and Fe

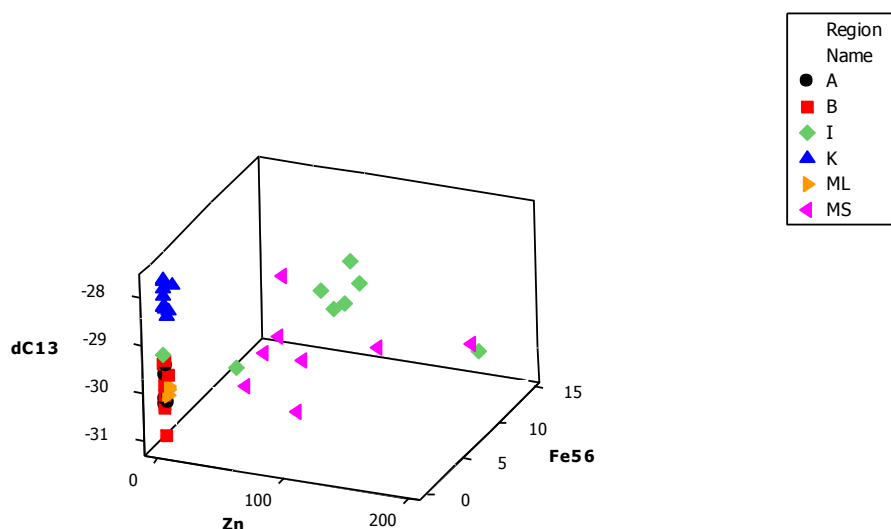


Figure 3.138. 3D-Scatterplot of $\delta^{13}\text{C}/^{12}\text{C}$ vs Fe vs Zn for geographical region.

3D-Scatterplot of vs Fe vs Zn for geographical region in Figure 3.138 was shown trace elements and $\delta^{13}\text{C}/^{12}\text{C}$ were evaluated with together olive oil of İzmir, Manisa and Edremit bay were separated each other and other regions.

A previous study which about isotope ratio and trace elements to show the results of the largest investigation ever carried out on multi-element isotope ratio and mineral composition using IRMS and ICP–MS in authentic PDO and PGI Italian extra-virgin olive oils. The capability to discriminate on the basis of minerals in PDOs could only be checked for few oils produced in specific areas with different geology and requires further research in order to be confirmed (Camin et al., 2010).

4. CONCLUSION

Olive oil is so important product in the sense of chemical and physical properties due to its sensory characteristic and its chemical composition show many variances which depend on olive ripening degree, harvesting time production methods, particularly extraction of oil, the climatic conditions and so on. In an attempt to increase the efficiency of the olive oil sector at international level, the compilation of data for the differentiation of different type of olive oils based on regional features might be beneficial and profoundly be critical.

New regulations on the quality of olive oils requires to the need to include geographical origin of principal quality foodstuffs on the label even as approval of trade mark steps. Many commercial products such as olive oil are produced in a well-defined region with specific physicochemical properties.

For this purpose, 49 samples which are deemed to be pure and-extracted from olives by known manufacturers were collected. These samples were collected from different regions including Marmara, North Aegean, Central Aegean and South Aegean regions. Since, these regions are quite critical in respect to oil production at which they accounted almost for 80.5 % of total of Turkey annual capacity overall. Minimum 6 maximum 12 samples were collected from each region.

The analyses carried out in this thesis include fatty acids methyl esters, sterols compositions, triacylglycerols, trace elements and $\delta C^{13}/C^{12}$ isotope ratio. Initial data were utilized in drawing column chart graphs and it was noticed that some important variances can be employed to determine their sole effect on the authentication and to find adulteration. PCA and HCA on the authentication was searched separately and the importance of to determine the adulterated oil was observed.

PCA analyses were evaluated so it was seen that Bursa (B), Manisa (MS) and İzmir (I) are clustered in score plot of sterols. Besides it was observed that oils from Bursa are constitute a regional cluster according to sterol composition. Although oils from İzmir and Manisa are present similar properties to each other, oils from İzmir are also grouped in itself according to heavy metals variances. Moreover, clustering is also seen in the dendograms drawn according to Ward and Single methods.

PCA and HCA analysis of sterol compositions of olive oils have revealed that phytosterols are important parameters for authentication and adulteration. FAME was investigated which have not dominant effect on the separation of regions according to results of PCA and HCA however, FAME was found more dominant variance for to determine adulterated oil.

While TAGs have not important role for authentication but TAGs have more power effect for adulteration. Trace element distribution, on the other hand, can be interrelated to the soil in which the olive tree partially transfers some elements. Consequently, trace element distributions of the samples were determined as another parameter. The region of İzmir (I) and Manisa (MS) are an excellent geographical area to study the importance of the soil composition for authenticity since they have rich hornblend, prit, calcoprit, sfalerit and barit minerals. Therefore, geographical structure has a significant influence on the trace element distribution.

The last parameter was the isotop ratio of the olive oil samples. As predictor variables in the PCA, $\delta^{13}\text{C}$ isotope ratio values showed the most significant discriminative power on Edremit Bay values.

Following the separate studies, the data of all oil samples were split into North and South regions to reveal any significant differences. By this means, Bursa and Edremit Bay constitute the North region, and İzmir, Manisa, Muğla and Aydın constitute the South region. For the sterol analyses, oils belonging to North region are forming a group according to score plot. In addition to this, some oils belonging to South region are present similar properties to oils from North region. As it was seen in the dendograms drawn according to Single and Ward methods, the North-South differentiation is clearer but some oils from South region are stay out of these two regions.

FAME analysis is basically for identifying quality and adulteration. However linoleic acid value singly indicates the addition of seed oils very clearly. For this reason, the linoleic acid column chart is enough for adulteration. It was observed that clustering is better even partially in PCA and cluster analyses without adulterated sample. According to North-South cluster and PCA analyses with the results of FAME analyses, clustering was not occurred very well.

