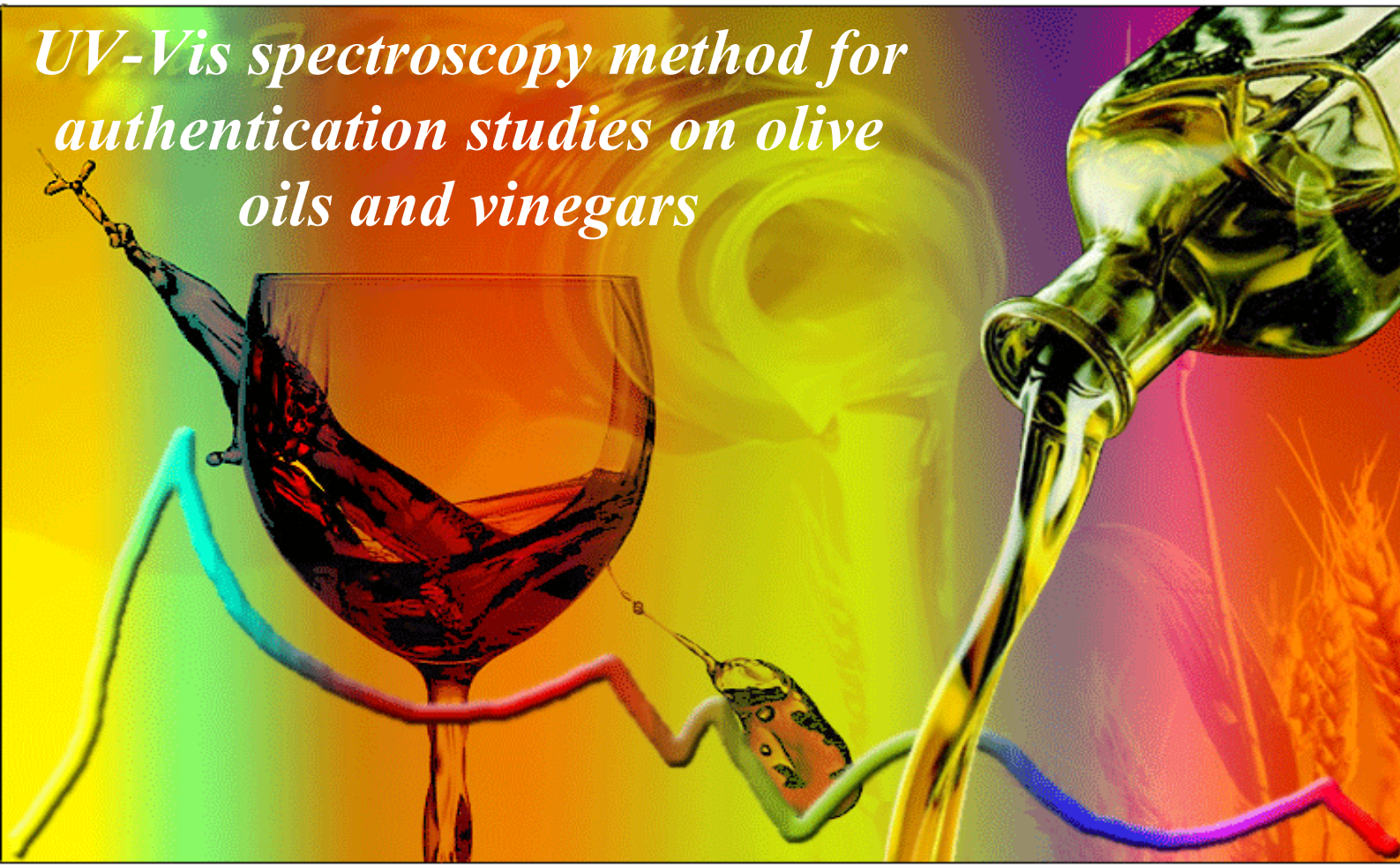

UV-Vis spectroscopy method for authentication studies on olive oils and vinegars

MARTA FERREIRO GONZÁLEZ

Puerto Real, March 2011

Education and Culture

Erasmus Mundus

Department of Chemical –Physics

Faculty of Sciences

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Marta Ferreiro González

EMQAL Thesis

March 2011

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This thesis is submitted by Marta Ferreiro González to the University of Cadiz as a partial fulfillment for the Degree of

EUROPEAN MASTER IN QUALITY ANALYTCAL LABORATORIES

Marta Ferreiro González

Puerto Real, March 2011.

This research work has been done entirely in the laboratories of the Department of Chemical -Physics, University of Cádiz, in the Faculty of Sciences under the supervision of Professor José Ángel Álvarez and Professor Jesús Ayuso.

Prof. José Ángel Álvarez Saura
March 2011

Prof. Jesús Ayuso Vilacides
March 2011

Acknowledgements:

I have been spent almost the 6 months of my thesis thinking about this part, if I should include it or not, if it would be too boring or not…
Actually I had decided not to write anything, but then thinking about it, maybe I will not be able to write anything else in the future and, since the person who will be reading this is because he/she will be mentioned here and it will probably be the only part he/she will read, so at the end I decided to write it and not forget even the smallest detail..

EMQAL, *Erasmus Mundus in Quality Analytical laboratories is not a master of any kind. It is a special master that makes the people special too.*

Erasmus *– the idea of being an exchange student who is required to study in one or more universities other than the home university. The Erasmus programme offers the opportunity of being able to compare the distinctive forms of work in different countries and in the same area of interest. It also gives you the chance to interact with people from different places with different cultures and languages, and as such, enable you to study and speak in a foreign language.*

My entire course group was sent to study at Gdansk University of Technology in Poland (GUT), where we had an unforgettable learning and socializing experience. There, all of us shared the experience of walking on the snow at 20 below 0ºC or trying to be able to say more than 3 words in Polish, the impossible language. It may sound exaggerated but let me just tell you something: I used to live in the Wrzeszcz neighborhood, 7 consonants and just 1 vowel!!!! "Impressive".

But we had very great time there apart from the hard study time. At GUT University all students of this Master´s received all the required theory classes which gave us the basics to defend our future thesis the following year. After finishing my first part of the master in Poland, I was designed to do my thesis in Cadiz, so I changed the snow boats and the scarf for a pair of sandals and shorts and I went to Cádiz. I must say that although Cádiz is in my own country the language was not much easier than Polish, as well as the culture and the weather which is pretty different from mine. So, that also was a new experience for me.

Mundus *– the idea of dealing with and spending time with friends, colleagues and professors from all over the world, allowing you to get to know other cultures and share your knowledge in a personal as well as professional level. This has brought me and my roommates from different countries and continents today to living together under the same roof. Aaron from Cameron, Umesh from Nepal, Yahia from Egypt and of course, Widi from Indonesia, I have never though before someone could love eating rice so much as you do Widi. I will miss you a lot and your music in the morning too!*

This is what makes this Masters programme so special, unlike others the purpose of which may be only educational. Personal growth, learning other languages, interacting with people from different parts of the world and sharing cultures with one another are the incentives that would make me do it again if I had to, and I am glad to have been given this chance.

Before I start to present my thesis, I would like to give special thanks to all the people who in one way or another have helped me get to this final stage of the master.

First of all, I would like to thank my supervisors: Jesús and Jose Ángel. They were much more than simple supervisors. They taught me everything what I know today about the world of olive oil, spectra and also about the culture of Cadiz. They were kind, helpful and available every moment, training me, teaching me, advising me and making me feel a full part of the group. My thesis would have been a very hard task without their help. Thank you for everything Jesús and Jose Ángel, it was my pleasure to work with you.

I would like to thank the rest of the group, starting with Charo and Daniel. Although we did not spend much time together, they were always very kind and close to me. Charo always had an anecdote to tell or some advice to give. And of course, to Antonio, what can I tell you, that you do not know yet.... You are funny, clumsy, get a good insurance because you really need it, avoid cables... but I had great time with you, thanks for your advice, for your help and for introducing Clarita to me, and for teaching me that the most important thing ...it is to never be the worst!!!

Last but not least to Miguel. Though he was not my supervisor, he behaved like one. Thanks for the shown interest and help since the moment I sent you an email telling you that I was a student from Asturias that had just been accepted in EMQAL until today, that you have offered me to work with you. Thank you for everything, because although sometimes was hard to find you, you always are there when it is necessary and you care of all us.

I would also like to express my gratitude towards the rest of the EMQAL professors and the staff from the host universities for being kind and caring, especially: Prfs. Bogdan, Wojciech and Kamila from GUT and Profs J. Maria and Enrique from UCA always available to help.

Y como no... a la pandilla del taper:

Al primero Darío, por la adicción compartida al café, y a los chistes macabros ou yeah! Por ser el divulgador de la historia de las arañitas y rey de los chismes junto a mi proyecto de Amiga Laura, la Gossip girl toa pava , ajín de

claro, quilla te voy a pedir pa´ reyes. A María, imagen viva de una Jerezana que me enseñó lo que era una ZAMBOMBÁ de las de verdad y un buen vestido de gitana, a Juan y su mirada, me imagino tu cara ahora mismo, sin molestarte en decir lo que piensas aunque seguramente ni te lo estés leyendo, a David y su título de ingeniero químico y su proyecto de celador de la UCA, como no encuentre trabajo te enteras… A Iván, el "repunante" que no repungante que aunque me llames montañesa y digas carajo, A Luis "el portugués", peculiar, diferente e independiente algún día llegarás a ser supervisor junto con el Ucraniano o "Fotoprens" como se hace llamar en el "facebook" sí hombre el que nunca estuvo en Ucrania y que tiene nombre pero que nadie lo usa, a Alejandro Montes de Jerez porque aunque te hayas cortado el flequillo siempre serás el madero de lady Gaga, A Jesús de Cádiz por culé y por los entrantes de queso, a Joaquín y a su coneja y a Nawal por sus miradas de" estas chicas están locas" y porque algún día iremos a Marruecos ☺

A Lucía "Pili", con la que he compartido prácticamente una parte de mi vida y una neurona, desde la carrera, horas de estudio, trabajos, prácticas, fiestas, cervezas, Erasmus, Erasmus Mundus, residencia ,casa, habitación, zulo, noches sin dormir comiendo patatitas o haciendo presentaciones, horas de ferry , limpieza de premisas, caminatas bajo la nieve, horas de aeropuerto , lloros y risas y risas y lloros, porque lo último que se pierde es el humor, porque fuimos Epi y Blas, Pili y mili y por lo nos queda…

A Fatjon porque si no fuera por ti hoy seguramente no estaría aquí, con esa carta de presentación en el único sitio que nos hubiéramos aceptado sería en segundo de la escuela de idiomas… gracias por todo, por estar ahí cuando te necesito, gracias por haberte leído todos mis assessments, por escuchar mis presentaciones y porque seguramente a estas alturas serías capaz de hacer la exposición por mí.

A mi primo Dani por ayudarme con la portada y porque hoy soy yo, pero estoy segura que pronto será el que esté escribiendo una tesis.

A mi tía Lucía, mi tío Josechu y a la tía Blanca por la ilusión con la que vivís todas mis cosas. A mi hermana Laura porque siempre está ahí para lo que sea y aunque ella no lo sepa es mi ejemplo a seguir.

Y por supuesto en especial doy las gracias a mis padres, por animarme y apoyarme siempre, porque a pesar de que no se enterarán de la mitad, hoy están aquí con toda la ilusión. Porque espero que estéis orgullosos de mí que sé que sí, pero que sepáis que parte del mérito es vuestro. Hommer que vuelvo a casa pero… esto me desgravará algunos años de la deuda no?

Thank you very much, Dziękuję bardzo, Muchas gracias!!!

Marta Ferreiro.

I would like to start my thesis by citing a few words that two of my dearest people once told me as a child (my grandfather and my uncle Josetxu)

"Nenina , Do not allow the one who sits next to you know more than you do. But you only deserve something if you get it fighting against yourself and the books, but not against the rest."

Me gustaría empezar mi tesis recordando unas palabras que dos personas muy importantes para mí me dijeron cuando era tan solo una niña (mi abuelo y mi tío Josetxu).

"Nenina, No permites que el de al lado sepa más que tú. Pero el mérito está en conseguirlo compitiendo contra ti misma y contra los libros, no contra los demás."

Abstract:

This research project addresses three independent studies that are related to the authentication of olive oil and sherry wine vinegar through the usage of UV-Vis spectroscopy.

In the first study, an adulteration study was carried out in olive oils by using colored vegetable oils with natural colorants.. Two different procedures were developed aiming at detecting the falsification.

The second study presents a novel method based on the standard deviation of normalized spectra at adequate wavelengths**.** This method has provided an accurate resolution of spectra at individual components in a two-component system which can be used for multicomponent systems with bigger sizes. The method then was effectively applied to different olive oil sets.

Finally, the third study was performed in order to determine the characterization of sherry wine vinegars. For the first time, chromatic parameters were used for characterizing sherry wine vinegar according to the type of the wine used and the acetification process followed during the elaboration process. Once again, the results were satisfactory.

The UV-Vis spectroscopic technique has been proven to be a versatile tool for a rapid and nondestructive analysis in oil and vinegar samples based on the study of their spectra.

