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CHROMATOGRAPHIC ANALYSIS AND COMPARISON OF CHEMICAL COMPOSITION OF THE SAMPLES BETWEEN OLIVE OILS IN EUROPE AND SOME CHINESE OILS

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Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Aknowledgement

The work here presented was carried out in two different phases, the first one, the experimental part, in the Central South University, China, and the second one, the writing part, in the University of Algarve, Portugal.

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To my father and my brother, I also want to say thank you for all the encouragement, and most specially, to my mother.

Resumo

O azeite é um óleo vegetal caracteristico da denominada "dieta Mediterranica", bem conhecida pelos seus efeitos benéficos para a saúde humana, tais como na prevenção de doenças cardiovasculares e na redução da incidência de cancro. Para além dos efeitos benéficos para a saúde, o azeite apresenta também uma elevada importância económica na região do Mediterrâneo, sendo esta responsável por mais de 90% da produção mundial de azeite. Tal como o azeite apresenta um forte impacto na economia dos países do Mediterrâneo, outros tipos de óleos vegetais apresentam também efeitos semelhantes em regiões distintas do Mundo, tal como o óleo de semente do chá em algumas províncias do sul da China. Para além do óleo de sementes do chá, vários outros tipos de óleo são também utilizados na preparação das refeições do dia-a-dia nas várias províncias da China, tal como o óleo de girasol, o óleo de colza, o óleo de sesamo, o óleo de milho e o óleo de amendoim, visto estes apresentarem preços bastante mais acessiveis quando comparados com o óleo de semente do chá.

As caracteristicas únicas de cada típo de óleo vegetal encontram-se directamente relacionado com a sua composição intrínseca, onde se encontra incluido tanto a distribuição dos ácidos gordos bem como as proporções entre eles. Visto a constituição em termos de ácidos gordos ser de tal forma importante para as caracteristicas dos óleos vegetais, o estudo da sua composição torna-se assim uma boa forma de caracterizar os diferentes óleos. Assim, a técnica de análise por CG-MS "fingerprinting" foi aplicada à caracterização dos diferentes tipos de óleo vegetal testados, tendo esta sido apta de identificar um total de 22 ácidos gordos entre os sete óleos analizados no laboratório da Central South University (CSU), onde 19 foram identificados no azeite, óleo de semente do chá, de milho, de amendoim e sésame, 20 no óleo de girasol e 22 no óleo de colza. Após a identificação dos ácidos gordos, métodos de quimiometria foram utilizados para a análise dos dados, nos quais se encontram incluidos o Principal Component Analysi (PCA) e o Partial Least Squares – Linear Discriminant Analysis (PLS-LDA). O principal objective da utilização destes métodos foi então o de observar se (1) é possível a formação de grupos entre as amostras de azeite de acordo com o seu local de produção na Peninsula Ibérica, e se (2) o método de "fingerprinting" utilizado pode ser validado para as análises de óleos vegetais através da comparação dos dados obtidos quer intra- quer interlaboratorialmente.

Através da utilização do método PLS-LDA foi-nos possível distinguir diferentes grupos de amostras de azeite de acordo com o seu local de origem na Peninsula Ibérica. Quando comparadas as amostras de azeite com as de óleo de semente do chá, foi notória a semelhança por estas apresentada em termos de “fingerprinting”, mas quando comparada estatisticamente, a sua composição em termos de ácidos gordos, foi observado a existência de diferenças significativas em quase todos os parâmetros analizados, apenas o ácido Oleico, o ácido Linoleico, o ácido Docosanóico e as MUFAs apresentaram valores de $P \geq 0.05$. Estando estas diferenças de acordo com os resultados obtidos através do modelo de PCA obtido, onde ambos os dois grupos de amostras conseguem ser perfeitamente separados.

De modo a verificar a validade deste método em termos da sua de repetibilidade, um novo conjunto de amostras voltou a ser preparado e analizado (2º batch), tendo sido posteriormente comparado com as primeiras amostras (1º batch) quer por modelos de PCA quer por PLS-LDA, tendo-se verificado que não existe separação entre os dois conjuntos de amostras, indicando assim que uma boa repetibilidade do método consegue ser obtida. No que diz respeito à reprodutibilidade, de modo a ser determinado este parâmetro as amóstras de óleo foram novamente preparadas e analizadas num outro laboratório, no Hunan Agricultural Product Processing Institute (HAPPI), mas visto que nem todos os parâmetros que definem a reprodutibilidade de um método puderam ser devidamente estabelecidos (tal como a utilização de um analista diferente), não será analizada a reprodutibilidade mas sim a precisão intermédia. Os resultados obtidos pelas análises efectuadas em ambos os laboratórios demonstraram assim que a precisão intermédia do método é bastante boa, conseguindo produzir resultados equivalentes em ambos os laboratórios.

Visto que no laboratório do HAPPI apenas foi possível a identificação de 12 ácidos gosdos para as amostras de azeite (em contraste com os 19 identificados no laboratório da CSU) e que mesmo com este reduzido número de ácidos gordos foi possível obter resultados semelhantes, resolvemos então testar todas as análises anteriormente efectuadas de modo a verificar se resultados semelhantes poderiam ser obtidos ao reduzir o número de ácidos gordos utilizados. Os resultados obtidos demonstram então que semelhantes conclusões podem ser produzidas quando recorrendo a um número mais restrito de ácidos gordos para as análises quimiométricas, demonstrando assim que estes 12 ácidos gordos possúem a informação relevante para o estudo em causa.

Sendo que por vezes nem sempre é possível a utilização de um padrão interno na preparação das amostras, decidiu-se também verificar se a ausência do mesmo iria alterar os as concluões obtidas pelas análises anteriormente produzidas, assim todos os resultados voltaram a ser analizados mas sem a utilização do padrão interno, tendo sido um ácido gordo que se encontra presente em todos os óleos vegetais escolhido para servir de pico de referência, o ácido Palmitico. Este pico de referência foi usado assim para o cálculo das áreas relativas dos restantes ácidos gordos. Os resultados obtidos com estas análises demonstraram que ao usar o ácido Palmitico como pico de referência em vez do padrão interno semelhantes conclusões podem ser obtidas, sendo esta uma possibilidade para a realização deste tipo de análises

Para além das análises efactuadas na CSU e no HAPPI, algumas amostras foram também preparadas na Universidade do Algarve (UAlg) através de um método diferente do anteriormente utilizado, mas onde apenas foi possível a análise de uma amostra e em diferentes condições de detecção (modo TIC, total ion current), tornando-se deste modo impossível a comparação destes resultados com os restantes dados, mas deixando uma porta aberta para uma possível continuação deste estudo.

Abstract

Olive oil is a well known vegetable oil due to its beneficial effects on human's health and its strong economic importance in the Mediterranean area, being this region alone responsible for more than 90% of the olive oil's world production. In other regions such as the Chinese one, olive oil is not that commonly used, and other vegetable oils such as tea seed oil, rapeseed oil, sesame oil, corn oil, sunflower oil and peanut oil are rather used to prepare the daily meals.

The unique characteristics of each type of vegetable oil is directly related to their fatty acid distribution, being this way the study of the fatty acid composition a good way to characterize them. So, GC-MS fingerprinting technique was applied for the characterization of the different oils, being able to identify a total of 22 fatty acids among the seven tested oils in the CSU's laboratory. Then, chemometrics were applied for data analysis, which included Principal Component Analysis (PCA) and Partial Least Squares – Linear Discriminant Analysis (PLS-LDA), in order to see if (1) it was possible to group the olive oils according to their region of production in the Iberian Peninsula, and (2) this fingerprinting method could be validated for the analyses of vegetable oils through its both inter- and intra-laboratorial comparison.

With PLS-LDA we were able to group the olive oil samples according to their region of production, and also a clear distinction could be made between olive oil and tea seed oil by means of a PCA model. In terms of repeatability and intermediate precision, good results were also obtained from the analyses performed both in CSU and HAPPI. The same analyses were then performed resorting to a group of 12 fatty acids, and similar results could be observed as when using all the fatty acids, meaning that these 12 fatty acids posses sufficient information to characterize the different types of oil.

The use of Palmitic acid as a reference peak instead an internal standard was also tested, proving to be a good way to perform these analyses.

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List of abbreviations

CRM – Certified reference material

CSU – Central South University

DAG – Diacylglycerol

EVOO – Extra virgin olive oil

FFA – Free fatty acid

GC – Gas chromatography

HAPPI – Hunan Agricultural Product Processing Institute

HPOO – Home production olive oil

ICS – International Chemometrics Society

IR – Infrared spectroscopy

IUPAC – International Union of Pure and Applied Chemistry

LC – Liquid chromatography

MUFA – Monounsaturated fatty acid

MS – Mass spectrometry

m/z – Mass-to-charge ratio

NMR – Nuclear magnetic resonance spectroscopy

PUFA – Polyunsaturated fatty acid

PC – Principal component

PCA – Principal component analysis

PLS – Partial least squares

PLS-LDA – Partial least squares - linear discriminant analysis

SIM – Single-ion monitoring

SFA – Saturated fatty acid

TAG – Triacylglycerol

TIC – Total ion current

UAlg – University of Algarve

UFA – Unsaturated fatty acid

UV-vis – Ultraviolet and visible

VOO – Virgin olive oil

1 Introduction

1.2. Olive oil background

Olive groves are a culture with deep roots implemented in the European Continent, mainly in the Mediterranean region. From ancient times that olive oil plays an important role in the Mediterranean diet, being abundantly used in the preparation of almost all the traditional dishes in this region. The most important components in olive oil are the fatty acids, and their proportion with each other will strongly influence the characteristics and nutritive value of the oil. Being known for its health benefits, among others working as a cancer prevention due to its high amounts of phenolic antioxidants and squaline,[1] olive oil has also a great importance in the economic sector of the Mediterranean countries.

1.2.1. Economic weight

From the middle of the 90s a world expansion of the olive oil sector has been observed, both in the production and consumption sector (**Figure 1**), being the Mediterranean Basin responsible for more than 90% of this world production.

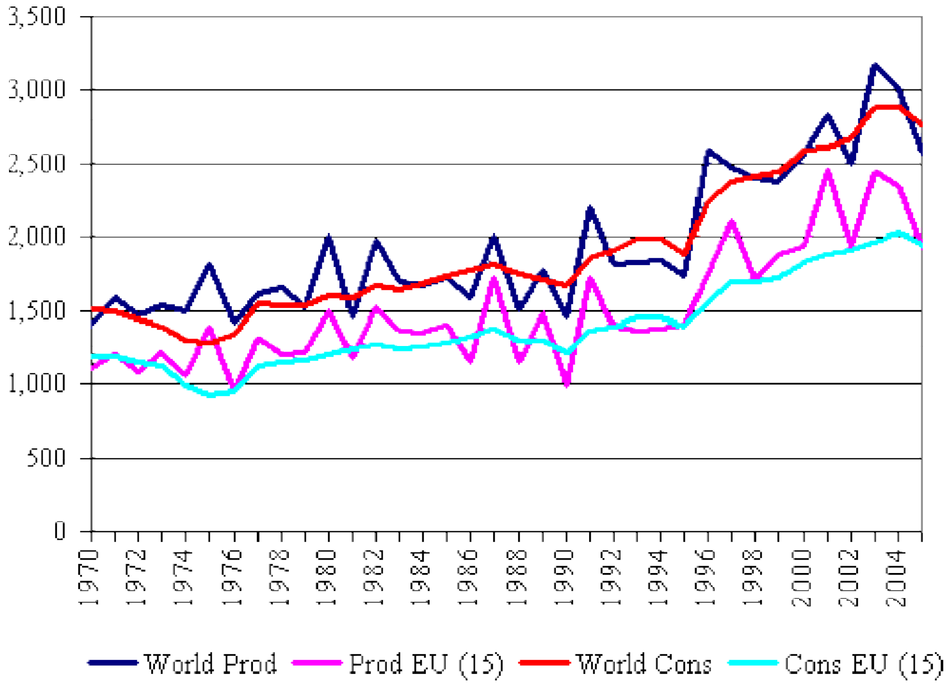

Figure 1. Evolution in the world's production and consumption of olive oil.[2]

According to **Figure 2**, it can be seen that Spain is by far the biggest world olive oil producer, being alone responsible for more than 40% of the word production (08/09 crop year), followed by Italy (with 19,5%) and Greece (12,9%). Portuguese olive oil represents about 2% of the world's production.[3]

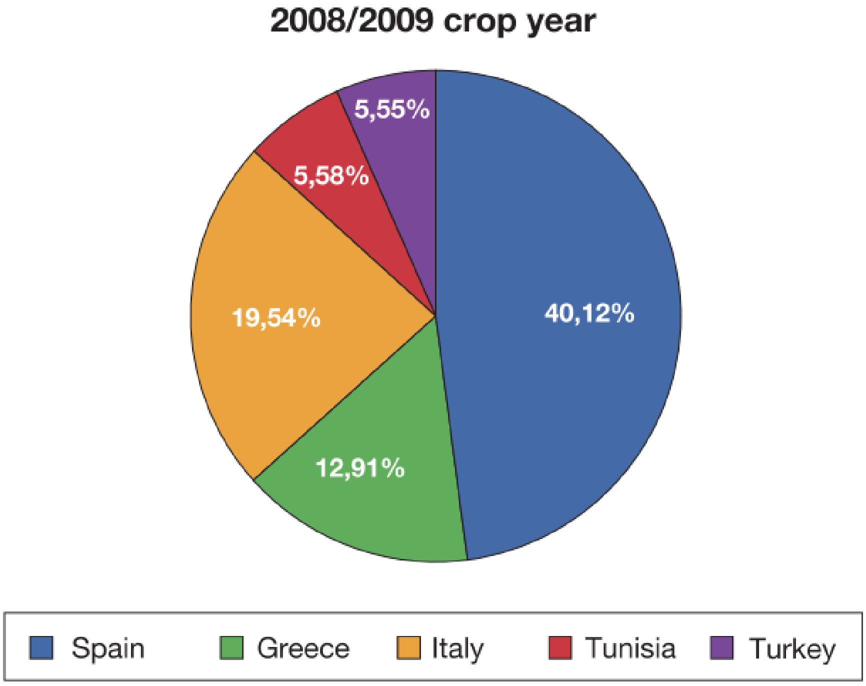

Figure 2. World olive oil production (2008/2009).[4]

By the amount of olive oil produced in the Mediterranean region, one can easily deduce that that this product represents a strong pillar in the economy of these countries. It is also the three main producing countries who possess the key markets of the EU, Jaén (Spain), Bari (Italy) and Heraklion/Messenia (Greece). The prices paid to producers of extra virgin olive oil on these markets (**Figure 3**) affect roughly 73% of the olive oil produced in the world, having also an impact on the prices paid in the other producing countries, particularly on exporting prices.

The world commercial flows (imports and exports) usually present themselves quite balanced, indicating that this sector does not generate surplus. From the non producing countries there are several countries, such as USA, Japan, Canada, Australia and Brazil, who have been registering a positive evolution in the consumption of olive oil, making them a target market of high interest. As emerging markets it can also be highlighted countries like China, Russia and South Korea.[4]

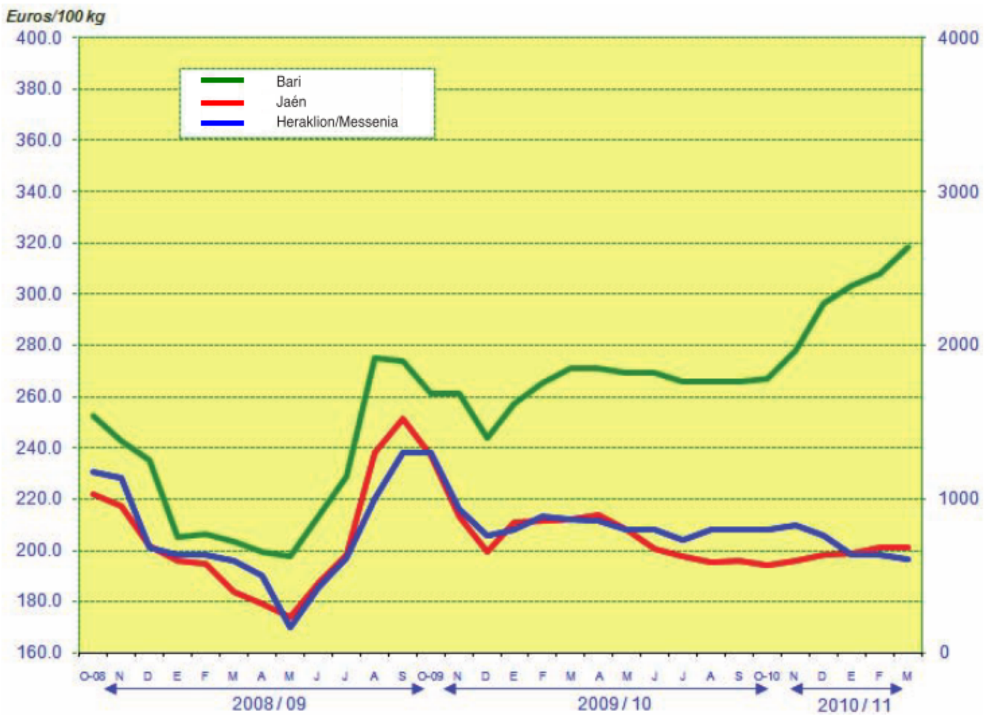

Figure 3. Movements in average monthly producer prices for extra virgin olive oil.[4]

As the olive oils has such a huge impact in the Mediterranean countries economy, also other kinds of vegetable oils possesses similar roles in other regions, such as the tea seed oil in some of the southern provinces of China, such as Hunan (roughly one-seventh of the country's population).

Tea seed oil is a high quality edible oil obtained by squeezing mature seeds of Camellia oleifera and Camellia sinensis. Its fatty acid composition (Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1) and Linoleic (C18:2)) is comparable to olive oil, as well as its high oleic acid content, low saturated fat, high antioxidants and excellent storage qualities, being even known around the world as “oriental olive oil”.

1.3. Edible fats and oils

Edible fats consist on a complex mixture that contains a wide range of compounds, being some of them essential for human's health and regularly present in a healthy diet. Dietary fats include all the lipids in plants and animal tissues that are ingested as food, and they may be either solid or liquid at normal room temperature, being usually called oils when they are liquid and fats when they are solid. These fats and oils are mainly composed by triacylglycerols (TAGs), diacylglycerols (DAGs), free fatty acids (FFAs), phospholipids and other minor compounds. All fats are derivatives of fatty acids and glycerol, being the TAGs and DAGs made up of fatty acids molecules, two and three, respectively, esterified to a glycerol backbone. Fatty acids constitute then the major component of TAGs and DAGs, and specially the first ones are required in human nutrition as a source of energy and for metabolic and structural activities.

1.3.1. Nomenclature of fatty acids

There are several systems of nomenclature for fatty acids, but the most complete and unambiguous is the systematic nomenclature recommended by the International Union of Pure and Applied Chemistry (IUPAC). As an example, let us take the oleic acid, which has the following structure (**Figure 4**):

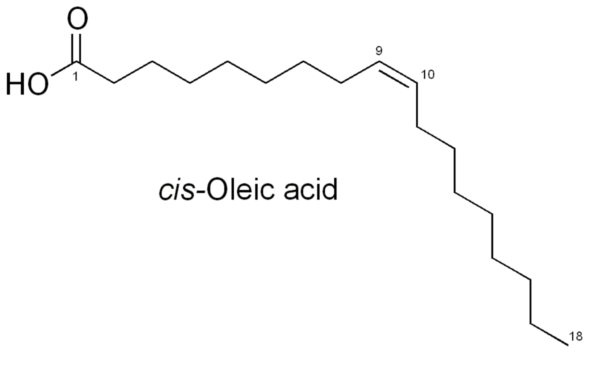

Figure 4. Oleic acid *cis* structure.

The commonly named oleic acid, in systematic nomenclature is the "*cis*-9-octadecenoic acid".

Although the IUPAC nomenclature is precise and clear, the names are long and not practical, therefore trivial names and abbreviations are frequently used. There are several abbreviated notations for dietary fatty acids and all of them adopt the C:D form, where C is the number of carbon atoms and D the number of double bonds in the carbon chain. A very often used abbreviated nomenclature for naturally occurring *cis* unsaturated fatty acids is the “n minus” system. This term refers to the position of the double bond closest to the methyl end of the carbon chain. In this nomenclature the “*cis*-9-octadecenoic acid” would be abbreviated to “18:1n-9”, since it has his double bound located at the 9th carbon counting from the methyl end.

Another widely used abbreviated system applicable to a large number of fatty acids is the delta system, which classifies according to the number of carbon atoms between the carboxyl group and its nearest double bond, specifying the position of the double bond as well as the *cis*/*trans* configuration. With this system “*cis*-9-octadecenoic acid” would be abbreviated to “*cis*-Δ9-18:1”, but in order to simplify the notation the “Δ” can be dropped being just “9c-18:1”.[5] In this work, whenever it is appropriate, each one of these different notation may be used.

1.3.1.1. Saturated fatty acids (SFAs)

Characterized by having only single bounds on their carbon chains, the SFAs have the general formula of R-COOH. They are further classified according to their chain length: short, medium, long and very long:

- Short-chain fatty acids: From 3-7 carbon atoms;
- Medium-chain fatty acids: From 8-13 carbon atoms;
- Long-chain fatty acids: From 14-20 carbon atoms;
- Very-long-chain fatty acids: From 21-above carbon atoms.

A list of some of the most common dietary SFAs, which are mainly provided by animals, is shown below (**Table 1**). Appreciable levels of SFAs are also present in some tropical oils, especially in palm oil and coconut oil.[5]

Table 1. List of the main saturated fatty acids and their typical sources.[5]

Trivial name	Systematic name	Abbreviation	Typical source
Butyric	Butanoic	C4:0	Dairy fat
Cproic	Hexanoic	C6:0	Dairy fat
Caprylic	Octanoic	C8:0	Dairy fat, coconut and palm kernel oils
Capric	Decanoic	C10:0	Dairy fat, coconut and palm kernel oils
Lauric	Dodecanoic	C12:0	Coconut oil, palm kernel oil
Myristic	Tetradecanoic	C14:0	Dairy fat, coconut and palm kernel oils
Palmitic	Hexadecanoic	C16:0	Most fats and oils
Stearic	Octadecanoic	C18:0	Most fats and oils
Arachidic	Eicosanoic	C20:0	Peanut oil
Behenic	Docosanoic	C22:0	Peanut oil
Lignoceric	Tetracosanoic	C24:0	Peanut oil

1.3.1.2. Unsaturated fatty acids (UFAs)

Characterized by having one (monounsaturated fatty acid, MUFAs) or more several (polyunsaturated fatty acids, PUFAs) double bounds in their carbon chain. The UFAs are further classified into three groups, according to their carbon chain length:

- Short-chain unsaturated fatty acid: From 19-below carbon atoms;
- Long-chain unsaturated fatty acid: From 20-24 carbon atoms;
- Very-long-chain unsaturated fatty acids: From 25-above carbon atoms.

1.3.1.3. Monounsaturated fatty acids

Oleic acid is the most common MUFA that occurs in nature and it is present in considerable quantities in both animal and plant sources (**Table 2**).[5]

Table 2. List of the main monounsaturated fatty acids and their typical sources.[5]

Common name	Systematic name	Delta Abbreviation	Typical source
Palmitoleic	*cis*-9-hexadecanoic	9c-16:1	Marine oils, macadamia oil, most animal and vegetable
Oleic	*cis*-9-octadecanoic	9c-18:1	All fats and oils, especially olive oil, canola oil and sunflower oil
***cis*-vaccenic**	*cis*-11-octadecenoic	11c-18:1	Most vegetable oils
Gadoleic	*cis*-9-eicosenoic	9c-20:1	Marine oils
Erucic	*cis*-13-docosenoic	13c-22:1	Mustard seed oil, rapeseed oil
Nervonic	*cis*-15-tetracosenoic	15c-24:1	Marine oils

1.3.1.4. Polyunsaturated fatty acids

These fatty acids have 2 or more *cis* double bonds that are separated from each other by a single methylene group. These methylene-interrupted double bonded *cis* configured fatty acids can be split into 12 families, ranging from double bonds located at the n-1 position to the n-12 position, being the most important in terms of human health and nutrition the n-3 and n-6 families (**Table 3 and 4**)

Table 3. Most important n-3 PUFAs.[5]

Common name	Systematic name	N minus Abbreviation	Typical source
α-linolenic	*cis*-9,*cis*-12,*cis*-15-octadecatrienoic	18:3n-3	Flaxseed oil, canola oil, soybean oil
Stearidonic	*cis*-6,*cis*-9,*cis*-12,*cis*-15-octadecatetraenoic	18:4n-3	Fish oil, blackcurrant seed oil, hemp oil
Eicosapentaenoic	*cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoic	20:5n-3	Fish oil
Docosapentaenoic	*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-16-	22:5n-3	Fish oil

	docosapentaenoic		
Docosahexaenoic	*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19-docosahexaenoic	22:6n-3	Fish oil

Table 4. Most important n-6 PUFAs.[5]

Common name	**Systematic name**	**N minus Abbreviation**	**Typical source**
Linoleic	*cis*-9,*cis*-12-octadecadienoic	18:2n-6	Most vegetable oils
γ-linolenic	*cis*-6,*cis*-9,*cis*-12-octadecatrienoic	18:3n-6	Borage and blackcurrant seed oils
Dihomo-γ-linolenic	*cis*-8,*cis*-11,*cis*-14-eicosatrienoic	20:3n-6	Animal tissues
Arachidonic	*cis*-5,*cis*-8,*cis*-11,*cis*-14-eicosatetraenoic	20:4n-6	Animal fats, liver, egg lipids and fish
Docosatetraenoic	*cis*-7,*cis*-10,*cis*-13,*cis*-16-docosapentaenoic	22:4n-6	Animal tissues
Docosapentaenoic	*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16-docosapentaenoic	22:5n-6	Animal tissues

1.3.2. Fatty acids

Fatty acids are carboxylic acids with a log chain that can be either saturated or unsaturated. The broadest definition of fatty acids includes their carbon chain length, which in the most commonly natural occurring fatty acids vary between C4 and C22. The chain is built from groups of two carbon units, and double bonds may occur at specific positions relative to the carboxyl group. This results in even-chain-length fatty acids with a characteristic pattern of methylene interrupted double bonds, originating a wide number of fatty acids varying in their chain length and unsaturation.

More than 1000 fatty acids are know, but only less than 20 are encountered in significant levels in fats and oils that justify commercial importance. The most common fatty acids are the C16 and C18 and, generally, below this chain length range they are characterized as short or medium chain and above as long chain-fatty acids. The fatty acid composition of the most widely traded oils and fats can be seen in the next table (**Table 5**).

Table 5. Fatty acid content of major oils and fats (values in wt%).[6]

	16:0	**18:1**	**18:2**	**18:3**	**Other**
Butter	28	14	1	1	4:0 (9), 6:0-12:0 (18), 14:0 (14) + odd chain and *trans*
Coconut	9	6	2	-	8:0 (8), 10:0 (7), 12:0 (48), 14:0 (18)
Corn	13	31	52	1	
Cottonseed	24	19	53	-	
Fish*	14	12	1	-	16:1n-7 (12), 20:1n-9 (12), 22:1n-11 (11), 20:5n-3 (7), 22:6n-3 (7)
Peanut	13	37	41	-	C_{20}-C_{24} (7)
Linseed	6	17	14	60	
Olive	10	78	7	-	
Palm	44	40	10	-	
Sesame	9	38	45	-	18:0 (6)
Soybean	11	22	53	8	
Sunflower	6	18	69	-	18:0 (6)

* Cod fish liver oil

Most plant oils contain the C18 fatty acids in higher amounts, while animal fats have a wider range of chain length.

Fatty acids may also be grouped based on the number of double bonds, where they can be SFAs, MUFAs or PUFAs. The double bonds of naturally occurring UFAs are very often to be with *cis* orientation, which means that the hydrogen atoms attached to the double bound are on the same side. If the hydrogen atoms are on opposite sides, the configuration term is *trans*.

1.3.3. Triacylglycerides

Fatty acids in oils and fats are found mainly esterified to glycerol. TAGs are the most important group of compounds present in dietary oils (from 95 to 98%); this group of compound is composed by trihydric alcohols (backbone) esterified with three fatty acids (**Figure 5**).

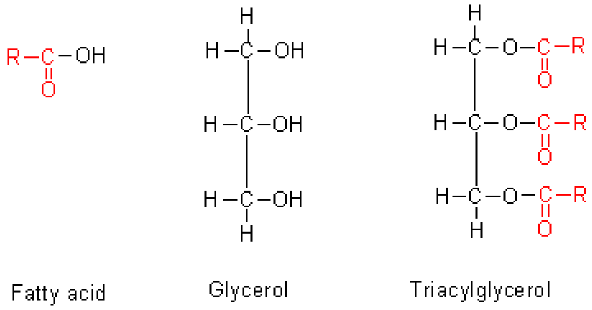

Figure 5. Schematic representation of a TAG structure.

Glycerol is a prochiral molecule, having a plane of symmetry, but if the primary hydroxyls are esterified to different groups, the resulting molecule is chiral and exists as two enantiomers. In Fisher projection of glycerol (**Figure 6**) the carbon atoms are numbered from 1 to 3 and the prefix *sn-* (for stereospecific numbering) denotes a particular enantiomer.[6]

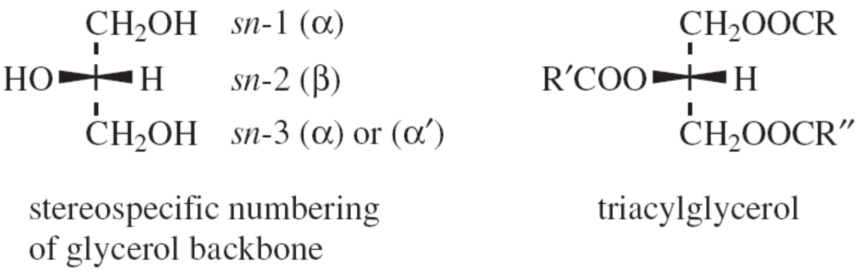

Figure 6. Fisher projection of glycerol and triacylglycerol.[6]

The molecular structure of each TAG species can be described by the following attributes:

- The total carbon number, which is the sum of the alkyl chain length of each of the three fatty acids;
- The degree of unsaturation in each fatty acid;
- The position and configuration (*cis*/*trans*) of the double bonds in each fatty acid.

Moreover, each TAG species may have and may be differentiated by its isomers according to the exact position of the three fatty acids on the glycerol backbone.[7]

Due to the vast number of fatty acids and the different combinations they may do on the backbone of the TAGs, the analysis of the TAG composition in oils or fats is always quite a challenging task. As an example, a simple seed oil composed by five different fatty acids may give 125 distinct TAG molecules (**Table 6**).