For the TAG analysis, LLL parameter is singly sufficient for the adulteration LLL is the fundamental component to identify adulteration. And also the PCA and cluster analyses results support this. When the TAG analysis was repeated after removing the adulterated oil, it was seen that Edremit Bay oils are clustered and separated from other oils in score plot. In the regional clustering performed after North-South grouping, Marmara and Aegean regions were differentiated very clearly. So the TAG analysis is more effective than the PCA analysis to differentiate oils from Marmara and Aegean regions. However, clustering was not occurred very well in the cluster analysis which was performed according to both Ward and Single methods.

Trace element analysis has not given a good indication for adulteration but, it provides a significant indication on authentication. İzmir and Manisa are differentiated from all other regions very clearly as mentioned above for column charts. When the PCA analysis was repeated after removing Fe56 and Zn, again it was seen that Manisa and İzmir are differentiated from other regions. Based on these results, it can be concluded that soil characteristics of İzmir and Manisa are different from other regions and it might be attribute to Spil mountain especially for Manisa. However it couldn't be seen clustering and differentiation in this manner in the cluster dendograms drawn.

For delta ^{13}C analysis, it was seen that each region has own delta ^{13}C values but the Edremit Bay region was differentiated from other regions only with this value. When the average values of each region are taken into consideration, it was seen that the adulterated oil (seed oil added) was on the top of the average delta ^{13}C value of its region.

After all possible productive combinations between $\delta^{13}\text{C}/^{12}\text{C}$ isotope ratio with sterol compositions, fatty acid methyl esters, triacylglycerols, heavy metals levels were investigated, then, the value of each critical parameters were choosen in order to establish to the link between $\delta^{13}\text{C}$ isotope ratio and other variances.

Based on all of these results, it was seen that all of the analyses used for adulteration and regional grouping were served the purpose properly depending on variances. It was also concluded that chosen parameters are sufficient to make clustering, to find out adulteration and to identify authentication.

The geographical traceability of virgin olive oil can be controlled by chemical species that are linked to the production area. Sterol composition, triacylglycerols and fatty acid methyl esters are among these species and these variances are the most effective to identify the addition of seed oils to olive oil (adulteration). Isotope ratio of $\delta^{13}\text{C}/^{12}\text{C}$ and trace element analyses facilitate regional grouping of olive oils (authentication).

Further studies will include the combination of trace element distribution and isotope ratio of $\delta^{13}\text{C}/^{12}\text{C}$ to be used PDO and PDI of the olive oil samples from any source for labelling purposes.

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APPENDIX

Appendix 1 Mean Values of Relative Percentage for Sterol Compositions

Appendix 2 Mean Values of Relative Percentage for Fatty Acid Methyl Esters

Appendix 3 Mean Values of Relative Percentage for Triacylglycerols

Appendix 4 Mean Values of Concentrations for Heavy Metals

Appendix 5 Mean Values for Delta C13

Appendix 1 Mean Values of Relative Percentage for Sterol Compositions

Region	CHOLESTRL	24-METILEN	CAMPESTRL	CAMPESTANL	STIGMASTRL	D7-CAMPESTL	D5-23 STIGMAS	CLEROS	BETASITO	D5AVENA	D5-24STIGMA	D7STIGMAS	D7-AVENA
A1	0,44	0,27	3,45	0,00	1,29	0,00	0,72	0,59	82,54	4,10	3,30	0,48	0,63
A2	0,28	0,44	0,94	6,86	0,50	0,99	0,19	0,00	78,93	0,22	0,60	4,32	2,42
A3	0,39	0,82	2,37	0,11	1,13	0,13	1,18	0,40	88,50	2,66	0,14	0,21	0,45
A4	0,90	0,25	3,42	0,00	1,14	0,00	0,80	0,51	86,39	2,49	0,92	0,40	0,62
A5	0,46	0,30	2,80	0,08	1,11	0,01	0,78	0,49	87,85	2,53	0,88	0,41	0,60
A6	0,42	0,28	3,10	0,05	1,20	0,00	0,70	0,42	84,20	3,00	0,70	0,44	0,62
A7	0,45	0,31	3,10	0,01	1,24	0,00	0,73	0,58	83,67	3,35	3,20	0,43	0,65
A8	0,78	0,23	3,35	0,01	1,10	0,00	0,80	0,52	85,20	2,28	1,02	0,41	0,59
B1	1,29	0,25	0,78	0,20	1,76	0,00	0,66	1,95	74,58	9,20	5,42	0,60	0,76
B2	1,20	0,28	0,82	0,10	1,65	0,00	0,70	2,05	76,45	8,55	3,86	0,10	0,85
B3	0,95	0,54	0,75	0,00	1,03	0,00	1,04	2,20	71,56	11,93	4,69	0,74	0,99
B4	2,90	0,19	0,17	1,38	0,00	0,00	0,72	1,26	76,95	8,03	5,00	0,34	0,56
B5	0,95	0,18	0,49	0,00	0,87	0,00	1,07	1,36	71,54	16,57	4,21	0,20	1,07
B6	1,23	0,17	0,88	0,07	1,47	0,11	0,84	1,50	79,48	10,68	0,21	0,39	0,76
B7	1,06	0,00	1,59	0,00	1,62	0,00	0,88	1,02	78,14	7,76	4,44	0,65	0,64
B8	1,47	0,27	0,79	0,82	0,00	0,00	1,04	1,52	70,11	15,55	4,94	0,55	0,74
B9	0,89	0,19	0,96	0,00	2,13	0,00	0,94	1,30	74,86	11,03	3,96	0,67	0,98

Appendix 1 Mean Values of Relative Percentage for Sterol Compositions (continue)