List of acronyms:

- **AEMO**…… *Asociación Española de Municipios del Olivo.*
- **ALS**…….. *Alternating least squares.*
- **AMD**…… *Age related macular degeneration.*
- **ANOVA**…….. *Analysis of variance*.
- **AU**…… *Absorbance units*.
- **CA**…….. *Cluster Analysis*.
- **CIE**……. *International Commission on Illumination.*
- **CR**…….. *Curve-resolution.*
- **DAD**…….. *Diode array detection.*
- **D.O**…….. *Designation of origin.*
- **EC**…….. *European Community Council of Regulation*.
- **EFA**…….. Evolving factor analysis.
- **EVOO**…….. Extra virgin olive oil.
- **FA**…….. *Fatty acids.*
- **FDA**…….. *Food and Drug administration.*
- **HCA** …….. *Hierarchical cluster analysis*
- **HELP**…….. *Heuristic evolving latent projections*.
- **HOVO**…….. *High oleic virgin oil*.
- **IOOC**…….. *International Olive Oil Council.*
- **LDA**…….. *Linear discriminate analysis.*
- **LDL**…….. *Low-density lipoprotein.*
- **MUFA**…….. *Monounsaturated fatty acids*.
- **NIR**…….. *Near Infra -red*
- **OIV**…….. *Office International de la Vigneet du Vin.*
- **OPA**…….. *Orthogonal projection analysis.*
- **PCA**…….. *Principal component analysis*
- **PCs**…….. *Principal components.*
- **PDO**…….. *Protected Designation of Origin.*
- **PUFA**…….. *Polyunsaturated fatty acids.*
- **PVA**…….. *Parallel vector analysis.*
- **RGB system**…. *Red, Green and Blue system.*
- **ROO**…….. *Refined olive oil.*
- **ROPO**…….. *Refined olive pomace oil*.
- **ROS**…….. *Reactive oxygen species.*
- **SD**…….. *Standard deviation.*
- **SFA**…….. *Saturated fatty acid*.
- **SIMCA**…….. *Soft independent modeling of class analogy***.**
- **TAGs**…….. *Triacylglycerols.*
- **SIMPLISMA**…… *Simple to use interactive self-modeling mixture analysis.*
- **SMCR**…….. *Self-modelling curve resolution.*
- **UPLC**…….. *Ultra Performance Liquid Chromatography.*
- **UV-Vis spectroscopy***Ultraviolet visible spectroscopy.*
- **VOO**…….. *Virgin olive oil.*
- **WFA**…….. *Window factor analysis.*

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Justification and objectives

Diet plays a key role in our health, and as such, much importance is given to the investigation of the quality in food, every day. As we all may know, the Mediterranean diet has been one of the most famous and valued ones worldwide not only for its quality and the variety of food, but also for its beneficial effects on health.

Virgin Olive Oil, VOO, and sherry wine vinegar, the objects of our study, are two essential components of the praised Mediterranean diet considered as products of high reputation, greatly appreciated in gastronomy. Both have been part of the Andalusia culture since ancient times. Nowadays, there are many studies that focus on demonstrating that a diet which is based on virgin olive oil and moderate amounts of wine can be a healthy dietetic model that leads to healthy aging and decreases the risk of cardiovascular diseases. The health benefits together with the fine aroma and a pleasant taste due to virgin olive oil composition, is the reason that makes the costs of virgin olive oil be high when compared to other commonly used vegetable oils. Hence, olive oil production is a big business subject to serious attempts at fraudulent marketing. Both facts, the one mentioned above and Andalusia´s reputation as the world's largest producer of olive oil being the main economic activity in some of its regions, have increased the interest of its study.

Due to the diversity of oils and vinegars on the market and their increase in demand, it has been considered necessary to investigate reliable analytical methods to establish criteria for determining quality and origin, since the objective authentication remains an unresolved issue.

Therefore, the aim of this thesis is to apply UV-Vis spectroscopy as a rapid, cheap and non-destructive technique for authentication studies on virgin oils and vinegars. Several spectroscopic and related techniques for determining adulterations based primarily on the search of real characteristics of samples will be proposed.

1. Development of a rapid method to detect adulteration in vegetables oils that were colored with chlorophyll pigments in order to simulate a better quality.

2. Development of a simple and rapid method for the resolution of curves applied to spectra in multicomponent systems.

3. Characterization of wine vinegars from local vineyards.

Introduction and literature review

I. Olive oil

I1. Introduction.

Olive oil is used since ancient times, and the ancient civilizations have used it, proof of this is the great cultural legacy that has come to our days on the olive tree, their cultivation, how to develop oil and all healing properties attributed to it , as many ancient civilizations gave it the name of liquid gold. The great flowering of olive growing came coupled with the expansion of all cultures. It was the Phoenicians and the Greeks who imposed their culture on the Iberian Peninsula. The first olive trees in the Iberian Peninsula were cultivated in Cádiz and Seville provinces. When the troops of Julius Cesar fought with those of Pompey in Hispania, they camped among trees in the Aljarafe region surrounding Sevilla, the traditional location of these trees, famous for its excellent olive oil. The word Córdoba means olive mill which indicates its olive groves and the quality of olive oil, as it was famous since Roman times for the oil produced in the region, even to the point that the Spanish-Roman poet Marcial called the Andalusian regions "Betis olifera". In Imperial Roma the olive tree and its branches were symbols of peace, fertility and prosperity.

Fig. I1 Roman amphora with olive oil (*www.sabor-artesano.com* 2010)

Virgin olive oil, VOO, pillar of the Mediterranean diet is considered as the main energy source of the fat matter. Moreover, being a source of essential fat acids, it also provides certain nutrients in low quantity such as antioxidants, phenolic compounds, E vitamins and carotenes). Olive oil has been generally considered to be nutritionally desirable for its health properties and oxidative stability which are due to not only the high content of oleic acid but also due to those components found in small amount as shown later on. Due to the health benefits, VOO is attracting increasingly the interest of scientists. [1]

Within the group of olive oil one can find different categories. The quality and type of the resulting olive oil depends not only on the olive fruit variety but also on all the steps that are involved in oil production together with other factors such as: clime, soil, culture…

I2. Olive oil production.

Olive oil production is carried out in several stages starting by the harvesting time, cleaning and storage of the olives. Olives must be harvested from the olive trees in late autumn or early winter when the olives have their highest level of fatty acids in pulp. The olive harvest is an important activity regarding costs of production but mainly the quality of the obtained olive oil since harvest time directly affects the composition of the oils and their sensory characteristics. Olives can be also harvested in many different ways, which can greatly affect the quality of the final product. In order to have the best quality oil, olives must be collected directly from the trees and at this phase all possible care must be taken to avoid damaging the fruit. It can be done by hand (hand-picking) with or without the aid of rakes or some mechanical devices.

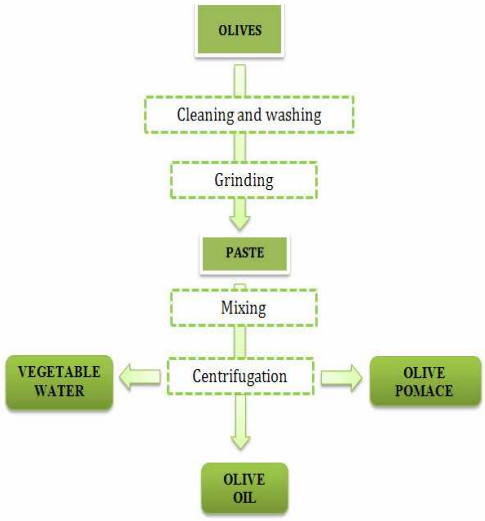


Fig.I2 Olive oil production diagram


Fig.I3 Mechanical harvesting
(Consorzio Nazionale degli Olivicoltori)

This manual harvesting accounts for the highest cost in olive oil production. Modern olive growing applies the use of mechanized harvesting, by means, primarily of shakers that can be attached either to the tree trunk or main branches. Though not always applicable, mechanical harvesting is man-powered and time saving and allows more competitiveness. Regardless of the method used, olives should always fall on collecting nets and if possible never touch the ground.Usually, the olive at the time of harvest contains: oil 18-32%, water 40-55% and olive pits and plant tissues 23-35 %.If olive **transportation** and storage is not properly carried out it can lead to loss of quality in the oil. Transportation, storage and milling of the olives must take place as soon as possible. Quality Standards for PDO set a maximum interval of 24-48 hours between harvesting and extraction. The longer the time between harvesting and oil extraction the worse the quality: increase of free acidity due to oxidation of the oil and organoleptic alterations (defects such as scalding, mouldiness, vinegary, etc) due to fermentation of cell sap and development of microorganisms. Once cleaned and washed, the olives are ready to go through the next steps.

- Grinding or milling, the aim of which is to break the cells where the oil content, Traditional stone grinders known as "**almazara**", or roller crusher, can be used as well as various types of metal crusher on order to grind the olives.

Fig.I4 Almazara s.XIX (Museo del Olivo)

- Mixing which is performed in order to form a continuous/homogenous oil base, suitable to be separated. During this phase the olive paste is slowly but continuously mixed to facilitate the aggregation of the oil in bigger and bigger drops in order to allow an easier separation from vegetable water. Mixers are made of a set of hollowed cylinders placed one on the top of the other and are opened in the upper part. They have a central axis where blades are assembled in order to remove the paste. Hot water circulates in order to heat the paste and help the separation of the olive oil. Time (over 15 min) and Tª (25-30º C) of the mixing phase are the key parameters as far as quality is concerned.

Fig.I5 Traditionally extraction system. (http://www.aceite-de-oliva.es)

Extraction: Nowadays, the technologies available for the extraction of olive oil from the olive paste, offer a great variety in equipment, systems and combinations. These different systems can affect the oil in terms of chemical composition, taste and aroma: Traditionally, **extraction system by pressure** has been used which separates the oily juice (a mixture of oil and olives residual water), from the olive pomace or solid part, through a filtration process aided by pressure. The olive paste coming out of the mixer or directly of the roller crusher is spread over olive mats (porous disks typically made of vegetable fibers). Olive mats are stacked up in a tower called load, which maintains the vertical position thanks to a metal needle through a central hole in the load. After placing the load in the press, pressure is applied to the lower part, pushing the olive mats against the upper bridge. This pressure facilitates the outlet of the oily juice that will be labeled as virgin olive oil, separating it from the pomace. **In continuous systems**, two and three-phase, the separation of the oil from the olive paste is carried out by the centrifugal force applied to olive paste that separates the different phases on the base of their different densities. After the centrigutation, what it is obtained is virgin olive oil, vegetable water ("alpechín") and pomace in the case of three phase system and virgin olive oil and two-phase pomace ("alperujo"). Depending on the extraction system used, the different percentages of by-product quantities are generated:

	Olive oil %	Pomace %	Vegetable water %	Alperujo %
Traditional system	21	47	32	-
Three –phase system	22	42	36	-
Two –phase system	23	-	-	67

Table I1 Percentages of oil extraction regarding the extraction method. *AEMO (Asociación Española de Municipios del Olivo, 2003).*

Once oil extraction is completed, the oil if not properly stored may become loses its aroma and the typical defect of rancidity may come up. Further problems may arise from anaerobic fermentation of the suspended particles or the sediment in the storage containers, which cause serious defects. In this case, if it is wanted to use as edible oil, refining procedure is required.

I3. Types of olive oil.

Based on the productions process, olive oils are classified as:

Fig.I6 *Olea europaea* *http://asusta2.com*

"Virgin olive oil" which is defined by the International Olive Oil Council (IOOC, 2003) as the oil obtained from the fruit of the olive tree (*Olea europaea*) by mechanical or other physical means under conditions that do not lead to alterations in the oil. Only minimal treatment such as washing, pressing, decantation, centrifugation and filtration is acceptable.

Additionally, "**extra virgin olive oil**", EVOO, must have a free acidity percentage of less than 0.8% as well as the clear flavor characteristics that reflect the original olive fruit. If the VOO contains a free acidity lower than 2% of free fatty acid, calculated as oleic acid, it means that this olive oil is suitable for consumption without any treatment.

Within this group an extra subdivision can be done regarding the fruit variety:

- *Monovarietal*: Obtained from a single variety of olive.
- *Coupage:* Obtained from a several varieties of olive.
- *Designation of origin (D.O)*: Generated from olives from particular geographical area, where it is also produced and bottled.

When virgin olive oil contains a higher free acidity, this oil is known as "**lampante olive oil"** and needs to be refined in order to become edible.

"**Refined olive oil"** is referred to those oils that had some defect (loss of aroma), and were processed in order to be edibles. The refining process can not alter its initial glyceride structure. "**Pure olive oils**", also sometimes labeled **"Olive oils"**, are blends of refined and virgin oils and must possess the free acidity of no more than 0.3% and conform to the standards within their category.

"Pomace olive oil "is obtained by solvent extraction, heat treatment, esterification, or refining. The composition of the oils is based on the fatty acids present in the triacylglycerols and their location on the glycerol backbone. The free acidity must be lower than 1%.

I3.1 Spanish olive oil.

Spain is the world's leading producer and seller of quality olive oil, followed by Italy and then Greece. The Spanish olive tree oil orchards design the landscape of each area and depend on the history of the specific place and on the variety of olive that has adapted better to the land conditions, climate and necessities of the farmer. Base on this fact, the following types of olive varieties can be found in Spain:

- Picual or Marteña (Jaén) -the largest producer ★
- Hojiblanca or Picuda (Córdoba y Málaga) ☆
- Arbequina (Lérida) ☆
- Empeltre (Aragón) ☆
- Cornicabra (Castilla la Mancha and Extremadura) ☆
- Royal (Jaén) ★
- Serrana (Castellón) ☆

Fig.I7 Olive varieties in Spain.

I4. Composition of VOO.

For better understanding of the importance of the studies involved in this thesis an introduction of VOO composition is needed. The composition of the olive oil varies not only with the type of olive fruit and extraction method but also with geographical origin and meteorological effects during the growth and harvest of the olives. VOO consists of complex mixtures consisting in:

- Saponifiable fraction (>98%): triacylglycerols-TAGs, phospholipids and fatty acids.
- Unsaponifiable fraction (<2%): hydrocarbons, tocopherols, sterols and alcohols.
- Minor components (ppm): pigments, volatile compounds and phenolic compounds.

VOO can be distinguished from the rest of oils due to the beneficial potential in human health of its pigments and some minor compounds [2]. There is the growing interest in the use of natural antioxidants as bioactive components in food, and such foods have been termed ‘‘functional foods’’ [3].

I41. TAG and FA.