Table 6. Number of TAGs in fat containing x different fatty acids.[7]

Fatty acids	Number of triacylglycerols		
	All isomers	No optical isomers	No isomers
x	x^3	$(x^3+x^2)/2$	$(x^3+3x^2+2x)/6$
2	8	6	4
3	27	18	10
4	64	48	20
5	125	75	35
10	1000	550	220
20	8000	4200	1540
40	64000	32800	11480

Most natural TAGs don’t possess a random distribution of fatty acids on their glycerol backbone. In plant oils, unsaturated acids predominate at the *sn*-2 position, whit more saturated ones at *sn*-1 and *sn*-3. For the positions sm-1 and sn-3, the distribution of fatty acids is often similar, though not identical. In animal fats, the type of fatty acids predominant at the sn-2 position is more variable.[6]

Only oils that are rich in one specific fatty acid may contain a high amount of monoacid TAGs, such as the olive oil with oleic acid (C18:1) and sunflower with linoleic acid (C18:2).

1.4. Vegetable oils characterization

1.4.1. Background

Vegetable oils are made up of a complex mixture of compounds, where fatty acids in the form of di- and triglycerides are the main components. A series of minor polar compounds are also present in these oils, and their distribution is characteristic of different plant species from which they were obtained. Moreover, in the same species, the abundance and composition of fatty acids and these minor compounds may vary, depending on the agronomic and climatic conditions.

The composition of theses oils and their characteristic health benefits will depend on the vegetable, seed or nut from which they were extracted. Olive oil is one of the most expensive vegetable oils, and perhaps the most associated to health benefic properties, and therefore frequently adulterated with lower priced oils.[8] To be sure about the authenticity of quality edible oils is of great importance both from the commercial value and health impact point of view. The organoleptic properties, high nutritional value and health benefits of quality oils are related to their intrinsic composition, including their unique fatty acid distribution, being the study of the fatty acid composition of the different vegetable oils a good way to characterize them.

Many analytical methods have been proposed to establish the authenticity of vegetable oils and to detect the occasional adulteration level. UV spectrophotometry,[9] Raman spectroscopy [10] and IR spectrometry [11] have been widely used for edible vegetable oil analysis. However, these techniques have limitations working as detection methods, since the spectral differences of most vegetable oils, which contain the same FAs (mainly C16 or C18 and their TAGs, C50, C52, C54), are quite small. But since each vegetable oil possesses its own characteristic fatty acid composition, determining the fatty acid “fingerprint” by chromatographic methods provides quite some useful information regarding authenticity and possible adulteration of vegetable oils. Usually, fatty acid determination is carried out by liquid chromatography (LC) or gas chromatography (GC), since these techniques, combined with mass spectrometry (MS), are the key techniques for lipid analysis, being GC nowadays extensively used for the compositional determination of fatty acids.[12]

1.4.2. Principles of chromatography

Chromatography is an extremely versatile technique for the analytical laboratories. It performs the separation of two sample components based on their different distribution between the stationary phase and the mobile phase. The stationary phase, a liquid or solid, is fixed in the system, while the mobile one, a fluid (a gas in GC and a liquid in LC), is streaming through the chromatographic system.[13] While the mechanisms of retention for various types of chromatography differ, all of them are based on the equilibrium between the analyte and both the stationary and the mobile phase. The distribution equilibrium of the eluting compounds through the phases is described by the distribution function:

$$K_m = \frac{[X]_s}{[X]_m}$$

Where $[X]_s$ is the concentration of the component X in the stationary phase at equilibrium, and $[X]_m$ the concentration in the mobile phase. So, solutes with a large K_m value will be retained more strongly by the stationary phase than those with a small value.[14]

1.4.3. Gas chromatography

In GC, the sample, usually in liquid state, is converted into vapor, being this way in the same physical state as the eluent used to carry it through the system. The stationary phase is usually a nonvolatile liquid supported on an inlet solid. The injection port, column and detector are heated to a temperature at which the sample has a vapor pressure of at least 10 torr, being the injection port and detector usually kept warmer than the column in order to promote a rapid vaporization of the injected sample, and prevent sample condensation in the detector.

The sample is automatically detected as it emerges from the column. By measuring the retention time and comparing this time with the one from a pure substance standard, it may be possible to identify the peak. The area under the peak is proportional to the concentration, and so the quantitative analysis may be performed.

When complex mixtures are applied, it is not a simple task to identify the many peaks present in the mixture. So, when dealing with complex mixtures, special detectors such as mass spectrometry may be applied to aid peaks' identification.[14]

1.4.4. Gas chromatography – mass spectrometry

Mass spectrometry is a sophisticated instrumental technique that produces, separates, and detects ions in a gas phase sample, being its basic components shown in the **Figure 7**. A sample with a moderate high vapor pressure is introduced in an inlet system, operated under vacuum and high temperature. It vaporizes and is carried to the ionization source, where analyte molecules, which are typically neutral, are ionized usually by high-energy electron bombardment, producing a positive ion called the molecular ion. The molecular ions are produced in different energy states and the internal energy is dissipated by fragmentation reactions, producing fragments of lower mass, which are themselves ionized or converted to ions by further ion bombardment. The ions are separated in the spectrometer by being accelerated through a mass separator. Separation is actually based on the mass-to-charge ratios (*m/z*) of the ions. The separated ions are then detected by means of an electron multiplier.[14]

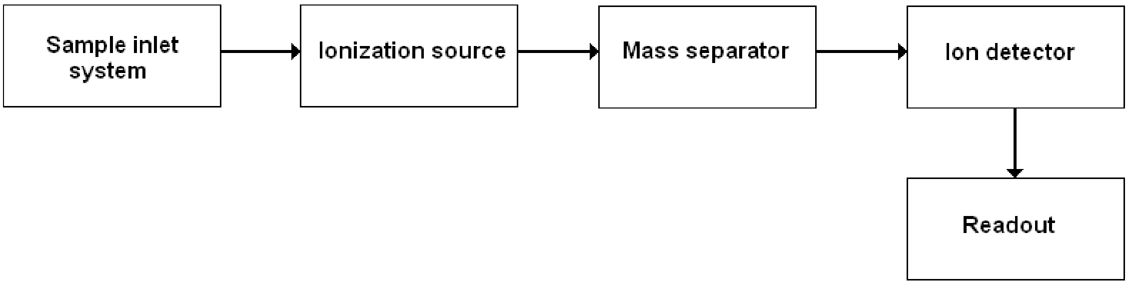

Figure 7. Block diagram of mass spectrometer.[14]

Hyphenated instrumentation, which is the name given to the technology that uses two or more quantitative measurement devices simultaneously, such as GC coupled with MS, became widely recognized in the 60’s as the most sensitive and versatile available technique capable of identifying volatile organic compounds. But it was only later, with the development of selected ion monitoring techniques, that the true potential of GC-MS for quantitative analysis was generally appreciated.

Not only can a GC-MS separate the volatile components of complex mixtures as also records the mass spectrum of each component. This instrument may provide two separate dimensions of information about the analytes, CG the retention time, which is related to specific chemical properties of the analyte (volatility, polarity, functional groups etc.), and MS the molecular weight, which is indicative of the atomic composition.

Despite of the great potential of this technique, it also possesses some serious limitation, such as: for a compound to be analyzed by GC-MS it must have sufficient volatility and thermostability to pass through the gas chromatographic column intact in the vapor state, or to be capable of conversion to a derivative which can do so. This requirement prohibits the analysis for most of the known organic compounds by this technique.[15]

Due to the relatively high boiling point of acids, the separation of fatty acids by this technique has been limited. So in order to carry the analysis on these compounds an additional step must be added to the procedure, the esterification of the fatty acids (**Figure 8**).

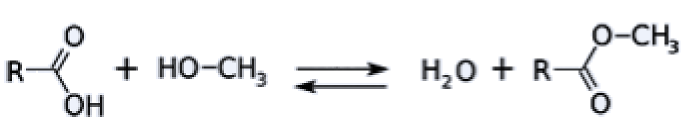

Figure 8. Schematic fatty acid esterification process.

The esterification of the fatty acids, where the ester bonds are hydrolyzed and the free fatty acids that are formed in the process are converted into the corresponding FAME, will make the CG-MS analysis possible, since FAMEs are moderately apolar and sufficiently volatile.

1.4.5. Fingerprinting

Fingerprinting technology is a highly sensitive and accurate detection method, which refers to the spectrum or image generated by certain analytical equipments. Fingerprinting classifies into three categories: electrophoresis fingerprinting, spectral fingerprinting and chromatographic fingerprinting.

Nuclear magnetic resonance spectroscopy (NMR),[16] infrared spectroscopy (IR),[17] ultraviolet and visible spectroscopy (UV-vis) [18] and MS [19] consist in the four main techniques of spectrum fingerprinting in organic analyses, and both GC [20] and LC [21] represent the main chromatographic ones.

MS spectrometry fingerprinting is a method from the spectral fingerprinting category, which is able to determine sample ions according to their *m/z*. First, the sample must be ionized, then the ions are separated when they pass through the electric and magnetic field, and finally the mass spectrometry of samples is obtained, allowing this way to obtain both qualitative and quantitative results. Organic MS is mainly used in food testing, and it is usually in tandem with GC or LC.[22] In this work since the separation is made by GC, the introduction of MS fingerprinting will have the chromatography fingerprinting incorporated. This technique has been previously applied for the analysis of vegetable edible oils [8, 19] with quite successful results.

1.5. Chemometrics

1.4.1 Background

The term chemometrics was firstly introduced in the early 70s by Svante Wold, who also established the International Chemometrics Society (ICS), and since then chemometrics has been developing, being nowadays widely applied to different fields of chemistry, especially analytical chemistry.

According to the ICS, chemometrics can be defined as “a new chemical discipline that uses the theory and methods from mathematics, statistics, computer science and other related disciplines to optimize the procedure of chemical measurement, and to extract chemical information as much as possible from chemical data”.[23]

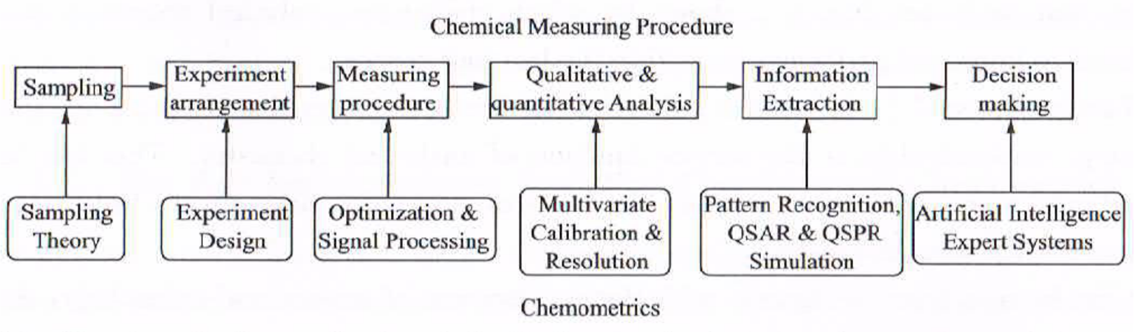

Figure 9. Relationship between chemical measuring procedure and chemometrics.[23]

Figure 9 shows a schematic representation of the close relationship between chemometrics and chemical measuring procedure, showing that at every step in the chemical measuring procedure, chemometrics can also provide some methodology for solving the problems in the procedure.[23]

1.4.2 Multivariate analysis

Hyphenated chromatographic systems, such as GC-MS and HPLC-MS, are extensively used to obtain detailed qualitative and quantitative information about the analyzed samples. With their high sensitivity, low limit of detection, possibility of analyzing a great number of analytes and identifying them with spectral dimension, makes hyphenated chromatographic systems a very strong analytical technique.

With the increasing amount and complexity of data obtained by modern hyphenated chromatographic instruments (multidimensional data), also more powerful data-processing techniques must be applied, so that we can extract useful information from this rich amount of data. In order to do so, multivariate data analysis is often applied.

The term multivariate analysis is used to describe analysis of data where numerous observations or variables are obtained for each individual or unit studied.[24] There is an advantage in this type of analysis over those that only construct a separator of the different categories, since multivariate analysis associates an *n*-dimensional bounded region to each category, they allow the extraction of hidden information and the characterization of redundant information source that often totally masks the relevant information contained in the chemical composition.[25]

Multivariate analysis may be divided into three main groups, description, classification and prediction, with distinct purposes from each other. In the present work, emphasis is given to both principal component analysis (PCA) and partial least squares (PLS) for classification and prediction analysis, respectively.

1.4.2.1 Principal component analysis

PCA is used with the aim of simplifying the description of a set of interrelated variables. This technique can be summarized as a method of transforming the original variables into new variables, uncorrelated ones. These new variables are called the principal components (PCs). Each PC is a linear combination of the original variables. PCs are arranged in order of decrease variance, being the first PC the major axis of the points in the n-dimensional space that possesses most of the information, the second PC, perpendicular to the first one, the second most informative, and so on.[24]

PCA is a well-documented multivariate method in the data analysis of edible vegetable oils obtained by only chromatographic methods (GC/LC) [25, 26, 27] or in tandem with mass spectrometry (GC-MS).[28]

1.4.2.2 Partial least square

PLS regression is a multivariate method that combines features from PCA and multiple linear regression. Its goal is to predict a set of dependent variables from a set of independent variables (predictors). This prediction is achieved by extracting from the predictors a set of orthogonal factors called latent variables, which have the best predictive power.[29]

The use of PLS methods has also been reported for data analysis with experimental data acquired by the means of a GC-MS equipment.[30]

1.5 Analytical performance parameters

The validation of a method consists in the investigation whether the analytical process of the method is achieved, which is obtaining analytical results with an acceptable uncertainty level.[31] In practice, method validation is done by evaluating a series of method performance parameters, such as precision, trueness, linearity and sensitivity, among others.

There are several factors that can affect the ability of a measurement system to discriminate among the units it measures. These factors can be categorized generally into those that affect central location and those that affect the variability (spread) of the measurement. The variability factors are measured by repeatability and reproducibility (referring to the precision of a measuring method), while the ones related to the central location of the measurement by bias, stability and linearity (referring to the accuracy of a measurement system).[32]

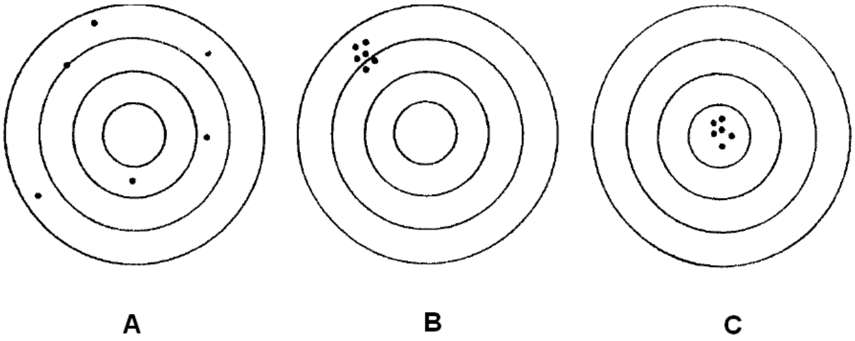

Figure 10. Comparison between precision and accuracy.[33]

As **Figure 10** clearly illustrates, being the target point at the center of each circle, for the case A it can be seen that both accuracy and precision are quite low, increasing the precision of the method in case B, but still with bad accuracy, and in case C both precision and accuracy can be noticed as high.

1.5.1 Precision, repeatability, reproducibility and intermediate precision

As exemplified above, precision is a term that describes an instrument's lack of random errors, being measured by repeatability and reproducibility.

The terms repeatability and reproducibility mean approximately the same, but they are applied in different contexts. Repeatability describes the closeness of output readings when the same input is applied repetitively over a short period of time, with the same measurement conditions, instrument and observer, in the same location and conditions of use maintained throughout. Reproducibility describes the closeness of output readings for the same input when there are changes in the method of measurement, observer, measuring instrument, location, conditions of use and time of measurement. So, the spread in the measurements are referred to as repeatability if the measurement conditions are constant and reproducibility if they vary.[33]

Intermediate precision expresses "within laboratory" variations; this means that it is considered when the repeatability criteria are not fully met.

1.5.2 Trueness and bias

Bias may be defined as the difference between the mean value determined for the analyte of interest and the accepted true value.[31] Trueness is defined as "the closeness of agreement between the average value obtained from a large set of tests results and an accepted reference value", according to ISO 3534, and is evaluated in terms of bias through the analysis of reference samples. Since not all the references have the same level of traceability, the selected reference should be the one that has the suitable level of traceability for the purpose of the analysis.[34]

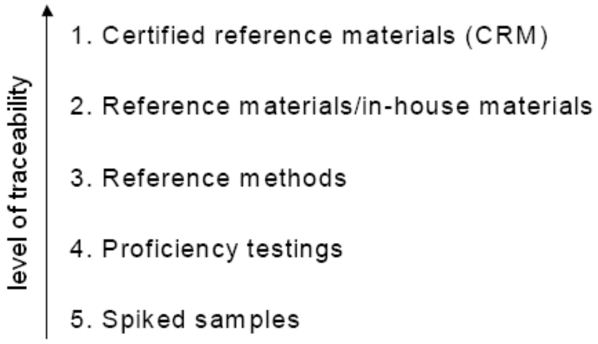

Figure 11. References commonly used to assess trueness in chemical measurements.[34]

The references commonly used in chemical analysis are listed in the figure scheme above (**Figure 11**), ordered according to their level of traceability.

1.6 Aims of the work

The main objectives presented with this work are:

- To check for patterns in olive oil samples from different regions of the Iberian Peninsula through their fatty acid composition by GC-MS fingerprinting technique coupled with PCA and PLS chemometric methods, and also between European olive oil and Chinese tea seed oil.
- To validate the used fingerprinting method for the fatty acid composition analysis of vegetable edible oils through its both inter- and intra-laboratorial comparisons coupled with PCA and PLS analysis.

2 Experimental

2.1 Samples and reagents

34 olive oil samples (19 from Portugal and 15 from Spain) were brought from Europe, while 15 more imported European olive oil samples were bought in China (8 from Spain, 4 from Greece and 3 more from Italy). The samples brought from Portugal were all purchased in local supermarkets and transferred from the original bottle to dark glass 50 mL flasks in such a way that the least possible air was left inside. The samples from Spain were provided by the University of Cadiz. All these samples were transported by plain from UAlg to the CSU.

Apart of olive oil, some local edible vegetable oils, such as tea seed oil (47 samples), rapeseed oil (11 samples), sesame oil (11 samples) corn oil (6 samples), sunflower oil (3 samples), and peanut oil (3 samples), were purchased from local supermarkets.

A 37-component FAME mix and the methyl tridecanoate internal standard solution were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. The solution of 0.4 M NaOH/CH3OH was freshly prepared in the laboratory by dissolving a reagent grade NaOH in methanol.

2.2 Sample preparation

For the analyses in both CSU and HAPPI, aliquots (50 µL) of sample were spiked with internal standard working solution (50 µL C13:0 methyl ester, 100 g/L). 1 mL 0.4 M of NaOH-CH3OH was added and reacted for about 10 min in an ultrasonic bath. After the solution turned clear and transparent the methyl esters were extracted with 1 mL of hexane twice and diluted to a final volume of 2 mL.

For the analysis in the UAlg, fatty acids were converted to the corresponding FAME according to a modified protocol of Pereira, H. et al.[35] where 50 µL of oil were measured and treated with 1.5 mL of derivatization solution (methanol/acetyl chloride, 20:1, v/v) in reaction vessels. The oil was disrupted with an IKA Ultra-Turrax disperser and then 1 mL of hexane was added and the mixture heated for 1 hour at 90 °C. Afterwords, samples were cool down in an ice bath and were then transferred into centrifuge tubes, 1 mL of distilled water and 3 mL of hexane were added and vortex for

1 min. Samples were then centrifuged at 1000 *g* for 5 min at 4ºC. The organic phase was then removed with a micropipette to new flasks. This process was repeted 3 more times. Samples were then filtered with 45 µm pore to chromatographic vials and dried with a nitrogen flow, resuspending them afterwards with a final volume of 500 µL with hexane. Previous to injection the sample was still dituted in a proportion of 1:4 (v/v) with hexane.

2.3 Replicate analyses

Due to time related issues, only in the analyses performed in CSU it was possible to prepare some replicate samples (2nd batch). True replicates were prepared for all 49 olive oil samples, nine for tea seed oil, three for corn oil, four for sunflower oil and three for rapeseed oil.

2.4 GC-MS instrument and analytical conditions

Three different sets of experiments were made, two batches performed in the Central South University (CSU) and one batch in the Hunan Agricultural Product Processing Institute (HAPPI). A few additional experiments were also performed in the University of Algarve (UAlg).

For the analysis performed in the laboratory from the CSU, all GC-MS analyses were performed by a Shimadzu GC2010A gas chromatography instrument coupled with a GCMS–QP2010 electronic impact quadrupole mass spectrometer (Shimadzu). In the gas chromatographic system, a DB-23 capillary column (30 m × 0.25 mm × 0.25 µm) was used. Column temperature was programmed from 120 ℃ to 160 at the rate of 20 ℃ /min, 160-190 ℃ at the rate of 6 ℃/min, 190-220 ℃ at the rate of 20 ℃/min, then hold 6 min at 220 ℃. The injection temperature was kept at 250 ℃, the carrier gas was Helium and the column flow 1.0 mL/min. A sample of 1 µL was injected with a split ratio of 500:1.

The analyses performed in HAPPI were carried out by an Agilent G1701EA GC/MSD Chemstation. All the conditions were set the same as the ones used before in CSU, including the capillary column that had the same characteristics, in order to have comparable results. Only the split ratio was set to 100:1.

The analysis performed in the UAlg were carried on an Agilent GC-MS (Agilent Technologies 6890 Network GC System, 5973 Inert Mass Selective Detector) equipped with a DB5-MS capillary column (25 m × 0.25 mm internal diameter, 0.25 μm film thickness, Agilent Tech) using helium as carrier gas with a flow rate of 0,8 mL/min. Samples were injected at 300 °C and the temperature profile of the GC oven was 60 °C (1 min), 30 °C min^{-1} to 120 °C, 5 °C min^{-1} to 250 °C, and 20 °C min^{-1} to 300 °C (2 min). A sample of 1 µL was used as the injection volume in splitless system.

2.4.1 MS conditions

The ion source temperature was 200 ℃ and the interface temperature 250 ℃. Ionization voltage was 70 eV; single-ion monitoring (SIM) at *m/z* 55, 67, 74, 79 and 87.

2.5 Fatty acid identification and data treatment

For the identification of the different fatty acids a 37-component FAME mix standard solution was used, conjugated with a fatty acid library. After the identification process, the peaks were integrated and the areas extracted to an excel file. Prior to the multivariate analysis all peak areas were divided by the correspondent internal standard (C13:0) in order to minimize the errors from sample preparation.

2.6 Multivariate Analysis

Principal component analysis (PCA) was performed without any prior knowledge about the sample set in order to get some information about the general trends of the samples (exploratory analysis). For classification and discrimination analysis partial least squares – linear discriminant analysis (PLS-LDA) was applied. Autoscaling was firstly used as a pre-treatment in order to preprocess the data before multivariate statistical analysis. All chemometric analyses were performed in Matlab, version 7.10.0 (R2010a), and the respective programming used was developed by the Central South University.

3 Results and discussion from the Central South University

3.1 Fatty acid identification in the CSU

In the table below (**Table 7**) there are presented the obtained results from for the fatty acid identification performed in the CSU, where a total of 22 fatty acids were identified from the 7 tested oils.

Table 7. Fatty acid identification from the different tested oils in CSU, represented as relative fatty acid value (fatty acid peak area/internal standard area) ± standard deviation.

	Olive oil	**Tea oil**	**Corn oil**	**Sunflower oil**	**Rapeseed oil**	**Peanut oil**	**Sesame oil**
Dodecanoic acid	0.000463± 0.000059	0.00072± 0.00024	0.000658± 0.000072	0.000585± 0.000060	0.00147± 0.00034	0.00069± 0.00014	0.00210± 0.00053
Myristic acid	0.00133± 0.00024	0.0055± 0.0017	0.00422± 0.00013	0.0075± 0.0013	0.0072± 0.0035	0.0051± 0.0021	0.00592± 0.00081
Pentadecanoic acid	0.00068± 0.00022	0.00122± 0.00039	0.00115± 0.00013	0.001720± 0.000065	0.00228± 0.00027	0.00133± 0.00033	0.00554± 0.00046
Palmitic acid	1.24±0.18	0.98±0.19	1.513±0.062	0.719±0.034	0.63±0.33	1.344±0.28	0.00134± 0.00032
7-Hexadecenoic acid	0.0062± 0.0011	0.00172± 0.00049	0.00273± 0.00095	0.000778± 0.000090	0.00192± 0.00030	0.00267± 0.00048	0.629± 0.084
Palmitoleic acid	0.040±0.017	0.00561± 0.00092	0.00453± 0.00032	0.00333± 0.00079	0.0091± 0.0014	0.00384± 0.00068	2.17±0.19
Heptadecanoic acid	0.0092± 0.0036	0.0069± 0.0018	0.00734± 0.00032	0.00492± 0.00062	0.0054± 0.0015	0.0092± 0.0014	0.057± 0.018
10-Heptadecenoic acid	0.0073± 0.0031	0.00307± 0.00039	0.00152± 0.00011	0.00128± 0.00036	0.00251± 0.00051	0.00188± 0.00051	2.37±0.24
Stearic acid	0.309±0.066	0.259±0.073	0.204±0.010	0.53±0.10	0.242±0.059	0.441±0.092	0.046±0.060
Oleic acid	3.9571±0.2806	4.01±0.93	1.597±0.051	1.25±0.10	2.72±0.58	2.10±0.45	0.06890.0040
11-Octadecenoic acid	0.143±0.026	0.095±0.020	0.0356± 0.0020	0.037±0.011	0.151±0.031	0.046±0.022	0.014±0.011
Linoleic acid	0.308±0.098	0.72±0.64	2.801±0.037	3.56±0.36	1.21±0.53	2.12±0.43	0.00095± 0.00018
r-Linolenic acid	nd	nd	nd	nd	0.042±0.024	nd	nd
Linolenic acid	0.0354± 0.0041	0.07±0.12	0.0393± 0.0054	0.0086± 0.0038	0.408±0.084	0.10±0.15	0.0169± 0.0038
Eicosanoic acid	0.0396± 0.0046	0.014±0.015	0.0446± 0.0026	0.0330± 0.0043	0.0627± 0.0078	0.136±0.050	0.00190± 0.00017

11-Eicosenoic acid	0.0118± 0.0015	0.027±0.012	0.0118± 0.0017	0.0074± 0.0012	0.119±0.062	0.036±0.012	0.0086± 0.0014
Heneicosanoic acid	0.00143± 0.00030	nd	0.00070± 0.00019	0.00069± 0.00022	0.00155± 0.00048	0.00228± 0.00050	0.00210± 0.00053
Docosanoic acid	0.0102± 0.0020	0.015±0.022	0.0131± 0.0023	0.0774± 0.0076	0.0350± 0.0056	0.215±0.097	0.00592± 0.00081
Erucic acid	nd	0.009±0.025	nd	0.0016± 0.0020	0.24±0.22	nd	nd
Tricosanoic acid	0.00162± 0.00034	0.0013± 0.0011	0.00123± 0.00020	0.00251± 0.00027	0.00227± 0.00077	0.00354± 0.00046	0.00554± 0.00046
Tetracosanoic acid	0.00379± 0.00081	0.0065± 0.0061	0.0138± 0.0014	0.0188± 0.0032	0.01433± 0.00087	0.088±0.040	0.00134± 0.00032
Nervonic acid	nd	nd	nd	nd	0.0093± 0.0049	nd	nd

As it can be found, rapeseed oil was the one where the highest amount of fatty acids was identified, 22 in total, then 20 were identified in sunflower oil and 19 in all the others, olive oil, tea seed oil, corn oil, peanut and sesame oil.

3.2 European Olive oil

As it was previously said, olive oil plays a very important role in European diet and economy, being Spain the producing leader of this vegetable oil. For this part of the study, 34 samples of olive oil from Europe (19 from Portugal and 15 from Spain) were analyzed, in order to see if some patterns related to their area of production could be found. Through the GC-MS analysis, 19 fatty acids were identified in these samples, as presented in **Table 7**.

In **Table 8,** it is shown the relation between each sample and its region of production, along with the type of oil.

Three of the Portuguese samples (P-2, P-11and P-12) and one from Spain (S-6) could not be related to any specific location, since their brands have several olive groves spread around the country, being these sample set apart from the geographical map (**Figure 12**), nor considered for this purpose. So for this section, a 31x19 matrix was created, considering only the 31 olive oil samples which their location was known, and the 19 identified fatty acids.

Table 8. Identification of the different olive oil samples.

Sample	Name	Type	Origin
Portugal			
P-1	Lagaretta	EVOO	Alentejo (Serpa)
P-2	Condstável	EVOO	*
P-3	Cabeça das Nogeiras	EVOO	Abrantes
P-4	Ouro d'Elvas	EVOO	Elvas
P-5	Azeite de Moura	EVOO	Moura and Barrancos
P-6	Herdade do Esporão	EVOO	Serpa
P-7	Relíquia da Vidigueira	EVOO	Vidigueira
P-8	Fundação EA (Cartuxa)	EVOO	Évora
P-9	Casa Grande	EVOO	Trás-os-Montes
P-10	Casa Aragão	EVOO	Trás-os-Montes
P-11	Oliveira da Serra	EVOO	*
P-12	Gallo	EVOO	*
P-13	Fonte dos Frades	EVOO	Alentejo (Beja)
P-14	Achan (Bio)	EVOO	Moura and Barrancos
P-15	Home production 1	HPOO	Alentejo (VVF)
P-16	Quinta do Pouchão	EVOO	Abrantes
P-17	Vilanova	EVOO	Valpaços
P-18	Arribas do Douro	EVOO	Moncorvo
P-19	Home production 2	HPOO	Guarda
Spain			
S-1	La flor de la Loma	EVOO	Úbeda (Jaén)
S-2	Guillen	VOO	Dos Hermanas (Sevilla)
S-3	Molino de Zafra	VOO	Zafra (Badajoz)
S-4	La flor de la Loma	VOO	Úbeda (Jaén)
S-5	Oro de Genave	EVOO	Genave (Jaén)
S-6	Carbonell	VOO	*
S-7	Sublime	VOO	Madrid
S-8	Oliveña	EVOO	Santa Fe (Granada)
S-9	Hipercor	VOO	Sevilla
S-10	La Española	EVOO	Sevilla
S-11	Borges/Hojiblanca	EVOO	Málaga and Jaén
S-12	Borges/Picual	EVOO	Jaén
S-13	Borges/Arbequina	EVOO	Cataluña
S-14	Los Remedios	VOO	Olvera (Cádiz)
S-15	Rocafort de Vallbona	EVOO	Catalunha

* - Oils with unidentified origin; HPOO - Home production olive oil; VOO - Virgin olive oil; EVOO - Extra virgin olive oil.