Region	CHOLESTRL	24-METILEN	CAMPESTRL	CAMPESTANL	STIGMASTRL	D7-CAMPESTL	D5-23 STIGMAS	CLEROS	BETASITO	D5AVENA	D5-24STIGMA	D7STIGMAS	D7-AVENA
I1	2,85	0,13	1,90	1,85	0,15	0,00	1,12	1,25	79,66	3,51	4,20	0,34	0,67
I2	0,29	0,02	3,11	0,14	0,77	0,91	0,02	0,70	87,41	3,65	2,00	0,27	0,24
I3	1,00	0,07	1,93	0,26	0,85	1,11	0,21	0,74	75,55	12,35	4,33	0,20	0,55
I4	0,79	0,01	2,58	0,21	1,34	1,29	0,24	0,97	80,55	3,81	6,81	0,27	0,34
I5	0,48	0,01	2,13	0,28	0,41	0,56	1,79	0,00	68,76	9,57	14,85	0,27	0,89
I6	2,66	0,00	2,85	0,35	1,63	0,48	2,00	0,00	68,83	7,02	13,51	0,17	0,21
I7	2,50	0,08	2,15	0,22	1,43	0,76	1,45	1,12	73,24	8,97	12,51	0,23	0,55
I8	0,80	0,01	2,48	0,18	1,50	1,24	0,20	0,05	72,78	8,79	13,28	0,24	0,78
K1	0,21	0,14	2,20	0,10	0,59	0,16	0,51	0,72	80,89	10,49	1,27	0,14	1,06
K2	0,94	0,16	2,15	0,91	1,90	0,76	0,57	0,00	74,87	9,50	1,68	1,02	1,47
K3	0,46	0,28	2,29	0,24	0,76	0,00	0,98	0,73	80,31	9,56	1,16	0,25	1,16
K4	0,08	0,24	3,07	0,65	0,57	0,52	0,94	0,30	78,78	9,73	2,63	0,26	0,98
K5	0,24	0,23	1,69	0,29	0,80	0,00	0,87	0,47	80,96	9,87	1,86	0,07	1,15
K6	0,25	0,18	2,55	0,12	1,53	0,27	0,91	0,47	77,14	10,96	2,30	0,42	1,20
K7	0,41	0,17	1,90	0,05	2,02	0,23	0,86	0,59	80,17	10,06	1,17	0,15	0,82
K8	0,24	0,11	2,72	0,12	0,50	0,18	0,92	0,21	82,42	8,64	1,20	0,15	1,04
K9	0,13	0,12	3,00	0,10	0,57	0,09	0,83	0,43	81,29	10,02	0,91	0,08	0,97
K10	0,23	0,16	1,83	0,09	0,84	0,09	0,79	0,43	82,35	10,01	1,04	0,15	0,83
K11	0,33	0,56	2,85	0,20	0,72	0,72	0,74	0,33	80,17	9,27	0,79	0,31	1,11

Appendix 1 Mean Values of Relative Percentage for Sterol Compositions (continue)

Region	CHOLESTRL	24-METILEN	CAMPESTRL	CAMPESTANL	STIGMASTRL	D7-CAMPESTL	D5-23 STIGMAS	CLEROS	BETASITO	D5AVENA	D5-24STIGMA	D7STIGMAS	D7-AVENA
ML1	0,80	0,17	3,71	0,19	2,11	0,16	1,27	0,74	83,78	2,54	1,02	0,31	0,43
ML2	0,78	0,16	3,25	0,15	1,87	0,18	0,98	0,68	79,89	2,23	0,95	0,27	0,51
ML3	0,81	0,18	3,42	0,17	1,90	0,20	1,05	0,70	81,20	2,45	1,14	0,29	0,46
ML4	0,73	0,15	3,80	0,16	2,20	0,16	1,33	0,72	82,35	2,67	1,03	0,33	0,41
ML5	0,83	0,14	3,70	0,20	2,06	0,14	1,29	0,64	83,73	2,31	0,98	0,35	0,39
ML6	0,75	0,19	3,81	0,18	2,15	0,13	1,11	0,60	85,02	2,72	1,17	0,26	0,50
MS1	1,17	0,17	3,28	0,00	1,55	0,00	0,93	0,60	80,39	12,75	3,62	0,28	0,45
MS2	2,50	0,07	2,10	0,40	1,39	0,81	1,10	1,22	76,85	11,45	7,84	0,86	0,32
MS3	1,42	0,03	1,72	0,34	0,90	0,71	0,07	1,69	71,63	12,64	6,66	0,25	0,26
MS4	1,76	0,12	1,84	0,48	0,91	0,53	0,00	0,51	72,55	13,28	6,24	0,16	0,38
MS5	1,45	0,09	1,88	0,50	0,89	1,17	0,21	0,72	75,88	12,31	3,66	0,89	0,34
MS6	2,65	0,00	2,19	0,68	1,20	0,98	2,97	0,95	74,45	11,00	6,78	0,22	0,19
MS7	1,56	0,15	1,78	0,41	0,97	0,87	0,18	0,80	72,21	12,87	7,02	0,18	0,32
MS8	1,68	0,13	1,87	0,39	1,09	0,94	0,10	0,65	71,98	13,32	6,89	0,21	0,35