Regarding the fatty acids it is important to distinguish between **SFA** (saturated fatty acid) and **MUFA or PUFA** (monounsaturated or polyunsaturated fatty acids).The healthiest fatty acids are those that contain high levels of MUFA. MUFAs and antioxidants, which not only reduce the risk of heart disease and cancer, but also provide a wide range of anti-inflammatory benefits that, can positively impact illnesses such as diabetes, hypertension, arthritis, and asthma. [4-5]. Even the **FDA** (Food and Drug administration) suggests that replacing just two tablespoons of saturated fat with olive oil in your daily diet can have measurable positive effects. The major fatty acids in olive oil triacylglycerols are:

- **Oleic Acid (C18:1 or 9-octadecenoic acid),** a monounsaturated omega-9 fatty acid. It makes up 55 to 83% of olive oil.
- **Linoleic Acid (C18:2 or 9, 12-octadecadienoic acid),** a polyunsaturated omega-6 fatty acid that makes up about 3.5 to 21% of olive oil. And it is consider as an essential fatty acid, since the human body is not able to make it.
- **Palmitic Acid (C16:0 or hexadecanoic acid),** a saturated fatty acid that makes up 7.5 to 20% of olive oil.

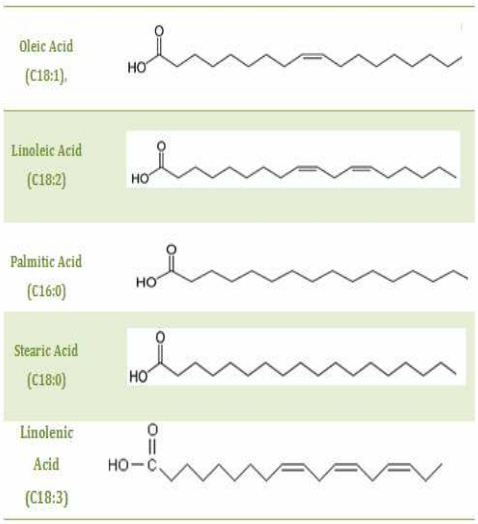


Fig.I.8 Major fatty acids in virgin olive oil.

- **Stearic Acid (C18:0 or octadecanoic acid),** a saturated fatty acid that makes up 0.5 to 5% of olive oil.

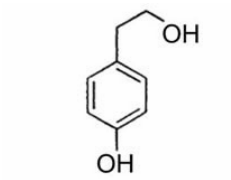
- **Linolenic Acid (C18:3 or 9, 12, 15-octadecatrienoico acid),** specifically alpha-Linolenic Acid), a polyunsaturated omega-3 fatty acid that makes up 0 to 1.5% of olive oil.

Olive oil contains more amount of oleic acid and less of linoleic and linolenic acids than other vegetable oils. That means, olive oil contains more monounsaturated than polyunsaturated fatty acids and this renders olive oil more resistant to oxidation because generally, the greater the number of double bonds in the fatty acid, the more unstable and easily broken down by heat, light, and other factors the oil is [6].

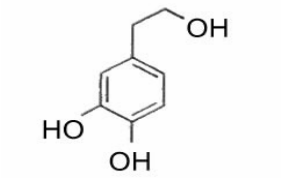
IR techniques are very suitable to distinguish VOO from other vegetable oils regarding the main composition. However, nowadays vegetable oils with high oleic content are found in the market. This makes not so easy the identification of oils by the determination of fatty acids composition. This has increased the interest of studying minor compounds.

I42. Polyphenols.

The flavonoid polyphenols in olive are natural antioxidants that have a host of health beneficial effects [7]. Hydroxytyrosol and tyrosol are some of the many phenol compounds in olives that contribute to bitter taste, astringency, and resistance to oxidation. [8]. [9]. Within the vegetables oils, VOO is considered the most resistant to the oxidation due to its polyphenol content. The antioxidant power of these polyphenols is mainly due to hydroxytyrosol.



Tyrosol



Hydroxytyrosol

Fig.I9 Main polyphenos in VOO

Some clinical evidence suggests that it is olive oil's phenolic content, rather than its fatty acid profile, that is responsible for at least some of its cardio protective benefits. They have been shown to have a host of beneficial effects from healing sunburn to lowering cholesterol, blood pressure, and risk of coronary disease. They are considered in olive oil as natural antioxidants. Due to their ability to scavenge reactive oxygen species (ROS), antioxidants are capable of inhibiting the process of low-density lipoprotein (LDL) cholesterol oxidation subsequently decreasing the risk of cardiovascular diseases. [10].

I43. Phytosteroles.

Phytosterols are a major portion of the unsaponifiable fraction of olive oils. Clinical studies have demonstrated that dietary intake of phytosterols, due to their structural similarity with cholesterol, can inhibit its intestinal absorption, thereby lowering total plasma cholesterol and low-density lipoprotein levels (Wong, 2001)[11]. Phytosterols have also been reported to present antioxidant, antibacterial and anti-inflammatory activities and may offer protection against cancers, such as breast, colon and prostate (Awad and Fink, 2000)[12]

I44. Tocopherols and tocotrienols.

This family of compounds is particularly important in preventing lipid oxidation processes in olive oils, mainly due to their antioxidant activity. There is a special interest of vitamin E that can be included within this group rather than in the vitamins group. A large range of biological activities have been ascribed for these compounds since vitamin E have been associated with a preventive action against reactive oxygen species in biological systems (Valdioli et al,1996)[13].However, there are some studies that suggests that the most important function of vitamin E is as a signalling molecule[14-15]. Vitamin E is a fat-soluble antioxidant, this means that is not broken down by cooking and it is stored in the liver and body fat for long periods so it is not essential to eat them with every meal. Although vegetables oil contain more amount of vitamin E , it is also found in VOO together with vitamin k.

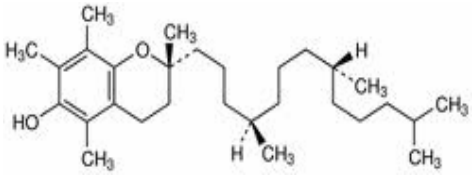


Fig I10 α-tocopherol (Vit. E)

Alfa-tocopherol was probably the most studied vitamin during the past years since it was considered the most active vitamin E isoform, nevertheless, nowadays several studies have shown that the other vitamins also have important roles in the human organism such as □ -tocopherol and tocotrienols which are correlated with the reduction of blood cholesterol levels [16] and may have a chemopreventive action [17].

I45. Pigments.

Color is an organoleptic parameter of virgin olive oil and an important attribute that affects the consumer's perception of quality. In general, the consumer identifies colored oil with virgin olive oil, while olive oils with little color recall refined seed oils. VOO presents colors ranging

from greenish-yellow to gold, depending on the fruit's ripeness. The color of olive oil results from 2 types of natural pigments groups present in olives, **chlorophylls and carotenoids**: Chlorophyll pigments account for the greenness of the oils, while the carotenoids for their yellowness. Both groups are the unique identify in fresh olives and include pigments found in the fresh olives and others formed during the oil extraction.

- **Chlorophylls Chlorophylls** are the most well-known bluish-green chlorophyll a and the yellowish–green chlorophyll b, but can also be found in other types of chlorophyll pigments such as pheophytins, chlorophyllides, pheophorbides, pyropheophytins, chlorines, rhodins, and purpurins.[18]. Although, there are some reports that hypothesize that chlorophyll pigments may be beneficial for human health, for now, there are no evidences apart from the well-known role of chlorophylls as natural pigments accounting for greenish colors and in photosynthesis activity.

- **Carotenoids**: have attracted the attention of scientists for decades due to their nutritional importance. Some carotenoids (β-carotene, α-carotene, β-cryptoxanthin, and so on) are precursors of vitamin A. Over and above the role of some carotenoids as provitamins, a large body of evidence exists indicating that they may be effective antioxidants [19] and beneficial in relation to the prevention or amelioration of serious human ailments like skin [20] and eye disorders [21], cancer [22], cardiovascular disease [23]. For over more than 30 years, the photo-protector role of carotenoid-rich food products has been known for its beneficial effects against skin damage caused by prolonged exposition to sun light and UV radiation. [20]

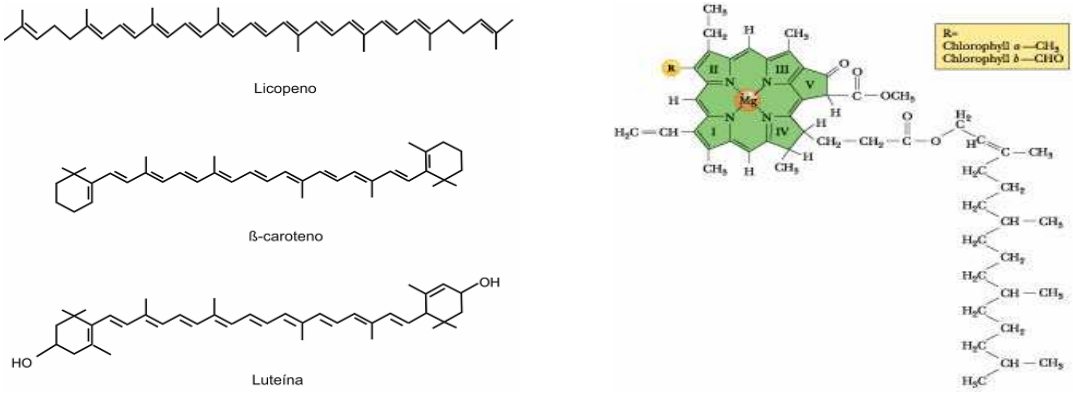


Fig I11 a) Main carotenes in VOO b) Chlorophylls molecular structure.

Regarding the health benefits of carotenoids, generally, it is important to bear in mind that they must be absorbed and delivered to the relevant tissues so they can exert their biological functions or actions. There are many source of carotenoids but from the

bioavailability point of view, VOO is one of the most considered, providing the most active biologically carotenoids such as beta carotenes, lutein etc…the lack of lutein causes blindness due to macular degeneration. Lutein provides also health benefits decreasing the risk of cardiovascular disease and some cancers [24, 25]. Similar results have been found for others VOO components such as polyphenols and vitamin E.

All VOO contain lutein perfectly dissolved and in the best bio- available conditions, therefore its diary ingestion can prevent age related macular degeneration (AMD).There are also evidences which prove that these natural antioxidants have other functions like protection and stability of the food that contains them, even after being subjected to sterilization processes [26].

Recently, possibility of a synergic effect between vitamin E and carotenoids has been suggested [27-28].

In the thesis special attention will be paid to the pigments.

I5. Adulteration Olive oil.

Adulteration of food products, involving the replacement of high-cost ingredients with lower grade and cheaper substitutes can be very attractive and lucrative for a food manufacturer or raw material supplier. In fact, adulteration of food products is not only a major economic fraud but can also have major health implications for consumers. In the 1980s, more than 400 deaths and 20,000 casualties occurred from the disease known as 'Spanish toxic syndrome,' caused by the consumption of adulterated oil [29]. Therefore the detection of adulteration is of so high importance.

As it was mentioned above there are evidences showing that virgin olive oil has positive effects on human health.The health benefits together with the fine aroma and a pleasant taste resulting of VOO composition, is the reason that makes the costs of VOO be high when compared to other commonly used vegetable oils, making it prone to falsify or adulterate with less expensive oils in order to increase profits. Most common adulterants found in virgin olive oil are seed oils, such as sunflower, soy, corn and rapeseed oils as well as nut oils, including hazelnut and peanut oils [30].

Several international regulations have been developed to protect consumers with uniform definitions, labeling, and a multiplicity of analytical techniques to identify genuine quality standards in many countries. For instance, several commercial categories of olive oil

are legally defined by the European Community Council of Regulation (EC, 2001), which are marketed with different prices.

In general, the consumer identifies colored oil with virgin olive oil, while olive oils with little color recall refined seed oils, therefore another common type of adulteration is by adding colorants European regulations do not permit the addition of colorants to oils and fats of animal or vegetable origin [18]. However, for other food products two natural colorants structurally related with the chlorophylls are authorized; those denominated E 140 and E 141. Because they are natural (pigments obtained from an animal or vegetable raw material), and although being subject to rigorous examination, they are not as a rule subject to regulatory specific criteria of purity. E 140 comprises direct chlorophyll derivatives; it is marketed according to its solubility: E 140 i is liposoluble, chlorophylls derivatives, and E 140 ii is hydrosoluble, composed of chlorophyllins. E 140i is obtained from natural sources: alfalfa, nettles, and other edible plant materials, via solvent extraction. During this process, part of the chlorophylls can be transformed into their magnesium-free derivatives: the pheophytins. This means a drastic change in the color, which goes from green to brown.

The colorant E 141 is composed of copper complexes of chlorophyll derivatives; that is, the corresponding copper derivative of E 140. The product is marketed as E 141i, which is liposoluble and known as “copper chlorophylls”, and E 141ii, which is hydrosoluble and known as “copper chlorophyllins”; each results from the addition of copper to the respective pigment solutions. There is also an artificial green colorant (E 142), which is authorized in Europe, but not in the United States. Being artificial, it has to pass strict toxicological controls. The FDA, with regard to green colorants in foods, allows the use of E 141 only in citrus-based dry beverage mixes, and never exceeding 2%.

Copper chlorophyllins (E 141ii) are the colorings most used in food technologies, due to their hydrophyllic character, easily soluble in ice creams, jellies, soft drinks, vegetables in vinegar, etc. Therefore, there is more bibliography for the detection of this colorant [18].But, the colorant used to boost the color in olive oils is E 141i; that is, the liposoluble form of the copper derivative of chlorophylls, making it readily soluble in the oil. The cupro-derivatives are used preferentially because they are much more stable than the original chlorophyll, as the insertion of the Cu^{2+} ion in the macrocycle of the chlorophyll generates a highly stable complex that remains green despite the processing and storage of the food. In contrast, the naturally chelated magnesium in the chlorophyll macrocycle is readily substituted by hydrogen during the storage or processing to form other, non-green chlorophyll derivatives (pheophytins).

I51. Analytical methods to establish the authenticity of olive oil and to detect adulteration level.

At the present state of art of this matter, according to different types of techniques and different adulterations, we can find limit of detection in adulterant oils very low indeed.