From the previous table (**Table 8**), we can see information regarding the type of olive oil, being the samples from Portugal majorly EVOOs, with the exception of two samples (P-15 and P-19) that are home produced olive oils, and so its type is unknown. From the Spanish ones, there is also a majority of EVOO samples, with only six VOO samples.

Looking to the origin of each oil, one can see that they can be more easily grouped by region of production than by their country, since we have Spanish samples very close to the Portuguese border (S-3), and so more easily grouped with Portuguese oils, and on the other hand some samples from Cataluña (S-13 and S-15) that may be as distinct from the Portuguese as they can be from the rest of the Spanish, due to the distance from the other sampling regions. So, in order to better group these samples, the whole set was divided into five regions, as shown in **Figure 12.**

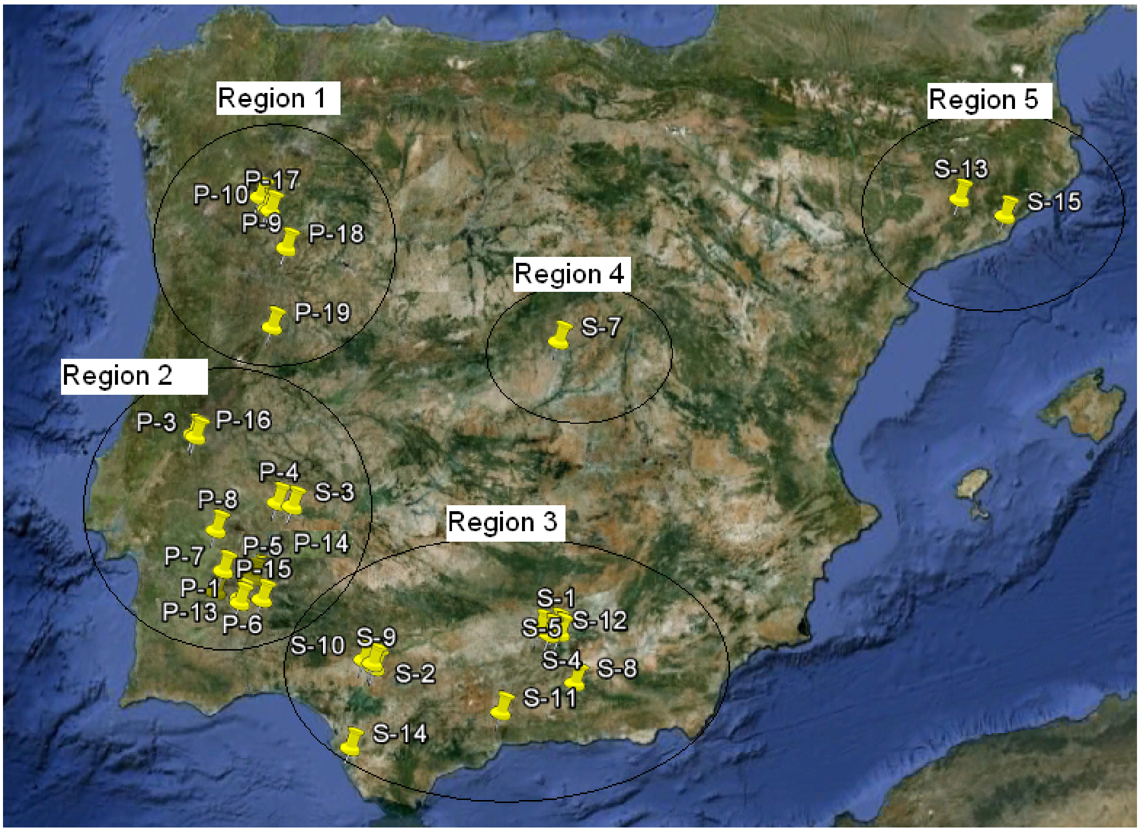

Figure 12. Geographic distribution of the samples and separation of the regions.

Considering these five regions, a PCA model was created (**Figure 13**) in order to have an overview of the capacity to distinguish the different region of production based on chromatographic data. This method is frequently employed to generate a reduced set of variables that may explain most of the variability in the original data.

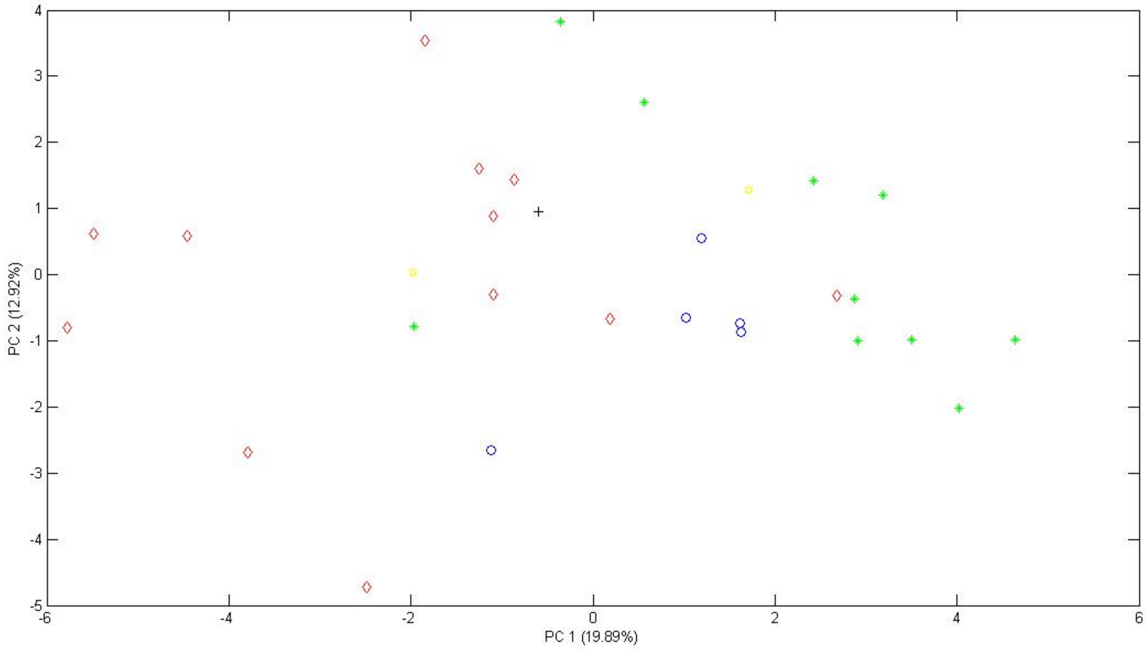

Figure 13. PCA scores plot of the European olive oil samples grouped by regions (region 1 – blue “o”; region 2 – red “◊”; region 3 – green “*”; region 4 – black “+”; region 5 – yellow “¤”).

It is easy to see that the data points obtained in **Figure 13** are not grouped by the regions proposed above, they seem rather randomly distributed than grouped by geographical proximity, even the two samples that were more separated geographically from the rest of the set (S-13 andS15, from Cataluña) were quite mingled among the others. By observing this scores plot, it could lead us to believe that between these two countries the geographical location of production has little to do with the fatty acids’ proportion in the olive oil. The lack of clarity and the lower percentage of variance explained by the first two PCs might be due to the fact that measurements often contain variables that may be irrelevant to the property under investigation, producing just noise to the model.

In order to further investigate the fatty acid proportion in the samples due to its geographical distribution, a PLS-LDA model was constructed. The PLS-LDA model can help us to determine whether two classes of samples are distinct or not, but since it can only analyze two classes at each time, it can not be used for the whole dataset, and so, three models were created, the first one between the regions 1 and 2 (**Figure 14**), the second between the regions 1 and 3 (**Figure 15**) and the last one regions 2 and 3 (**Figure 16**). Only the first three regions were used for classification purposes due to the lower number of samples in the other two regions.

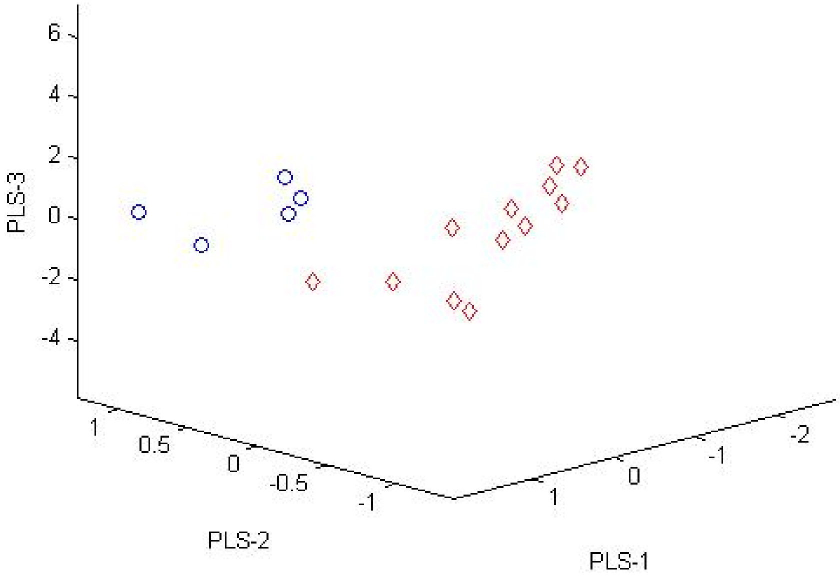

Figure 14. PLS-LDA scores plot of regions 1 (blue "o") and 2 (red "◊").

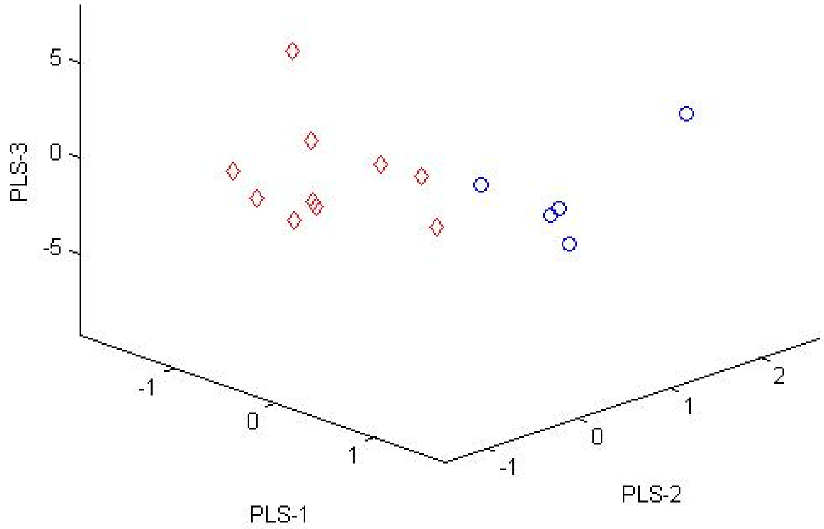

Figure 15. PLS-LDA model scores plot of regions 1 (blue “o”) and 3 (red “◊”).

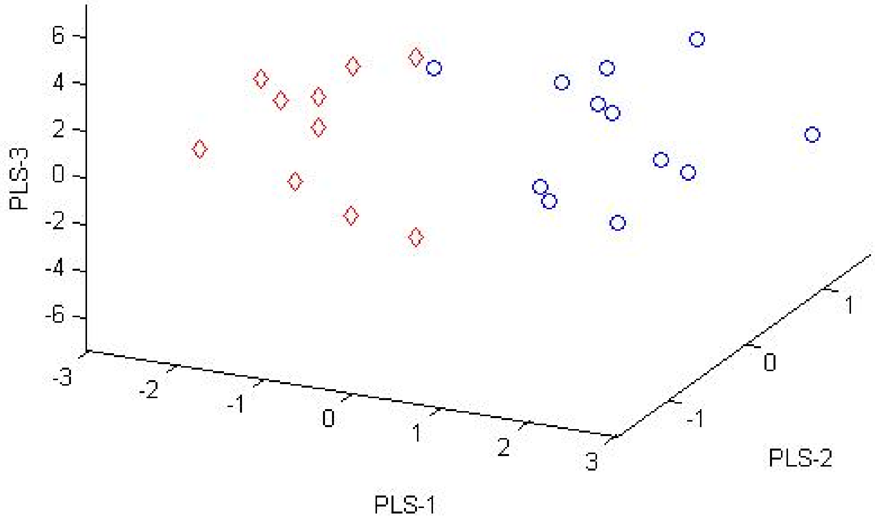

Figure 16. PLS-LDA model scores plot of regions 2 (blue “o”) and 3 (red “◊”).

By looking at these scores plots, one can see that by using a classification method, such as PLS, to analyze the different regions, some distinctions may be obtained. Furthermore, the classification results shown in **Table 9** also indicate a good classification and prediction ability of the performed models.

Table 9. Classification results between regions one, two and three by PLS-LDA method.

Groups	Recognition rate	Sensitivity	Specificity
Region 1 versus region 2	0.9412	1	0.9167
Region 1 versus region 3	0.9333	1	0.9000
Region 2 versus region 3	0.9545	0.9167	1

Recognition rate is the correct classification of the training set

Sensitivity is the number of true positives classified as positive.

Specificity is the number of true negative classified as negative.

3.3 European olive oil vs olive oil commercialized in China

3.3.1 Preliminary considerations

At this stage, it was intended to see the relation between the olive oils brought from Europe and the European olive oils bought in China. So, for this, 15 European olive oil samples were acquired in China. All of them were EVOO imported from different European countries (eight from Spain, four from Greece and three from Italy), as it can be seen in **Table 10**. A data matrix of size 49x19 was then obtained, containing the quantitative information of the 49 samples measured on 19 variables.

Table 10. Origin and type of olive oil samples purchased in China.

Sample	Type	Origin
C1	EVOO	Spain
C2	EVOO	Spain
C3	EVOO	Spain

C4	EVOO	Spain
C5	EVOO	Spain
C6	EVOO	Spain
C7	EVOO	Spain
C8	EVOO	Spain
C9	EVOO	Greece
C10	EVOO	Greece
C11	EVOO	Greece
C12	EVOO	Greece
C13	EVOO	Italy
C14	EVOO	Italy
C15	EVOO	Italy

3.3.2 Chemometric analysis

For the analysis of these samples, first a PCA model was constructed (**Figure 17**) with all the samples, so that we could see the general distribution of the samples bought in different countries.

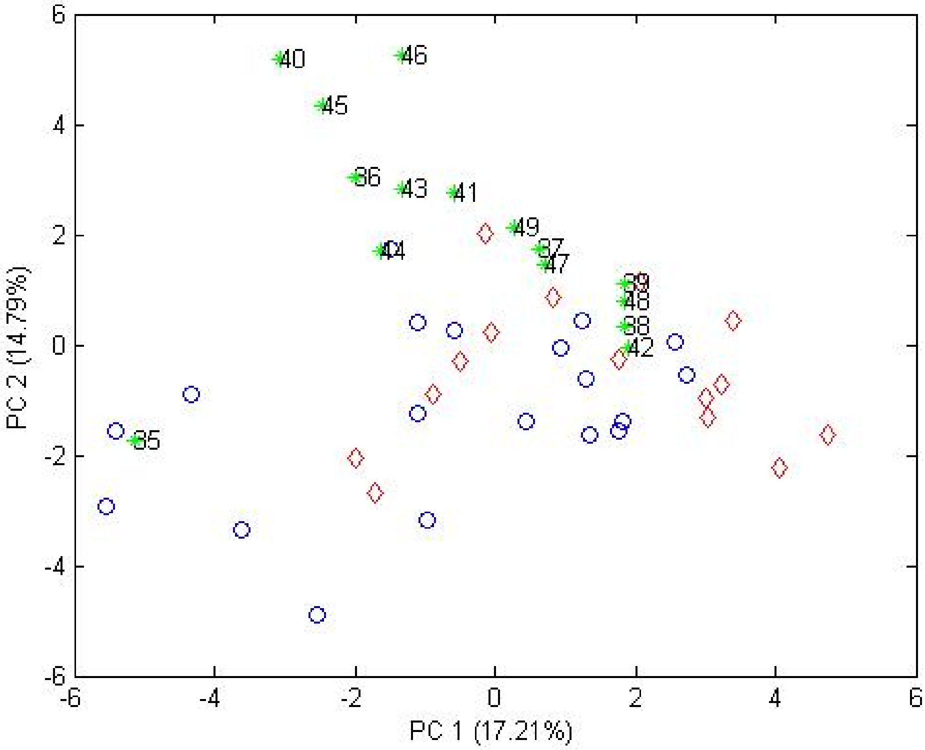

Figure 17. PCA scores plot of Portuguese olive oil (blue "o"), Spanish olive oil (red "◊") and European olive oil bought in China (green "*").

As it can be seen from the scores plot presented above (**Figure 17**), the samples bought in China are located rather in the superior half of the plot, unlike the general distribution of the Portuguese and Spanish samples, but even though, it is not clear the formation of two separate clusters, and only with this plot one can not say that the two sets of samples constitute different groups.

So, in order to further analyze these two sets of olive oil samples, a PLS-LDA model was constructed (**Figure 18**). In this model, the samples brought from Europe were compared with the ones bought in China.

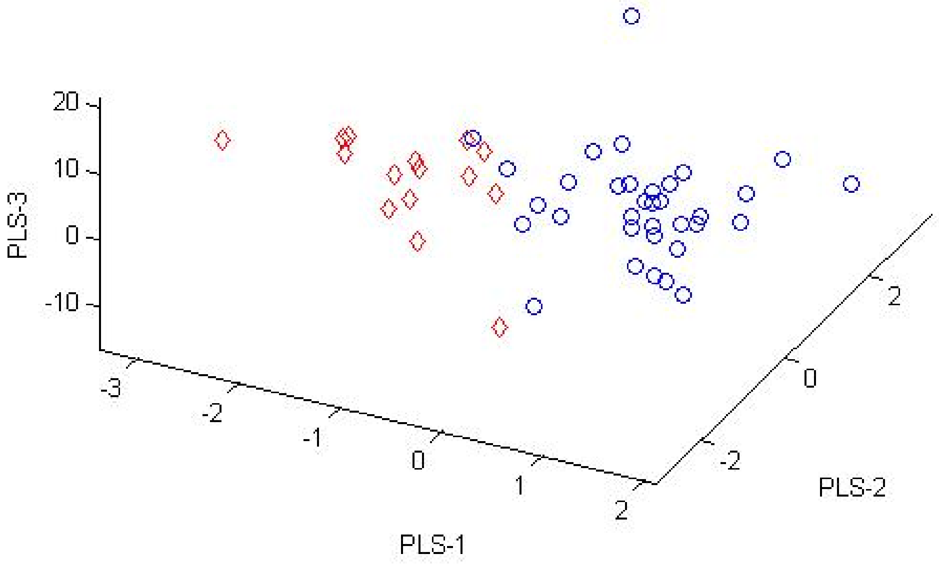

Figure 18. PLS-LDA scores plot of European olive oils (blue "o") Vs European olive oils bought in China (red "◊").

As it may be observe, there are some samples near the center of the plot, and so with doubtable classification, but in general it is clear that two main clusters were formed, each one belonging to a different class of samples. These two separated clusters were not expected to be formed, since most of the samples bought in China were produced in Spain, and therefore similarities were expected to exist. With the obtained results one could say that the European olive oil bought in China is not equal the olive oil bought in

Europe. An explanation for these results could be due to the olive oil producers who export some lower quality products to these countries where olive oil has no traditional roots. In countries like China, where olive oil is considered a luxury product, one bottle of this oil can cost quite a high amount of money, ranging from 80 – 150 Yuan/L, which is equivalent to 10 – 18 Euros. So this could be a very good opportunity for the olive oil producers to sell lower quality products for higher prices. Another hypothesis could be due to some adulteration of the olive oil with lower priced oils, such as sunflower and corn.[36]

3.4 Tea seed and olive oil

As mentioned in the first section, the health benefits of tea seed oil are quite often compared to the olive oil ones, being even known as the "oriental olive oil". Since these health benefits are mostly related to the fatty acid composition and their relative proportions, it is of great interest to analyze the fatty acid composition of this oil in light of the olive oil one.

In **Figure 19** it is shown the typical TIC (total ion current) chromatograms of both olive oil (A) and tea seed oil (B). In these profiles a total of 20 fatty acids were identified, being erucic acid (18) only detected in tea seed oil and heneicosanoic acid (16) in olive oil.

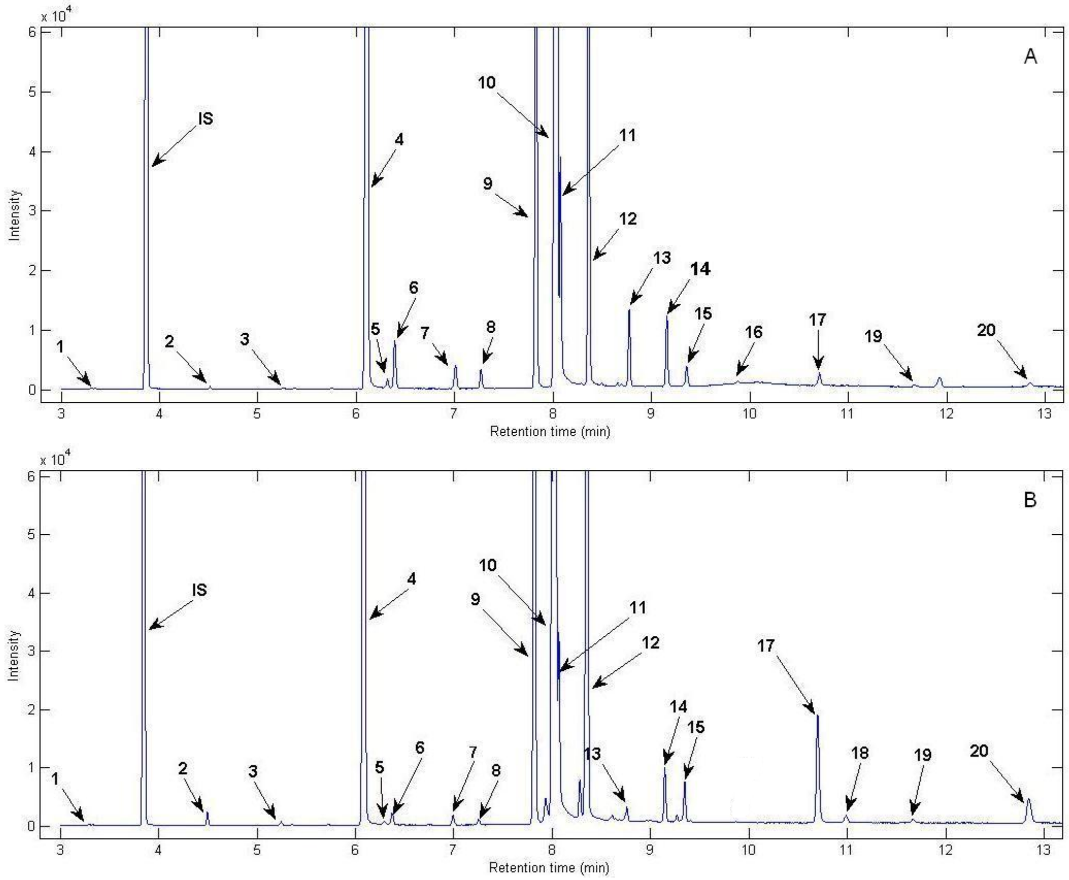

Figure 19. Typical GC-MS total ion chromatograms (TICs) of olive oil (A), and tea seed oil (B). Peaks' identification: 1 = Dodecanoic acid, 2 = Myristic acid, 3 = Pentadecanoic acid, 4 = Palmitic acid, 5 = 7-Hexadecenoic acid, 6 = Palmitoleic acid, 7 = Heptadecanoic acid, 8 = 10-Heptadecenoic acid, 9 = stearic acid, 10 = oleic acid (Z)-,11 = 11-Octadecenoic acid, 12 =Linoleic acid, 13 = Linolenic acid, 14 = Eicosanoic acid, 15 = 11-Eicosenoic acid, 16 = Heneicosanoic acid, 17 = Docosanoic acid, 18 = Erucic acid, 19 = Tricosanoic acid, 20 = Tetracosanoic acid, IS = Internal Standard (tridecanoic acid).

Table 11. Relative fatty acid values (fatty acid peak area/internal standard area) of olive and tea seed oil, indicating the fatty acids with significant difference between the two oils by T-test.

Component	Olive oil	Tea oil	P value
Dodecanoic acid	0.000463±0.000059	0.00072±0.00024	7.36e-11 *
Myristic acid	0.00133±0.00024	0.0055±0.0017	2.68e-31 *
Pentadecanoic acid	0.00068±0.00022	0.00122±0.00039	4.39e-13 *
Palmitic acid	1.24±0.18	0.98±0.19	2.99e-10 *
7-Hexadecenoic acid	0.0062±0.0011	0.00172±0.00049	7.39e-44 *
Palmitoleic acid	0.040±0.017	0.00561±0.00092	6.17e-24 *
Heptadecanoic acid	0.0092±0.0036	0.0069±0.0018	1.90e-04 *
10-Heptadecenoic acid	0.0073±0.0031	0.00307±0.00039	8.71e-15 *
Stearic acid	0.309±0.066	0.259±0.073	6.92e-04 *

Oleic acid	3.9571±0.2806	4.01±0.93	0.70
11-octadecenoic acid	0.143±0.026	0.095±0.020	2.18e-16 *
Linoleic acid	0.308±0.098	0.72±0.64	3.01e-05 *
Linolenic acid	0.0354±0.0041	0.07±0.12	0.05
Eicosanoic acid	0.0396±0.0046	0.014±0.015	1.12e-19 *
11-eicosenoic acid	0.0118±0.0015	0.027±0.012	1.21e-13 *
Heneicosanoic acid	0.00143±0.00030	nd	-
Docosanoic acid	0.0102±0.0020	0.015±0.022	0.14
Erucic Acid	nd	0.009±0.025	-
Tricosanoic acid	0.00162±0.00034	0.0013±0.0011	2.92e-02 *
Tetracosanoic acid	0.00379±0.00081	0.0065±0.0061	2.57e-03 *
18:2n-6cc/18:3n-3ccc	8.8±3.2	41±87	1.03e-02 *
18:1n-9c/18:2n-6cc	14.4±5.7	8.6±3.7	4.23e-08 *
SFA	1.62±0.15	1.29±0.21	6.31e-14 *
PUFA	0.344±0.098	0.79±0.75	8.59e-05 *
MUFA	4.17±0.27	4.15±0.93	0.93
MUFA/PUFA	13.3±4.6	8.50±3.71	1.97e-07 *

Note: Data are presented as mean ± SD

* Indicates significant difference of the corresponding fatty acid or parameter between olive oils and tea seed oils. T-test (P<0.05)

By looking at the chromatograms presented in **Figure 19**, it is clear that there are some resemblances between the two oils. Both are mainly composed of oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), palmitic acid (C16:0) and stearic acid (C18:0). Nevertheless, there can be found great significant differences in the fatty acid composition between the two types of oil, **Table 11** gives an idea of these differences, measured by a T-test on the difference between the average concentration of each fatty acid. These results show that mainly all fatty acids have a significant difference between the two classes of samples, for a confidence level of 95%; only oleic acid, linolenic acid and docosanoic acid are present in both samples with no significant difference between them. Heneicosanoic acid is only present in olive oil and erucic acid in tea seed oil. Also MUFAs were observed to have no significant difference between these two oils.

The T-test assumes a Gaussian behavior of the random variable, which is not necessarily the case for the average concentration of each fatty acid. As such, **Table 11** must be seen only as an indication of possible differences.

Essential fatty acids such as linoleic (18:2n-6) and linolenic (18:3n-3) acids, belonging to the n-6 and n-3 families, respectively, are the starting points for the elongation and desaturation mechanisms, meaning that they can produce longer and more unsaturated fatty acids. It is of great nutritional interest to maintain a well balanced linoleic/linolenic ratio intake, since both are involved into similar metabolic systems for the synthesis of their active metabolites, any alteration in the concentration of n-3 fatty acids will affect the metabolism of the n-6 fatty acids. The total daily calories as linoleic acid is recommended to be between 1-2%, with a contribution of linolenic acid to reach a ratio of around 4/1 to 10/1.[37] As it was observed by the performed T-test, the linoleic/linolenic ration is quite unstable for the tea oil, presenting a very high standard deviation. This is mainly due to the linolenic acid, which presents a very wide variation, provoking this way a very inconstant linoleic/linoleni ration among the tea oil samples.

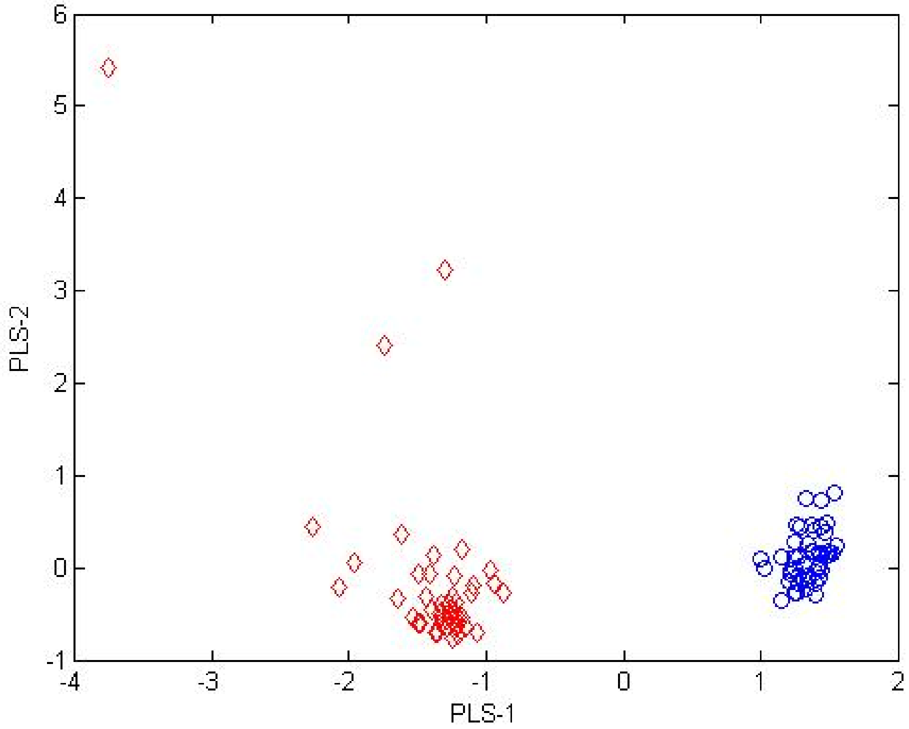

Figure 20. PLS-LDA scores plot of olive oil (blue “o”) and tea seed oil (red “◊”).

As it can be seen, **Figure 20** displays the PLS-LDA scores plot between tea seed and olive oil, being notorious that the two oils are well separated from each other, indicating that in fact they can be well distinguished and classified by PLS method.