Appendix 2 Mean Values of Relative Percentage for Fatty Acid Methyl Esters

Region	PALMITIC	PALMITOLEIC	HEPTADECNOIC	STEARIC	OLEIC	LINOLEIC	ARACHIDIC	GONDOIC	LINOLENIC	HENEICOSONOIC	BEHENIC	ARACHIDONIC	LIGNOCERIC	NERVONIC
A1	8,50	0,27	0,00	2,96	76,09	8,16	0,83	0,38	0,60	0,10	0,36	1,92	0,20	0,07
A2	5,61	0,18	0,07	3,48	49,32	37,48	0,59	0,39	0,44	0,02	0,91	0,33	0,42	0,03
A3	7,03	0,29	0,05	3,18	75,08	10,66	0,73	0,57	0,70	0,04	0,22	1,08	0,11	0,03
A4	8,78	0,46	0,04	3,27	77,28	7,45	0,59	0,44	0,66	0,02	0,18	0,59	0,08	0,01
A5	8,25	0,40	0,04	3,10	77,34	8,79	0,61	0,45	0,68	0,02	0,20	1,05	0,06	0,02
A6	7,90	0,45	0,05	3,23	76,10	8,48	0,58	0,51	0,66	0,03	0,25	0,95	0,08	0,01
A7	7,50	0,38	0,05	3,02	76,90	8,25	0,64	0,52	0,70	0,02	0,18	0,68	0,08	0,01
A8	8,15	0,32	0,06	2,98	75,68	9,45	0,73	0,49	0,64	0,04	0,30	0,75	0,07	0,02
I1	8,50	0,46	0,08	3,26	74,63	9,03	0,76	0,46	0,74	0,06	0,28	1,28	0,17	0,05
I2	10,88	0,71	0,04	2,19	75,57	7,81	0,50	0,35	0,81	0,03	0,14	0,80	0,08	0,03
I3	12,96	0,93	0,10	3,16	72,67	7,54	0,48	0,27	0,58	0,02	0,13	1,01	0,06	0,01
I4	10,68	0,56	0,04	2,60	75,08	8,44	0,45	0,35	0,71	0,02	0,13	0,80	0,06	0,01
I5	10,26	0,46	0,14	2,95	72,36	11,62	0,45	0,33	0,67	0,02	0,12	0,03	0,06	0,01
I6	11,36	0,66	0,05	2,63	73,31	9,38	0,50	0,38	0,69	0,03	0,15	0,03	0,08	0,01
I7	9,65	0,41	0,14	2,95	72,52	12,11	0,46	0,35	0,67	0,03	0,13	0,02	0,06	0,01
I8	10,25	0,58	0,08	2,86	72,80	10,28	0,47	0,34	0,68	0,03	0,12	0,03	0,07	0,01

Appendix 2 Mean Values of Relative Percentage for Fatty Acid Methyl Esters (continue)

Region	PALMITIC	PALMITOLEIC	HEPTADECNOIC	STEARIC	OLEIC	LINOLEIC	ARACHIDIC	GONDOIC	LINOLENIC	HENEICOSONOIC	BEHENIC	ARACHIDONIC	LIGNOCERIC	NERVONIC
ML1	10,05	0,03	0,07	2,73	73,46	9,09	0,60	0,42	0,70	0,03	0,21	1,01	0,08	0,02
ML2	9,85	0,03	0,08	2,85	73,95	9,20	0,62	0,42	0,68	0,03	0,20	1,05	0,09	0,02
ML3	10,15	0,03	0,09	2,70	72,85	10,05	0,61	0,44	0,71	0,03	0,22	0,95	0,10	0,02
ML4	9,75	0,04	0,10	2,90	73,00	9,25	0,63	0,43	0,69	0,04	0,20	1,00	0,08	0,03
ML5	10,21	0,03	0,08	2,68	73,55	9,04	0,64	0,40	0,72	0,03	0,21	0,88	0,07	0,03
ML6	9,84	0,04	0,11	2,86	72,78	10,32	0,61	0,41	0,70	0,03	0,23	0,91	0,09	0,02
MS1	10,35	0,48	0,05	3,21	74,50	9,84	0,61	0,40	0,79	0,04	0,18	0,54	0,08	0,02
MS2	12,64	0,92	0,15	1,68	72,84	9,62	0,35	0,28	0,69	0,03	0,08	0,51	0,06	0,04
MS3	11,47	0,77	0,15	2,90	76,27	5,89	0,45	0,29	0,60	0,02	0,13	0,92	0,07	0,01
MS4	12,51	1,02	0,15	2,79	75,40	5,72	0,42	0,26	0,62	0,02	0,12	0,84	0,06	0,01
MS5	12,20	1,06	0,12	2,39	76,07	5,61	0,39	0,28	0,58	0,02	0,11	1,03	0,06	0,01
MS6	12,37	1,01	0,14	3,03	74,18	6,51	0,51	0,30	0,61	0,02	0,13	0,99	0,07	0,01
MS7	14,37	1,19	0,11	1,45	68,32	12,22	0,38	0,30	0,74	0,03	0,12	0,56	0,06	0,01
MS8	12,18	0,90	0,12	2,24	72,73	9,49	0,41	0,33	0,67	0,02	0,13	0,03	0,07	0,01

Appendix 2 Mean Values of Relative Percentage for Fatty Acid Methyl Esters (continue)