For example, Torrecilla et al.[31] can detect VOO adulterated with the refined olive oil (ROO) or refined olive pomace oil (ROPO) when the adulterating agent concentration is less than 10%, by using chaotic parameters calculated from UV-vis spectra of oils. As well, Saba [32] by GC-MS and GC-MS/MS, can detect addition of deodorized olive oil to extra virgin olive oil. Such a blending may not significantly affect the parameters usually checked as quality indicators, although the organoleptic properties may change. Downey et al. [33] using NIRS and the multivariate classification method SIMCA (soft independent modeling of class analogy) can successfully discriminate between authentic extra virgin olive oils and the same oils adulterated with sunflower oil at levels as low as 1%, and Ozturk et al. [34] use NIRS to determinate olive oil adulterations with vegetable oils (cotton, corn, canola) at levels down to 2% except for soybean oil, that the limit for quantitative determination was minor amounts of 5%. Mannina et al. [35] based on ^{1}H NMR measurements combined with a suitable statistical analysis, can detect down to 10% adulteration of olive oils with refined hazelnut oils, and Agiomyrgianaki et al. [36] can down the limit to 5% using also data of ^{31}P NMR.

Lee et al. [37] can identify olive oil samples adulterated with < 1% of other seed oils by using a UPLC (Ultra Performance LC) equipped with UV photodiode array and mass spectrometer detectors. Contiñas et al. [38] can differentiate adulterations of 1%, 2% and 10% of refined sunflower oil, refined soybean oil and refined olive pomace oil, respectively, in extra virgin olive oil by applying discriminant analysis techniques and using typically spectrosphotometric parameters as ΔECN42, ΔK and the sum of the trans-linoleic and trans-linolenic isomers as variables. Poulli et al. [39] using Synchronous fluorescence can prove adulterations of olive-pomace, corn, sunflower, soybean, rapeseed and walnut oil in virgin olive oil at levels of 2.6%, 3.8%, 4.3%, 4.2%, 3.6%, and 13.8% respectively . By FTIR spectroscopy combined with multivariate calibrations and discriminant analysis, Rohman et al. [40] can quantify levels of adulterations of extra virgin olive oil with palm oil down to 1%, and Lerma-Garcia et al. [41] has been able to detect a low cost oil content in VOO as low as 5%. Zou et al. [42] investigated the potential of Raman spectroscopy and can distinguish the genuine olive oils from the olive oils containing 5% or more of other edible oils, such as soybean oil, rapeseed oil, sunflower seed oil, or corn oil. However, El-Abassy et al. [43] achieved a quantitative detection limit down to 0.05% of adulteration of extra-virgin olive oil with sunflower oil.

Detection of adulteration is often complicated when oils with chemical compositions similar to VOO are employed. Attempts to counterfeit VOO using colored high oleic vegetable oils are well known [44]. In such cases, determination of the adulteration of VOO is not simple task; efforts to detect and determine adulteration traditionally demand monitoring of organic compounds to establish a comparison with typical unadulterated oils in order to identify change of composition that could be related to adulteration.

Regarding to the adulteration by coloring vegetable oil, Roca et al [18] has proposed a method, based on a pigment extraction and HPLC, for determining olive oil colored with commercial copper complexes of chlorophylls, E141i. Roca concluded that the presence of cupro-chlorophyll derivatives in an olive oil sample implies that the oil has been adulterated. Another conclusion is that the maximum of the band of largest wavelength of the main cupro-chlorophyll derivative, Cu-pyropheophytin a, is about 650 nm, significantly lower than the chlorophyll derivatives in olive oil, around 670 nm.

Normally, commercial high oleic vegetable oils are pale yellow or very pale green, because they significantly do not have pigments. That makes them easily distinguishable from VOO. Others kinds of adulterations consist in additions of green pigments to these cheaper vegetable oils in order to mimic a VOO. In this case, it is not easy to detect such adulterations of colored oils by simple spectroscopic methods. Although in one case just a simple solution is required, in most of these papers involve a tedious sample preparation, which has an economic and ecological cost in organic solvents. In addition, chemical methods mentioned above for the control of authenticity of VOO are time-consuming and require skilled operators.

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II. Sherry Wine Vinegar

II1. Introduction.

Vinegar has been made and used for thousands of years. Traces of it have been found in Egyptian urns dating from around 3000 BC.

Nowadays, vinegar is considered a product of high reputation, much appreciated in gastronomy. Vinegar is used not only as condiment but also as ingredient in many food products, particularly sauces and dressings. Vinegar quality is heavily influenced by flavour compounds. Several hundred of compounds from different families contribute to vinegar flavour. It's chemical and organoleptic properties are determined by the acetification system, the raw material used as substrate and, in some cases, by the wood aging [1]. A brief introduction about vinegar properties is required for better understanding of the interest in its study.

II2. Sherry wine vinegar.

Sherry wine vinegar is gourmet vinegar made from sherry wine. It is produced in the Spanish province of Cádiz and inside the triangular area between the city of J*erez de la Frontera* and towns *of Sanlúcar de Barrameda* and El *Puerto de Santa María* , known as the "sherry triangle".

The production and quality of sherry vinegar is monitored and controlled by the Consejo Regulador of Sherry vinegar has its own Denomination of Origin, which is protected by Spanish and EU law. This denomination is referred to vinegars exclusively obtained from wines made with grapes cultivated in the production zone of "Jerez-Xérès-Sherry" and "Manzanilla de Sanlúcar de Barrameda" Denominations of Origin, with a minimum alcoholic graduation of 9.5º and a total minimum acidity of 2.5 g/L expressed as tartaric [2].

Fig.II1.Sherry Triangle.

The word "vinegar" derives from the Latin words "vinum aegrum" meaning "feeble wine". Therefore, vinegar from sherry has been around since sherry wine was first produced in and around Jerez and bodegas wines which had undergone acetic fermentation and turned to vinegar used to be considered failures; however since the 1950s winemakers started to view

sherry vinegar as a product in its own right and now even encourage it. They also began to carefully age their vinegars in the same way as their wines and brandies.

II3. Sherry Vinegar production.

Sherry wine vinegar is one of the finest vinegars in the world. It is made from sherry wine which is exclusively produced from grapes cultivated in the Jerez area vineyards. Therefore, the style of sherry vinegar will depend firstly on the grape variety used to produce the wine it is made from:

- ***Palomino*:** Most sherry vinegar is produced from wines which were made from the Palomino grape. The wine being used to produce the vinegar can be young wine or can be a wine which has already aged.
- ***Pedro Ximénez** :* Wines produced from *the Pedro Ximénez* grape that are usually sweet, often very sweet, and consequently the vinegars produced from these wines are usually sweeter than other sherry vinegars.
- ***Moscatel*:** Small amounts of sherry vinegar are produced from the Moscatel grape, that is a highly flavored grape, mainly due to terpenic compounds



Fig.II2 a. Palomino grapes b. Pedro Ximénez grapes c. Moscatel grapes.

In early September, when it is soft and sweet, the "Palomino" grape is harvested in the Jerez triangle. "Moscatel" and "Pedro Ximenez" grapes go through a drying process before going to the collection centers. In this process the grapes are left out in the sun on "esparto" or wicker mats from 48 hours to several days, but covered at night to protect them from the morning dew.

Sherry wine is obtained by the traditional *'Criaderas y Solera'* system making it unique in which it adopts the qualities from the oldest wine once they are combined. The solera system has been developed to benefit from this. This traditional system works as follows :

The oldest wine is held in the '*Solera*', the bottom row of oak barrels. From it, from time to time, a quantity of wine is drawn for bottling (<30%). The quantity removed is replaced with somewhat younger wine from the '*first Criadera'*(Spanish term that translates as "nursery"), the row of barrels stored above in the Solera. In turn this wine must be replenished with still younger wine from the second "criadera", replaced in turn with the young wine named "*sobretabla*". This way, each wine cask is "*rocío*" (washed down) at least three times a year. No other wine is combined in this way.

Fig.II.3 Solera and criadera wine aging system.

The wine becomes vinegar through a natural process of fermentation that takes in stainless steel tanks. Essentially, vinegar is a product from a double fermentation process: first, the alcoholic fermentation carried out by the action of yeasts, transforming sugars into alcohol, and then the acetic fermentation that results from the action *of Acetobacter* bacteria that oxidizes the alcohol to make acetic acid and water [1]. It is allowed the usage of nutrients such us ammonium phosphate, sodium or potassium as well as the addition of malt or yeast in order to promote acidification or forced oxidation by air and pure oxygen. Subsequently, the vinegar is then subject to clarification and filtration to remove impurities. [2]

Sherry wine vinegar is aging using either a static system called "añadas", in which the vinegar remains always in the same cask, or the traditional dynamic system "criaderas and solera". Though the "Consejo Regulador", admits the static system of ageing, the "criaderas and soleras "is much more widespread and appreciated in gastronomy [3]. The aging process must take at least 6 months for non-aged vinegars, and more than 2 years for aged vinegars. According to the ageing process, the vinegar is labeled as:

- *Vinagre de Jerez* if it has a minimum of 6 months ageing in wood.
- *Vinagre de Jerez Reserva* if it has a minimum of 2 years ageing in wood.
- *Vinagre de Jerez Gran Reserva* when it has a minimum of 10 years aging in wood.

II4. Vinegar Characterization.

Due to the diversity of vinegars in the market and the increase in demand, it has been considered necessary to investigate reliable analytical methods to establish criteria for determining quality and origin, since objective authentication remains an unresolved issue. Vinegar quality is heavily influenced by flavor compounds. Several hundred of compounds from different families contribute to vinegar flavor.

As important as obtaining vinegar of specific quality is the need to determine objectively the appropriate parameters that allow us to characterize and differentiate one vinegar from another. Various studies can be found in the literature with the objective of characterizing or differentiating this product [2]: Antonelli et al. [4] used the polyalcohols content to differentiate vinegars from various raw materials; García – Parilla et al. [5] employed polyphenolic compounds; Casale at al. [6] and Pizarro et al. [7] used volatile compounds in order to differentiate vinegars from different raw materials and production process (with or without aging in wood). With the same objective, Natera et al., [8] developed several pattern recognition approaches that permit the classification of vinegar samples according to raw material and production process, using different analytical parameters, such as polyphenol content, organic acids, and volatile compounds. Duran et al., [9] employ methods for the determination of volatile compound in vinegar by Gas Chromatography. However, although these methods are rapid, they require very expensive instruments and also expertise workers at wineries.

Rapid methods of the spectroscopic type have been developed for the control of the vinegar process on the industrial scale. In some, only the consumption of ethanol by the bacteria is determined in-line, in others the near infrared (NIR) technique has been employed for the simultaneous control of ethanol and other compounds. In fact, it has been demonstrated that the NIR technique is valid for the estimation of various parameters of interest in samples of vinegar. In recent years numerous methods have been also developed for the individual estimation of compounds of interest in wine and related drinks, employing the technique of mid infrared as the system of measurement, usually using Fourier Transform based instruments. The basic reasons for interest in this methodology are the rapidity of the analysis, the lack of need for sample preparation, and the absence of residues generated by not employing reagents for the determinations. Additionally, they do not need specially trained workers, but only during calibrations steps. These characteristics make it an appropriate technique for developing vanguard/rearguard analytical strategies in the control of productive processes such that the need for particular corrective actions can be judged in the shortest possible time [10].

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III. UV-Vis Absorption Spectroscopy

III1. Introduction.

In absorption methods the sought-for information is the magnitude of the radiation power from an external source that is absorbed by the analyte species. Here the absorption of radiation is accompanied by excitation of the molecules. For absorption to occur, the frequency of incident radiation must correspond to the energy difference between the two states involved in the transition [1].

The absorption of radiation follows Beer´s law:

$$A = -\log T = \text{Log}_{10}(I_0/I_1) = \epsilon \cdot l \cdot c$$

Where A is called the absorbace, T the transmittance, I_1 is the intensity of light at a specified wavelength λ that has passed through a sample (transmitted light intensity) and I_0 is the intensity of the light before it enters the sample or incident light intensity (or power), ϵ extinction coeffiencitent, l is the pathlength of absorption, c the concentration of the absorbing species.

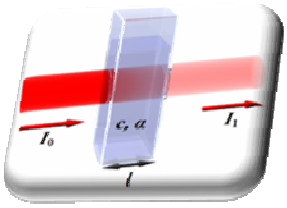

Fig.III1. Absorption process

Absorption methods involve measuring the ratio of two radiant powers, calculating the absorbance, and relating the absorbance to concentration.

III2. UV-VIS spectroscopy.

Very briefly, UV-Vis spectroscopy is a technique based on measuring the absorption of near –UV or visible radiation by molecules. Radiation in this wavelength region causes electronic transitions at wavelengths characteristics of the substance. The UV-Vis region of the electromagnetic spectrum is the wavelength range from 190 nm to 800 nm.