The fatty acid composition of vegetable oils has been widely studied due to the beneficial health effects associated mainly with olive oil, but also similar studies have been conducted on tea oil. These studies show that its unsaturated fatty acid rich composition also induces great health benefits, such as: anti-tumor effects, Siegel *et al.*[38] reported that unsaturated fatty acids are significantly more effective killers of tumor cells *in vitro* than the corresponding saturated fatty acids of the same carbon length; coronary heart disease prevention, Deng *et al.*[39] showed that the consumption of tea seed oil rich in MUFAs could increase the ability to prevent coronary heart diseases; delaying atherosclerosis , Chen *et al.*[40] showed that in rabbits and rats tea oil can significantly delay atherosclerosis formation; immune function regulation, Feng *et al.*[41] studied the effect of different fats and oils on the immune function of mice, reporting that based on the all immune indexes, the positive regulatory immunological function of tea oil was the strongest.

Since unsaturated fatty acids are essential substances that can not be synthesized by the human body, they must be supplied with food. In tea oil the content of unsaturated fatty acids is high [42] and therefore it makes it a good source for these essential substances.

3.5 Analysis on all vegetable oils

3.5.1 Preliminary considerations

For this section, it was intended to compare the fatty acid composition of olive oil, one of the most popular and used edible oils in Europe, with several traditionally used Chinese vegetable oils. In order to do so, seven different vegetable oils were considered, 49 samples of olive oil, 47 of tea seed oil, 11 of rapeseed oil, 11 of sesame oil, 6 of corn oil, 3 from sunflower oil, and 3 of peanut oil. Among the seven different types of oils, 22 different fatty acids were identified, being some of them only present in specific oils, as it was shown above in **Table 7**.

As it can be seen, erucic acid was only identified in tea, sunflower and rapeseed oils, and heneicosanoic acid was only absent from tea oil. Also it was found that r-linolenic acid and nervonic acid are only characteristics of rapeseed oil.

After the fatty acid identification, a data matrix of size 130x22 was built, in order to perform the chemometric analyses.

3.5.2 Chemometric analyses on all vegetable oils

In order to have a first view of the distribution of the different oils, some exploratory analyses were performed, creating a PCA model that is shown below (**Figure 21**).

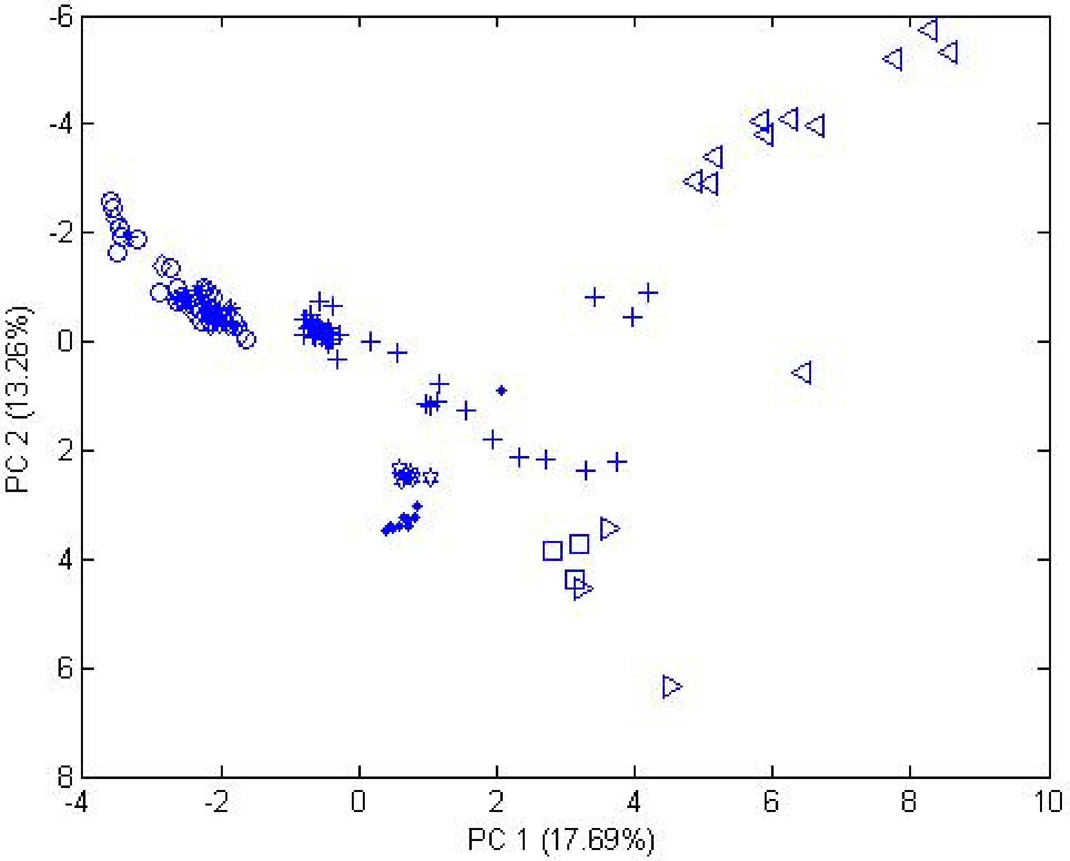

Figure 21. PCA scores plot for all the oils, where Portuguese olive is "o", Spanish olive oil "◊", olive oil bought in China "*", tea oil "+", corn oil "¤", sunflower oil "□", rapeseed oil "◄", peanut oil "►" and sesame oil "●".

As it can be observed in this plot, all the olive oil samples are clustered together, being only some of the Portuguese olive oil samples somewhat shifted from the main cluster, but still presenting very small angles between the origin of the graphic and the main cluster, and thus not considered a different group. For the tea seed oil samples, it can be seen a main cluster near the origin of the plot, in the same quadrant of the olive oil samples, showing that its fatty acid composition has more similarities with the olive oil than with all the other oils. Though we can see this main group, some of the tea oil samples were somehow spread along the plot, near to the clusters formed by other oils. This occurrence is rather curious and may generate some speculation related to the quality of the samples. If we have in mind that the tea seed oil is one of the most expensive vegetable oils sold in China, with a price ranging from 5 to 10 time higher than the other common vegetable oils, the adulteration of this oil with lower priced ones might present a valid explanation for the wide spread of the tea oil samples. Though to prove this hypothesis further investigation on the subject should be performed. Another explanation could be due to some bad storing conditions of the samples, provoking a partial degradation of their fatty acid composition.

3.6 Analyses using only 12 selected fatty acids

From what it will be observed in the next Chapter (Chapter 4 – Results and discussion from the Hunan Agricultural Product Processing Institute), we will here proceed to the chemometric analyses of the previously reported results, but recurring only to the use of 12 fatty, the ones identified in HAPPI’s laboratory for the olive oil samples. The main goal of these analyses is to see if only using the more intense and easily identified peaks (the ones detected in HAPPI’s laboratory), it is possible to obtain similar results as when using all peaks identified in CSU’s laboratory, and therefore to conclude if there is useful information in the lower peak that justify the use of an equipment with better detection and identification limits.

The fatty acids used for these analyses were: Myristic acid, Pentadecanoic acid, Palmitic acid, 9-Hexadecanoic acid, Heptadecanoic acid, 10-Heptadecanoic acid, Stearic acid, Oleic acid, Linoleic acid, Linolenic acid, Eicosanoic acid, 11-Eicosanoic acid and Tridecanoic acid (as internal standard).

3.6.1 European olive oil

First, we'll see if the separation of the five regions shown previously in **Figure 12** can be obtained. To do so, a first PCA scores plot was produced (**Figure 22**), in order to see the distribution of the different regions. Here we have also to take in mind the obtained results from the analyses performed with all fatty acids (**Figure 13**), where no distinction between the regions was possible to make only with a PCA model.

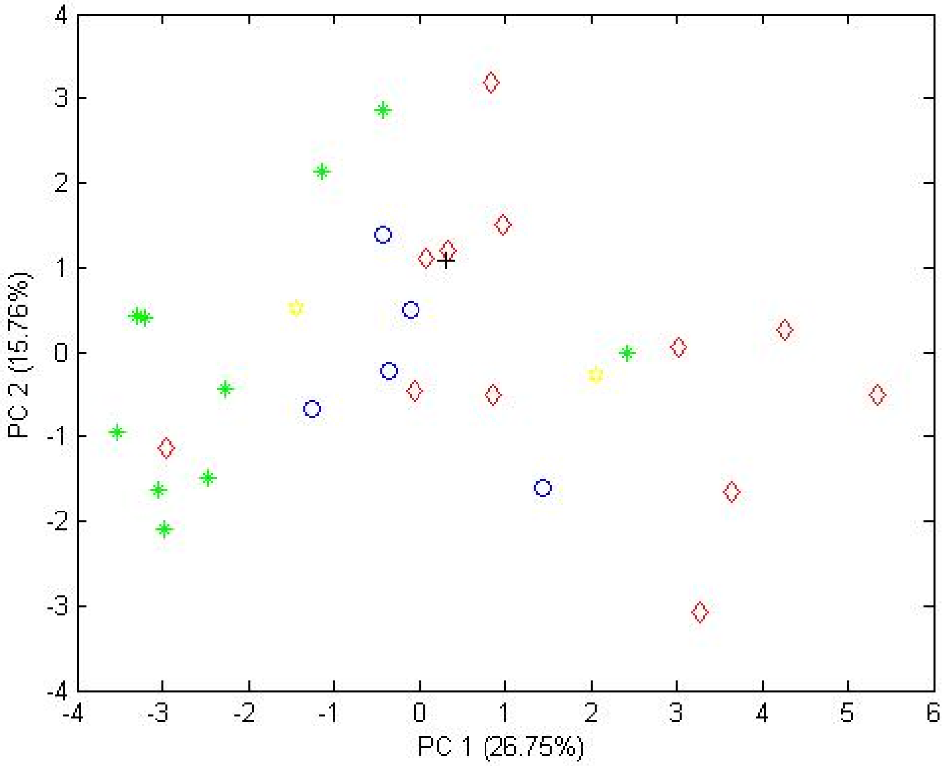

Figure 22. PCA scores plot of the European olive oil samples grouped by regions with 12 fatty acids (region 1 – blue "o"; region 2 – red "◊"; region 3 – green "*"; region 4 – black "+"; region 5 – yellow "¤").

By observing this plot, one can not see any clear relation between the samples and the regions proposed. The same was found also in **Figure 13**, and so, at this stage, we can see no differences by reducing the number of fatty acids used for the analyses.

To further investigate this subject, more analyses must be performed and as previously done with all the fatty acids, three PLS-LDA models were constructed to compare the first three regions with each other, excluding regions four and five due to the reduced number of available samples.

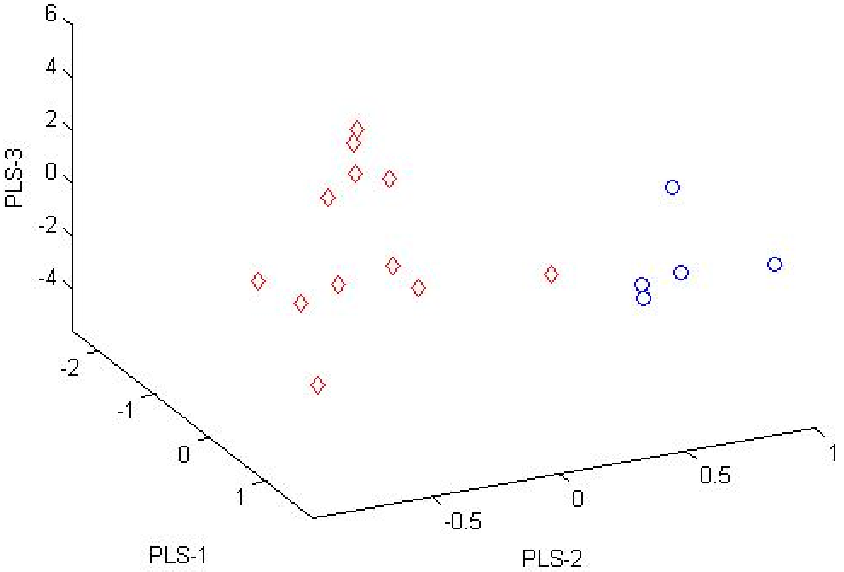

Figure 23. PLS-LDA scores plot of regions 1 (blue “o”) and 2 (red “◊”) for 12 fatty acids.

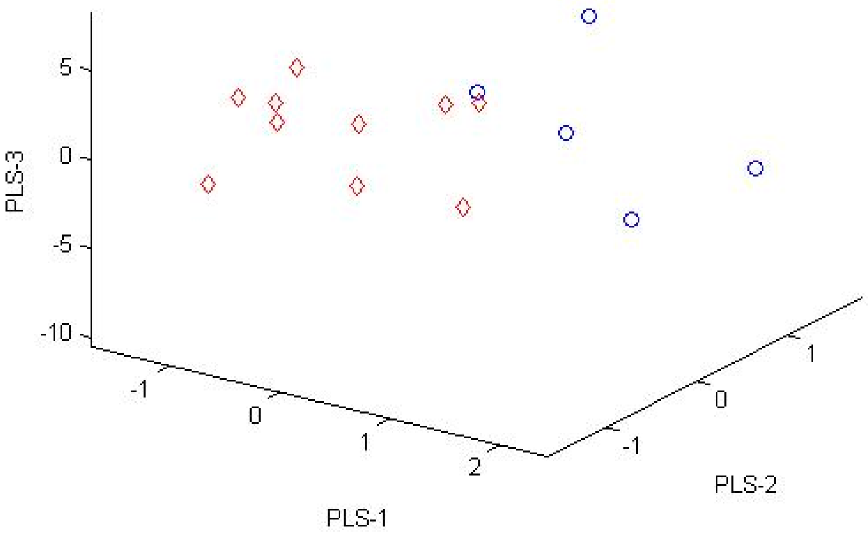

Figure 24. PLS-LDA scores plot of regions 1 (blue “o”) and 3 (red “◊”) for 12 fatty acids.

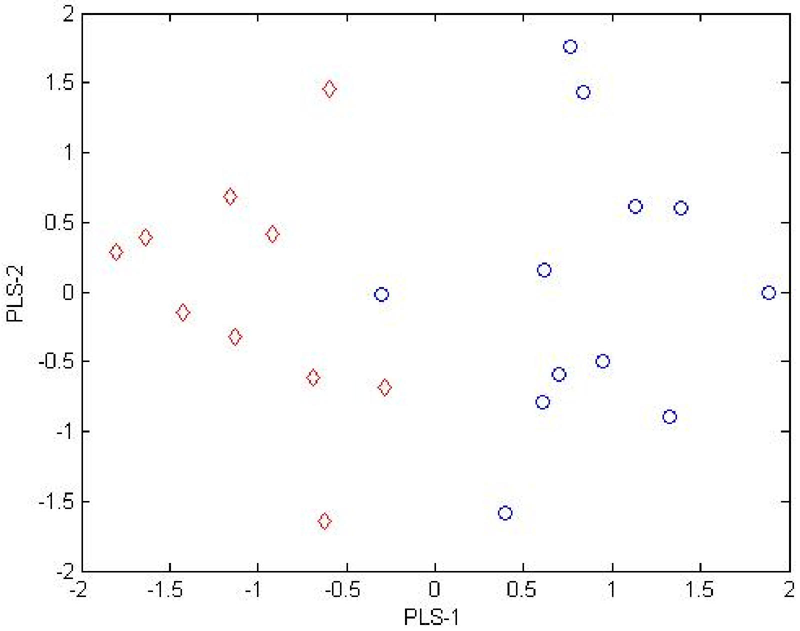

Figure 25. PLS-LDA scores plot of regions 2 (blue "o") and 2 (red "◊") for 12 fatty acids.

By looking at these plots (**Figures 23, 24 and 25**), we can clearly see the separation between the different classes, although some of the samples are located quite near the center of the plot, which may lead to an eventual misclassification in that region. By comparing the classification results present in **Table 12** with the ones obtained with all fatty acids (**Table 9**), it can be seen that both results are exactly the same, a good separation may be achieved also when only using 12 fatty acids. This may mean that when we do the variable selection, the most relevant variables belong to this group of 12 fatty acids, and so no significant information is lost with the reduction of fatty acids.

Table 12. Classification results between some of the regions by PLS-LDA method, using 12 fatty acids.

Groups	Recognition rate	Sensitivity	Specificity
Region 1 versus region 2	0.9412	1	0.9167
Region 1 versus region 3	0.9333	1	0.9000
Region 2 versus region 3	0.9545	0.9167	1

Recognition rate is the correct classification of the training set

Sensitivity is the number of true positives classified as positive.

Specificity is the number of true negative classified as negative.

These results may lead us to conclude that by using only these 12 fatty acids, we are able to classify the different regions as good as when using all the fatty acids.

3.6.2 European Vs China bought olive oil

For a first view of the European and China bought olive oil samples' distribution, a PCA scores plot was created with all the olive oil samples (**Figure 26**), using only the selected 12 fatty acids.

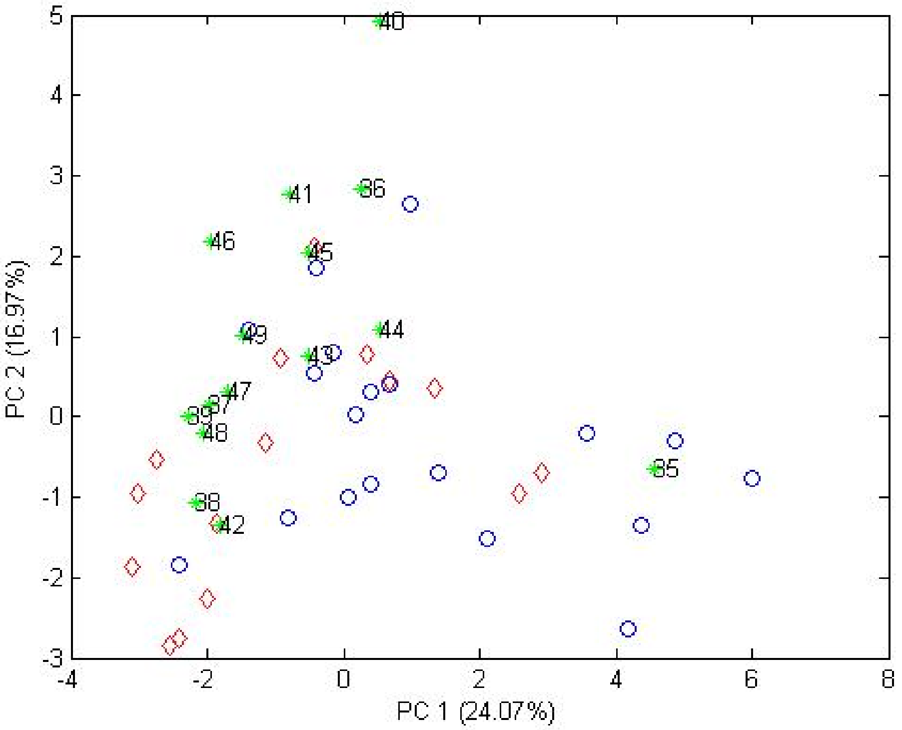

Figure 26. PCA scores plot with 12 fatty acids of Portuguese olive oil (blue "o"), Spanish olive oil (red "◊") and China bought olive oil (green "*").

As also previously observed when using all fatty acids (**Figure 17**), with only a PCA model it is not possible to distinguish from the samples bought in Europe and the ones bought in China. To do so, a PLS-LDA model was then constructed (**Figure 27**).

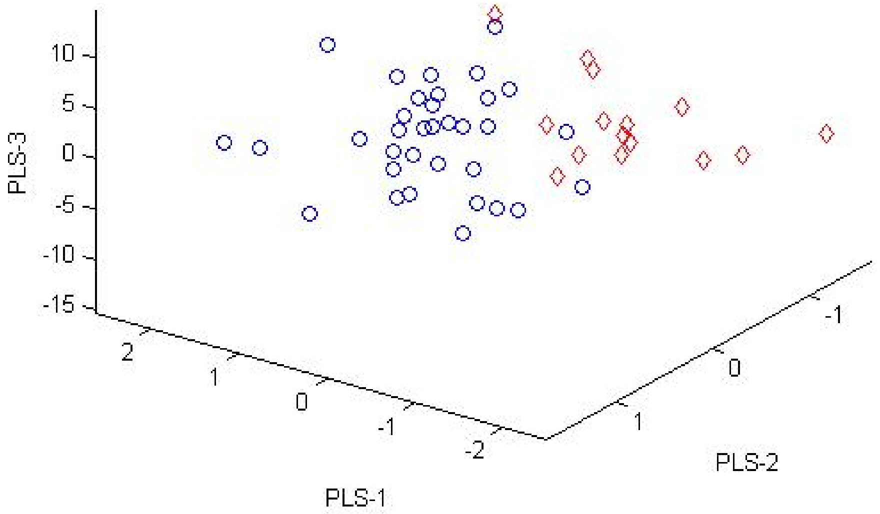

Figure 27. PLS-LDA scores plot of European olive oil (blue "o") and European olive oil bought in China (red "◊").

In accordance with the previously obtained results, when testing with all fatty acids (**Figure 18**), there are some samples located near the center of the plot, and so with doubtable classification, but in general two different clusters may be observed. So, once again, with the use of a lower number of fatty acids, similar results may be obtained.

3.6.3 Tea seed and olive oil

As previously said, tea seed and olive oil have quite similar characteristics, both regarding fatty acid composition and nutritional values. So, at this part, it is intended to see if we can have a good classification between these two different classes of samples. To do so, a PLS-LDA model was built, as it is shown below in **Figure 28**.

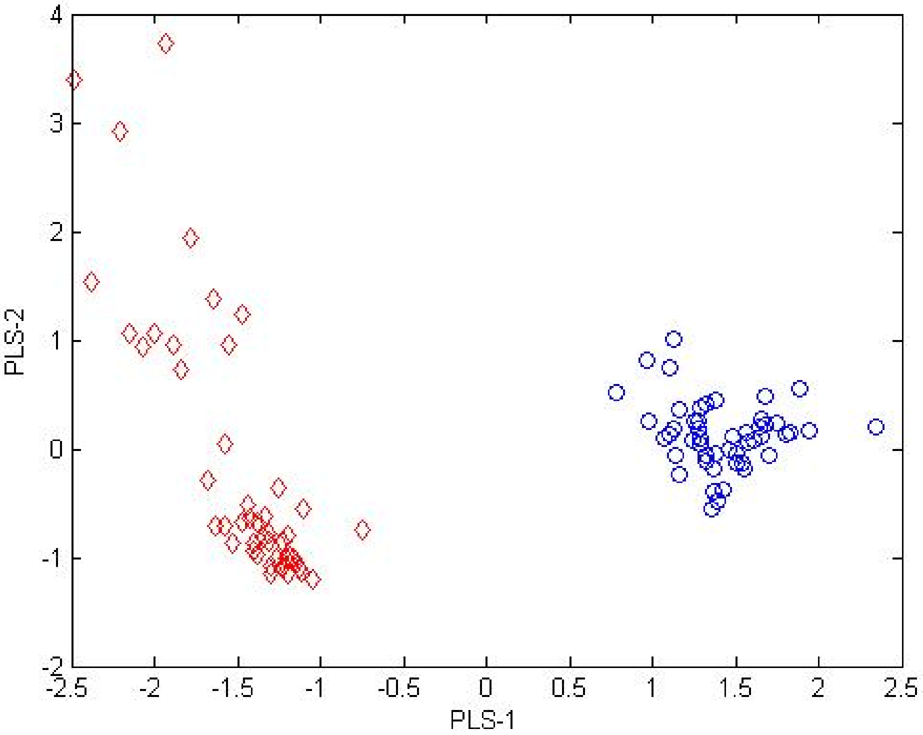

Figure 28. PLS-LDA scores plot of olive oil (blue “o”) and tea seed oil (red “◊”).

As it can be observed, and in accordance it the results presented with all fatty acids (**Figure 20**), when using only these 12 selected fatty acids, a good separation between these two classes may also be obtained.

3.6.4 Analyses on all vegetable oils

In order to see the general distribution of all vegetable oils using only the selected 12 fatty acids, a PCA scores plot was built and it can be seen in **Figure 29** presented below.

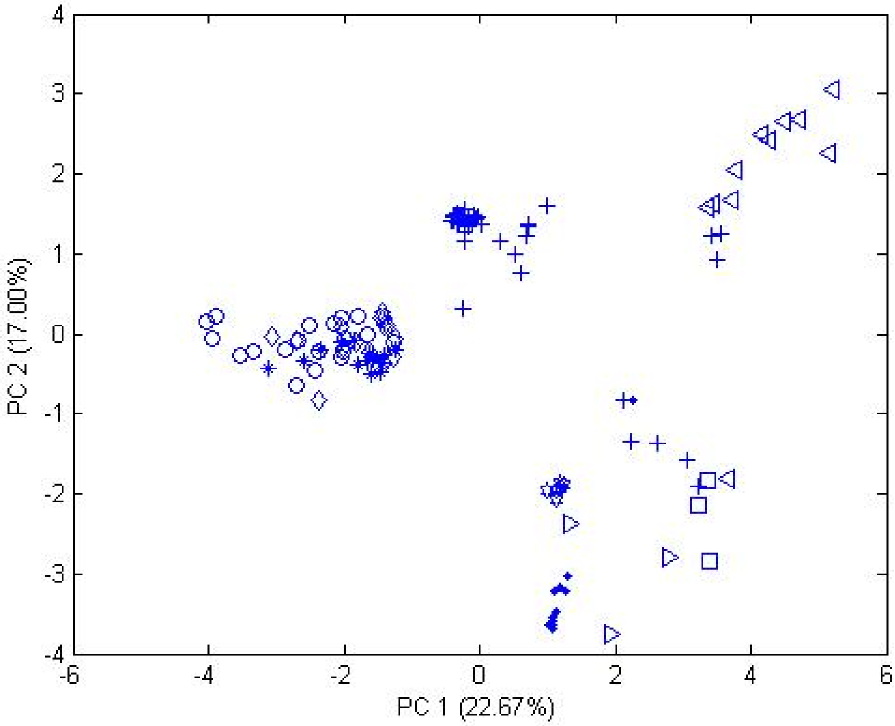

Figure 29. PCA scores plot for all the oils, where Portuguese olive is "o", Spanish olive oil "◊", olive oil bought in China "*", tea oil "+", corn oil "¤", sunflower oil "□", rapeseed oil "◄", peanut oil "►" and sesame oil "●".

By comparing this plot with the one previously obtained with the use of all fatty acids (**Figure 21**), we can see that the final results are basicaly the same, all classes of samples well separated (with the exeption of peanut oil, but also there are too few samples), with some tea oil samples drifting along the plot in direction to the rapeseed oil and sunflower oil. So, no information was gain by performing the analyses with all fatty acids.

3.6.5 conclusions

As a final conclusion of this part of the study, where only 12 selected fatty acids were used for the chemometric analyses, we can say that there are very few improvements when all the identified fatty acids are applied, same conclusions could be taken when running the analyzes with this selected few fatty acids. So, we may conclude that, for these analyses, there is no need to increase the detection and identification limits of the method, since these extra detected peaks do not bring much useful information to the model.

3.7 Intra laboratory validation of the method

3.7.1 General considerations

For the method validation, the intra laboratory precision of the method may be expressed as repeatability. Repeatability is defined in ISO 5725-1986E as “the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time”.

In this part of the work, in order to determine the repeatability of our method, duplicates of some samples were made, following all the requirements described above. With the duplicate analysis some analysis were also performed and then compared with the ones from the first replicate, in order to see if the method would prove to have a good repeatability.

3.7.2 Olive oil samples

All the 49 samples were analyzed in duplicate and a PCA model was constructed (**Figure 30**) with these second batch of data, in order to compare the results with the performed analyses on the first batch.

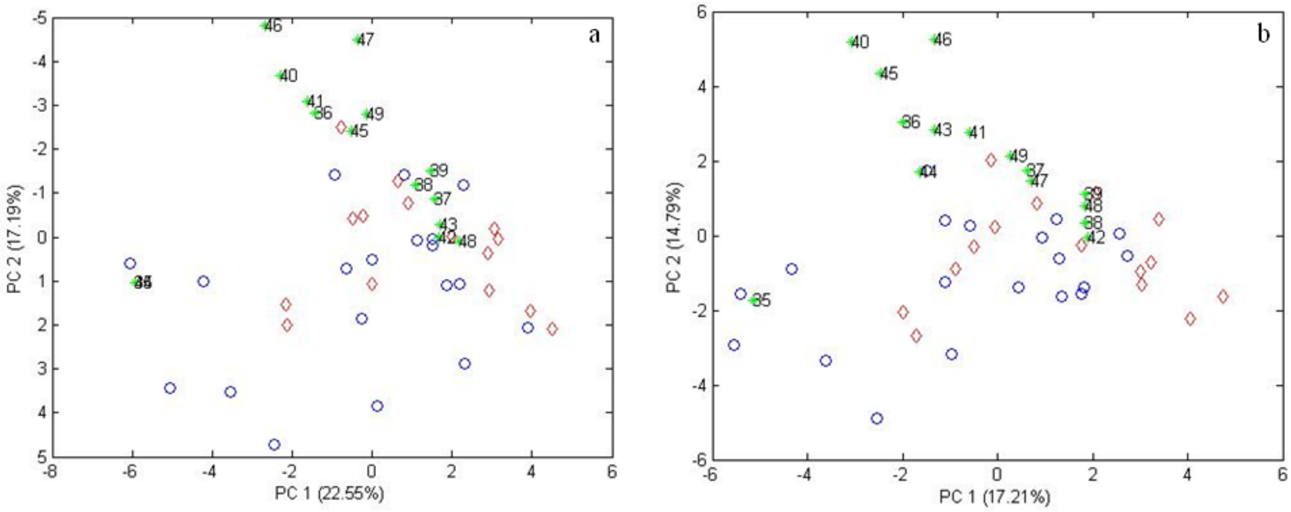

Figure 30. PCA scores plot comparison for the duplicate analysis on olive oil (a – 2nd batch and b – 1st batch), where Portuguese olive oil (blue “o”), Spanish olive oil (red “◊”) and European olive oil bought in China (green “*”).

As it can be seen, the PCA scores plot obtained in the second batch (a) is relatively similar to the one produced in the first batch (b), but because there is no separation of groups it is difficult to compare the two batches.

To further investigate the repeatability of the method, a PLS-LDA model between the first and second batch of olive oil samples was created (**Figure 31**), in order to see if distinct classes could be formed between the two batches. The formation of distinct classes would suggest the existence of clear differences between the two batches of samples, and lead to the conclusion that the method might not have a good repeatability.