Region	PALMITIC	PALMITOLEIC	HEPTADECNOIC	STEARIC	OLEIC	LINOLEIC	ARACHIDIC	GONDOIC	LINOLENIC	HENEICOSONOIC	BEHENIC	ARACHIDONIC	LIGNOCERIC	NERVONIC
K1	10,29	0,47	0,12	2,86	72,78	10,40	0,63	0,41	0,58	0,03	0,21	0,78	0,10	0,02
K2	10,63	0,49	0,12	2,84	72,29	10,61	0,59	0,42	0,61	0,02	0,20	0,77	0,10	0,02
K3	10,65	0,50	0,12	2,71	73,10	9,93	0,59	0,40	0,57	0,03	0,20	0,78	0,09	0,02
K4	9,89	0,51	0,15	2,91	72,74	10,61	0,65	0,44	0,63	0,03	0,22	0,77	0,10	0,02
K5	9,25	0,40	0,12	2,89	73,97	10,30	0,63	0,43	0,59	0,02	0,22	0,79	0,09	0,02
K6	10,52	0,54	0,12	2,69	72,04	11,26	0,55	0,41	0,64	0,04	0,19	0,59	0,09	0,02
K7	10,24	0,46	0,12	2,96	71,99	11,31	0,63	0,41	0,60	0,03	0,21	0,66	0,09	0,01
K8	9,00	0,40	0,12	2,83	74,74	9,33	0,71	0,46	0,59	0,04	0,23	1,05	0,12	0,02
K9	9,93	0,43	0,12	2,91	72,93	10,46	0,64	0,44	0,64	0,05	0,23	0,82	0,10	0,02
K10	8,50	0,37	0,13	3,05	74,24	10,27	0,71	0,47	0,65	0,04	0,26	0,90	0,11	0,02
K11	10,26	0,64	0,10	2,55	70,75	11,98	0,61	0,44	0,74	0,04	0,22	1,21	0,12	0,02
B1	9,95	0,68	0,11	3,58	75,19	6,99	0,61	0,36	0,61	0,03	0,20	1,25	0,10	0,02
B2	7,36	0,43	0,11	3,90	76,69	7,14	0,78	0,43	0,62	0,03	0,24	1,84	0,12	0,03
B3	9,41	0,51	0,14	3,97	76,09	6,94	0,63	0,33	0,51	0,03	0,18	0,85	0,08	0,02
B4	7,28	0,42	0,13	3,43	75,95	8,80	0,81	0,50	0,70	0,07	0,27	1,16	0,15	0,02
B5	7,46	0,41	0,16	4,27	75,61	7,66	1,08	0,47	0,57	0,04	0,29	1,40	0,15	0,04
B6	10,06	0,54	0,13	3,41	74,19	8,62	0,53	0,35	0,57	0,03	0,15	0,00	0,07	0,01
B7	10,33	0,56	0,09	3,11	75,33	7,59	0,53	0,36	0,64	0,03	0,15	0,94	0,08	0,01
B8	8,62	0,40	0,15	3,97	77,36	6,31	0,62	0,00	0,51	0,03	0,18	1,13	0,08	0,01
B9	9,62	0,54	0,13	3,44	76,86	6,14	0,55	0,33	0,51	0,03	0,16	1,19	0,08	0,02

Appendix 3 Mean Values of Relative Percentage for Triacylglycerols

Region	LLL	OLLn+PoLL	PLLn	OLL	OOLn+PoOL	PLL+PoPoO	POLn+PoPoPo+PPoL	OOL+LnPP	PoOO	SLL+PLO	PoOP+SPoL+SOLn+SPoPo	PLP	OOO+PoPP	SOL	POO	POP	SOO	SOS+SLS
A1	0,08	0,13	0,10	1,48	1,28	0,49	0,53	15,12	1,25	5,64	0,59	0,99	42,65	1,20	20,85	2,44	4,95	1,27
A2	12,11	0,24	0,14	18,99	1,08	5,47	0,32	14,32	2,93	6,24	0,34	0,82	20,17	2,46	9,51	1,54	2,43	0,90
A3	0,22	0,23	0,10	2,47	1,40	0,91	0,55	15,33	1,29	6,27	0,70	0,73	39,92	1,23	19,77	2,74	4,77	1,37
A4	0,08	0,14	0,10	1,43	1,35	0,46	0,58	12,16	1,23	4,77	0,48	1,00	45,31	1,20	20,90	2,12	5,46	1,24
A5	0,08	0,13	0,10	1,46	1,29	0,49	0,57	12,75	1,28	6,02	0,52	0,78	44,75	1,20	21,10	2,82	4,86	1,29
A6	0,09	0,14	0,10	1,47	1,30	0,47	0,55	15,05	1,25	4,25	0,49	0,98	42,20	1,23	19,79	2,46	5,13	1,49
A7	0,08	0,14	0,11	1,49	1,33	0,46	0,58	13,22	1,28	5,78	0,64	0,79	43,10	1,22	20,76	2,27	4,77	1,33
A8	0,07	0,13	0,11	1,50	1,39	0,49	0,55	12,78	1,26	6,06	0,59	0,91	41,24	1,20	22,05	2,08	5,22	1,14
B1	0,27	0,23	0,22	1,34	1,21	0,42	0,52	9,36	1,89	4,45	1,09	0,71	39,51	1,01	23,74	5,16	5,51	2,35
B2	0,30	0,32	0,22	3,35	1,45	1,26	0,88	15,82	1,25	7,49	0,51	0,80	37,02	1,11	20,50	2,59	4,83	1,41
B3	0,16	0,17	0,04	1,42	1,23	0,56	0,58	10,08	1,83	4,56	1,04	0,44	42,34	0,84	23,16	3,56	6,20	1,78
B4	0,09	0,12	0,07	1,28	1,12	0,38	0,46	10,23	1,49	4,31	0,80	0,67	42,02	0,93	23,79	4,29	6,04	1,93
B5	0,24	0,20	0,12	2,16	1,24	0,70	0,56	11,80	1,62	5,30	0,83	0,61	40,55	1,05	22,89	3,58	5,08	1,45
B6	0,08	0,14	0,04	1,55	1,21	0,56	0,53	11,00	1,58	5,03	0,68	0,62	40,14	1,12	23,76	3,76	6,39	1,80
B7	0,18	0,17	0,08	2,14	1,19	0,68	0,42	12,04	1,74	5,24	0,96	0,47	41,05	0,98	21,67	3,96	5,36	1,67
B8	0,11	0,15	0,05	1,49	1,37	0,51	0,55	11,34	1,36	4,35	0,44	0,35	43,66	1,16	22,64	3,80	5,34	1,32
B9	0,16	0,20	0,07	2,88	1,23	0,62	0,52	11,67	1,42	4,26	0,69	0,60	38,97	1,06	22,90	3,82	5,63	1,59