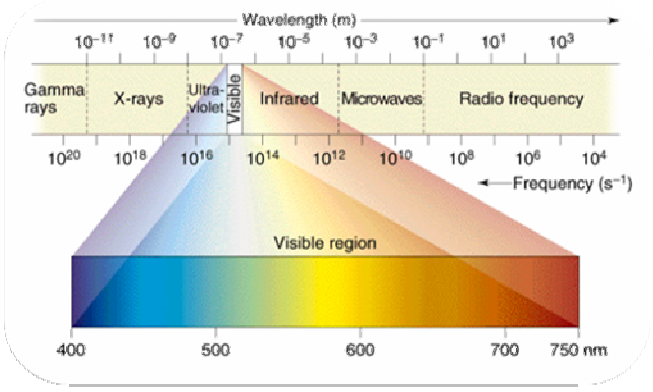

Fig. III2. Electromagnetic Spectrum (http://cyberchalky.wordpress.com/page/2/)

The diagram below represents a typical UV-VIS molecular absorption spectrophotometer:

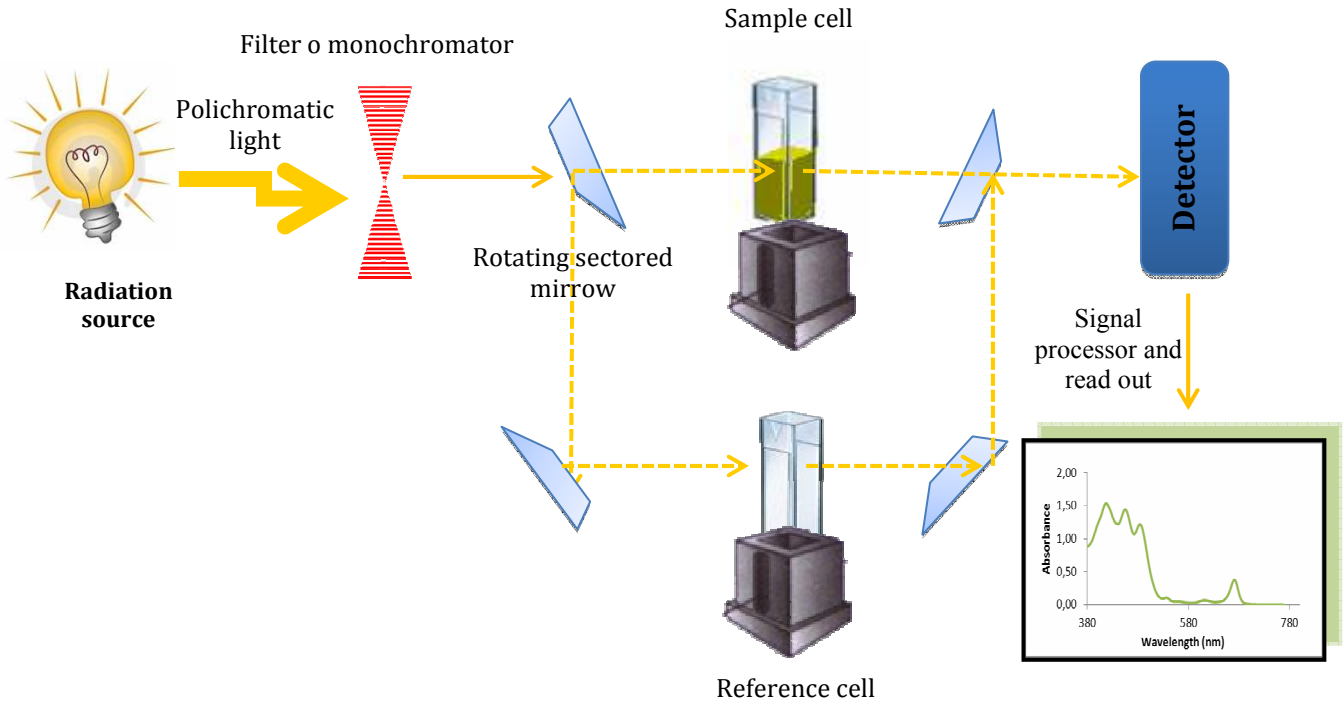

Fig.III3. Diagram of a UV- visible molecular absorption spectrophotometers

In the visible spectrum some samples can show certain bands owing to the presence of compounds which are responsible of the color. Samples can be spectroscopically differentiated one from another by the intensity of these bands.

III21. Applications.

The spectral position of an absorption band is indicative of the presence or absence of certain structural features or functional groups in a molecule. Also compilations of UV-Vis absorption spectra of many compounds are available to compare to the absorption spectrum of pure unknown. Usually, a match between the reference and unknown spectra is not sufficient proof of the identity of the compound because the positions affected by minor difference in the structure, particularly for large molecules, therefore spectrophotometry is not considered as a major tool for qualitative analysis, but it is for studying chemical equilibrium and kinetics.

The use of spectroscopy as a quantitative tool in fundamental and analytical applications is very common.

Often it is possible to choose appropriate wavelength to monitor the absorbance of one or more reactants, products, or intermediates in the presence of other species. The concentration is then determined by applying Beer´s law and known molar absorptivity's. For equilibrium studies, known concentrations of reactants are mixed and the absorption

spectrum of the reaction mixture is obtained after equilibrium is reached. The final concentrations of the reactants and products are determined from the measured absorbance at selected wavelengths and stoichiometric relationships.

This technique can be also used as a screening procedure, where the absorbance of the analyte is measured directly to provided that it is the predominant absorbing species at the analysis wavelength or if it is separated from potential absorbing species before detection. (For instance, nitrate in natural water is determined by measuring the absorbance at 220nm)[1]. It is widely used to determine metals, cationic species, anionic species and complex ions too. Furthermore, presently UV-Vis spectrophotometry is the most popular detection technique for HPLC due in part to the fact that UV-Vis absorption is a reasonably universal phenomenon for the majority of compounds and provides good to excellent detections limits in most cases. The basic reasons for interest in this methodology are apart from the wide applicability, the rapidity of the analysis, the lack of need for sample preparation, the absence of residues generated by not employing reagents for the determinations. Besides, it has high sensitivity, moderate-high selectivity, and good accuracy and is easy to handle. All of these characteristics make it an appropriate technique for developing vanguard/rearguard analytical strategies in the control of productive processes [2]. Therefore UV-Vis spectroscopy is frequently used to monitor and assess the composition and quality of products in food, beverage, water, and pharmaceutical, refining industries, biological samples… [3-4]. Also, the on-line analysis of the composition of a sample based on selective absorption UV/VIS has been used for monitoring and controlling processes [5]. And recently this technique has been becoming popular for the characterization [2] and detection of adulteration in food, as is the case virgin olive oil [6].

For instance, Fig.III4 shows the visible spectrum for virgin olive oil exhibits. In the visible spectrum of a virgin olive oil, certain bands in the ranges 430–480 nm and 660–670 nm stand out owing to the presence of various carotenoid and chlorophyllic pigments. Oils can be differentiated spectroscopically one from another by the intensity of these bands. The strong band with several peaks between 380 and 500 nm result from overlap of the bands for the constituent carotenoids. The presence of chlorophyll pigments is signaled by two typical bands, one at approximately 420 nm, which is quite strong and overlapped with the carotenoid band, and the other at approximately 670 nm [7].

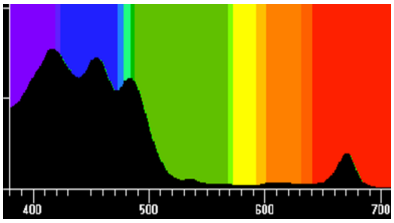

Fig.III4. Visible spectrum for virgin olive oil.

In some cases, enough information can be obtained only by visualizing the spectra. However, nowadays, there has been an exponential increase in the availability of analytical instrumentation capable of acquiring huge amounts of spectra in a short time. As a result, advanced and efficient strategies for the analysis of the data are needed in order to interpret the information involved behind the spectra.

III3. Resolution of curves.

The resolution and identification of the components in complex mixtures continues representing a difficult analytical task in chemistry in spite of improvements in instrumentation and methodology. Generally, the determination of compounds comprising complex mixtures is carried out using chromatographic techniques which require time and sample preparation making these techniques ill-suited to real time analysis. Moreover, these powerful techniques have their limitations, mostly when the profile from a component is overlapped with bands from neighboring components or background [8-10]. The field of curve resolution was born in response to the need for a tool to analyze this multivariate experimental data from multicomponent systems. The curve resolution of a multicomponent system involves the description of the variation of measurements as an additive model of the contributions of their pure constituents. [10-11].

Normally, in order to apply some curve resolution methods, spectral data are organized into a matrix "D" containing raw measurements about all of the components present in the data set. The aim of any curve resolution method is the optimal decomposition of the D matrix into the product of two wanted matrices S and C, which contain as much information as possible about the pure component spectra and their concentrations, respectively. Combinations of the above matrices enable the application of multivariate statistical analysis (curve resolution techniques) for the resolution of overlapping spectral bands. It is not an easy task to obtain S and C from D, as requires the application of adequate methods base on complex algorithms.

In particular, self-modelling curve resolution methods (SMCR) [12-13] are a group of chemometrical approaches suited for the treatment of multicomponent data matrices. SMCR techniques utilize a certain mathematical decomposition to deconvolute spectra from unresolved multicomponent mixtures into factors for single species. [14-16]

Examples of these techniques involve evolving factor analysis (EFA)[17-18]alternating least squares (ALS) [19-21], heuristic evolving latent projections (HELP) [22-23], simple to use interactive self-modeling mixture analysis (SIMPLISMA)[24-25], window factor analysis (WFA) [26], orthogonal projection analysis (OPA)[27-28], and parallel vector analysis (PVA) [29]. Compared with curve-fitting methods, such as the Gaussian–Lorentzian function and polynomial or linear least squares procedures, SMCR, in principle, does not need to model the recorded data in order to resolve pure variables. Among other things, SMCR requires some generic knowledge about the pure variables, such as non-negativity, unimodality, and closure of the concentration or spectral profiles. Furthermore, SMCR has sometimes experienced difficulties in yielding sufficient resolution performances. One of the main reasons stems from the poor initial estimate of pure variables. SMCR procedure starts with the estimation of initial pure variables, such as concentration profile and pure spectra. [16]

Nowadays, Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) has become a popular chemometric method used for the resolution of multiple component responses in unknown unresolved mixtures. MCR-ALS is a method that solves the MCR using a constrained ALS algorithm [10, 30-31]. The flexibility in 'where-and-how' applying constraints and the capability to treat the most diverse multi-set structures are the main assets of this method. However it requires the proper selection and application of the constraints that are really fulfilled by the data set and the ability to predict how to design and to deal with the most informative multi-set structures. Moreover, the correct performance of any curve-resolution (CR) method depends strongly on the complexity of the multicomponent system [10].

Spectroscopy curve resolution is very suitable, since it may save time, money and ecological costs.

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IV. COLOR

IV1. Introduction.

Color can be defined as the sensation produced by light rays that strike the visual organs and depends on the wavelength. So, the color is an attribute of things that results from the light they reflect, transmit, or emit in so far as this light causes a visual sensation that depends on its wavelengths. The science and technology used to quantify and describe physically the human color perception is known as ***Colorimetry.***

IV2. Colorimetry.

There is no doubt that, in the first place, consumers judge foods according to their external appearance (color, texture, and so on), so this first assessment is going to influence decisively their choices. This is partly due because the color of foods in general is frequently related to their stage of maturity, the presence of contaminants or microorganisms, the conditions of the industrial processing and and other characteristics of foods [1-2].

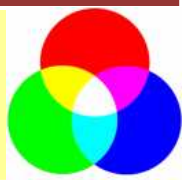
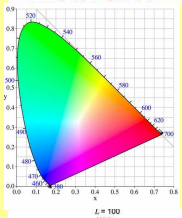
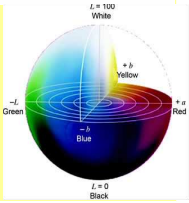
One of the main advantages of the objective measurement of color is that several parameters can be obtained in a matter of seconds; so these color assessments can be very appropriate to obtain a rapid estimation of the effects of different commercial practices [1]. Therefore, the analysis of the pigment responsible for color is of great importance, since it can be used to prove the authenticity and quality of food [3].

The human eye has photoreceptors (called **cone cells**) for medium- and high-brightness color vision, with sensitivity peaks in short (*S*, 420–440 nm), middle (*M*, 530–540 nm), and long (*L*, 560–580 nm). Thus, in principle, three parameters describe a color sensation: "The **tristimulus values** of a color" that can be defined as the amounts of three primary colors in a three-component additive color model needed to match that test color. Any specific method for associating tristimulus values with each color is called a color space. The main color spaces are represented in Table IV.1.

Nowadays the description of the color is expressed numerically according to the chromatic coordinates a^* and b^*, the lightness L^*, and the chroma C of the CIE$L^*a^*b^*$ standardized system of the International Commission on Illumination [4].

This has led to the establishment of a maturity index based on measurements of color and other physicochemical properties for some foods. Similarly, a color index has been developed as a formula based on chromatic coordinates [3].

Table IV.1

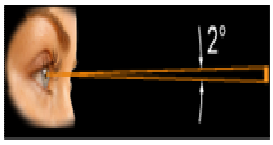
Color Space	Definition	Color diagram
CIE RGB	Any color (source C) can be matched by a linear combination of three other colors (primaries Red, Green, Blue), provided that none of those three can be matched by a combination of the other two" C = Rc(R) + Gc(G) + Bc(B)	
CIE XYZ (1931)	It translated the RGB tristimulus values into a different set of all positive tristimulus values, XYZ called ideal primary colors: C= X+Y+Z	
CIE LAB	The CIE L*a*b* color space is based on the concept that colors can be considered as combinations of red and yellow, red and blue, green and yellow, and green and blue.	

IV21. CIE LAB system.

The 1976 CIE L*a*b* Space is the one of the most overspread method for measuring color objectively. According to the International Commission on Illumination CIE color model was developed to be completely independent of any device or other means of emission or reproduction and is based as closely as possible on how humans perceive color. In order to eliminate this variable, the CIE defined 2 key elements to describe the color:

- ***Standard (colorimetric) observer***. Regarding the observer it can be distinguished between the original 1931 specification and a revised 1964 specification.) The difference between the 1931 and 1964 standard observers was the field of vision used to view the screens.

  - The 1931 observer had a 2° field of vision (i.e., the amount taken in by the fovea alone). This was later considered inadequate in many cases since it did not take in enough of the observer's peripheral vision.



- The 1964 specification widened the observer's field of vision to 10° in order to get tristimulus values that reflect a wider retinal sensitivity (Stiles and Burch and Speranskaya [5]).

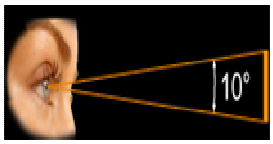


Fig. IV1. 2° and 10°field of vision *http://www.binder-muc.de (2010).*

❖ ***Standard sources :***

Table IV.2 Standard sources:

Illuminants	*Simulates to :*	*Color Tª (ºk) of:*
A	A tungsten-filament lamp	2856
C	Average daylight	6750
D65	Natural daylight	6500
F2	White fluorescent lamp	4200
F7	Fluorescent lamp>> daylight	6500

**Illuminants are not physical sources; they are models of light defined by a spectral power distribution*

CIE L*a*b* defines colors more closely to the human color perception; this system is often used in the quality control of colored products. For instance, once the color of a production sample is located within the CIE L*a*b* color space, it is compared to the color quality control production standard. Color differences between the production sample and the standard are then determined and compared to predetermined acceptance tolerances. The CIE L*a*b* color space is based on the concept that colors can be considered as combinations of red and yellow, red and blue, green and yellow, and green and blue. To determine the exact combination of colors of a product, coordinates of a three dimensional color space are assigned. The three colors coordinate:

- ***L* – the lightness coordinate***

The L* coordinate of an object is the lightness intensity as measured on a scale from 0 to 100, where 0 represents black and 100 represents white. It is the central vertical axis. **L** represents a type of **"psychometric brightness"** (or lightness).