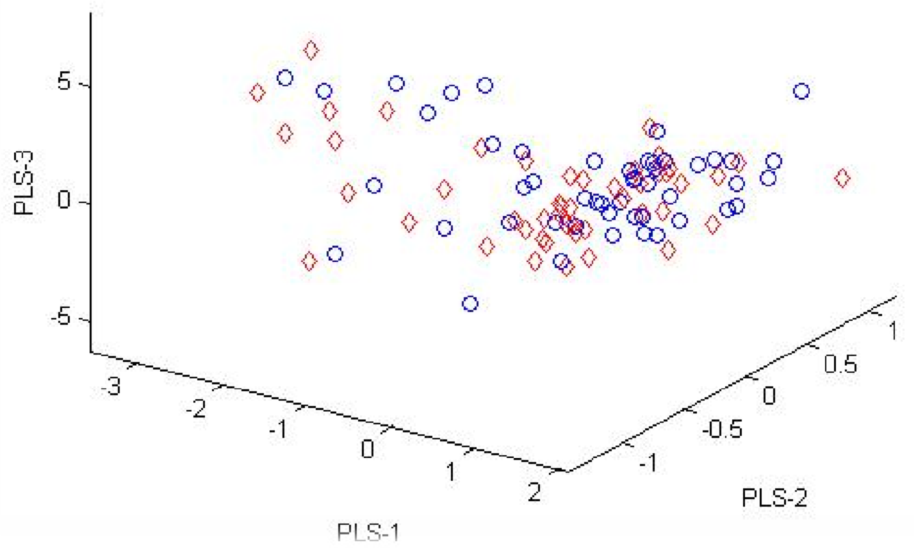

Figure 31. PLS-LDA scores plot between the first batch (blue o) and the second batch (red ◊) of olive oils samples.

By observing **Figure 31**, it is quite clear that there is no distinction between the two classes in this model, all the samples are rather mixed and the separation of clusters did not occur. These results show that, in fact, the analysis performed by this method has a good repeatability.

3.5.2 All oils from the first and second batch

At this point, it was intended to see the distribution of the second batch of samples in light of the first one, so, first the PCA for all the oils from the second batch was performed, giving, as it can be seen in **Figure 32,** quite a similar display of samples when compared with the results from the first batch.

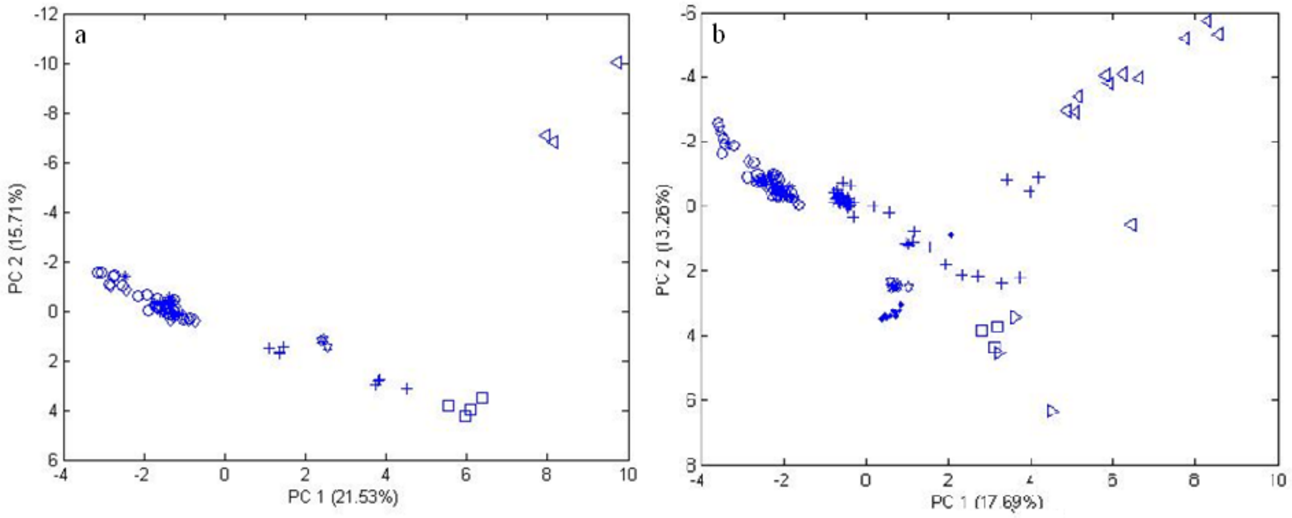

Figure 32. PCA scores plot for all the oils from the second batch (a – 2nd batch, b – 1st batch), where Portuguese olive is "o", Spanish olive oil "◊", olive oil bought in China "*", tea oil "+", corn oil "¤", sunflower oil "□" and rapeseed oil "◄".

To better compare the two batches of samples a PCA plot was created (**Figure 33**) where the PCs from the first batch were used to display both the first and the second batch. So we calculated the PCA for the first batch and then input the data from the second.

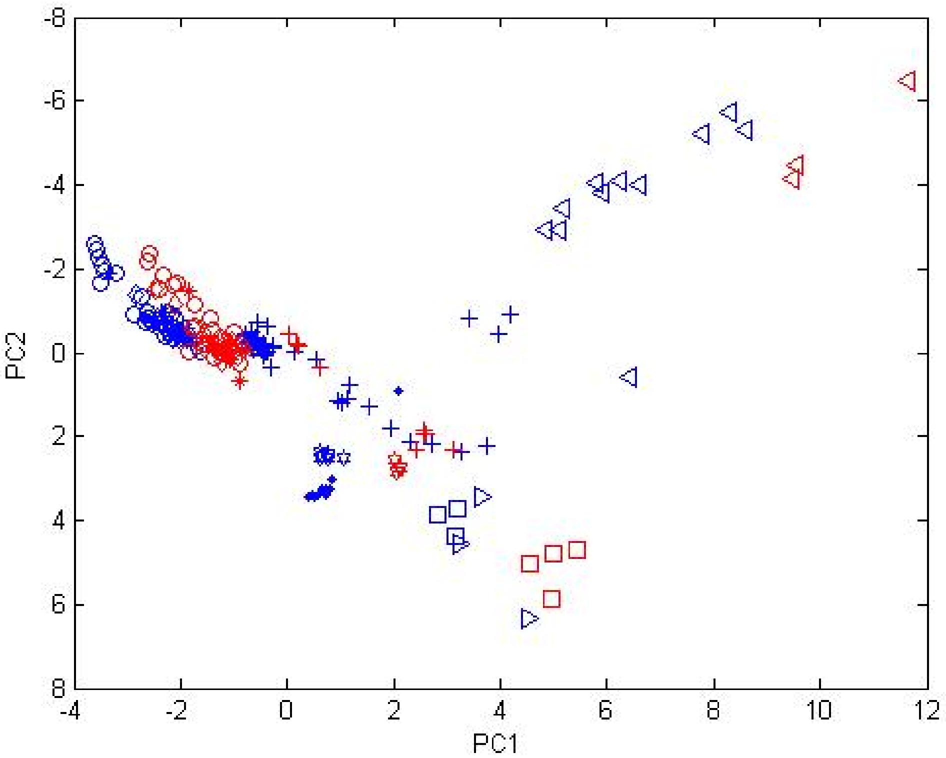

Figure 33. PCA scores plot for all the oils for both first (blue) and second (red) batches, where Portuguese olive is “o”, Spanish olive oil “◊”, olive oil bought in China “*”, tea oil “+”, corn oil “¤”, sunflower oil “□”, rapeseed oil “◄”, peanut oil “►” and sesame oil “●”.

By observing this plot it can be seen that the relative position of the different samples from both batches is the same, only the second batch has a small shifting to the right side in comparison to the first batch, but still, the samples are clustered together and more or less in the same region of the plot.

3.8 Conclusions

From this Chapter we can conclude that it is possible to well distinguish olive oil samples from different region in the Iberian Peninsula by its fatty acid constitution coupled with chemometric methods, such as PLS-LDA; when comparing olive oil samples produced and bought in Europe with samples produced in Europe but bought in China some differences may be found when applying PLS-LDA; even though the fatty acid chromatographic profile of both olive oil and tea seed oil are quite similar, differences may be found in their relative proportions, giving them this way some similar characteristics, such as the health benefits, but each one possessing a distinct pattern of fatty acids’ relative proportion; By the means of PCA, it was possible to well distinguish all seven different types of vegetable oil; by using only a selected group of fatty acids, similar results may be obtained as when analyzing with all the identified fatty acids; the results for the repeatability of the method were proven to be quite good.

4 Results and discussion from the Hunan Agricultural Product Processing Institute

4.1 Fatty acid identification in the HAPPI

In the table below (**Table 13**) there are presented the obtained results from the fatty acid identification performed in HAPPI, where a total of 15 fatty acids were identified from the 7 tested oils.

Table 13. Fatty acid identification from the different tested oils in HAPPI, represented as relative fatty acid value (fatty acid peak area/internal standard area) ± standard deviation.

	Olive oil	Tea oil	Corn oil	Sunflower oil	Rapeseed oil	Peanut oil	Sesame oil
Dodecanoic acid	nd	0.00144±0.00030	0.00154±0.00014	0.00179±0.00053	0.00243±0.00046	0.00127±0.00014	0.00136±0.00020
Myristic acid	0.00152±0.00039	0.0057±0.0019	0.00489±0.00055	0.0084±0.0012	0.0085±0.0043	0.0051±0.0020	0.00282±0.00093
Pentadecanoic acid	0.00078±0.00026	0.00105±0.00035	0.001121±0.000095	0.00172±0.00035	0.00222±0.00034	0.00115±0.00025	0.00047±0.00035
Palmitic acid	1.46±0.31	1.01±0.17	1.525±0.085	0.748±0.026	0.71±0.33	1.316±0.072	1.23±0.16
Palmitoleic acid	0.057±0.029	0.0056±0.0014	0.0063±0.0028	0.0046±0.0022	0.0091±0.0031	0.00371±0.00055	0.0070±0.0018
Heptadecanoic acid	0.0113±0.0051	0.0064±0.0019	0.00742±0.00069	0.00487±0.00096	0.0059±0.0015	0.0087±0.0013	0.00603±0.00090
10-Heptadecenoic acid	0.0093±0.0045	0.00323±0.00087	0.00160±0.00011	0.0022±0.0021	nd	0.00191±0.00039	0.0019±0.0012
Stearic acid	0.361±0.077	0.251±0.077	0.214±0.025	0.50±0.11	0.249±0.062	0.43±0.10	0.692±0.074
Oleic acid	3.14±0.37	2.70±0.45	1.51±0.12	1.41±0.51	2.40±0.46	1.73±0.28	2.08±0.24
Linoleic acid	0.38±0.14	0.65±0.44	2.04±0.12	2.11±0.36	1.12±0.33	1.60±0.17	1.96±0.20
Linolenic acid	0.0452±0.0086	0.07±0.13	0.048±0.012	0.09±0.17	0.44±0.11	0.10±0.16	0.060±0.078
Eicosanoic acid	0.0422±0.0064	0.012±0.014	0.0415±0.0082	0.037±0.013	0.054±0.011	0.121±0.054	0.0702±0.0051
11-Eicosenoic acid	0.0178±0.0035	0.026±0.014	0.0133±0.0038	0.023±0.033	0.122±0.066	0.038±0.017	0.018±0.016
Docosanoic acid	nd	nd	nd	0.065±0.020	0.0165±0.0057	0.20±0.11	0.0122±0.0028
Tetracosanoic acid	nd	nd	nd	nd	nd	0.070±0.040	nd

Comparing these results with the ones obtained in the CSU (**Table 7**), one can see that the fatty acid identification in HAPPI was much lower than the performed in CSU, with 22 fatty acids identified from the same 7 vegetable oils.

4.2 Inter laboratory validation of the method

For the validation of a method it is important to see the contribution of the random errors, and at this part of the work we want to test the precision of the method expressed as reproducibility. Reproducibility is defined in ISO 5725-1986E as “the closeness of agreement between test results obtained with the same method on identical test material in different laboratories with different operators using different equipment”.

In order to fulfill most of these requirements, we went to a different laboratory, the Hunan Agricultural Product Processing Institute (HAPPI), and analyzed all the samples in their GC-MS equipment. Since that in this case the reproducibility conditions could not be fully met, as the operator was the same for both analyses, an intermediate precision was measured.

4.2.1 Fatty acid identification in olive oil

As previously said, in the CSU’s laboratory, for the olive oil samples, there were identified 19 peaks correspondent to fatty acid methyl esters. Comparing this to the results obtained in the HAPPI, where only 12 peaks were identified, one can say that this method, when applied to this new system, did not have the same performance in the fatty acid identification. The fingerprinting of an olive oil sample obtained in HAPPI is presented below, in **Figure 34**, with its correspondent fatty acid identification.

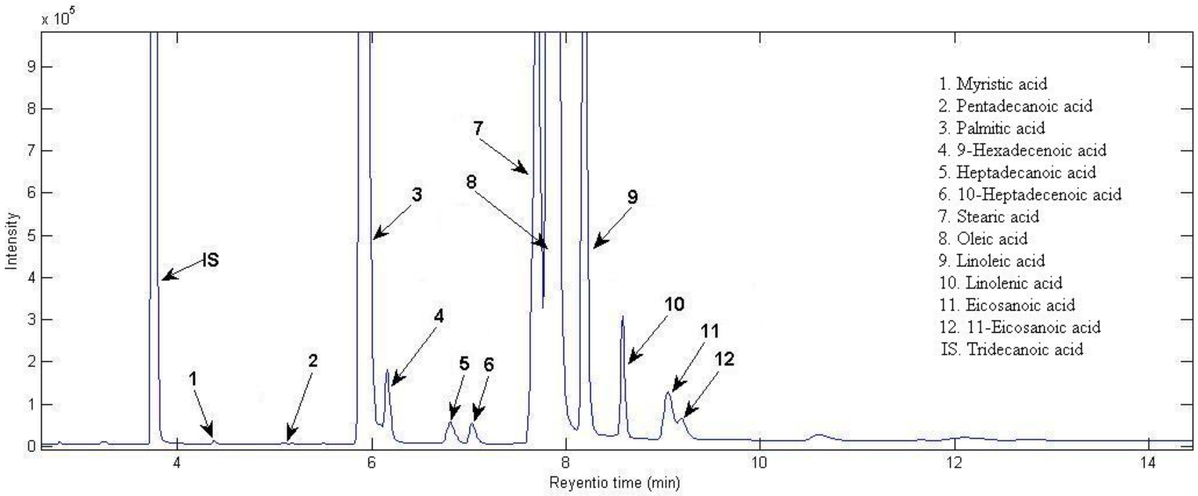

Figure 34. Typical GC-MS single ion monitoring (SIM) chromatogram of an olive oil sample (P-10) performed in Hunan Agricultural Product Processing Institute.

As it can be observed, this chromatogram is not as clear as the ones obtained from the CSU's laboratory (**Figure 19**), many fatty acids were not identified, peaks are less thin and, in some of the cases, not as well separated. On the other hand, in this laboratory it was detected one fatty acid that was not detected in the CSU's laboratory. The resuming table of fatty acid identification between these two laboratories for olive oil is presented below in **Table 14**.

Table 14. Fatty acids identified in the two different laboratories for olive oil samples.

	CSU	HAPPI
Dodecanoic acid	0.000463±0.000059	nd
Myristic acid	0.00133±0.00024	0.00152±0.00039
Pentadecanoic acid	0.00068±0.00022	0.00078±0.00026
Palmitic acid	1.24±0.18	1.46±0.31
7-Hexadecenoic acid	0.0062±0.0011	nd
Palmitoleic acid	0.040±0.017	0.057±0.029
Heptadecanoic acid	0.0092±0.0036	0.029±0.0051
10-Heptadecenoic acid	0.0073±0.0031	0.0093±0.0045
Stearic acid	0.309±0.066	0.363±0.077
Oleic acid	3.9571±0.2806	3.13±0.37
11-Octadecenoic acid	0.143±0.026	nd
Linoleic acid	0.308±0.098	0.38±0.14

Linolenic acid	0.0354±0.0041	0.0452±0.0086
Eicosanoic acid	0.0396±0.0046	0.0422±0.0064
11-Eicosenoic acid	0.0118±0.0015	0.0178±0.0035
Heneicosanoic acid	0.00143±0.00030	nd
Docosanoic acid	0.0102±0.0020	nd
Tricosanoic acid	0.00162±0.00034	nd
Tetracosanoic acid	0.00379±0.00081	nd

By analyzing these results, it is clear that the conditions used for the equipment from the CSU are not the best ones for the equipment in HAPPI, leading to a worst separation and therefore many of the low intensity fatty acids were not detected. The last eluting compounds verified in the CSU were not even detected in the HAPPI, so in order to optimize the fatty acid separation and detection, the application of different conditions might be necessary.

4.2.2 Chemometric analysis of olive oil

For the chemometric analysis on the olive oil, a matrix of size 49x12 was constructed. As a first step, a PCA model with all olive oil samples was created in order to see if the results would be in agreement with the ones obtained at the CSU. This model is shown below in **Figure 35**.

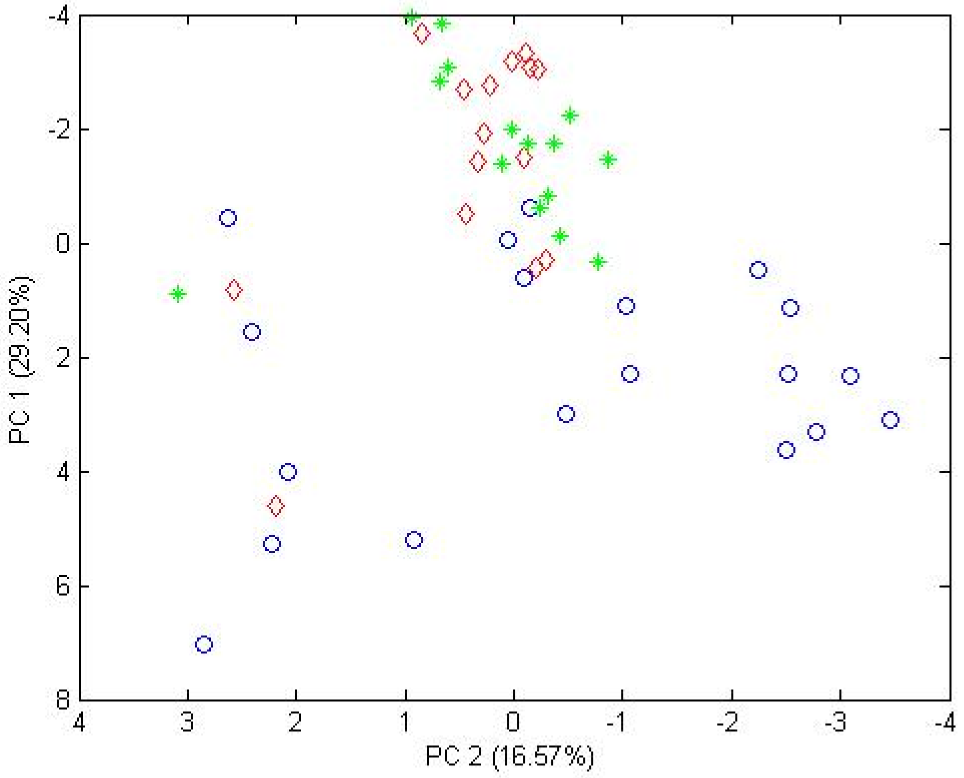

Figure 35. PCA scores plot for the olive oil samples analyzed at HAPPI's laboratory, where Portuguese olive oil (blue "o"), Spanish olive oil (red "◊") and European olive oil bought in China (green "*").

As it was previously observed in **Figure 17**, for the PCA analysis of olive oil samples at the CSU, it is not totally clear the formation of separate cluster for the different samples, but it can be noticed that the Portuguese samples are more spread in the lower part, in opposition to the Spanish and Chinese, which are grouped in the upper part of the graphic. It is expected that the Spanish and Chinese are more related with each other since most of the olive oil samples bought in China were imported from Spain.

4.2.3 Chemometric analysis for all vegetable oils

After processing the fatty acid identification to all the different vegetable oils, a 130x15 sized matrix was constructed, and in order to first see the distribution of the different oils, a PCA plot was built, as shown below in **Figure 36**.

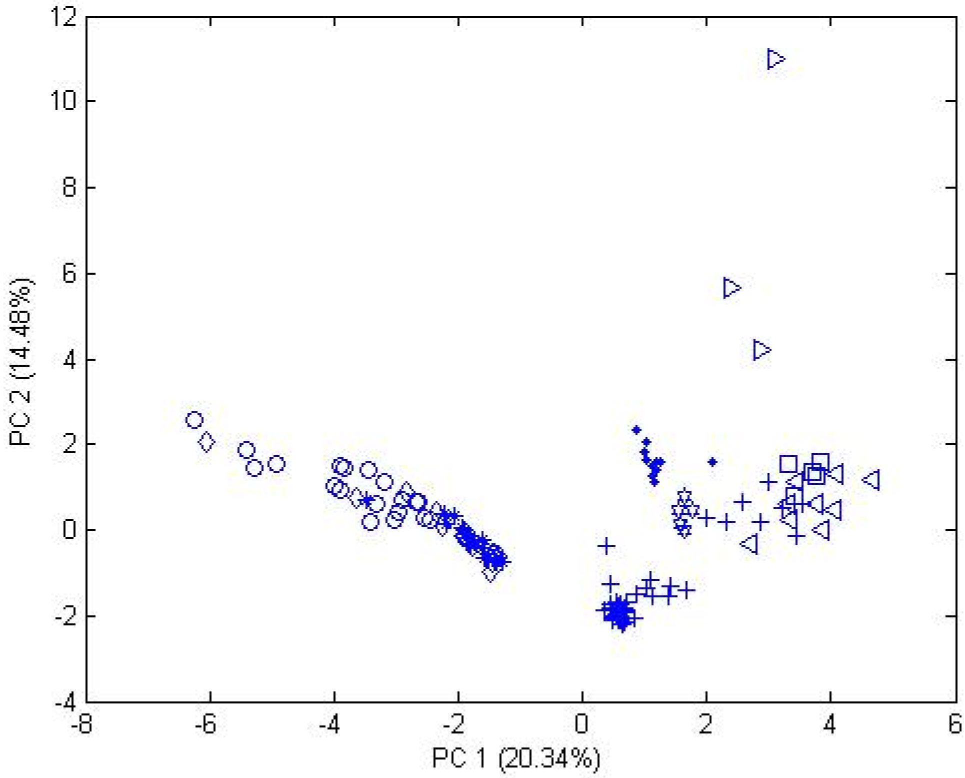

Figure 36. PCA scores plot for all the oils analyzed in HAPPI, where Portuguese olive is "o", Spanish olive oil "◊", olive oil bought in China "*", tea oil "+", corn oil "¤", sunflower oil "□", rapeseed oil "◄", peanut oil "►" and sesame oil "•".

Comparing this plot with the one obtained from the CSU (**Figure 21**), we can find some notorious differences, such as that here olive oil and tea seed oil samples are also well separated but in this one located in such a position that indicates that there is no correlation between them, forming an approximate 180º angle with the origin of the graphic. This conclusion though should not be overvalued since in both cases tea oils samples are very close to the origin, and so very small shifting to one side could produce opposite conclusions. Also in here rapeseed samples are clustered together with sunflower oil, which didn't occur in the plot from CSU, where it was shown a negative correlation.

In order to better see the relation between these two analyses, a PCA plot with both data from the CSU and HAPPI was created (**Figure 37**), using the PCs obtained from the CSU and plotting then the data from the HAPPI.

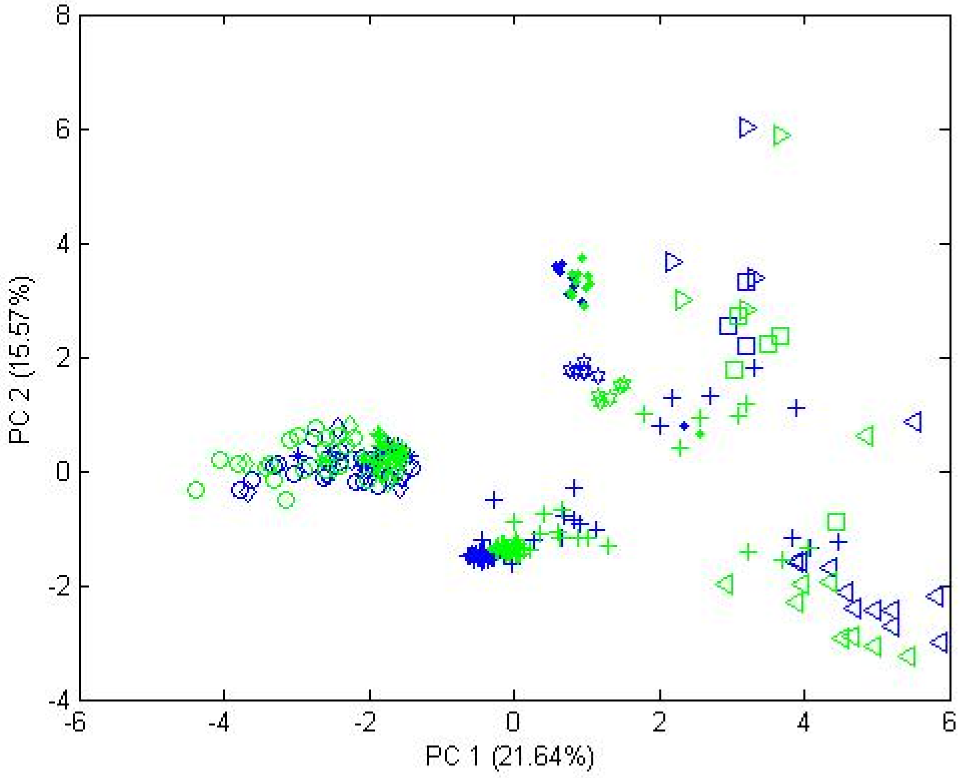

Figure 37. PCA scores plot for all the oils analyzed in CSU (blue) and HAPPI (green), using PCs obtained from the CSU model, where Portuguese olive is "o", Spanish olive oil "◊", olive oil bought in China "*", tea oil "+", corn oil "¤", sunflower oil "□", rapeseed oil "◄", peanut oil "►" and sesame oil "●".

By observing this plot, one can see that the clusters from different classes of samples from both analyses match almost perfectly, it exists a small shifting but not too significant. So with these results we can conclude that both analyses are quite similar and that we can obtain a quite good reproducibility, in terms of intermediate precision, with this method.

4.3 Conclusions

From this Chapter we can conclude that when applying the used method in HAPPI's laboratory, a much worst peak detection was observed, leading this way to the detection and identification of only 12 fatty acids in this laboratory; Even thought the number of identified peaks was much smaller, the reproducibility of the method, in terms of intermediate precision, was quite good.

5 Results and discussion from the University of Algarve

5.1 Fatty acid identification

At this part of the work, it was intended to see if the use of a different method, in a different laboratory and analyzing the same samples could produce similar results. To see this, an olive oil sample (C9) was analyzed at the UAlg. In **Figure 38** it is presented the obtained CG-MS TIC from this analysis, as well as the respective fatty acid identification.

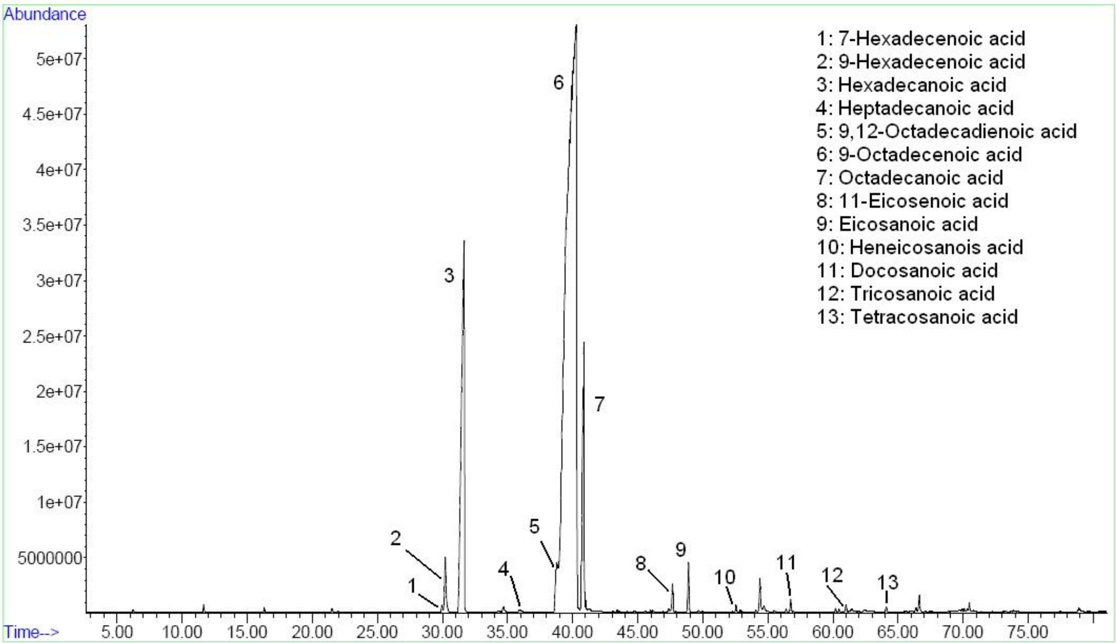

Figure 38. Typical GC-MS total ion chromatogram (TIC) of olive oil (sample C9) from the laboratory at UAlg.

As we can see from the previous figure, only 13 fatty acids were detected and identified in this analysis and no internal standard was used. If we compare this analysis with the ones performed in the CSU's laboratory and at the HAPPI's laboratory, we can see that all the fatty acids identified in the olive oil samples at the UAlg were also identified at the CSU but not at the HAPPI, where only 8 fatty acids were identified in common. To better see these relations, **Table 15** presents the peak identification from the three laboratories.

Table 15. Fatty acid identification in olive oil for the three different laboratories, CSU, HAPPI and Ualg.

Fatty acids	CSU	HAPPI	UAlg
Dodecanoic acid	✓		
Tetradecanoic acid	✓	✓	
Pentadecanoic acid	✓	✓	
Hexadecanoic acid	✓	✓	✓
7-Hexadecenoic acid	✓		✓
9-Hexadecenoic acid	✓	✓	✓
Heptadecanoic acid	✓	✓	✓
10-Heptadecenoic acid	✓	✓	
Octadecanoic acid	✓	✓	✓
9-Octadecenoic acid	✓	✓	✓
11-Octadecenoic acid	✓		
9,12-Octadecadienoic acid	✓	✓	✓
9,12,15-Octadecatrienoic acid	✓	✓	
Eicosanoic acid	✓	✓	✓
11-Eicosenoic acid	✓	✓	✓
Heneicosanoic acid	✓		✓
Docosanoic acid	✓		✓
Tricosanoic acid	✓		✓
Tetracosanoic acid	✓		✓

Since only one sample was analyzed under these conditions, no chemometrics could be applied, but the results from this sample were compared with the other laboratories' analyses, with the results present in Chapter 6.

6 Results and discussion without the use of an internal standard

6.1 Choosing the best fatty acid to use as reference peak

For this study, we also found relevant to check if similar results could be obtained without resorting to an internal standard, calculating the relative areas of the peaks by selecting a reference peak, a fatty acids present in all the vegetable oils. A possible disadvantage to this might be the lost of one degree of freedom, since we are using one compound present in the samples to calculate the relative areas, but, on the other hand, if we can obtain similar results without resorting to an external compound, this would eliminate one step on the procedure, saving us time and money.