Appendix 3 Mean Values of Relative Percentage for Triacylglycerols (continue)

Region	LLL	OLLn+PoLL	PLLn	OLL	OOLn+PoOL	PLL+PoPoO	POLn+PoPoPo+PPoL	OOL+LnPP	PoOO	SLL+PLO	PoOP+SPoL+SOLn+SPoPo	PLP	OOO+PoPP	SOL	POO	POP	SOO	SOS+SLS
I1	0,17	0,21	0,09	1,94	1,46	0,77	0,82	12,10	1,57	6,28	0,79	0,96	36,93	0,96	24,23	4,29	4,95	1,48
I2	0,09	0,22	0,07	1,64	1,72	0,60	0,73	12,41	1,47	5,18	0,54	1,09	42,77	0,89	21,79	2,89	4,56	1,32
I3	0,15	0,24	0,05	1,50	1,33	0,60	0,68	11,28	2,12	5,86	1,44	0,50	39,08	0,98	23,54	4,14	5,04	1,44
I4	0,15	0,22	0,04	1,89	1,49	0,49	0,66	13,83	1,18	5,64	0,59	0,72	42,24	1,04	22,08	2,50	4,40	0,84
I5	0,19	0,23	0,10	3,39	1,32	0,86	0,64	16,98	1,06	6,72	0,46	0,82	38,76	1,65	18,92	2,46	4,39	1,05
I6	0,21	0,22	0,05	2,28	1,51	0,76	0,70	13,83	1,54	6,16	0,79	0,85	39,51	1,10	21,64	3,28	4,45	1,10
I7	0,22	0,28	0,12	3,57	1,32	0,95	0,60	17,70	1,01	6,97	0,51	1,09	38,35	1,92	17,92	2,19	4,49	0,79
I8	0,21	0,24	0,10	2,78	1,54	0,82	0,61	17,04	1,02	5,57	0,61	0,85	39,24	1,25	19,25	2,61	4,43	0,86
K1	0,17	0,27	0,07	2,01	1,06	0,63	0,64	13,53	1,32	6,92	1,02	0,38	37,82	0,93	24,08	3,97	4,14	1,24
K2	0,23	0,23	0,12	2,46	1,15	0,89	0,73	14,29	1,24	7,40	0,70	0,89	35,64	1,08	23,58	3,85	4,21	1,32
K3	0,17	0,22	0,05	2,38	1,27	0,83	0,72	14,86	1,41	7,12	0,89	0,95	36,64	1,32	22,16	3,43	4,34	1,24
K4	0,15	0,17	0,06	2,26	1,16	0,79	0,64	13,88	1,28	6,88	0,90	0,99	37,30	1,13	23,18	3,80	4,04	1,38
K5	0,18	0,19	0,09	2,52	1,28	0,89	0,68	14,64	1,16	7,00	0,53	0,88	36,37	1,14	22,98	3,83	4,35	1,30
K6	0,15	0,19	0,05	2,35	1,15	0,76	0,64	14,88	1,24	6,96	0,62	0,53	38,32	1,03	22,65	2,83	4,19	1,45
K7	0,24	0,26	0,09	2,79	1,18	0,95	0,60	15,49	1,23	7,49	0,66	0,72	35,27	1,19	22,18	3,60	4,69	1,37
K8	0,31	0,31	0,12	3,23	1,50	1,26	0,80	14,82	1,80	9,26	1,31	1,32	30,45	1,02	23,45	4,67	3,25	1,10
K9	0,11	0,16	0,05	1,89	1,05	0,60	0,56	12,90	1,28	6,63	0,97	0,84	38,37	1,04	24,23	4,35	3,99	0,98
K10	0,17	0,24	0,05	2,34	1,07	0,79	0,57	14,59	1,34	7,16	0,86	1,05	36,76	1,25	22,54	3,71	4,15	1,36
K11	0,14	0,21	0,08	2,29	1,21	0,67	0,60	14,85	1,12	6,55	0,70	0,94	38,45	1,30	21,66	3,57	4,69	0,99

Appendix 3 Mean Values of Relative Percentage for Triacylglycerols (continue)

Region	LLL	OLLn+PoLL	PLLn	OLL	OOLn+PoOL	PLL+PoPoO	POLn+PoPoPo+PPoL	OOL+LnPP	PoOO	SLL+PLO	PoOP+SPoL+SOLn+SPoPo	PLP	OOO+PoPP	SOL	POO	POP	SOO	SOS+SLS
ML1	0,11	0,19	0,07	1,86	1,44	0,64	0,60	13,21	1,31	6,58	0,55	0,47	38,75	0,96	23,92	4,02	4,08	1,22
ML2	0,07	0,12	0,04	1,44	1,31	0,53	0,59	12,77	1,07	5,00	0,42	0,60	43,96	1,06	21,97	3,46	4,64	0,85
ML3	0,09	0,13	0,07	1,77	1,42	0,58	0,61	12,87	1,23	6,22	0,56	0,58	39,75	0,98	23,35	3,81	4,23	1,03
ML4	0,08	0,13	0,06	1,54	1,33	0,60	0,61	12,17	1,09	5,99	0,49	0,51	41,10	1,05	22,90	3,90	4,09	1,19
ML5	0,08	0,12	0,05	0,94	1,41	0,60	0,62	12,93	1,30	5,69	0,49	0,52	39,65	1,02	22,66	4,05	3,94	1,27
ML6	0,08	0,12	0,08	1,52	1,36	0,60	0,59	12,43	1,18	6,11	0,52	0,53	39,05	1,00	23,05	4,13	4,18	1,21
MN1	0,14	0,18	0,08	2,26	1,48	0,60	0,52	14,62	1,32	5,95	0,35	0,41	41,05	1,38	21,65	2,08	4,82	1,09
MN2	0,17	0,28	0,06	2,27	1,57	0,83	0,94	14,00	1,78	6,32	0,98	0,64	37,81	0,79	22,81	4,11	3,46	1,19
MN3	0,12	0,12	0,03	1,00	1,32	0,33	0,51	9,61	1,79	4,10	1,15	0,54	44,06	0,91	24,45	3,42	5,34	1,20
MN4	0,04	0,12	0,03	0,84	1,19	0,52	1,47	8,97	2,80	5,16	3,10	0,77	41,05	0,81	23,84	3,91	4,75	1,44
MN5	0,11	0,14	0,08	0,93	1,33	0,32	0,61	8,92	2,35	4,14	1,49	1,17	42,84	1,22	25,14	3,92	4,06	1,21
MN6	0,12	0,16	0,13	1,22	1,31	0,54	0,68	9,20	2,03	4,41	1,05	1,24	40,55	1,21	25,37	4,07	4,91	1,79
MN7	0,29	0,30	0,12	3,29	1,66	1,24	0,89	15,00	1,93	8,58	1,19	1,17	31,32	0,97	23,38	4,12	3,19	1,34
MN8	0,15	0,24	0,09	2,20	1,42	0,60	0,65	13,97	1,80	6,41	1,16	1,14	37,78	1,04	22,93	4,00	3,52	0,89