- ***a* – the red/green coordinate***

The a* coordinate of an object represents the position of the object's color on a pure green and pure red scale, where -127 represents pure green and +127 represents pure red.

- ***b* – the yellow/blue coordinate***

The b* the coordinate represents the position of the object's color on a pure blue and pure yellow scale, where -127 represents pure blue and +127 represents pure yellow.

These color values have no dimension, they are just units. They describe an exact position in the CIELAB color space. The color coordinates describe the color in the color space for a specific light source and a specific observer (e.g. D65/10°).

The resultant CIE L*a*b* diagram is sometimes also called the "Psychometric Color Diagram". The color axes are based on the fact that a color cannot be both red and green, or both blue and yellow, because these colors oppose each other. Therefore, values are only needed for two color axes and for the lightness or gray scale axis (L*), which is separate (unlike in RGB, CMY or XYZ where lightness depends on relative amounts of the three color channels).

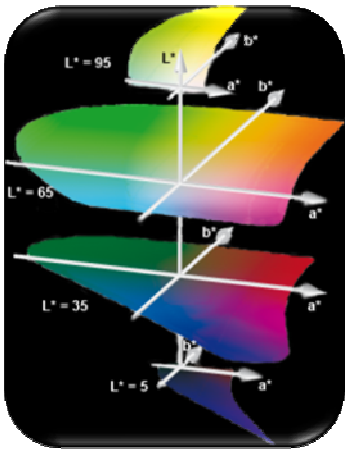


Fig. IV2. Psychometric Colour Diagram *http://www.binder-muc.de (2010).*

The orthogonal L*a*b* color system is easy to use, but not always corresponding to our perception. If we have for e.g. two orange colors where one is stronger than the other one, we will never describe the color distance as redder and more yellow, but it is stronger or weaker.

For this reason **a polar coordinate system** can be used to describe the color in the CIELAB color space.

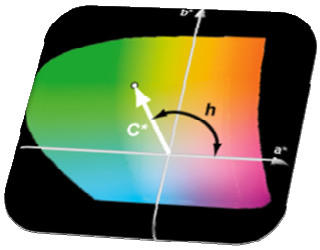


Fig. IV3 a polar coordinate system *http://www.binder-muc.de (2010).*

The chroma is represented by the letter C*, h describes the hue and as in the orthogonal system L* is the value for the lightness. The transformation of (a *, b *) to (C *, h) is given by:

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}}$$
(chroma, as radius vector);
$$h_{ab} = \text{Arctan}\,\frac{b^*}{a^*}$$
(hue angle, as polar angle).

No matter which system is used, L*a*b* or L*C*h, it is just a different way of the description how to get to a position in the CIELAB color space. The recommendation is, that for neutral colors (C*<10) the L*a*b*-system is used, because they are really redder, greener, more yellow or bluer. For higher chroma the L*C*h-system is the better choice.

IV22. Applications.

The information involved in the visible spectra can be very high. A way to reduce such amount of information is by transformation of absorbance values in color measurements. Visible transmittance or absorbance measurements allow color to be quantified by calculating the chromatic coordinates of the sample concerned in a CIE-1976 color system such as CIE-L*a*b* [6] this entails using as large a number of spectral data as possible (i.e. measuring the absorbance or transmittance at short wavelength intervals) in order to minimize errors. J. Ayuso et al. [7] developed software in order to obtain the chromatic parameters according to the CIE lab system, from the visible absorbance values.

Once the chromatic coordinates are calculated, the samples can be plotted in the color space. The way the samples are distributed in that color space can give very interesting information. Various studies of food color characterization based on the chromatic coordinates can be found in the literature. Escolar et al. [8] studied the relationship be tween a number of transmittances values measured and the color characterization of olive oil based on CIE 1931, CIELUV and CIELAB 1976 spaces. The same author developed a method to study the decolorizing effects of different quantities of activated carbon adsorbent on the color of sherry samples by means of chromatic parameters [9]. Color characterization make possible link chromatic coordinates to other physic –chemical processes. However, apart from the color characterization the CIELAB system yield the concentration of a solution quickly and easily through the usage of the calibration line for chromatic parameters [10]. Chromatic coordinates are useful to define the color of the products, but also to estimate the content of pigments, such as carotenes and chlorophylls in oil, which is very interesting and can be applied in quality control and other situations [11]. The function for representing the boundaries of the color space for virgin olive oil in the CIE 1976 color diagram has been already developed by Escolar et al [6]. By establishing the color space occupied by every possible variety of virgin olive oil or extra virgin olive allows one to define the entire acceptable color range that those oils can span. Therefore if the chromatic coordinates for a given oil sample fall outside the color space the sample should be studied deeper or rejected. Similar color spaces can be established for other food fit for human consumption by using the same procedure.

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V. CHEMOMETRICS

V1. Introduction.

In recent decades, there has been an exponential increase in the availability of analytical instrumentation capable of acquiring huge amounts of data in a short time and, as a consequence, the need for advanced and efficient strategies for the analysis of the data has emerged.

Chemometrics is considered as part of analytical chemistry and it has been applied to different purposes: chemical analysis, safe research, nutrition, pharmaceutical development, environment … In its arsenal we can find methods that help analytical chemists to deal with all steps of analytical procedures, starting from the design of an experiment to extraction of information and to the final decision making [1]. A majority of chemometrics methods are general, this means, they can be applied to any type of analytical experiment and to any type of instrumental signal. However, there are problems associated with the specific types of instrumental signals or with the particular analytical techniques that need special treatment. Only with the knowledge of the system studied and of the principles of the performed measurements, the well suited methods can be chosen.

Chemometric techniques applied to analytical data have proved to be important tools in food analysis, because they can be used for exploratory analysis and classification [2]. Multivariate statistical analysis has been used to recognize the parameters able to discriminate the food varieties [3-4]. Depending on the requirement of the analyses and the nature of the information, these data can be analyzed by techniques based on linear (principal component analysis, multivariable regression techniques, etc) or nonlinear algorithms in more complex situations. Among multivariate statistical analysis, principal component analysis (PCA), cluster analysis (CA) and linear discriminant analysis (LDA) occupy a very important position.

V2. Principal component Analysis.

Exploratory data analysis such as PCA is used primarily to determine general relationships between data. Principal component analysis (PCA) is a statistical tool commonly used for classification of data. The main aim of PCA is to reduce a large number of variables into new, uncorrelated variables called principal components (PCs) that capture the vast majority of variance in the data. PCA is a projection and dimension reduction method that reduces the

dimensionality of the data considerably, enabling effective visualization, regression and classification of multivariate data [5].

The starting point is a data matrix consisting of "n" rows of samples and "m" columns of variables, called an n x m matrix. Each principal component is a linear combination of the original measurement variables. It is important to recognize that the aim of PCA involves finding mathematical functions which contain certain properties which can then be related to chemical factors, and in themselves PCs are simply abstract mathematical entities. The original data are said to be mathematically modeled by the PCs. These Pcs can have important structural information by themselves as can be seen in fig. V1.

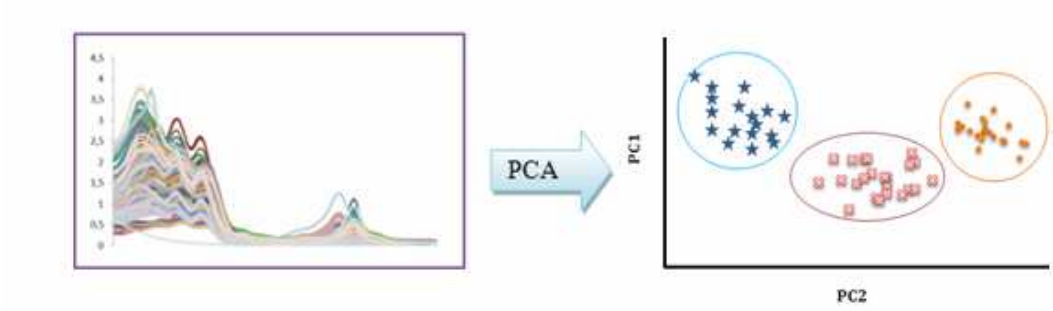

Fig. V1. The first figure displays a body of spectra of a system. By projecting the PCA according to PCs a classification is obtained.

V3. Cluster analysis (CA):

Clustering techniques are used frequently in chemistry to show and to interpret similarities or dissimilarities between objects. Clustering can be applied also to variables, to find groups of similar variables, frequently with the objective of variable selection for calibration techniques. The results of a clustering technique are generally reported in a plot (the dendrogram of similarities/dissimilarities) where the ordinate is the similarity between groups and the abscissa has no specific meaning, but it is used only to separate the clusters. Hierarchical agglomerative (HCA) methods are the most popular [6]. They are based on the similarity between two objects (or between two clusters), given by equation:

$$s_{ij} = 1 - \frac{d_{ij}}{d_{\max}}$$

where *dij* is the distance between the two objects and *d*max the maximum distance between two objects in the dataset.

The two objects with the maximum distance have similarity 0. The distance between two objects depends on the used metric. These distances can be based on a single dimension or multiple dimensions, with each dimension representing a rule or condition for grouping objects, see table V1. Hierarchic agglomerative techniques start from as many clusters as objects are. Gradually objects are joined into clusters, up to the final cluster with all the objects. In each step, two objects or one object and one cluster or two clusters are merged. In the first step, the two objects with the largest similarity are merged. Then in each step, the two most similar clusters are merged. The value of their similarity (or of their distance) is retained. It will be used to draw the typical result of these techniques, the dendrogram.

With N objects, the final cluster is obtained after $(N - 1)$ steps. The hierarchy is a consequence of the fact that larger clusters are always obtained by the merger of smaller ones (with all their objects). In chemometrics, the chemical significance of a cluster is based on the possibility of interpretation. Interpretation means that we compare the clusters suggested by the dendrogram with external information, not used to compute the dendrogram. Frequently, the real problem suggests that some categories exist and the interpretation step is to compare the number and the composition of the clusters with these categories. The category index is a discrete variable. In other cases the interpretation uses one or more continuous variables.

Table V1.

Distance Function	Equation
Euclidean distance	$\text{Distance}(x, y) = \{\Sigma_i (x_i - y_i)^2\}^{1/2}$
City-block (Manhattan distance)	$\text{Distance}(x,y) = \Sigma_i \lvert x_i - y_i \rvert$
Chebychev distance	$\text{Distance}(x,y) = \text{Maximum} \lvert x_i - y_i \rvert$
Power distance	$\text{Distance}(x, y) = (\Sigma_i \lvert x_i - y_i \rvert^p)^{1/r}$

V4. Linear discriminant analysis a (LDA):

Linear discriminant analysis is a technique for classifying a set of observations into predefined classes. The purpose is to determine the class of an observation based on a set of variables known as predictors or input variables. The model is built based on a set of observations for which the classes are known. This set of observations is sometimes referred to as the training set. Based on the training set, the technique constructs a set of linear functions of the predictors, known as discriminant functions. The aim is to analyze which are the variables that contribute most to discrimination between subjects in the different groups established a priori [7]. To do this most discriminating variables are reduced to canonical variables that are a linear combination of independent originals variables. This linear

combination is the discriminant function, where the dependent variable is membership in either group.

In particular, LDA is used to find a linear combination of variables which characterize or separate two or more classes of objects or events. See in fig.V2 a LDA plot. The resulting combination may be used as a linear classifier or, more commonly, for dimensionality reduction before later classification [8]. A linear function of the variables is sought, which maximizes the ratio of between-class variance and minimizes the ratio of within-class variance. LDA is closely related to ANOVA (analysis of variance) and regression analysis, which also attempt to express one dependent variable as a linear combination of other features or measurements. In the other two methods however, the dependent variable is a numerical quantity, while for LDA it is a categorical variable (i.e. the class label). Discriminant analysis can be used only for classification but not for regression.

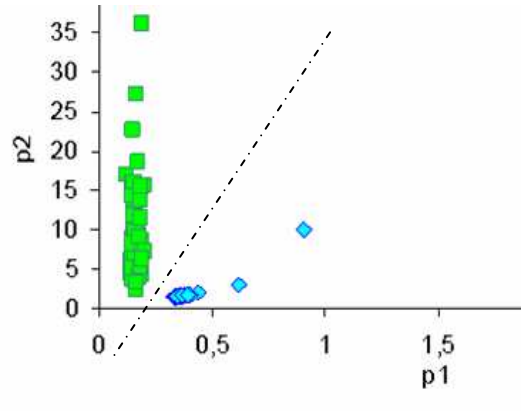

Fig. V2. Linear classification for several oil samples.

V5. References.

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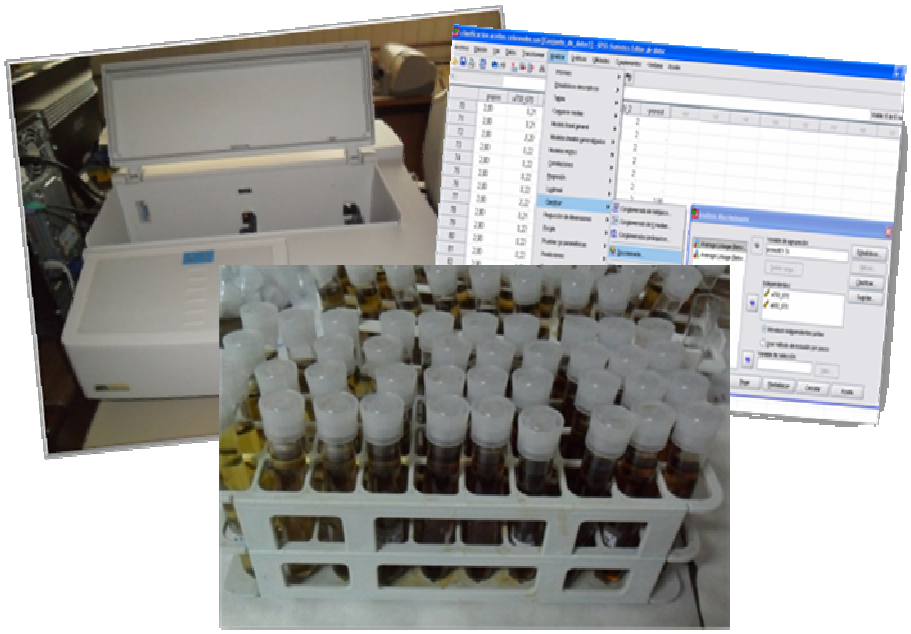

Materials and Methods

VI. Olive oil adulteration study

VI1. Samples.