So, to do these analyses, first the fatty acid to be used as reference peak had to be chosen, and to do so, some considerations must be taken, such as that this fatty acid must be present in all types of the studied vegetable oils, must be clear and without interferences from other peaks and must also be of and intermediate height between the smaller and larger peaks.

To better analyze the choosing criteria, in **Figure 39** is illustrated the fatty acid chromatogram of each vegetable oil. So, based on all the different oils, Palmitic acid was chosen to perform the ratios, since it is present in all oils, is isolated and it has the same order of magnitude as the internal standard for all the cases.

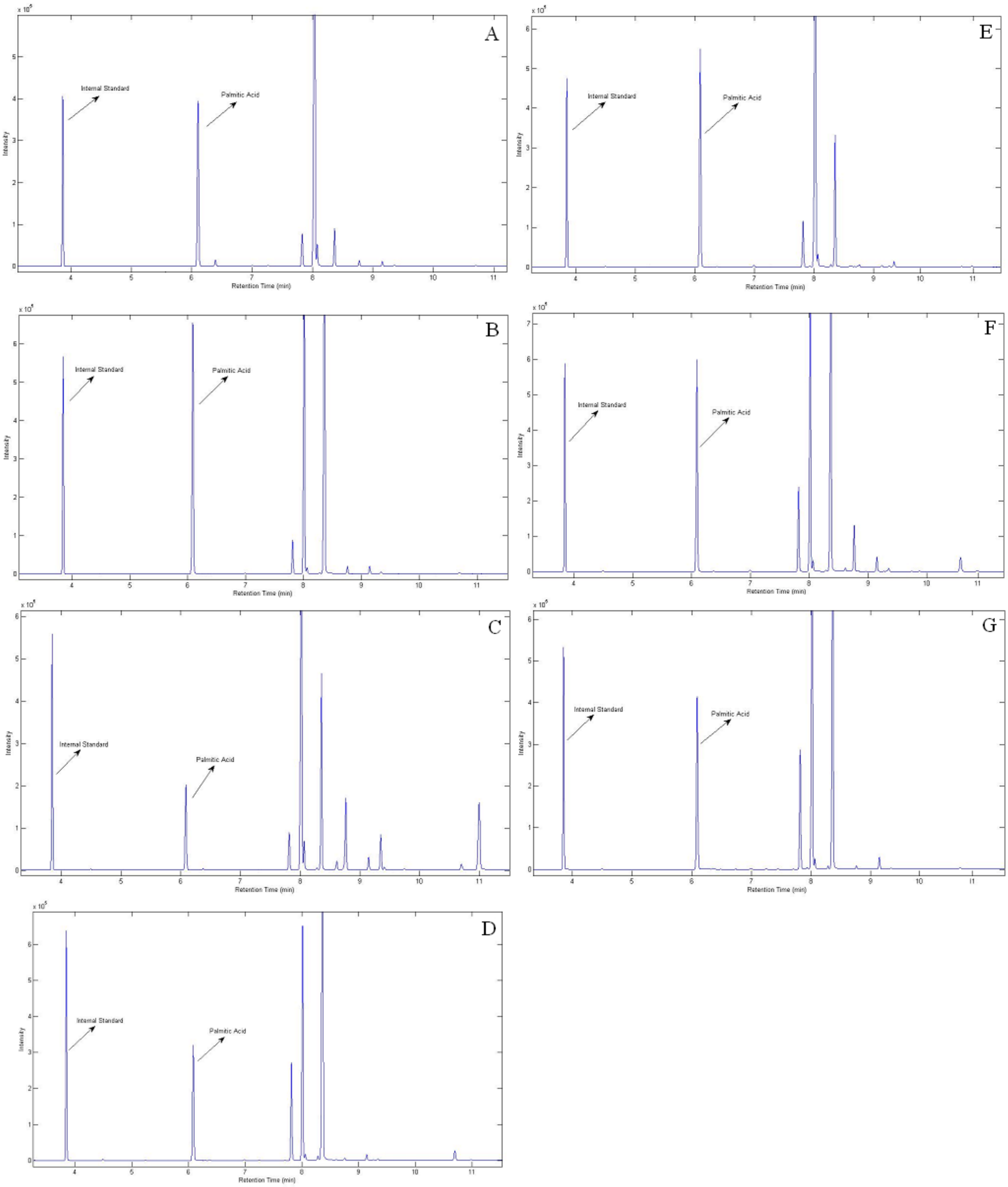

Figure 39. Chromatogram representation of every used type of oil, identifying the internal standard and palmitic acid, where A: olive oil, B: corn oil, C: rapeseed oil, D: sunflower oil, E: tea oil, F: peanut oil, G: sesame oil.

6.2 Results and discussion from the CSU’s laboratory

6.2.1 European Olive oil

As previously said, with the use of Palmitic acid to perform the ratios, one degree of freedom is lost, and so the matrix formed with the European olive oil samples will have a 30×18 dimension.

The study to find patterns in the different regions of the olive oil’s production was carried out by performing a PCA scores plot separating these five regions, and it is presented below in **Figure 40**.

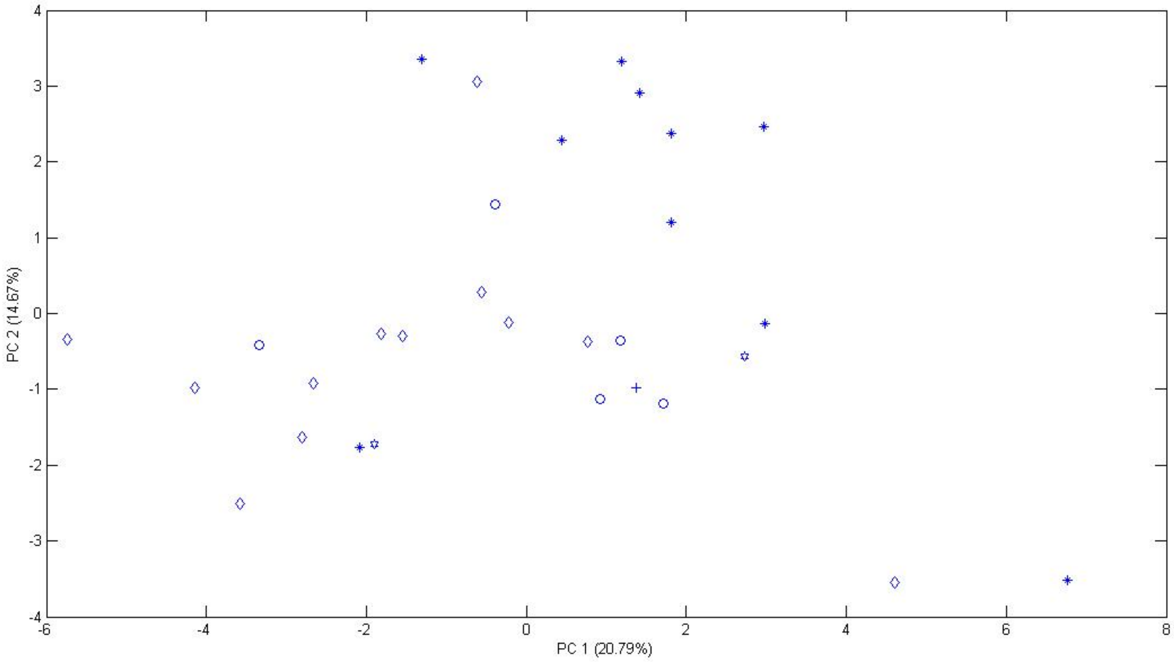

Figure 40. PCA scores plot of the European olive oil samples grouped by regions with Palmitic acid as reference peak (region 1 – “o”; region 2 – ”◊”; region 3 – “*”; region 4 – ”+”; region 5 – “¤”).

As in the previous study, without the use of Tridecanoic acid as internal standard it is also not possible to distinguish the five different regions with only a general PCA, and so PLS-LDA models were performed (**Figures 41, 42** and **43**).

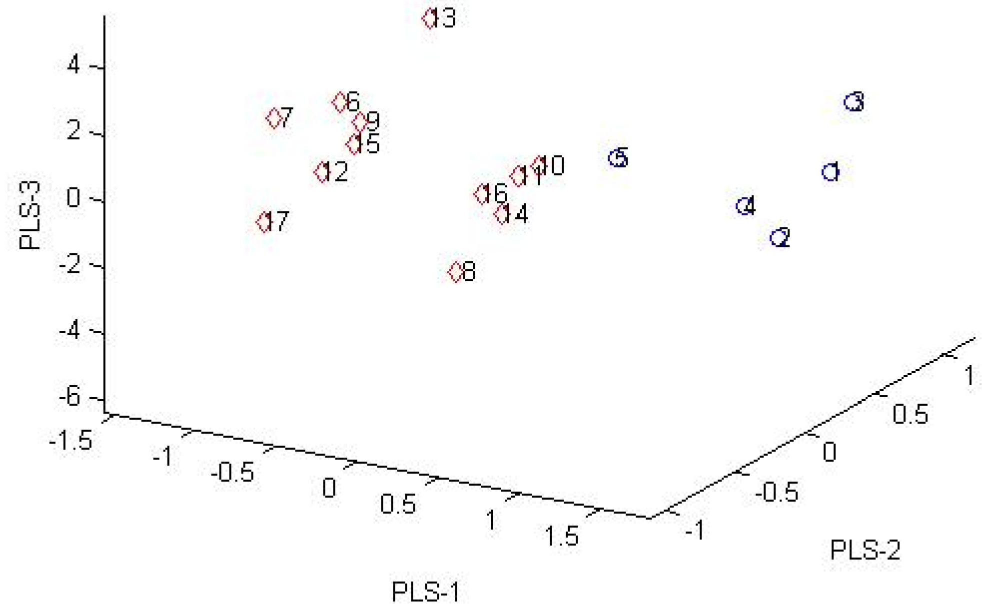

Figure 41. PLS-LDA scores plot of regions 1 (blue “o”) and 2 (red “◊”) with Palmitic acid as reference peak.

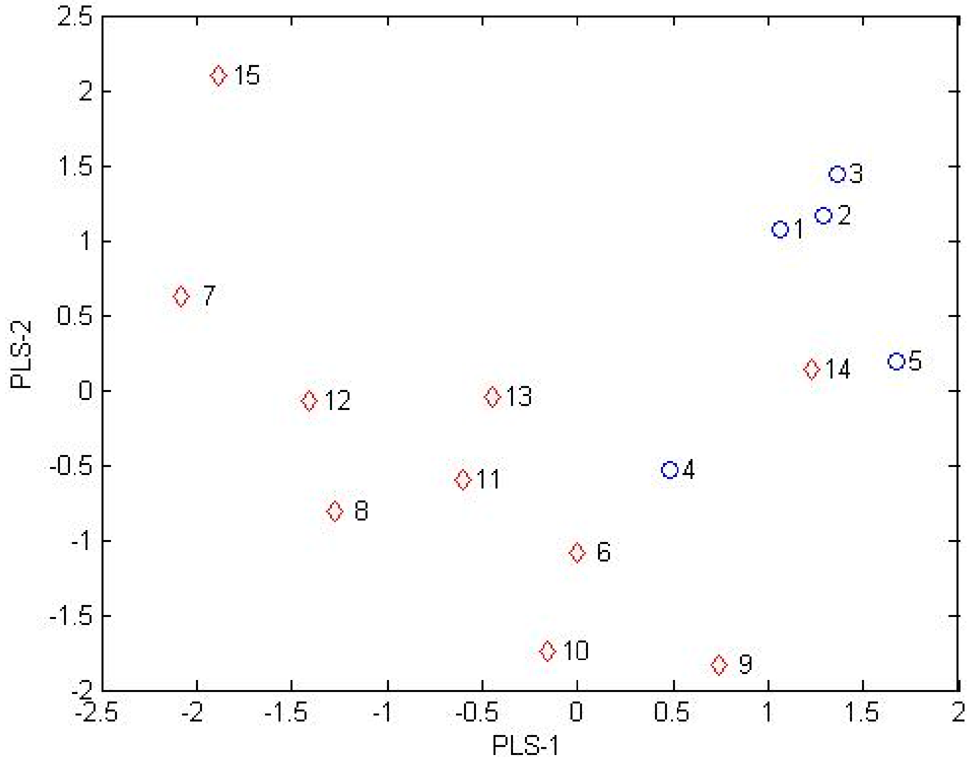

Figure 42. PLS-LDA scores plot of region 1 (blue “o”) and 3 (red “◊”), with Palmitic acid as reference peak.

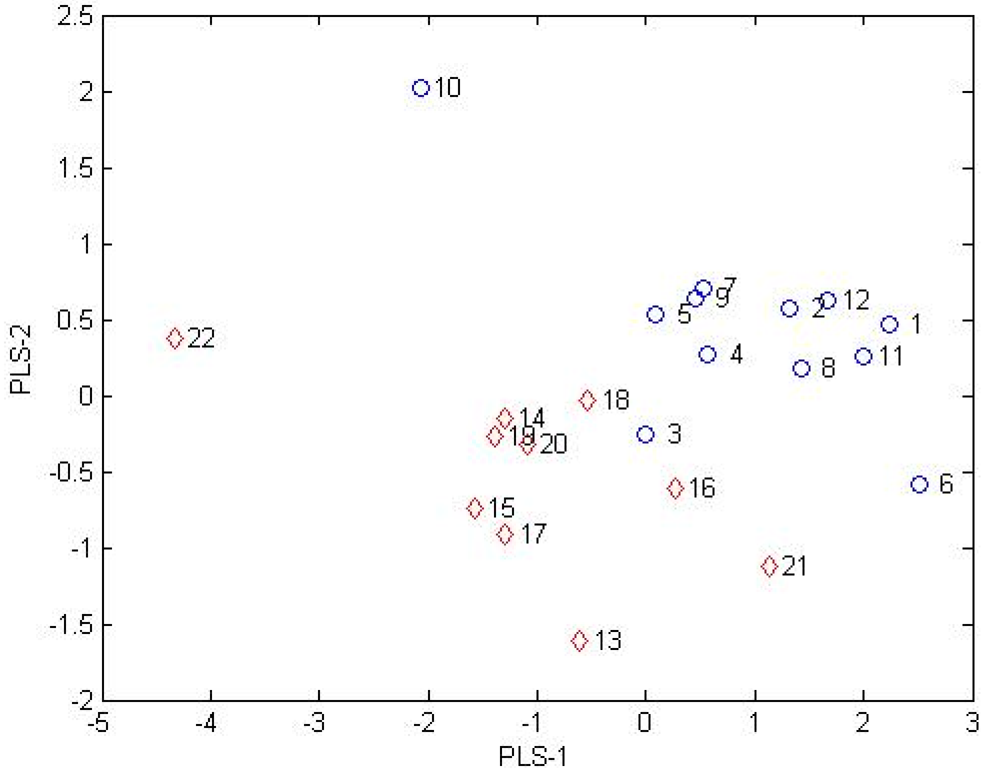

Figure 43. PLS-LDA scores plot of regions 2 (blue “o”) and 3 (red “◊”), with Palmitica acid as reference peak.

As one can see by observing these three scores plots and the information provided by **Table 16**, the use of PLS-LDA can well distinguish between regions when using Palmitic acid as reference peak. The recognition rate gave values higher than 90% in all three comparisons as well as the specificity and sensitivity.

Table 16. Classification results between three of the regions by PLS-LDA method.

Groups	Recognition rate	Sensitivity	Specificity
Region 1 versus region 2	1	1	1
Region 1 versus region 3	0.9333	1	0.9
Region 2 versus region 3	0.9545	0.9167	1

Recognition rate is the correct classification of the training set;

Sensitivity is the number of true positives classified as positive;

Specificity is the number of true negative classified as negative.

6.2.2 European Vs Chinese bought olive oil

First, a PCA modes was performed to compare it with the one obtained by using the Tridecanoic acid as internal standard. In **Figure 44** we can see this comparison.

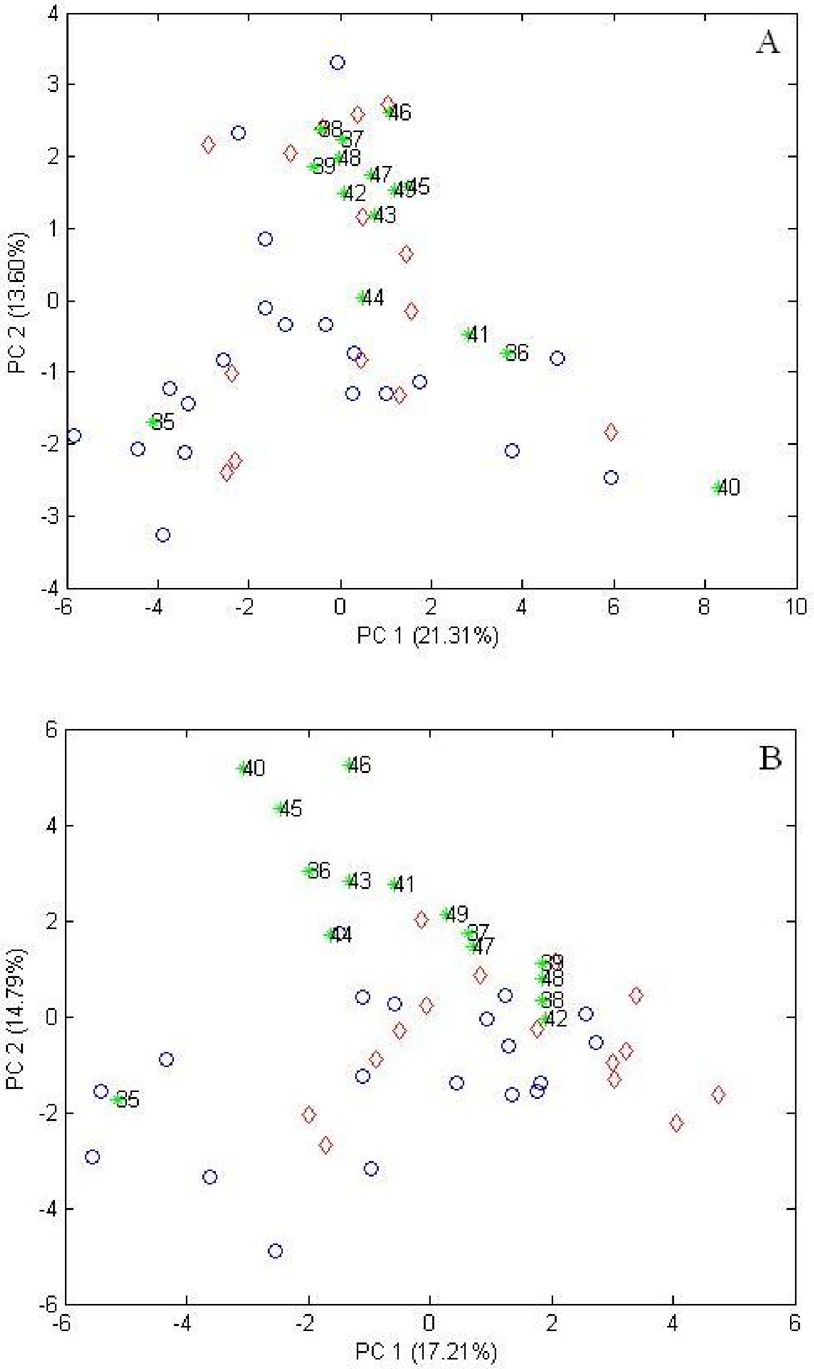

Figure 44. PCA scores plot of Portuguese olive oil (blue "o"), Spanish olive oil (red "◊") and European olive oil bought in China (green "*"), where A: using Palmitica acid as internal standard and B: Tridecanoic acid as internal standard.

As it was previously observed, there is no clear separation according to the region of origin between the two sets of samples, and so, further analyses are required. A PLS-LDA model was then constructed in order to better investigate this matter.

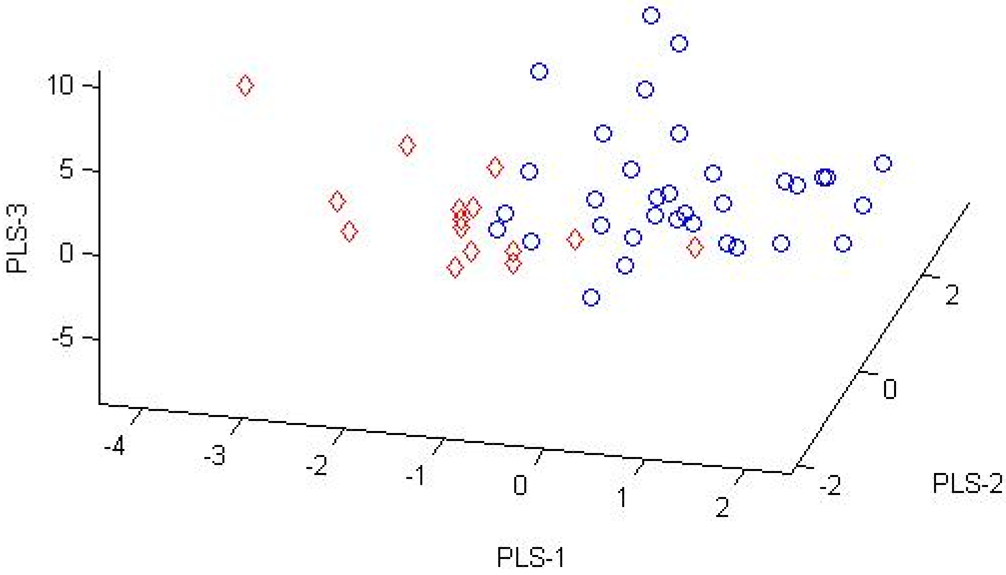

Figure 45. PLS-LDA scores plot of European olive oil (blue “o”) and European olive oil bought in China (red “◊”), whith the use of Palmitic acid as internal standard.

As it can be observed in **Figure 45**, one can see that it exists a separation between the two types of samples, even though that some samples are located around the central region of the graphic, making the classification doubtable in this region. By comparing this plot with the one obtained with Tridecanoic acid as internal standard (**Figure 18**), we can see that there are no great differences between the two plots, even the prediction rate, sensitivity and specificity (**Table 17**) are quite similar to one another, showing this way that when using Palmitic acid as reference peak, it works as good as with Tridecanoic acid as an internal standard.

Table 17. Classification results between European olive oil and China bought olive oil by PLS-LDA method, both Tridecanoic acid as internal standard and Palmitic acid as reference peak.

Internal standard/reference peak:	Recognition rate	Sensitivity	Specificity
Palmitic acid	0.8367	0.7941	0.9333
Tridecanoic acid	0.8571	0.7941	1

Recognition rate is the correct classification of the training set;

Sensitivity is the number of true positives classified as positive;

Specificity is the number of true negative classified as negative.

6.2.3 Tea seed Vs Olive oil

When using the Tridecanoic acid as internal standard, tea seed and olive oil were perfectly separated, forming two perfectly distinct clusters (**Figure 20**). With Palmitic acid, the same PLS-LDA model was constructed in order to see if tea seed and olive oil could be distinguished. The results are shown below in **Figure 46**.

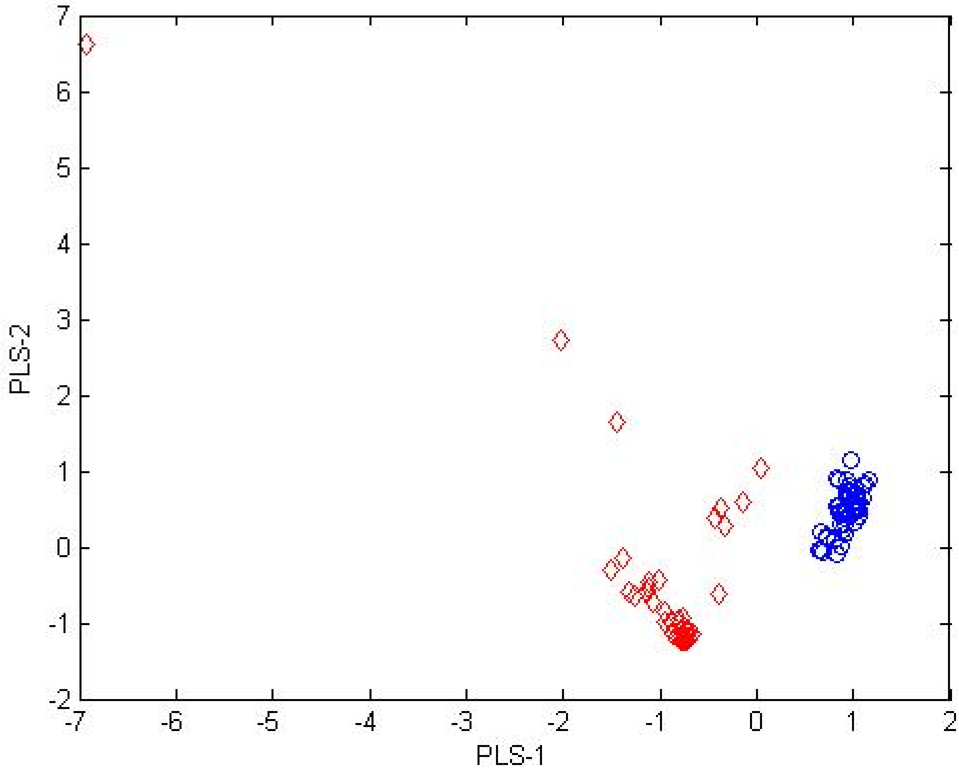

Figure 46. PLS-LDA scores plot of olive (blue “o”) and tea seed (red “◊”) oils with Palmitic acid as reference peak.

By observing these results, it is clear that with Palmitic acid used as reference peak, a good separation between tea seed and olive oil may be obtained, by PLS-LDA model, meaning that there are no great advantages of using an internal standard for these analyses.

6.2.4 All vegetable oils

In order to see a general distribution of all vegetable oils, a PCA scores plot was created (**Figure 47**), and in the light of the one obtained previously with the use of Tridecanoic acid as internal standard (**Figure 21**), we can see that when resorting to Palmitic acid as a reference peak, also all the different types of oils are generally clustered together. As it was also observed in **Figure 21**, some of the tea oil samples are somehow spread along the plot, and also one rapeseed sample located further from its cluster.

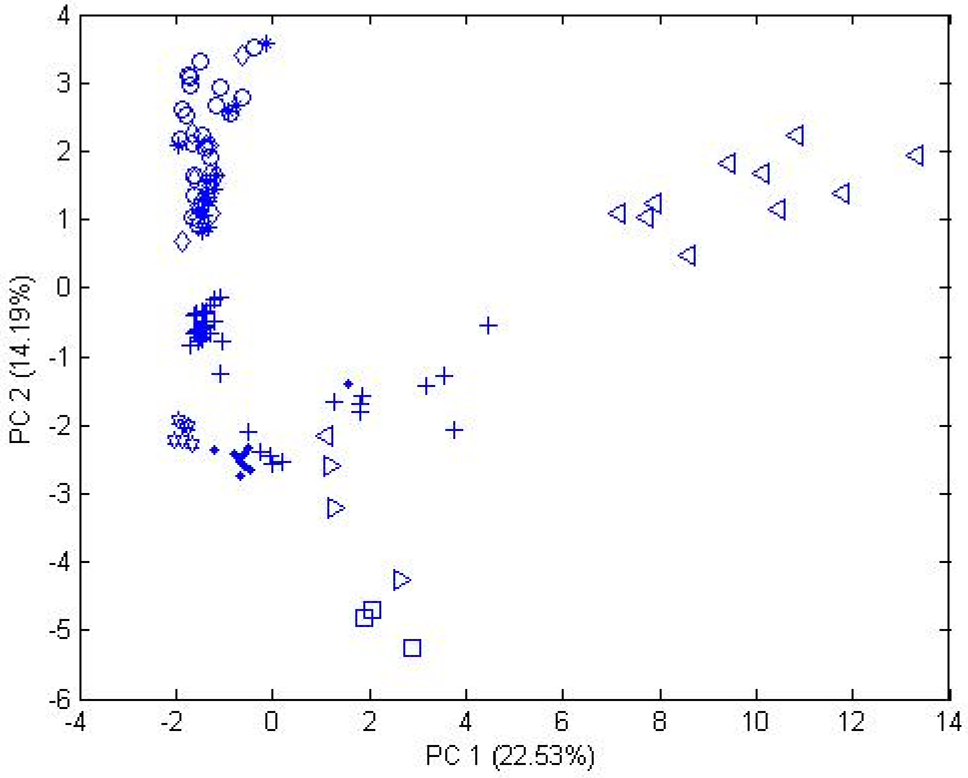

Figure 47. PCA scores plot for all the oils with Palmitic acid as reference peak, where Portuguese olive is o, Spanish olive oil ◊, olive oil bought in China *, tea oil +, corn oil ¤, sunflower oil □, rapeseed oil ◄, peanut oil ► and sesame oil ●.

In conclusion, for the study of all vegetable oils by PCA, the use of Palmitic acid as a reference peak for the calculation of peak ratios produces quite similar results as when Tridecanoic acid was applied as internal standard.

6.3 Results and discussion from HAPPI's laboratory

In this part, it is intended to see if the obtained results from HAPPI's laboratory using Palmitic acid as a reference peak are similar to the ones obtained with Tridecanoic acid as internal standard from Chapter 4.

6.3.1 Analyses on olive oil

First, a PCA scores plot was constructed to check for the general distribution of the olive oil samples (**Figure 48**).

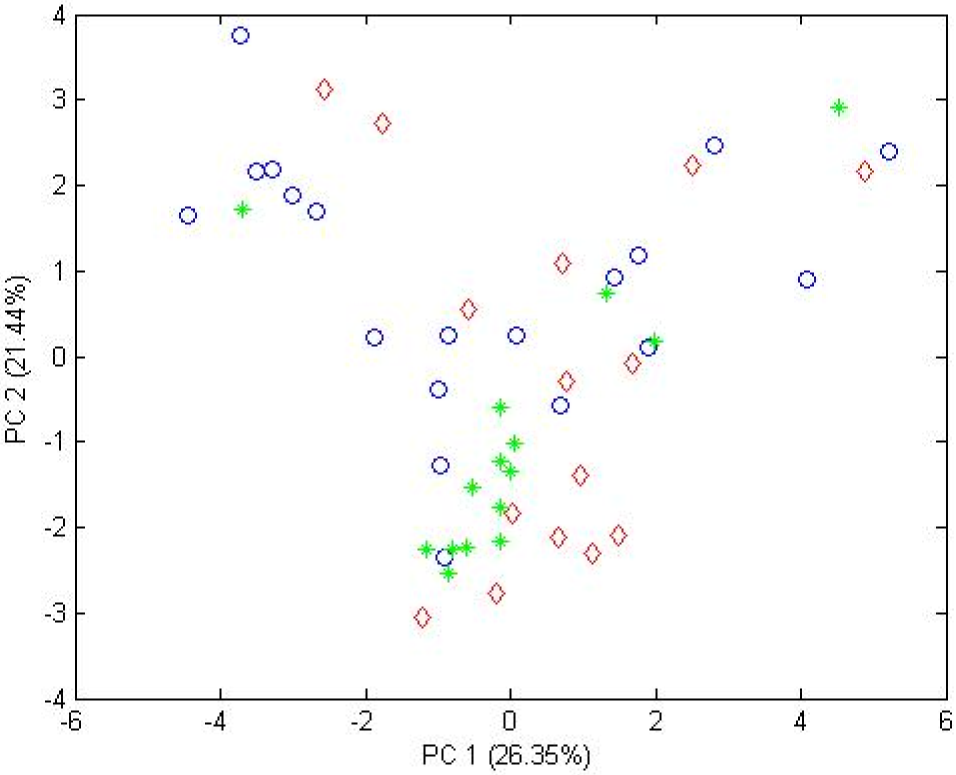

Figure 48. PCA scores plot for the olive oil samples analyzed at HAPPI's laboratory with Palmitic acid as reference peak, where Portuguese olive oil (blue "o"), Spanish olive oil (red "◊") and European olive oil bought in China (green "*").