Appendix 4 Mean Values of Concentrations for Heavy Metals

Region	V	Cr	Mn	Co	Ni	Cu	Zn	As	Ba	Pb208	Na	Mg	K	Ca	Fe56	Zn
A1	0,130	0,320	0,180	0,010	1,135	0,450	3,694	0,060	0,325	0,240	0,092	0,021	0,027	0,220	0,011	3,694
A2	0,022	0,230	0,130	0,002	0,300	0,330	1,000	0,036	0,260	0,043	0,075	0,019	0,000	0,150	0,008	1,000
A3	0,120	0,300	0,220	0,010	1,216	0,450	3,615	0,022	0,426	0,530	0,089	0,022	0,023	0,300	0,012	3,615
A4	0,020	0,070	0,090	0,000	0,490	0,250	1,607	0,020	0,180	0,050	0,075	0,013	0,000	0,180	0,005	1,607
A5	0,140	0,300	0,170	0,010	1,120	0,410	3,567	0,020	0,350	0,080	0,080	0,020	0,020	0,180	0,040	3,567
A6	0,130	0,250	0,140	0,005	1,150	0,420	1,876	0,030	0,300	0,500	0,072	0,018	0,018	0,250	0,011	1,876
A7	0,120	0,350	0,090	0,010	1,160	0,350	3,450	0,025	0,450	0,550	0,090	0,020	0,000	0,180	0,016	3,450
A8	0,116	0,240	0,160	0,000	1,120	0,320	1,800	0,020	0,400	0,480	0,075	0,014	0,020	0,220	0,001	1,800
I1	0,022	0,053	0,100	0,000	0,300	0,420	1,239	0,026	0,180	0,060	0,074	0,013	0,000	0,130	0,006	1,239
I2	0,000	0,000	0,160	0,000	0,636	0,112	17,640	0,000	0,232	0,067	0,075	0,000	0,007	0,612	6,999	17,640
I3	0,000	0,345	0,229	0,000	0,905	0,825	75,430	0,000	0,398	0,329	0,023	0,000	0,000	0,532	10,650	75,430
I4	0,000	0,030	0,262	0,000	0,345	0,544	50,380	0,000	0,329	0,326	0,087	0,001	0,010	0,690	13,150	50,380
I5	0,000	0,340	0,330	0,000	0,541	1,827	96,200	0,000	0,437	0,426	0,038	0,000	0,009	0,682	8,691	96,200
I6	0,054	1,471	0,348	0,000	0,294	0,754	180,400	0,000	0,289	0,506	0,087	0,000	0,000	0,730	12,440	180,400
I7	0,000	0,098	0,787	0,000	0,674	2,020	72,570	0,000	0,565	0,751	0,046	0,012	0,014	0,608	14,670	72,570
I8	0,001	0,045	0,368	0,000	0,568	1,750	76,780	0,000	0,385	0,345	0,064	0,000	0,000	0,675	12,750	76,780

Appendix 4 Mean Values of Concentrations for Heavy Metals (continue)

Region	V	Cr	Mn	Co	Ni	Cu	Zn	As	Ba	Pb208	Na	Mg	K	Ca	Fe56	Zn
ML1	0,120	0,300	0,170	0,008	0,810	0,510	4,159	0,029	0,480	0,310	0,085	0,025	0,013	0,250	0,011	4,159
ML2	0,100	0,280	0,150	0,007	1,024	0,610	3,245	0,013	0,300	0,210	0,079	0,015	0,017	0,180	0,009	3,245
ML3	0,120	0,240	0,190	0,008	1,321	0,580	4,577	0,028	0,350	0,270	0,080	0,026	0,020	0,610	0,012	4,577
ML4	0,110	0,260	0,120	0,007	0,750	0,340	3,900	0,023	0,260	0,200	0,078	0,020	0,016	0,150	0,009	3,900
ML5	0,085	0,200	0,150	0,004	1,000	0,500	4,150	0,015	0,280	0,160	0,054	0,018	0,017	0,340	0,008	4,150
ML6	0,100	0,250	0,160	0,000	0,900	0,450	4,400	0,022	0,300	0,210	0,070	0,020	0,018	0,300	0,006	4,400
MS1	0,000	0,000	0,128	0,000	0,328	0,115	16,080	0,000	0,174	0,100	0,024	0,000	0,011	0,316	13,960	16,080
MS2	0,000	0,000	0,168	0,065	0,646	1,410	30,540	0,000	1,128	1,307	0,057	0,000	0,025	0,359	8,550	30,540
MS3	0,000	0,001	0,150	0,000	0,400	0,160	18,900	0,000	0,176	0,100	0,030	0,000	0,010	0,300	12,750	18,900
MS4	0,000	0,416	0,322	0,000	0,959	0,320	129,000	0,000	0,944	0,834	0,106	0,005	0,104	0,719	7,560	129,000
MS5	0,019	0,000	0,143	0,000	0,225	0,774	30,610	0,000	0,276	0,172	0,028	0,000	0,030	0,250	5,980	30,610
MS6	0,000	0,151	0,373	0,000	0,383	1,062	69,910	0,000	0,327	0,277	0,104	0,000	0,015	0,319	6,315	69,910
MS7	0,000	0,760	0,160	0,000	0,180	0,785	197,600	0,000	0,306	0,533	0,058	0,000	0,027	0,470	8,650	197,600
MS8	0,010	0,120	0,200	0,000	0,275	0,800	72,200	0,000	0,275	0,350	0,040	0,000	0,025	0,380	6,750	72,200