The first study was carried out on a set of 81 of virgin olive oil and extra virgin olive oils, VOO-EVOO, 3 high oleic sunflower oils, 1 seed oil and 1 rapeseed oil. All of them were purchased at different market in the area.

Four commercial food colorants were used: Lutein (E-161b), β-carotene (E-160a), copper complexes of chlorophylls (E-141i) and magnesium complexes of chlorophylls. The oil samples were obtained from freshly opened packages, which, however, were also used to fill five topaz bottles of which three were stored frozen for use as reference and in other types of tests, and the other two refrigerated for subsequent checks.

VI2. Spectra.

The spectra were obtained with an ATI Unicam UV4 spectrophotometer connected to PC computer. Olive oils spectra data were obtained from pure, undiluted samples. The spectroscopic cells used were largely of the Dispolab Kartell 1937 disposable polystyrene type and 10 mm thick. Spectra of all samples were obtained by measuring the absorbance spectra in the range of 350-770nm by 1 nm interval. All spectra were obtained with an empty cell in the reference beams the same cell for all samples that was also used to record baselines. With such a convenient reference, the spectra exhibited a negative absorbance of less than -0.03 AU in the zone from 750 to 770 nm; therefore, the absorbance obtained with it were all corrected in order to avoid negative values in the visible range. The spectrum for each sample was recorded at least 3-4 times in order to minimize the effect of operational errors and changes in sample condition. The differences between the records of the same sample were always very similar to the baseline. The highest noise level encountered was 1 $x10^{-3}$ AU, and the mean square noise level was less than 0.5 $x10^{-3}$ AU.

VI3. Color.

The color of pure liquid samples was determined from spectral data and characterized in terms of CIE $L^*a^*b^*$ chromatic coordinates by using illuminant D65 with the 10° supplementary observer. The calculation of the chromatic parameters as it is specified by CIE was performed using a computer program developed in our laboratory [1] on the basis of absorbance values from the entire visible spectrum.

VI4. Experimental procedure.

In order to imitate VOO, with an alleged fraudulent purpose, vegetable oils were colored with one of the commercial colorants: Lutein (E-161b), β-carotene (E-160a), copper complexes of chlorophylls (E-141i) and magnesium complexes of chlorophylls. 146 seed oil samples were colored as follows: 16 seed oil samples were colored with different amounts of Lutein and Magnesium Chlorophyll, 66 seed oil samples colored with different amounts of β-carotene and Magnesium Chlorophyll, 21 seed oil samples colored with different amounts of Lutein and Cupper Chlorophyll and 43 seed oil samples were colored with different amounts of β-carotene and Cupper Chlorophyll. From here, the latter two will refer briefly as Cu-*Chl* and Mg-*Chl*, respectively. The reason for using these colorants is that the color of VOO is due to two groups of natural colorants: chlorophylls and carotenoids, Ayuso [2] Lutein and β-carotene are the red-orange pigments most abundant in VOO and are very scarce in high oleic vegetable oils.

In order to prepare the colored oil, the oils were stirred during 12 hours with the colorants.

VII. Curve resolution study

VII1. Samples.

The second study was carried out on 3 different sets:

- "Validation set" formed by 79 commercially available samples of VOO most of them extra grade from a variety of origins in Spain, Greek, Italy and Portugal. The samples spanned a variety of colors (i.e., a wide range of pigment compositions). Most were mixtures of oils from different olive varieties. Also, those marketed as single-variety had actually been obtained from mixtures of the same olive variety but in different degrees of ripeness. Spectra were obtained from freshly opened packages, which, however, were also used to fill five topaz bottles of which three were stored frozen for use as reference and in other types of tests, and the other two refrigerated for subsequent checks.

- Ni^{2+}, Co^{2+} and Cu^{2+} set, formed by 3 samples with the following ratios of concentrations Ni $(NO_3)_2$, Co $(NO_3)_2$ and Cu $(NO_3)_2$, 1:1:2, 1:2:1, and 2:1:1 respectively.

- Data set composed of 108 commercially available oil samples: 81 VOO-EVOO samples, 17 olive oil samples and 10 pomace olive oil samples.

VII2. Spectra.

Spectra were recorded as it is specified in VI2. Spectra of ionic solutions were run by measuring the absorbance spectra in the range of 350-900 nm by 1 nm intervals.

VIII. Sherry wine vinegar study

VIII1. Samples.

86 samples obtained from different Sherry vinagers Denomination of Origin, and produced by different methods of acetifications analyzed. All vinegar samples were taken from local winery in Jerez, the last February by a specific sampling procedure done by the group.

The samples were divided in different categories, according to wine variety (Palomino, Pedro Ximénez and Moscatel), according to acetification method (industrial or traditional in oak barrel), and according to the size of the wood oak barrel and the level in barrel´s stack. All the barrels which were sampled had a capacity of 500 L, except the ones used for the volume comparison study and from each sampled oaks were taken nearly 50 ml.

VIII2. Sampling.

All samples are "Reserva" vinegars, i.e., more than 2 years aged in oak barrels. They were taken from different buildings, different positions, and different starting grape/wine and different barrel volumes.

Fig.VIII1 Sampling rooms: room 1(upper left), room 2 (upper left), 3 (bottom left) and 4 (bottom right).

According to this, the samples were name as a code of 6 characters: *AABCCD*

- AA: Grape Variety: PF (Palomino Fino), MO (Moscatel),or PX (Pedro Ximénez).
- B: Room number: 1-4.
- CC: Acetification Process: OB (Oak barrel), IA (Industrial acetification).
- D: Barrel row level.

Room 1: Traditional acetic fermentation in barrels. Grape variety: Palomino Fino

- 11 samples from the 11 small oak barrels (250 L), located at 5th level, coded as PF1OB5.
- 12 samples from the 12 regular (500 L) oaks situated at level 4th, coded as PF1OB4.
- 15 samples from the solera (1st level) oak barrels coded as PF1OB1.

Room 2: Traditional acetic fermentation in barrels. Grape variety: Palomino Fino

- 6 samples from the 1st level coded as PF2OB1.
- 6 samples from the 2nd level coded as PF2OB2.

Room 3: Traditional acetic fermentation in barrels. Grape variety: Moscatel and Pedro Ximénez

- 6 samples from the 1st level coded as MO3OB1.
- 6 samples from the 2nd level coded as MO3OB2.
- 6 samples from the 1st level coded as PX3OB1.
- 6 samples from the 2nd level coded as PX3OB2.

Room 4: Industrial acetic fermentation in stainless steel deposits:

- 6 samples from the 1st level, coded as PF4IA1.
- 6 samples from the 2nd level, coded as PF4IA2.

VIII3. Spectra:

Spectral data were obtained from pure samples, using 1-cm-thick disposable polystyrene cells and an ATI Unicam UV4 interfaced to a PC computer. 20 ml from vinegar samples were filtered by using 0.45 μm filters before being subjected to UV-Vis spectrophotometer, in order to reduce turbidity and likely impurities. Spectra of all samples were obtained by measuring the absorbance spectra in the range of 360-800 nm by 1 nm intervals.

VIII4. Color:

The color of vinegar samples was determined from spectral data and characterized in terms of *CIE L*a*b** and *CIE L*C** chromatic coordinates by using illuminant D65 with the 10° supplementary observer and *CIE 31*. Once again, the calculation of the chromatic parameters was performed using a computer program developed in our laboratory [3] on the basis of absorbance values from the entire visible spectrum.

IX. Equipements, chemicals and softwares

IX1. Equipement.

- ATI Unicam UV4 spectrophotometer connected to and controlled by a personal computer.
- Polystyrene disposable cells of 1 cm thickness. (Dispolab 1937; Kartell, Noviglio, Italy).
- Multigrade Glassfiber Nylon 0.45µm filters.
- 10 ml Plastics seringes without needle.
- 50 ml BD Falcon® Conical-Bottom Disposable Plastic Tubes.

IX2. Chemicals and reagents.

- Commercial food colorants: Lutein (E-161b), β-carotene (E-160a) from Sigma-Aldrich, and copper complexes of chlorophylls (E-141i) and magnesium complexes of chlorophylls from Soquiber-Lab. Cosp, S.A.
- Reactives from Aldrich p.a >99%.

IX3. Softwares.

- Microsoft Excel 2010 .
- SPSS 17.0 [3] (SPSS Inc., Chicago, IL, USA) software.
- Vision standard software version 3.5© Unicam.
- Spectrocolor software designed by research group [1].

IX4. References.

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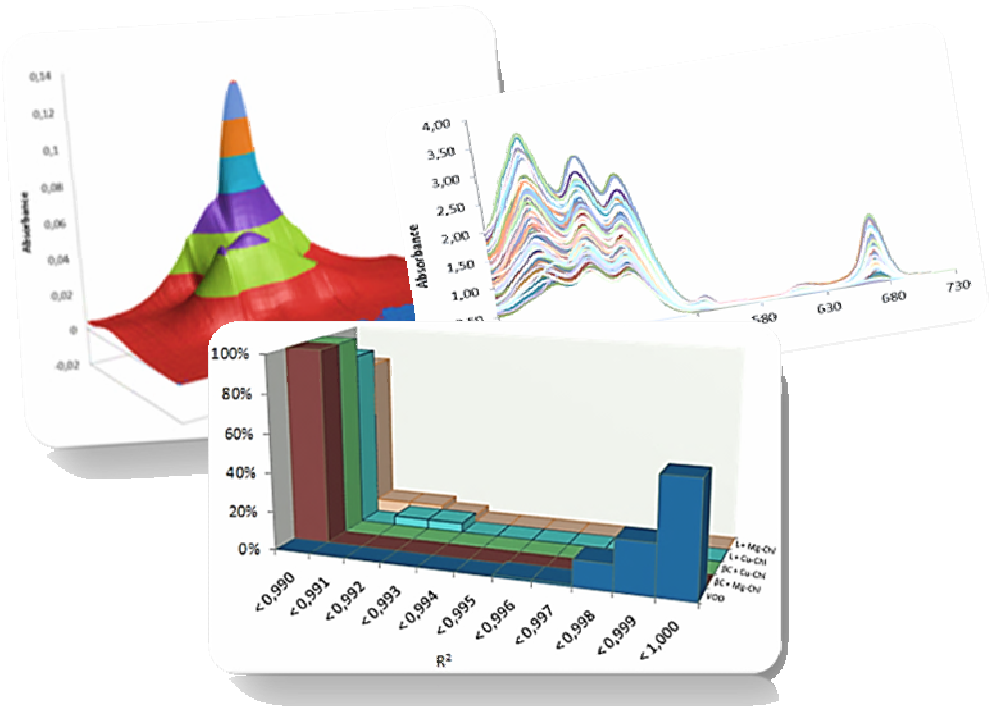

Results and discussion

X. Olive oil adulteration Study:

Chlorophyll pigments added to vegetable oils in order to simulate a better quality:

A rapid method of identification.

X1. Introduction.

The adulteration of olive oil is not only an economic fraud but can also be damaging to our health. Most of the conventional methods such as chromatographic and mass spectrometry or HPLC are expensive, destructive, time-consuming, require skilled operators and have a high environmental impact. Therefore, this work proposes a fast, economic and simple alternative for determination of adulterations with high oleic vegetable oils (rapeseed, sunflower and seed) colored with commercial pigments. The work was carried out based on the group experience in the study of UV-Vis spectra of pigments in olive oils, Escolar [1] and Ayuso [2]. In addition, this technique does not require tedious sample preparation nor economic and ecological cost in organic solvents.

It will be presented a justification of how these colorants are very well to simulate VOO-EVOO by comparing the global color space of colored seed oil with the global color space of the VOO-EVOO. Then, two ways to discern the colored seed oils from VOO-EVOO are presented. The first way is achieved by using the determination coefficient, R^2, of the spectra for mimic VOO correlated to the spectra for the colored seed oil. This method will allow to distinguish between seed oils colored with Cu-Chl from those colored with Mg-Chl. The second way is done by using two spectroscopic parameters that can discriminate between VOO-EVOO, colored seed oils with Cu-Chl and colored seed oils with Mg-Chl, at the same time.

Due to the satisfactory results this study will be presented *in the technical – scientific symposiums Expoliva, XV interantional fair of olive oil and allied industries* that will takes place in Jaén, Andalusia.

X2. Results and discussion.

Speaking in visual terms the consumer can be easily confused if food colorants which are similar to the VOO-EVOO pigments are added to a nearly colorless oil seeds (green or pale yellow). But not all colorants are equally good at mimicking realistic colors Fig. X1 shows the normalized visible spectra for 4 colorants: β-carotene and lutein as carotenoid pigments, and Mg-Chl or Cu-Chl as chlorophylls pigments. They were chosen because their visible spectra are

similar to the visible spectra for real pigments found in VOO-EVOO. Also the amounts of the colorants must be adequate in order to get a good falsification.