As it may be observed, and comparing with the one obtained using Tridecanoic acid as internal standard (**Figure 35**), there are no clear distinctions from the different classes of samples, and so, similar results may be obtained when using both Tridecanoic acid or Palmitic acid to calculate the peak ratios.

6.3.2 Analyses on all vegetable oils

A PCA scores plot (**Figure 49**) was built with the information from all vegetable oils obtained from the HAPPI and using Palmitic acid to calculate the rations.

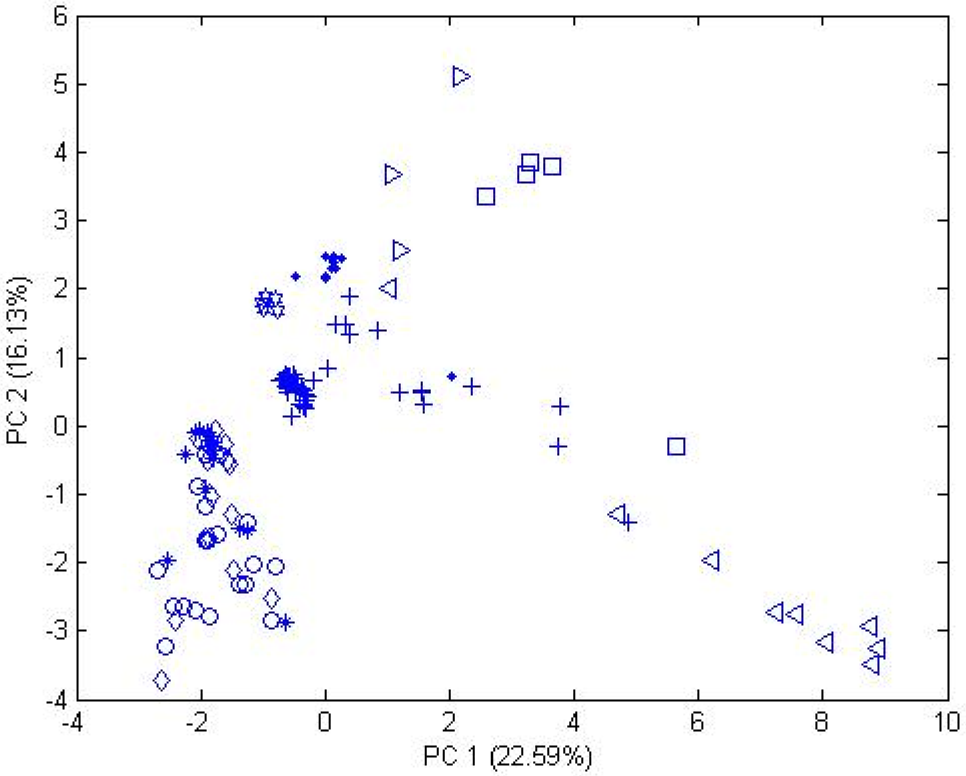

Figure 49. PCA scores plot for all the oils analyzed in HAPPI using Palmitic acid as reference peak, where Portuguese olive is “o”, Spanish olive oil “◊”, olive oil bought in China “*”, tea oil “+”, corn oil “¤”, sunflower oil “□”, rapeseed oil “◄”, peanut oil “►” and sesame oil “•”.

By observing **Figure 49** and comparing it with the one obtained with Tridecanoic acid as internal standard (**Figure 36**), we can conclude almost the same from both, but in **Figure 40** we can see both rapeseed and sunflower oils clustered separately one from another, which was not clear in **Figure 36**. So, in conclusion, we were able to obtain more clear results when using Palmitic acid to calculate the peak ratios than with Tridecanoic acid.

6.4 Results and discussion for the comparison between the analyses performed at UAlg and CSU

In order to see if the method used to perform the analysis at the UAlg's laboratory gives similar results with the one from CSU, a PCA scores plot was firstly performed (**Figure 50**), including all the fatty acids identified at CSU, to see if the sample from UAlg would be mixed among the other olive oil samples or forming a separate cluster.

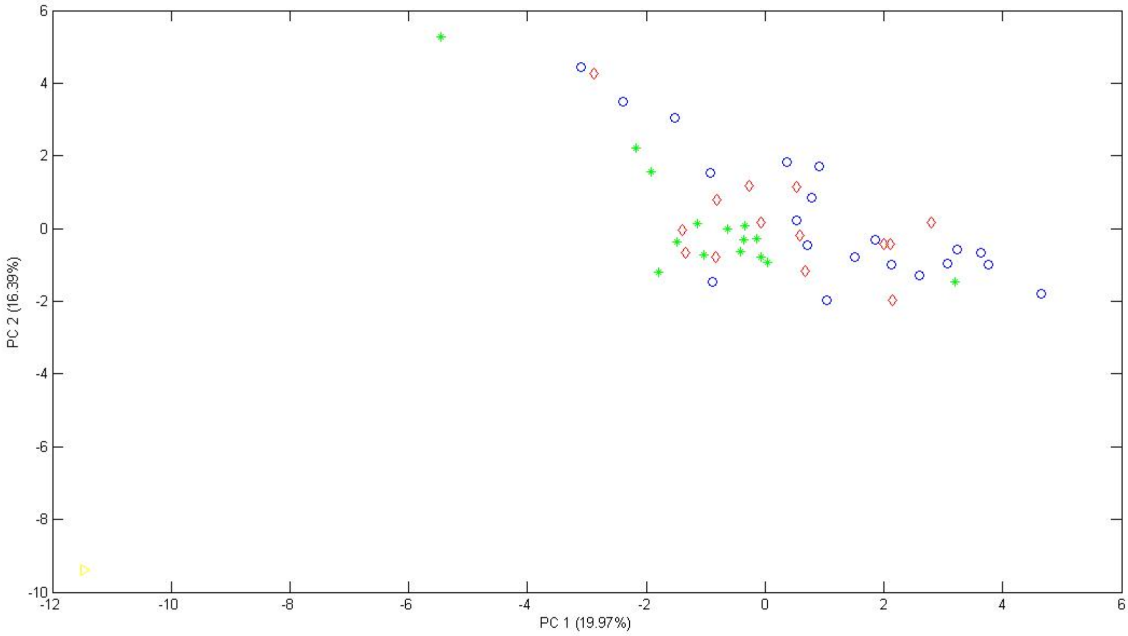

Figure 50. PCA scores plot for olive oils analyzed at CSU (Portuguese olive, blue "o"; Spanish olive oil, red "◊"; olive oil bought in China, green "*") and UAlg (Olive oil bought in China, yellow "►").

As it can be seen, the sample analyzed at the UAlg's laboratory was found to be quite distinct from the ones analyzed at CSU's laboratory, this might mean that the different procedures used at the different universities affected the fatty acids' detections, producing then distinct results. Also, since we could only analyze one sample at UAlg and since this sample was analyzed in total ion mode, opposite to the analyzes in the other laboratories where selective ion mode was applied, these conclusions might not be actually true, and so, it would be interesting for a future work to analyze more vegetable oil samples, using the same detection mode, to have more and more comparable data to better substantiate the conclusions from this Chapter.

6.5 Conclusions

To conclude this chapter, it can be said that the results obtained when using Palmitic acid as a reference peak do not vary significantly when comparing to the ones where an internal standard was added to the samples. So, to perform the analyses presented in this work, it may be considered the use of Palmitic acid as internal standard, avoiding this way the addition of one extra step to our method, which does not bring any significant improvements to the final results.

As for the sample analyzed at UAlg, we may say that more data should be collected in order to better conclude thistopic.

7 Conclusions

By performing the work here presented, it was possible to conclude that:

- With the data obtained on the fatty acids' composition of the olive oils from different regions of the Iberian Peninsula by GC-MS, it is possible to distinguish the samples according to their region of productions with the help of PLS-LDA method;
- Besides the clear similarities that exist between tea seed and olive oil, it is possible to distinguish these two oils by their fatty acid composition;
- A good repeatability may be obtained with the proposed method, and also its reproducibility, in terms of intermediate precision was good;
- By only detecting the 12 major fatty acids in olive oil the same results could be obtained as when all 19 fatty acids were used, so the use of a method with higher detection ad identification limits may be enough to perform these analyses;

- Palmitic acid may be used as a reference peak to produce these analyses, since there were no significant differences between these results and when Tridecanoic acid was used as internal standard;
- More data from the UAlg should be obtained before drawing any conclusions to this topic.

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9 Annexes

Annexe I

Table1. Olive oil’s GC data from the first batch at the CSU.

Table2. Olive oil’s GC data from the second batch at the CSU.

Table 3. Olive oil’s GC data from the HAPPI.

Table 4. Tea 6oil’s GC data from the first batch at the CSU.

Table 5. Tea oil’s GC data from the second batch at the CSU.

Table 6. Tea oil’s GC data from the HAPPI.

Table 7. Corn oil’s GC data from the first batch at CSU.

Table 8. Corn oil’s GC data from the second batch at CSU.

Table 9. Corn oil’s GC data from the HAPPI.

Table 10. Sunflower’s GC data from the first batch at the CSU.

Table 11. Sunflower oil’s GC data from the second batch at the CSU.

Table 12. Sunflower oil’s GC data from the HAPPI.

Table 13. Rapeseed oil’s GC data from first batch at the CSU.

Table 14. Rapeseed oil’s GC data from second batch at the CSU.

Table 15. Rapeseed oil’s GC data from the HAPPI.

Table 16. Peanut oil’s GC data from the CSU.

Table 17. Peanut oil’s GC data from the HAPPI.

Table 18. Sesame oil’s GC data from the CSU.

Table 19. Sesame oil’s GC data from the HAPPI.

Annexe II

PCA codes forMATLAB

PLS-LDA codes for MATLAB

Annexe I

Table 1. Olive oil's GC data from the first batch at the CSU.

	13:0	12:0	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c	21:0	22:0	23:0	24:0
P1	487867	229	990	583	805577	3371	37201	6765	5935	92563	1643686	87294	226625	15416	18014	5698	744	5306	741	2263
P2	473270	233	545	384	443983	3175	9686	6888	4220	171664	1877532	45798	180925	15270	18763	6669	664	4559	694	1188
P3	473085	191	802	439	749495	3438	33678	5400	5178	109560	1774272	89539	161931	17474	18454	5822	726	5263	902	2214
P4	489329	221	450	270	603904	2608	19215	2403	2020	159751	2075849	72169	71948	16657	17967	4984	574	4437	734	1721
P5	478425	175	667	430	650227	2703	26894	4345	3957	117151	1868496	75423	117809	18696	17446	5578	672	4612	766	1781
P6	463088	236	662	286	605497	3052	19345	5090	3820	154051	1789286	64837	148027	17896	19478	4930	590	4962	837	1858
P7	496174	232	708	585	766599	2167	47373	5984	6564	101433	1840310	99659	102400	17944	15497	5007	772	4021	837	1453
P8	496627	196	626	313	676630	3241	24367	5722	4508	175687	1879751	73300	177370	20524	21275	5076	794	5162	912	2007
P9	486625	260	615	301	569456	2440	10423	3688	2497	132797	1709653	45771	223160	19031	15954	4929	779	4072	701	1402
P10	482101	265	528	331	559167	2446	13284	6883	5228	145606	1869734	60390	143769	18149	17901	5183	585	4296	600	1518
P11	465112	278	515	253	445718	2659	9195	6176	3813	176433	1861174	45341	153079	15048	18285	5979	522	4084	602	1522
P12	535681	241	538	371	593001	3739	16122	7188	4947	183450	2086389	61735	167888	19238	21439	6221	607	5279	780	1830
P13	475656	202	967	450	768906	3831	33287	4596	4379	84026	1652577	92116	246943	15606	17940	6244	675	5407	804	2149
P14	473317	237	661	323	598655	2151	21554	3842	3316	116043	1833502	69358	113745	18468	17069	5377	545	4658	709	1732
P15	491217	218	758	472	550153	2747	13439	6033	4552	135837	1979200	60565	156892	20060	21532	7152	784	5838	810	2283
P16	489341	259	738	580	770570	2914	41258	6395	6820	111426	1769342	94244	116805	19667	17333	5094	712	4737	896	1867
P17	483675	226	563	321	561416	2786	11138	5965	3885	162823	1873777	53167	201139	20951	18516	5062	504	4393	700	1312
P18	477810	247	513	293	584524	2471	16454	3384	2608	113070	1914078	67624	117596	16406	16652	5662	591	4528	630	1479

P19	439040	207	580	348	603905	2041	27931	5784	6399	98784	1774989	82304	92780	14736	15262	4596	595	4190	689	1265
S1	613339	266	707	204	665236	3798	20847	2821	2057	226063	2290398	79131	117720	17806	21056	5438	1769	4697	828	1522
S2	471431	194	667	256	565720	3442	13974	3810	2516	148744	1915539	61544	151478	13907	21435	6096	787	5637	787	1926
S3	478722	196	627	404	670534	2284	24333	4981	4007	180608	1712972	74149	208543	17295	21285	5021	604	4733	672	1851
S4	508416	189	653	224	580674	3506	18812	2334	2029	218586	2164612	74160	99611	18387	20006	5343	746	4328	922	1651
S5	505209	268	506	255	605955	2280	16715	2066	1668	132028	2112234	67690	60002	15646	16975	5112	515	3879	443	1608
S6	482239	159	698	284	551299	2704	15019	5203	3844	147661	1792653	56835	177059	18854	18729	5953	595	4682	740	1898
S7	472290	253	673	401	576895	3327	18463	4185	3301	144065	1787628	66800	163254	18604	18480	5685	603	4603	696	1837
S8	481338	261	472	169	500675	2366	11984	2646	2113	154949	2009786	60524	84561	15060	16948	4891	496	4101	268	1392
S9	469869	182	503	162	512275	2131	15275	2294	1811	121790	1817524	62053	100921	16350	15313	4895	510	3822	752	1452
S10	475628	229	483	187	496652	2337	13966	2381	1676	162165	1945008	57115	89840	13747	19530	5505	644	4470	701	1622
S11	477715	234	520	278	507213	2376	13326	3032	2278	149108	1879742	56459	103558	15602	17100	5241	579	4006	662	1485
S12	473562	231	725	447	660828	3039	20277	5973	4882	102197	1698637	73319	205716	13586	17606	5523	523	4716	651	1432
S13	540318	185	621	345	561939	3539	14204	4232	3092	152956	2111638	61844	148454	18649	20092	6582	694	5038	582	1780
S14	479318	228	608	301	457115	3280	8335	6514	4455	151841	1880487	49672	160634	18289	18943	6923	618	4697	625	1687
S15	473703	190	738	367	666944	4219	20448	6348	5352	99453	1799383	71110	199188	11634	17356	5547	735	4648	571	1422
C1	707066	345	1167	676	1202796	5900	49005	7598	6985	139887	2447706	141179	381184	21821	27496	8749	902	7842	1297	3415
C2	654365	293	984	621	773057	4937	21238	7425	5544	237107	2780458	91676	201817	27862	28736	9302	1297	7183	1440	3101
C3	571992	300	718	288	721832	4184	21363	3472	2928	196959	2595693	93308	131615	20948	23368	6732	839	6053	1146	2331
C4	693609	370	806	355	835025	4543	26366	3731	3085	265424	2892702	106749	153694	23136	26694	7232	1026	6306	1233	2526
C5	544948	232	668	317	674480	2815	19965	3309	2588	224509	2432592	78424	124737	18992	22230	6081	869	5245	883	1976
C6	695458	311	980	625	738158	4593	15591	12719	7939	277993	2946796	82331	277241	28882	33995	10843	1085	11304	1506	4593
C11	665101	253	790	489	776241	4707	18115	8683	5988	251934	2948408	90284	212782	25899	29031	9610	892	7255	1293	2932
C14	709061	338	798	409	816944	4235	25221	3843	3062	268961	2882732	97737	239222	22377	25773	7004	1048	5686	1179	3068

C7	618479	250	972	282	804777	4521	22271	3361	2422	181501	2395644	86856	217791	21789	29245	7707	1105	8825	1244	2642
C10	628585	288	964	373	821469	4233	24476	4680	3123	195638	2335727	90220	305534	22374	28262	8001	1004	7456	1246	2808
C12	716017	317	1170	455	948569	5343	27368	3091	2400	207762	2908146	113350	199392	29372	36242	10119	1297	10793	1577	3912
C13	530163	236	769	176	714626	3431	19102	2561	1919	164111	2422478	83280	146875	19403	27601	7628	889	8876	1225	2750
C8	723804	338	983	429	868302	4709	26492	4579	3449	286129	3077273	112572	185662	26323	29789	8375	1085	7106	1388	2895
C9	700198	373	861	377	845116	3441	24531	4156	2935	286695	2933334	101901	203083	23888	29347	7342	1020	7012	1207	2633
C15	621561	335	769	352	760675	4219	21712	4917	3539	235926	2624743	93087	181713	21535	27625	7871	934	7127	1257	2526

Table 2. Olive oil's GC data from the second batch at the CSU.

	13:0	12:0	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c	21:0	22:0	23:0	24:0
P1	556352	118	1118	701	878247	4202	40264	7251	6275	103829	1773033	102690	246509	16775	20008	6149	894	5957	898	2349
P2	662176	326	723	440	643183	5008	14029	9785	6054	250487	2765149	72745	265474	22227	27255	9369	1005	6694	1055	2057
P3	548571	297	899	551	890052	3834	39485	6336	6035	129687	2092390	110921	192644	20362	21859	6810	1016	6072	1023	2476
P4	581737	263	590	305	674278	2250	21223	2682	2174	179374	2343830	82554	80898	18568	20145	5575	655	4808	773	1872
P5	580170	289	794	488	799604	3398	32924	5319	4884	143462	2307771	99299	147510	22879	21545	6577	701	5710	882	2195
P6	330397	104	449	108	432224	1656	13909	3677	2725	111007	1331583	47384	110927	13155	14668	3653	293	3699	527	1174
P7	532860	281	767	615	855944	2360	52530	6667	7261	114349	2059619	113867	114794	19971	17745	5534	856	4500	819	1798
P8	660280	290	833	510	934616	4170	32790	7613	6104	242442	2547739	118722	242942	27452	28931	6816	838	6680	1114	2425
P9	586301	300	673	280	706783	2342	12725	4583	3052	167132	2171758	58996	282761	23911	20353	7022	813	5063	792	1636
P10	646420	308	675	464	708488	3685	16714	8503	6588	186100	2379297	79694	182645	22870	22731	6781	663	5349	644	1969
P11	400240	214	435	207	397671	2500	8541	5544	3393	158793	1743992	38294	142914	14005	16951	4939	532	3912	633	1134

P12	364720	173	406	215	409165	2037	11197	4936	3416	127832	1490305	42205	120959	13599	15299	4290	463	3591	522	1178
P13	351138	144	783	343	588016	2998	25615	3518	3492	65400	1303916	73320	195022	11860	14375	5057	561	4277	671	1570
P14	606825	297	776	479	813623	3363	29110	5120	4357	158296	2524892	98466	155897	24605	23661	7146	792	6172	1046	2430
P15	597305	303	815	531	657658	3105	15938	7199	5318	163574	2372661	75533	188367	23817	25720	8966	938	7068	1016	2541
P16	562892	248	845	715	910346	3811	49183	7523	7995	131668	2091408	115342	139199	23228	20574	6133	855	5356	920	2158
P17	561364	277	607	412	679452	2314	13084	7175	4698	198870	2287587	64293	244715	25273	22720	6823	696	5505	766	1761
P18	602308	280	665	361	726543	3621	20554	4055	3300	141493	2414572	78934	145527	20504	21113	7526	767	5677	812	1991
P19	334162	173	377	298	438035	1559	21210	4333	4706	70264	1343368	58208	71051	11500	11167	3397	448	3117	494	900
S1	542523	273	626	316	650277	3461	20046	2682	2194	225567	2311022	79756	117785	17635	21344	5503	760	4565	777	1489
S2	613028	316	921	426	752010	4491	18574	4864	3457	198614	2559155	83539	201202	18301	28794	8888	917	7417	1118	2631
S3	655977	306	810	410	902859	3581	32667	6492	5340	244316	2328574	99388	280625	23179	28748	6691	817	6543	970	2575
S4	529764	201	587	276	596613	3207	19199	2363	1960	227388	2255367	76492	103186	18673	20633	5541	757	4279	866	1578
S5	557808	277	458	247	652473	1956	17926	2372	1806	143354	2291385	73583	65473	16990	18652	5599	606	4414	783	1760
S6	586985	295	881	476	684215	3310	18738	6415	4838	184727	2267148	73767	222713	23347	23502	7532	823	6127	1059	2308
S7	586999	317	833	397	723837	4038	22934	5251	4006	182192	2274738	77219	203946	22992	23336	7368	834	5916	1032	2344
S8	602620	303	566	406	601173	2804	14241	3126	2448	187469	2409003	79872	103415	17893	20530	6042	704	5003	862	1782
S9	596556	257	611	279	672681	3378	19911	2873	2419	161318	2409556	82009	134732	21103	20394	6536	791	5111	817	1863
S10	634040	288	642	250	656071	2910	18115	3073	2272	213564	2551456	94207	121132	17849	25387	7305	864	5918	996	2092
S11	576426	290	624	417	652460	3749	17062	3910	2999	192061	2427320	78312	134720	19791	22059	6730	778	5188	892	2022
S12	657841	318	1038	549	976391	5021	29974	8630	7153	149334	2524063	106622	302902	19827	25827	8078	911	6922	1019	2263
S13	552757	236	644	384	607563	3894	15417	4575	3308	166409	2289827	72770	162724	20136	21708	7050	837	5362	988	1831
S14	579538	273	655	408	570806	4856	10486	8109	5380	189891	2337960	61501	198704	22545	23662	8591	715	5877	912	1996
S15	643711	279	1031	580	931186	5627	28628	8853	7493	139011	2564295	108649	280442	16270	24421	7864	961	6635	860	1968
C1	588111	304	1114	664	1067287	3647	42525	6866	6212	124572	2173724	126169	336031	19166	24778	7830	913	7151	1176	3076

C2	679758	333	965	578	789288	5006	21288	7548	5486	243755	2853417	92057	207675	28401	29786	9339	1069	7531	1576	3221
C3	529659	200	619	310	651120	2866	19174	3160	2520	177625	2371132	78809	119549	19049	21057	6185	771	5415	920	2019
C4	699781	344	856	446	882919	4950	28219	3898	2948	282537	3065687	115115	163819	24342	28666	7617	1031	6680	1405	2641
C5	565802	271	700	255	702720	3880	21042	3382	2710	232981	2519194	89139	133552	19766	23215	6373	844	5559	1080	2114
C6	744746	351	1040	671	759194	5625	16256	12915	8083	284663	2980917	79114	279389	29427	34706	10988	1073	11643	1321	4358
C7	658460	340	1017	363	863094	4490	23460	3642	2725	198235	2611346	95955	237734	23359	31802	8762	1156	9501	1260	3014
C8	594386	269	770	398	679501	4007	20833	3668	2820	224783	2395879	87646	146983	20653	23443	6333	760	5529	1000	2045
C9	638779	268	858	413	757699	3813	22442	3682	2640	258104	2627553	93291	182415	21305	26298	6620	874	6209	1059	2116
C10	588111	304	1114	664	1067287	3647	42525	6866	6212	124572	2173724	126169	336031	19166	24778	7830	913	7151	1176	3076
C11	641454	276	796	517	750667	4605	17302	8317	5634	244388	2822530	87125	216993	24698	28276	9332	914	7376	1169	2776
C12	620616	268	986	323	824239	4512	23719	2717	2133	181255	2530949	100959	173836	25610	31637	9177	1136	9246	1418	3496
C13	540088	215	817	252	716399	3318	18940	2559	1992	163180	2366479	38354	146772	19295	27110	7485	863	8560	988	2637
C14	714438	306	883	433	829045	4293	24899	3836	2983	273074	2966039	106015	153211	23276	27152	7340	957	6317	1212	2620
C15	540675	255	705	380	706191	3043	19859	4524	3200	220053	2492057	82143	171515	20105	25737	6880	950	6355	1008	2193

Table 3. Olive oil’s GC data from the HAPPI.

	13:00	**14:0**	**15:0**	**16:0**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**
P1	298029265	848314	466049	664844288	40098411	6572451	5711245	85420619	1057306706	202806113	14749999	14680112	5826935
P2	323703162	493230	296515	445231580	12532652	6612910	4342735	172615603	1189410799	189603990	19081837	17374806	8287828
P3	319348111	667066	401980	665673218	38135734	5438121	5481249	105496058	1168537198	159905574	17485880	15690016	7258691
P4	357429297	518574	272789	629841015	25861544	2992221	2623246	175688468	1391956308	87622633	18771939	17594503	8187519
P5	268526352	479989	282922	503637894	25872675	4104227	3696519	94584129	1004992241	101090902	15877278	13207310	4916614

P6	277146429	485105	269218	506091522	19803141	4573988	3470205	133147345	1033452715	132097292	16307753	15252773	6873973
P7	244331813	439645	356311	505210983	37660793	4145681	4848574	70781145	884552209	75701763	13128854	9586401	4435873
P8	192424856	278578	143317	286860306	12179545	2429493	2031377	75854376	548656158	77575546	9329977	8723356	2717799
P9	289761947	471412	220830	505302257	11703221	3427778	2312688	122451926	1052908230	203179105	18457793	13986573	6469508
P10	180922909	215068	144983	236992143	5093599	2972744	2296315	62578767	539687382	62717704	8409339	7090164	3028655
P11	252422690	331645	200346	320583862	7000762	4321592	2834309	124992465	867810381	111669764	11707192	11892442	4997181
P12	381613393	594155	374176	622662577	22030086	7867517	5533607	195448864	1429524108	189289910	22284800	20092904	8873151
P13	241374222	678674	263186	514596713	28573984	3193649	3282657	58935820	849475321	173289781	11592828	11155177	5530781
P14	272721612	470247	251642	483071079	21705298	3262531	2940409	96081069	1012856780	100500628	16081495	13107995	5888104
P15	251536712	408441	237579	317204396	9381236	3484425	2713575	77276176	759727501	91972855	12383503	10488044	5866645
P16	187558744	292899	240561	317214654	20149317	2927625	3211011	47494086	530989727	52150593	9014468	7173329	3157945
P17	373331914	524687	287504	605015630	15458402	6738506	4626092	183461344	1330362546	225476688	24720795	18808090	7545085
P18	338133958	485445	247071	573936272	20619907	3361513	2824147	115332082	1253801816	124869569	17432809	15459492	7481033
P19	233233020	305086	231134	337496470	18759027	3264305	3767605	54177249	699093985	55555935	9103194	7338021	3316268
S1	17311208	19761	7904	20822973	809831	84909	77241	7198295	50200575	4156710	595161	631756	239393
S2	16570159	26060	9914	20931382	612191	139548	99318	5565800	48254183	5763196	546831	731344	301285
S3	23563027	32560	19022	35395340	1507684	284868	238710	10239184	65907328	11372963	1032685	1083681	423594
S4	16211631	19246	8381	18545739	730007	73127	65772	6940604	47006522	3468425	644642	587983	224568
S5	17031944	15249	7957	21095885	660535	83743	70712	4648770	49236835	2321794	608924	558653	272790
S6	19415858	32693	17266	24705609	802318	257798	189036	6735492	55632928	8067184	947236	857822	411240
S7	18784399	29660	14934	24422904	962827	188705	150982	6248420	53028351	7086070	901198	745561	335892
S8	22836471	25078	11113	25263516	701886	141033	118589	7813137	66598511	4626912	876057	826794	364605
S9	21055132	25748	11330	25843953	856873	121348	103066	6287445	61375914	5451606	887584	746803	323616
S10	21421211	23790	11310	24593601	734881	123271	96014	8049758	62985835	4774231	714864	863272	344783
S11	23508175	28055	14188	27332834	811505	176754	130049	8063147	68292443	5916463	874332	867823	359272
S12	22352387	41979	19315	33766019	1237720	320355	270962	5470272	63414180	10821050	849362	881407	360792
S13	21886483	28685	13439	25362267	786806	209920	159523	6963204	63840952	6988057	891670	815199	374437
S14	16742412	20114	12039	17024934	363572	234265	162842	5623842	46678553	5962918	747306	618298	311938

S15	290431550	631868	291400	557884824	21776922	6032514	5093168	87335027	1070877539	177534244	11078798	13700654	5664012
C1	32134043	57444	29982	55028263	2749202	390815	364484	6785121	86336224	18175562	1177046	1205964	532502
C2	429607226	662911	354309	556078676	19307892	5260069	4043672	169310228	1287596066	149821574	21481365	16510171	6763969
C3	463254373	546730	249883	593319003	21741861	2682996	2393218	160726097	1373238339	116981696	18180299	15485432	5481774
C4	30107081	30973	15066	35583392	1289394	144500	118851	11105703	82022065	6946571	1014594	956537	395403
C5	16310337	18216	8255	18796030	666779	87219	70699	6055816	45304433	3670500	522452	519247	175599
C6	26442973	35481	21686	28557503	662780	473050	307890	10333891	73287885	10462121	1145294	1055394	450759
C7	347258066	575333	178507	488938594	16582533	1966116	1526226	110930027	994793629	138355629	14181823	16132136	5554746
C8	505051552	747993	324738	667427169	25760570	3433477	2766193	221257867	1518996638	152585746	21762481	19720050	7365343
C9	27052346	41113	16702	37720478	1361487	182112	138557	12924764	86338385	9501145	1137272	1124034	378285
C10	20251964	34069	14411	31408484	1073141	181171	125214	7564993	62671271	11663963	917753	1017173	359434
C11	26603406	31094	17571	30698256	823749	321012	232542	9612118	75321832	8575058	1074561	976564	436839
C12	537309775	1036138	322602	784270619	29649790	2600967	2127528	179426660	1579051111	178996054	26450180	27669777	10784064
C13	21433753	31067	10147	29068343	904451	98589	80530	6613351	63900252	6445601	818470	1016362	391021
C14	519594899	674104	309402	701495819	26877163	3123297	2572199	235507963	1604344051	140249470	20915344	18875588	7746848
C15	15546716	18425	8429	18600406	624519	110559	82681	5631771	44053354	4610005	531344	572317	205725

Table 4. Tea oil’s GC data from the first batch at the CSU.