Appendix 4 Mean Values of Concentrations for Heavy Metals (continue)

Region	V	Cr	Mn	Co	Ni	Cu	Zn	As	Ba	Pb208	Na	Mg	K	Ca	Fe56	Zn
K1	0,140	0,260	0,150	0,006	0,920	0,390	2,379	0,017	0,430	0,200	0,080	0,022	0,015	0,260	0,009	2,379
K2	0,120	0,260	0,150	0,007	0,860	0,370	2,542	0,020	0,300	0,200	0,081	0,018	0,021	0,290	0,011	2,542
K3	0,120	0,320	0,260	0,010	0,970	0,415	1,897	0,026	0,310	0,320	0,086	0,018	0,018	0,200	0,034	1,897
K4	0,110	0,300	0,200	0,008	0,680	0,410	2,573	0,020	0,350	0,150	0,080	0,017	0,100	0,230	0,009	2,573
K5	0,130	0,340	0,170	0,007	0,600	0,380	2,181	0,018	0,420	0,190	0,077	0,021	0,020	0,280	0,010	2,181
K6	0,100	0,280	0,150	0,007	1,024	0,610	1,843	0,013	0,300	0,210	0,079	0,015	0,017	0,180	0,009	1,843
K7	0,120	0,240	0,190	0,008	1,321	0,580	9,844	0,028	0,350	0,270	0,080	0,026	0,020	0,610	0,012	9,844
K8	0,110	0,260	0,120	0,007	0,620	0,340	1,307	0,023	0,260	0,200	0,078	0,020	0,016	0,150	0,009	1,307
K9	0,083	0,200	0,150	0,004	1,232	1,277	4,546	0,015	0,280	0,160	0,054	0,018	0,017	0,340	0,008	4,546
K10	0,130	0,290	0,190	0,007	1,901	0,450	5,643	0,020	0,380	0,300	0,081	0,023	0,019	0,370	0,011	5,643
K11	0,100	0,350	0,910	0,007	0,490	1,101	2,546	0,025	0,920	0,950	0,087	0,019	0,098	0,240	0,013	2,546
B1	0,130	0,290	0,220	0,008	2,586	0,610	5,427	0,036	0,480	0,430	0,081	0,021	0,014	0,410	0,013	5,427
B2	0,125	0,280	0,180	0,010	0,610	0,480	1,143	0,060	0,390	0,260	0,083	0,019	0,014	0,150	0,010	1,143
B3	0,010	0,030	0,570	0,009	0,860	0,380	2,431	0,022	0,230	0,510	0,079	0,015	0,017	0,230	0,009	2,431
B4	0,010	0,011	0,065	0,000	0,330	0,370	0,800	0,006	0,220	0,032	0,073	0,011	0,000	0,120	0,004	0,800
B5	0,009	0,033	0,090	0,000	0,750	0,310	1,682	0,010	0,280	0,030	0,072	0,017	0,000	0,210	0,005	1,682
B6	0,014	0,044	0,100	0,000	0,330	0,400	1,822	0,015	0,170	0,110	0,071	0,010	0,000	0,160	0,006	1,822
B7	0,021	0,150	0,150	0,000	1,181	0,440	1,942	0,045	0,340	0,120	0,075	0,019	0,000	0,220	0,009	1,942
B8	0,110	0,260	0,180	0,009	0,700	0,440	1,959	0,012	0,330	0,200	0,084	0,022	0,017	0,200	0,015	1,959
B9	0,014	0,070	0,100	0,000	0,580	0,380	1,575	0,003	0,290	0,083	0,071	0,016	0,000	0,190	0,010	1,575

Appendix 5 Mean Values for Delta C13

Region Name	Delta C13	Region Name	Delta C13	Region Name	Delta C13	Region Name	Delta C13	Region Name	Delta C13	Region Name	Delta C13
ML1	-29,99	A1	-30,24	I1	-29,27	MS1	-29,63	B1	-29,69	K1	-27,75
ML2	-30,06	A2	-28,13	I2	-30,50	MS2	-30,40	B2	-29,90	K2	-27,70
ML3	-30,10	A3	-29,49	I3	-29,60	MS3	-30,70	B3	-30,95	K3	-27,89
ML4	-29,90	A4	-30,32	I4	-29,70	MS4	-29,70	B4	-29,47	K4	-28,26
ML5	-30,00	A5	-30,10	I5	-29,10	MS5	-30,70	B5	-30,10	K5	-28,06
ML6	-29,95	A6	-29,69	I6	-30,30	MS6	-31,10	B6	-30,29	K6	-28,28
								B7	-29,90	K7	-27,76
								B8	-29,40	K8	-28,26
								B9	-30,36	K9	-28,44
										K10	-28,32
										K11	-28,32