	13:00	**12:00**	**14:0**	**15:0**	**16:0**	**16:1n-9c**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:1n-7c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**	**22:0**	**22:1**	**23:0**	**24:0**
T1	576644	414	3012	918	402744	995	3477	2886	1523	195096	2778425	58514	328678	4428	13989	11142	38318	2571	1473	12660
T2	543547	355	2722	804	356053	867	3064	2745	1360	183166	2553905	35953	287540	9212	13399	10208	34686	1956	1338	10978
T3	711311	424	3883	803	708893	942	3968	4965	2267	154195	3047491	58430	289093	8952	3522	18178	1670	1938	372	2496
T4	730147	493	3681	1065	362997	1276	5792	2094	1585	200067	3153956	48301	327739	1574	18170	9356	53724	140	2188	19846

T5	547785	411	2565	761	379262	762	2608	3108	1490	242056	2565856	50753	302867	3982	15149	9674	40858	887	1175	10720
T6	680123	411	3275	967	455470	982	3845	3289	1782	223309	2955005	42863	323679	5506	14807	12072	38876	2189	1543	12070
T7	574173	363	3695	1019	849064	1165	2701	8772	2318	173433	2091606	40310	436379	9517	5463	22718	3110	6495	527	2969
T8	584064	394	2683	681	578654	901	3264	4394	2087	129775	2619660	46715	250839	9041	2821	16111	1200	2050	260	1942
T9	734998	447	3369	772	721612	1788	3729	5459	2631	172685	3276274	59556	300114	9352	3866	19351	2583	2138	556	2487
T10	584950	500	4875	999	721838	1192	2787	5043	1334	216437	906060	50133	1452126	149201	19908	7166	22978	0	2230	6717
T11	729482	454	3338	759	731545	1894	5216	4740	2219	166223	3233327	94707	384229	44252	8203	24094	3821	15217	0	2974
T12	628546	403	2610	651	617455	1382	3639	4820	2284	153011	2882453	74021	252854	12699	3705	18341	1813	8310	502	2080
T13	610431	404	2827	598	636443	1514	3498	3948	2031	130249	2802265	66614	261038	9898	2515	15478	868	1001	330	1488
T14	604883	357	2731	528	602019	799	3222	3747	1960	121627	2763623	65208	260924	10177	2711	16270	937	1826	415	1591
T15	622864	366	3065	559	611273	901	3497	3665	2008	125036	2758258	65473	260950	9726	2364	14856	787	788	332	1475
T16	668496	400	2954	625	637613	1454	3639	3952	2112	127069	2926667	72356	256321	9863	2708	17366	924	2331	642	1763
T17	629334	373	3317	662	626997	925	3498	3792	1864	126261	2685646	63926	269890	8517	2572	15454	1061	1026	531	1597
T18	634578	333	2824	593	647883	718	3316	4002	2172	121703	2831167	64848	277423	10310	2554	16822	954	1302	397	1798
T19	626877	364	2906	603	590399	794	2776	3759	1757	125645	2654917	59316	246014	9590	2371	15277	764	897	314	1665
T20	727464	401	2997	714	728243	1007	3652	4529	2263	139528	3181496	79776	312673	11327	2630	17864	1071	1508	967	1672
T21	632197	417	3049	539	613337	913	3256	3864	1893	124590	2692941	64002	260892	10947	2908	17660	1170	6697	440	1954
T22	638833	422	3329	545	635840	884	3567	3926	2026	130918	2894853	66033	267427	10007	2451	15798	853	1010	418	1477
T23	642957	396	2981	583	635339	1705	3382	3998	1981	134509	2906102	64958	262295	9947	2751	17178	904	1085	465	1611
T24	622039	372	2997	475	626189	1322	3389	3539	1772	129157	2675187	67067	264098	449	9068	14924	1125	1320	420	1650
T25	606692	402	2909	511	599338	773	2994	3788	1893	125715	2746569	61572	248373	9141	2398	15600	865	950	340	1456
T26	625425	360	2667	515	619414	788	3221	3866	1972	124394	2810950	65767	256659	9585	2398	15823	826	957	372	1684
T27	629008	392	2966	554	654457	1556	3571	3883	2109	130224	2895048	68454	263757	10548	2649	17364	911	2869	372	1644
T28	748405	707	3442	752	725716	2146	5225	4744	2377	167212	3237326	96932	281331	8287	4027	19352	1845	1970	700	2602

T29	638089	380	2906	660	652436	877	3078	4658	2193	144626	2840519	66974	284096	14722	3576	17247	1633	2554	410	1871
T30	628363	415	2999	718	598964	1295	3453	4477	2004	154696	2688808	50254	262335	17057	5254	19868	2840	12880	315	2833
T31	656072	500	3450	707	653204	1393	3935	4370	1941	160496	2685569	54746	422592	34783	8902	22944	5889	20730	560	3407
T32	613384	361	2736	711	599867	803	3042	4680	2145	151576	2800021	50040	225470	6971	3529	17207	1525	3305	340	1933
T33	728629	418	3199	691	720335	1534	3739	5149	2506	162630	3286122	57169	293066	12503	3392	19273	1611	2795	217	2081
T34	665117	760	4236	1461	418402	1087	5941	4251	2052	180732	1944764	107117	856480	314387	40260	32425	22220	1629	1564	8370
T35	709438	444	3524	704	683579	900	3782	4856	2240	159481	2977641	55159	263809	4914	3715	17839	1850	1448	296	2654
T36	629264	378	2790	699	608945	828	2987	4816	2113	153162	2773703	51180	239801	11474	4619	17232	2325	3410	300	2463
T37	591178	1009	5149	1302	448333	870	4673	3723	1622	161073	1554733	82278	801411	232767	32354	23954	19129	543	1362	7086
T38	603102	404	5728	990	752781	1130	3218	5562	1651	230751	1359025	43950	1179143	127011	16143	9360	15973	345	1676	5189
T39	666068	479	4926	1142	831630	494	2840	6677	1965	289853	1335330	50557	1540091	185971	21015	9378	21571	570	2204	6772
T40	567865	421	2924	602	569715	847	3082	4152	1838	132218	2505078	49840	247749	18742	4383	16138	1945	2824	353	2144
T41	592773	516	5226	1162	762402	1187	2802	6337	1364	291015	783716	45789	1625233	227292	23346	8936	23592	6488	2240	6911
T42	658953	428	2853	701	641196	1465	3615	4875	2226	161073	2971675	51351	242620	10547	3483	17198	1576	1268	364	2065
T43	572518	530	4170	664	623392	680	3442	3975	1723	143316	2239673	46682	393561	26463	7812	17552	4940	12279	501	2910
T44	638414	429	3592	625	669007	864	3632	4609	2131	151470	2788935	51429	271728	9044	3342	16259	1371	1099	333	2098
T45	581943	363	3247	667	611861	845	3352	4359	1996	138199	2633662	47414	263903	10764	3023	15410	1282	1188	400	1933
T46	572554	877	6965	1039	800311	1400	2992	5239	1268	221950	804637	45439	1475767	143669	20461	6907	22849	1090	2349	6815
T47	617463	799	4393	1277	439938	861	4348	3844	1705	177470	1610978	71885	873474	213068	30033	57560	17129	107223	1480	7378

Table 5. Tea oil's GC data from the second batch at the CSU.

	13:00	12:00	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c	22:0	22:1	23:0	24:0
T1	543005	402	2632	750	352722	805	3090	2506	1398	171057	2430466	37972	281156	3690	12338	9708	33159	2130	1246	10647
T2	550336	381	2667	754	351271	773	2934	2718	1338	182253	2533805	39398	282198	8953	13435	10180	34455	1886	1340	10728
T3	593741	401	3314	647	611460	815	3410	4335	2073	133564	2616343	47606	245885	7753	3288	15919	1435	1685	371	2040
T4	742641	475	3854	1133	368830	1213	5836	2106	1390	198580	3064998	48136	318331	1384	17881	8985	51571	0	2094	18360
T5	538252	359	2407	785	355331	726	2506	2895	1402	225790	2399881	31438	282680	3979	14101	9197	37190	967	1087	9252
T6	559305	404	2933	798	395560	792	3217	2916	1483	193837	2562117	38610	280617	4846	12785	10469	33495	1927	1322	10590
T7	533003	353	3397	929	807860	1972	2736	8329	2170	165421	1989303	39292	415282	8991	5354	21606	2814	5988	663	2863
T8	643850	438	2711	694	565891	909	3160	4215	2045	125233	2489413	47563	245607	8137	2900	15718	1247	3235	278	1759
T9	538398	388	2556	641	556358	1583	3152	4122	1872	122580	2502763	44691	239869	8741	2645	15101	1112	1885	376	1540

Table 6. Tea oil's GC data from the HAPPI.

	13:00	12:00	14:0	15:0	16:0	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c
T1	420408430	625704	2245189	575797	294746367	2207556	1854581	1002779	132878030	1215170694	231674298	3054212	8012834	7145482
T2	194691709	237809	1091422	249003	143013868	1067550	920708	587940	68912822	631373349	111455330	3683629	4581099	4653080
T3	147919057	168207	760345	131563	145701299	990733	899537	458278	29469942	414854607	59922022	1802815	772877	3600608
T4	591634087	1002439	3942013	997095	402837491	5114417	2020450	1383196	205944232	1852100117	345498407	1746663	14948013	8836212
T5	420074323	562719	2028994	538012	304520116	2015661	2286243	1172863	178430526	1223725262	233075484	3279145	9719419	7268946
T6	307451949	375347	1546356	384561	221266686	1800015	1450025	709219	100165640	890396249	154413654	2580575	5664220	5461462
T7	177229091	248733	1205104	305285	260092018	1302819	2718459	802557	52050724	464774484	131091501	4089723	2118395	7119438
T8	143937295	181192	669106	144612	140265210	1029808	956716	479198	28752144	412252483	62076208	2387612	812871	3894208

T9	222329232	307754	1094552	245744	232472096	1870785	1632689	825060	48158766	660739103	101731742	3707552	1435504	6788837
T10	574535527	934059	3174315	637750	649708968	3829068	4731737	2371300	154058334	1746321709	296829638	4500863	11526337	3148903
T11	236811551	366546	2120404	333090	306612432	1614450	2018085	571236	89759206	355141192	445253572	67035390	7459466	3495001
T12	249330744	324037	1109157	183424	257718949	1655192	1469152	719402	50224147	708324232	107991547	4311656	656341	6279592
T13	239605219	305649	960810	190739	233187118	1464721	1624766	797603	53232750	692728829	94560959	4959244	1453801	7130856
T14	210886569	244641	907741	153877	213847711	1232545	1192574	586477	39976559	601525853	87957636	3601525	617902	5315118
T15	203270698	248460	900433	157562	196182281	1487114	1069856	605227	34647423	573805833	81263755	3652326	563556	5073119
T16	202919494	257528	946900	141686	198971384	1164448	1039230	642759	36311042	575017984	84201990	3119421	373999	4428205
T17	262032372	337128	1041185	192889	240946908	1179359	1331261	664199	43936467	717827131	95439136	3964049	664034	6373905
T18	336679329	482438	1853515	272659	333472544	1833939	1825188	958118	62607749	912833828	142439995	4628386	419403	6871136
T19	352415907	499330	1562714	267989	353886536	1437243	1997018	1109964	61592841	966542046	149668061	5682183	737186	8553806
T20	466354108	661300	2619155	404885	524205870	2187912	2959691	1609696	106220497	1407956686	220841215	8984533	993977	11950247
T21	362843220	511140	1565321	291946	378573703	1585228	2116107	1134370	68455675	1044459716	163031340	5956104	816323	9101087
T22	385359521	548848	2128668	335540	413918448	1958179	2461227	1326520	78477108	1113016642	174613705	7981906	942101	11275798
T23	482166220	702497	2840675	417571	524904771	2576046	2950322	1632433	102354224	1440803550	221964084	9026290	778130	12199187
T24	218052409	276242	888569	146223	200487638	1052163	1105603	589205	37943370	595945956	80662603	2981378	369357	5059459
T25	222463883	283479	1094721	162224	225814012	966138	1216555	660929	42748868	628648452	93309905	3260432	400809	5086451
T26	207913311	252889	949896	148643	206034088	1108581	1197951	665452	38699728	599668275	84289009	3067750	292636	4616556
T27	224219515	275455	984743	172502	231821796	1163345	1236232	695384	41887743	655510835	94895086	3420761	346926	5363340
T28	349169356	486765	1682894	265353	365406527	2050872	2013523	1074924	68124404	1010348532	147197964	6477601	863624	9500705
T29	211717017	340201	937040	158352	208845986	929062	1199923	602736	44137142	607404826	82140073	2239071	643040	4496658
T30	213055347	267509	920083	171911	218713058	932902	1384282	673018	44345747	610655145	95287595	4815921	769511	5034119
T31	217458160	270988	1026629	182092	213797280	1260155	1417301	672429	50943008	623281963	96361771	6128305	1818494	7046177
T32	211815420	279141	1130668	170149	217573807	886954	1324504	670490	49211213	593441767	139293372	12862646	2472974	7517004
T33	222174527	284594	936733	184243	220579950	818619	1444690	699551	50792616	649289411	87298638	2463433	1000140	5568220
T34	221943796	244197	1019011	187745	232152577	823610	1450444	816474	48081810	662446236	95973191	3895634	852846	5925975
T35	248407768	480606	1592179	440343	164864745	2396041	1475143	1532236	64887011	578820682	281770227	119386626	13035680	14190520
T36	235424915	305321	1235323	217844	244414152	1162513	1560527	732339	53350382	687021865	97596495	1762703	1593655	5958132

T37	234030794	307822	1025074	215188	234243697	1287282	1691241	886337	55861323	687649392	100360177	4602994	1545060	5679173
T38	448063478	1273493	4783941	903452	408081833	3582305	3178924	2923755	138030314	1065557955	585486800	208588141	24669771	24266848
T39	365715297	515460	3740794	527157	455775044	1603461	3168374	848974	138686097	684909252	555051713	84660358	9016364	5476917
T40	395067255	607919	3303804	588245	489851741	1618127	3659409	1037463	169200699	681113277	666999304	17188266	10213581	4808851
T41	204418854	264308	1042362	181212	206038596	1170479	1281695	620598	43533560	585748711	90939430	6756536	1336966	5377282
T42	355030354	580986	3434356	563137	446767932	1630053	3496784	850638	170076156	447267357	666576320	139879167	11634252	4785123
T43	402164336	598021	2009101	418299	418044197	2330758	2948987	1478535	98753331	1183132096	161680132	7717737	1728280	10476909
T44	387098012	755128	3113181	445370	441853506	2392289	2661892	1231062	97935476	1058322422	266704127	21620948	4628715	10665476
T45	211335655	250716	1163275	174237	217432636	911549	1299872	574170	44647494	584709989	88901396	3033615	1024854	4826981
T46	373756505	555295	2318809	397241	412168144	2527228	2740860	1324696	88045585	1099833181	177346495	7844536	1564342	10079815
T47	210738728	406582	1547464	369011	158673576	1270667	447166	1060019	58138551	453141722	263075326	76629855	8657091	21402589

Table 7. Corn oil’s GC data from the first batch at CSU.

	13:00	**12:00**	**14:0**	**15:0**	**16:0**	**16:1n-9c**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:1n-7c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**	**21:0**	**22:0**	**23:0**	**24:0**
Corn1	681580	441	2734	675	1042997	1363	2872	4835	910	128018	1092862	21966	1884126	25980	29699	6551	411	7025	711	8809
Corn2	548466	350	2349	720	864630	2253	2594	4113	847	107743	891575	20054	1554341	21829	25093	6811	267	6314	595	7913
Corn3	611013	332	2663	660	887815	1157	2558	4193	931	127360	973152	23150	1678240	27706	25226	5943	477	7638	621	7026
Corn4	637855	432	2778	694	977976	2010	3095	4905	933	138841	1028338	23304	1792792	27006	30283	8349	657	10495	912	9891
Corn5	644197	439	2670	836	1008521	2171	3131	4875	1028	130189	1060273	22332	1809666	18949	30424	8688	465	7886	907	9569
Corn6	749435	572	3140	838	1065755	1376	3246	5485	1230	156446	1123944	26720	2127525	30346	31807	9253	446	11504	1048	10245

Table 8. Corn oil's GC data from the second batch at CSU.

	13:00	12:00	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c	21:0	22:0	23:0	24:0
Corn1	743934	519	3168	981	1184680	2829	4023	5710	1348	149098	1305383	27701	2077165	29609	33928	10360	700	8564	1093	11341
Corn2	520096	363	2309	666	859564	2391	2685	4092	783	106704	892468	18642	1551672	21437	24755	6770	406	6143	688	7606
Corn3	760891	503	3137	822	1038362	1385	3167	5027	1093	149327	1135680	29089	1948922	32937	29781	10255	521	9109	772	8293

Table 9. Corn oil's GC data from the HAPPI.

	13:00	12:00	14:0	15:0	16:0	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c
Corn1	513839290	852704	2504784	618165	832646005	4596732	4023668	826756	112438152	837162159	1034444321	26227989	21640921	8433379
Corn3	312437200	446914	1455689	321672	452924084	1076027	2078197	500139	63640364	461801218	616545111	16969466	9440253	2336036
Corn4	556915116	955605	3192984	682631	894236963	5439061	4665567	945831	138095962	903348852	1146984732	31021634	29563302	8933459
Corn5	156998772	216507	662363	160378	225655430	767803	1092519	221847	27994558	211505709	301169109	4023205	6220236	1788350
Corn6	236940554	359268	1174046	266595	359129772	1097836	1723120	394757	52268324	354600925	527756440	12203213	10067055	3606990

Table 10. Sunflower's GC data from the first batch at the CSU.

	13:00	12:00	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c	21:0	22:0	22:1	23:0	24:0
SF1	738921	393	5285	1237	526899	572	2290	3268	653	400908	932435	23813	2318128	8620	24916	5624	492	54319	2830	1634	11410
SF3	558990	320	3631	1003	384906	386	1493	3142	765	353004	637232	16801	2117619	5429	20632	3384	511	48185	471	1446	10657

SF4	555508	361	4974	939	419882	483	2339	2626	888	236671	742998	27458	2078548	2445	15820	4712	267	40305	0	1520	12115

Table 11. Sunflower oil's GC data from the second batch at the CSU.

	13:0	**12:0**	**14:0**	**15:0**	**16:0**	**16:1n-9c**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:1n-7c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**	**21:0**	**22:0**	**22:1**	**23:0**	**24:0**
SF1	729020	450	5214	1220	521956	1210	2659	3423	814	393032	913641	23474	2266306	9325	24867	6381	582	54275	3466	1685	12982
SF2	617237	769	4310	1014	401280	459	2003	2655	653	328767	753193	18468	1939912	4821	19539	4311	517	44773	729	1431	10853
SF3	546056	326	3369	957	369681	497	1413	2984	675	342718	617135	14953	2042410	5129	20171	3346	478	47674	412	1344	10519
SF4	554815	360	4636	990	397175	461	2236	2506	848	222880	699929	21112	1957742	2301	15064	4524	340	37689	0	1530	11072

Table 12. Sunflower oil's GC data from the HAPPI.

	13:00	**12:00**	**14:0**	**15:0**	**16:0**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**	**22:0**
SF1	151395396	250438	1492911	236502	117121607	569108	671935	172263	83413409	183493628	329077892	2034815	5098349	1390550	11253727
SF2	192224311	423919	1616587	309243	146548930	778339	883374	236272	113576235	247100896	443321528	1764090	6755394	1835746	15502641
SF3	162892751	204157	1053876	252328	115331337	471076	798708	197520	98227274	174129188	381759691	1523897	4956524	1178776	12394398
SF4	304309908	412218	2592294	452731	230697225	1183358	1195394	429561	121537868	362832103	684313202	1503020	8260026	2485228	19195539
SF5	566654764	1401316	4932677	1316162	418725517	4744275	3669382	3435595	214983033	1307653179	838993085	220716756	33863245	46276922	18198447

Table 13. Rapeseed oil’s GC data from first batch at the CSU.

	13:0	12:0	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-6ccc	18:3n-3ccc	20:0	20:1n-9c	21:0	22:0	22:1	23:0	24:0	24:1n-9c
RS1	655765	829	3739	1347	317280	1022	5906	2833	1188	134424	1699244	86541	607242	32552	263146	45508	126898	860	28829	343154	1125	10527	7358
RS2	652936	849	3799	1427	418794	1399	6010	3453	1696	145403	1874212	97232	768808	58659	182979	41377	63852	1232	18508	101370	1268	9397	5119
RS3	676443	1032	4302	1745	326927	1143	5865	2991	1757	170422	2014669	91052	628828	23981	250817	40859	104284	686	17730	174111	1377	9165	5833
RS4	753301	980	4204	1700	345277	1380	7423	3595	2053	152349	2284739	124370	705571	21072	352418	50525	94075	1046	25808	151016	1273	10026	7206
RS5	832960	1202	5646	2073	421003	1678	8990	4057	2201	172571	2573805	139098	821839	22590	406602	53619	87528	1093	27746	165504	1731	12325	9703
RS6	706515	959	4603	1660	412072	1189	6987	4201	2403	181464	2272627	124777	863955	25000	335969	47858	51619	1040	25728	43337	1533	10238	4641
RS7	711314	886	4132	1489	387825	1175	7042	3930	2005	155533	2102777	124799	805147	35525	321459	44221	44420	1003	24429	18011	1415	9247	4877
RS8	611806	920	3991	1416	312269	1149	6237	3271	1858	137028	2021787	121701	627098	20136	292685	41577	47708	903	20800	41845	1253	8590	4086
RS9	610499	1511	10844	1113	993122	1371	3439	5866	1376	249891	863552	55121	1681659	46894	152368	24752	10296	1749	27091	9805	2749	8684	0
RS10	622581	920	3530	1740	286013	1137	5892	2494	1274	128368	1627796	93892	591325	2682	294219	39512	111652	873	19870	315516	1544	9467	12050
RS11	776148	1130	5424	1707	501395	1976	5975	3891	1341	196839	1484387	92117	956359	27692	276332	48662	170438	1184	29446	487556	1783	11293	10997

Table 14. Rapeseed oil’s GC data from second batch at the CSU.

	13:0	12:0	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-6ccc	18:3n-3ccc	20:0	20:1n-9c	21:0	22:0	22:1	23:0	24:0	24:1n-9c
RS1	678154	929	3642	1384	303521	1097	5635	2725	1498	128866	1612153	85053	577072	33552	255840	43753	120553	784	27847	329124	1168	10723	10473
RS2	595897	889	3560	1396	392013	1228	5523	3239	1682	136785	1754042	85064	721651	55138	171650	38793	60137	644	17430	95914	1224	9182	4743
RS3	628377	950	3818	1564	292627	1048	5398	2635	1395	152167	1794250	79562	549419	19367	223152	36322	92584	593	15423	154407	1113	7949	5325

Table 15. Rapeseed oil’s GC data from the HAPPI.

	13:00	12:00	14:0	15:0	16:0	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c	20:2n-6c	22:0
RS1	193465060	397447	1235866	408341	101157506	1532332	902500	1379918	39470129	417175970	171098432	81657138	10934646	43528406	1423766	3511718
RS2	634721823	1638903	4878673	1536701	532012086	9856622	4693556	4582461	173795362	1627705260	794004182	232366946	43930933	89599517	2345108	10816110
RS3	269706063	621424	1824207	621649	135365070	1890540	1202082	1851011	63881688	611955213	225028979	99457165	12174676	45305877	1237076	2627007
RS4	436004707	1030391	2981702	983463	239627502	4291990	2316196	3726974	95607228	1106845172	412120448	229582313	25978166	69325979	1511137	8111754
RS5	271594436	698414	2673251	793972	191532432	3206777	1712562	3086764	71144101	824966813	321495878	174773185	18308546	43523334	1499704	5848240
RS6	33617719	63127	186888	59750	16345210	239400	158978	219033	6386361	88146499	32917636	13257734	1671750	2113177	97182	158491
RS7	532567266	1247770	3732480	1177638	350322902	4890278	3283579	4254381	127990702	1355564660	603196031	278765008	29476678	42773933	1616764	11083638
RS8	401315009	919062	2724557	860748	221759071	3649949	2134696	3158706	88016828	999129125	372850898	196266378	21581847	36559922	1285519	6549667
RS9	362144000	1270672	7124135	648750	557191009	1711003	3269566	876046	144838757	488086557	693389954	96071979	11927389	3733789	425235	7898976

Table 16. Peanut oil’s GC data from the CSU.

	13:00	12:00	14:0	15:0	16:0	16:1n-9c	16:1n-7c	17:0	17:1n-7c	18:0	18:1n-9c	18:1n-7c	18:2n-6cc	18:3n-3ccc	20:0	20:1n-9c	21:0	22:0	23:0	24:0
PN1	642627	447	2609	719	861091	1912	2748	5552	1337	216218	1592459	24276	1218007	10590	82100	27028	1145	129211	1981	62520
PN2	624281	343	2298	718	822943	1823	1908	5086	812	296399	1387615	18329	1162734	3979	118015	26816	1412	197790	2213	76581
PN3	675012	556	5048	1155	926709	1427	2834	7282	1524	345139	1078346	48043	1765447	187163	61002	14623	1881	84365	2697	29470

Table 17. Peanut oil’s GC data from the HAPPI.

	13:00	**12:00**	**14:0**	**15:0**	**16:0**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**	**22:0**	**24:0**
PN1	154724136	207814	613375	139082	193658980	539006	1181923	298654	47717939	287462742	223514794	2355132	16335310	6942594	26634009	10570610
PN2	179231141	198223	716849	206551	249919634	593796	1509680	269565	88538547	345692355	281197817	859616	32639750	9046130	57381900	19818236
PN3	270591929	367729	2018917	378801	352405698	1176745	2737752	620509	129295078	380985743	480033068	75885123	20769642	5036987	30201563	8462755

Table 18. Sesame oil’s GC data from the CSU.

	13:00	**12:00**	**14:0**	**15:0**	**16:0**	**16:1n-9c**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:1n-7c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**	**21:0**	**22:0**	**23:0**	**24:0**
SES1	719203	420	1819	441	772414	1663	4408	4170	1068	422435	1457775	41898	1783354	42076	45681	8286	696	12028	1629	5733
SES2	647037	339	1525	237	737036	1562	4036	3895	832	447712	1440364	35178	1563416	18270	46144	7666	543	9721	1233	5198
SES3	684250	372	1514	274	764621	1102	3957	4008	944	478947	1520610	35608	1624455	16737	52857	8604	954	10153	1217	5258
SES4	819037	405	1813	352	805561	1744	4289	4230	884	507674	1619054	39025	2010298	18196	52650	8168	727	19954	1445	8290
SES5	609827	307	1185	185	651145	1680	3434	3107	670	426145	1315086	30035	1442132	12056	43125	5728	513	8478	1077	4320
SES6	727416	406	1755	345	774378	931	4048	4313	880	445886	1499317	38052	1692440	35516	48666	7659	691	13825	1393	6601
SES7	689927	361	1327	220	722078	1827	3847	3771	898	471171	1511614	35360	1710734	14037	48520	6794	612	10195	1416	5456
SES8	691704	370	1306	208	715225	1612	3614	3661	882	471642	1525625	33093	1692907	14591	48505	6777	522	10308	1195	5619
SES9	704556	401	1940	434	909533	1791	3965	4208	931	363603	1375261	31613	1922310	18356	45523	8167	640	10487	1374	7759
SES10	764700	715	3080	998	641254	1376	6254	4392	1703	340152	2034699	83980	1316655	169993	52092	36592	882	18091	1574	8112
SES11	608743	394	1210	260	653405	770	3614	2787	679	416142	1320288	39883	1398968	11432	42760	5466	496	8620	1065	4465

Table 19. Sesame oil’s GC data from the HAPPI.

	13:00	**12:00**	**14:0**	**15:00**	**16:0**	**16:1n-7c**	**17:0**	**17:1n-7c**	**18:0**	**18:1n-9c**	**18:2n-6cc**	**18:3n-3ccc**	**20:0**	**20:1n-9c**	**22:0**
SES1	221382964	246361	640043	103083	268612430	1316965	1370657	400596	139016046	426987190	440029670	15269621	13771159	3257263	2836146
SES2	461867302	707152	1182802	162807	556369561	2716353	2784215	661368	330606199	907281251	838724617	16021657	33766505	6775633	5017525
SES3	242224765	318698	585514	95357	303259650	1598392	1518273	371525	181407039	503389049	474995549	7476369	18364553	3315141	2493251
SES4	240480500	304900	639073	92637	278329866	1055783	1414136	337166	166971856	466466903	488661494	6446961	16386521	3185008	4229766
SES5	410967263	552610	780738	82608	442506797	2581033	1970339	460979	280290439	742689422	699776353	9415939	27277750	4538357	2832430
SES6	294774853	375569	991803	154146	424947067	2645488	2260817	612332	237465822	688264289	655436580	22272649	22691171	3516775	4283744
SES7	250990488	324697	541867	63754	298291326	1590605	1460541	377658	185121378	517132508	507525416	5911838	17949146	3302374	2980660
SES8	226562421	275812	465494	65322	252227079	1363944	1166265	292650	156163780	445763253	434402248	5297376	14111887	2102652	2773350
SES9	259533660	333358	925265	168586	411806149	2251644	1836563	421037	162419840	546011797	614780187	9991605	19638304	3639144	3317236
SES10	264548546	487507	1365483	383166	285516463	2816355	1764042	1467938	141838367	705054807	459854743	76628299	18624159	17685337	3769042
SES11	511154330	762292	1179605	129500	616632660	4037624	2476348	615246	385262718	1020370623	914221927	13067589	35743551	6452404	5056096

Annex II

PCA codes:

```
[ U S V]=svd(X);
u=U*S;
```

PLS-LDA codes:

（1）select the significant variable

```
X=X1(1:A,:);
Y=[ones(1,B),ones(1,C)*-1]' ;
F=carsplslda(X,Y,D,E,'autoscaling',F,G)
```

（2）data modeling using the significant variable

```
F.vsel
X4=X1(1:A,[H]);
CV=plsldacv (X1, Y, D, E, 'autoscaling')
LDA = plslda (X, Y, I, 'autoscaling')
```

Where:

A – total number of samples;

B – the number of samples for the first set;

C – the number of samples for the second set;

D - the maximal number of PLS components to extract;

E - number of folds for cross validation;

F - the number of Monte Carlo Sampling;

G - 0, regression coefficients(default); 1, selectivity ratio;

H – the values obtained by the F.vsel function;

I – optimal number of components to build the PLS-LDA model determined by plsldacv.