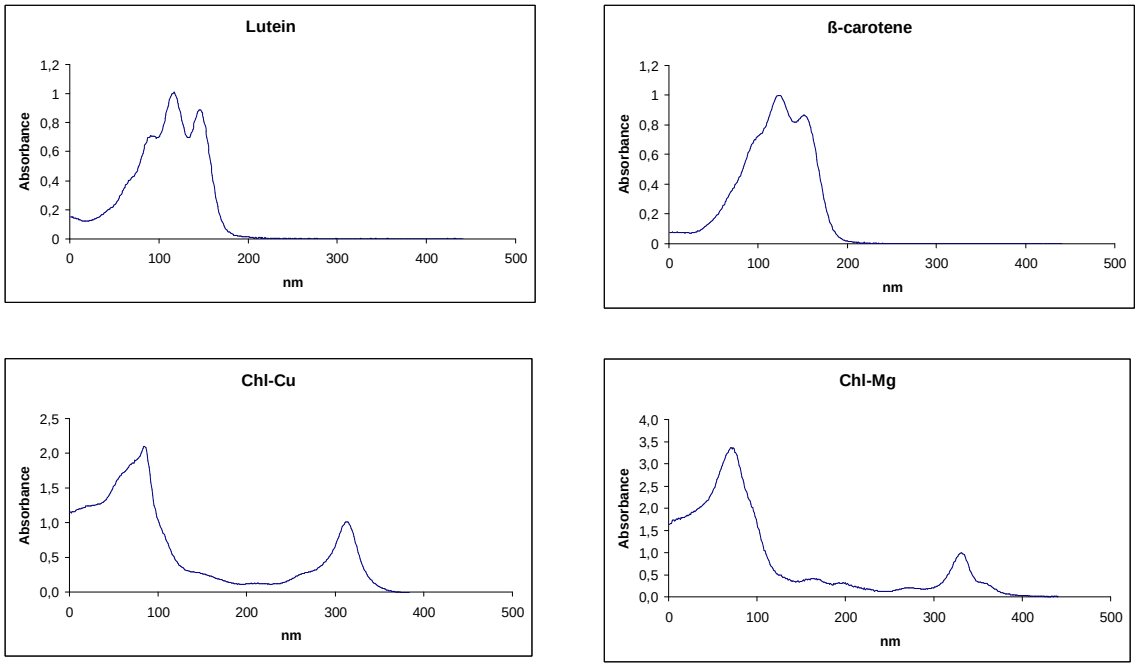

Fig. X1: Visible spectra for carotenoids and chlorophylls pigments. Lutein and β-Caroteno spectra are normalized at its maximum at λ= 460 nm. Cu-Chl and Mg-Chl are normalized at the maximum of the band at λ=670nm.

The same procedure described by Escolar et al. [1] regarding the obtaining of the whole color space for VOO-EVOO was used for our case. Thereby, CIE L*a*b* color spaces for the different combinations of the four colorants when added to sunflower oil, were obtained. The

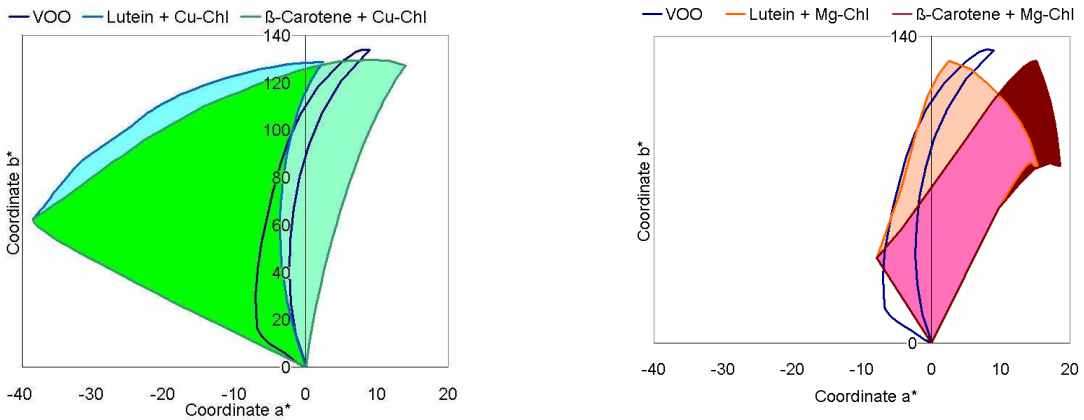

Fig. X2. Color space for VOO-EVOO oil samples (dark blue) and for sunflower oil colored with the 4 different combinations of chlorophyll and carotenoid pigments. Lutein+Cu-*Chl* (light blue), β-caroteno+Cu-*Chl* (green), Lutein +Mg-*Chl* (orange) y β-caroteno+Mg-*Chl* (violet).Illuminant D65 and 10 ° standard observer.

resulting color spaces are combinations of one carotenoid pigment (lutein and beta-carotene) and one chlorophyll pigment (Mg-Chl or Cu-Chl) added to free- pigment vegetable oil. Four different combinations are possible when these four colorants are combining in pairs: β-carotene+Cu-*Chl*, β-carotene+Mg-*Chl*, Lutein+Cu-*Chl* and Lutein+Mg-*Chl.*. Every color space was calculated by using 49 spectra, each one generated by adding different amounts of the colorant spectra. Dark green on fig.X2a and pink on fig.X2b represents the area where the color spaces overlap. In order to do comparison studies, color space for the virgin olive oil was also displayed in fig.X2 and fig.X3.

Fig. X3 shows an alternative representation for color spaces by using L* coordinate and C* parameter (Chroma= $(a^2 + b^2)^{1/2}$). Notice how the four color spaces overlap with almost the whole color space for VOO-EVOO. As far as choosing appropriate quantities of the mentioned colorants, sunflower oil can mimic VOO. On fig.X3 chromatic parameters for 146 imitations VOO samples made from several high-oleic canola oil, sunflower oil, high oleic sunflower oil and high oleic seed oil that have been colored in our laboratory are represented. As it can be noticed most of the fake samples fall within the color space for VOO-EVOO.

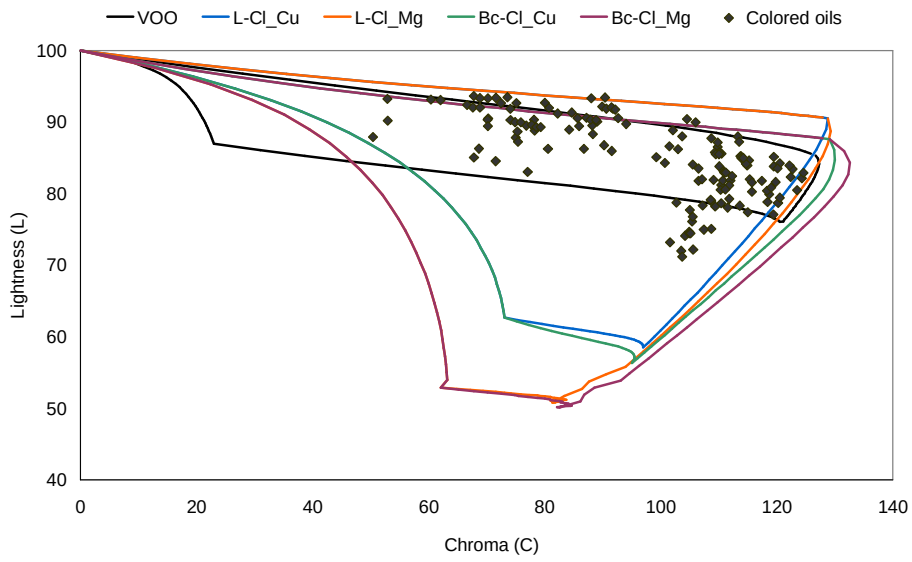

Fig. X3 L* and C* color space for colored oil obtained with the illuminant D65 and 10°.

The combinations of ß-carotene with Cu-Chl or lutein with Mg-Chl are those that overlap the color space for VOO–EVOO the most. However, according to fig.X2 the color space for Cu-Chl falls further from the VOO-EVOO space than Mg-Chl. Consequently, the latter would be a more effective green colorant when looking for a counterfeit based on the color. Nevertheless, fig.X2 shows how low quality oils cannot be easily distinguished from VOO-EVOO if they have been colored with similar colorants.

In spite of the color space that may vary when different oil or colorants from another source of origin are used, the boundaries of such spaces will not differ so significantly.

In order to detect colored high-oleic vegetable oils, two procedures have been developed. The first one is based on the linear fitting between real spectrum and simulated spectrum obtained through the combination of VOO-EVOO pigments isolated by photodegradation by Ayuso et al. [2]. According to Ayuso, the correlation measured by the coefficient of determination obtained in the linear least-squares fitting of the real UV-Vis spectrum for a VOO-EVOO versus simulated spectrum using carotenoids and chlorophyll as a base, was always greater than 0.995. Based on this study, it was proposed to usage of this coefficient of determination as an indicator to discern VOO-EVOO in a quick and effective way.

Fig.X4 shows the histogram as a percentage of the R^2 for 81 VOO-EVOO samples and 146 colored oils samples with the four colorants versus their simulated spectra respectively. The coefficient of determination for real and simulated spectra for VOO-EVOO samples is indeed higher than 0.995. On the contrary, real and simulated spectra for colored oils showed lower R^2 values, usually less than 0.990; therefore this procedure can be effectively used to distinguish between real VOO-EVOO and fake colored seed oil.

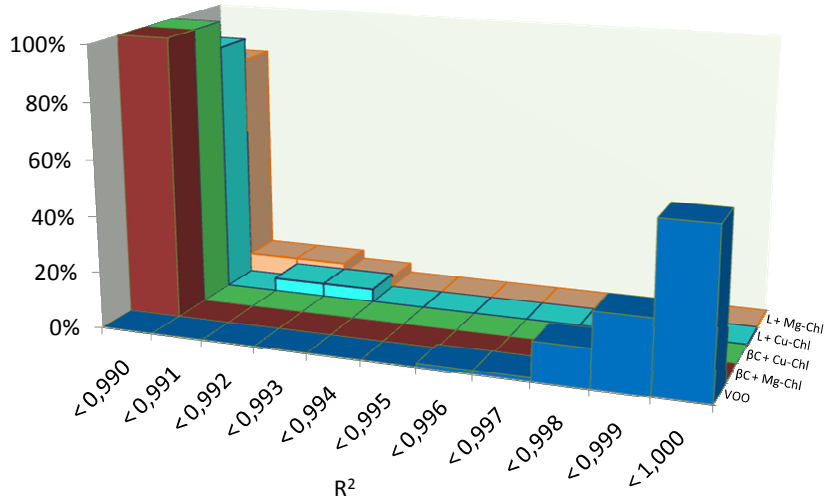

Fig.X4: Histogram of R^2 values for VOO and colored high oleic vegetal oils.

Despite its usefulness, this procedure is rather limited in terms of its incapacity to distinguish the type of colorant used for coloring. Since the oil used in the forgeries may have a yellowish color, it seems to have a priority in distinguishing between colored oils with Cu-Chl and those colored with Mg-Chl, regardless of whether carotenoid colorant is used or not. In order to do so, we proceed with the development of a procedure based on the visual examination of the visible spectra.

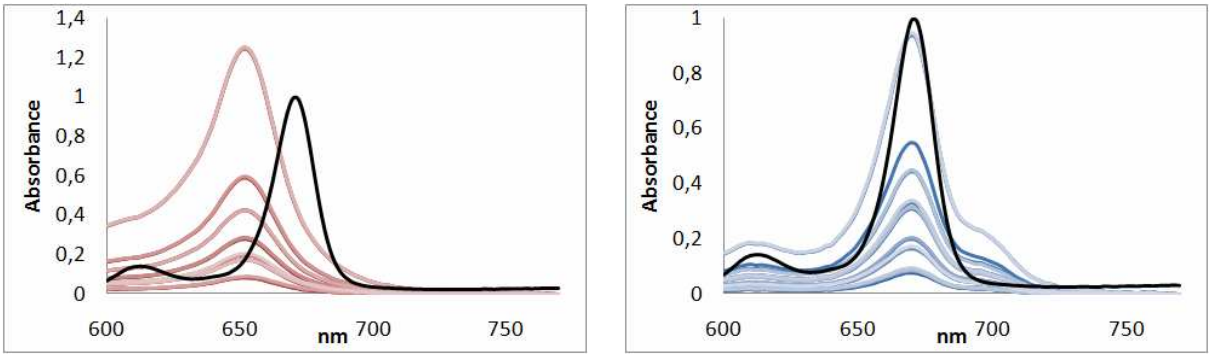

Fig. X5. Spectral range from 600 nm to 770 nm for colored oils: a) with Cu-Chl , b) with Mg-Chl; Spectrum for VOO-VOO is overlapped and highlighter

Fig. X5 shows the spectral range from 600 nm to 770 nm for colored oils with *Cu-Chl* (Fig. X5a) or *Mg-Chl* (Fig. X5b). Black spectrum on fig. X5 represents VOO-EVOO. This spectral range was selected because the spectrum for VOO-EVOO contains the most characteristic band, at approximately 670nm due to chlorophyll pigments. The spectra for oils colored with *Cu-Chl* shows the maximum of its most intensive band at 650 nm, while if they colored with *Mg-Chl,* the colorants with the most intense band overlap with the VOO-EVOO. When *Mg-Chl* colorant is used, the spectrum shows a shoulder at 700 nm. Therefore, the colorant used can be distinguished by measuring the absorbance ratios: A650/A670 and A700/A670. In order to avoid movement of the baseline, the usage of ratios resulting from the subtraction of absorbance values at 650, 670 and 700 nm respectively, and the absorbance at 770 nm where the baseline value is considered null, was more convenient. As other quantitative estimators used in the literature [3], parameters p1 = (A650 - A770) / (A670 - A770) and p2 = (A700 - A770) / (A670 - A770) were also used with the purpose of distinguishing among colored oils with Cu-Chl and/or Mg-Ch on and VOO-EVOO.

In order to test the usefulness of these estimators, HCA (Hierarchical Cluster Analysis) available in SPSS was performed. As the cluster analysis method is based on the assignation of the samples into subsets or clusters with similar values of certain qualifiers parameters, being in this case, *p1* and *p2*, Cosine measure was used in order to determine the similarity between samples of each cluster. On the other hand, the NNS method (Nearest neighbor search) was used to decide the grouping of points in the metric space, in which the distance between two clusters is defined as the lower distance between two samples from different clusters.

The samples used were 146 colored oils and 81 VOO-EVOO colored oils. Fig. X6 shows a dendrogram with the distances at which obtained clusters are combined; This way, a 100%

correct classification of samples into three clusters was obtained; one containing VOO-EVOO, and the other two containing Cu-Chl and Mg-Chl colored oils, respectively.

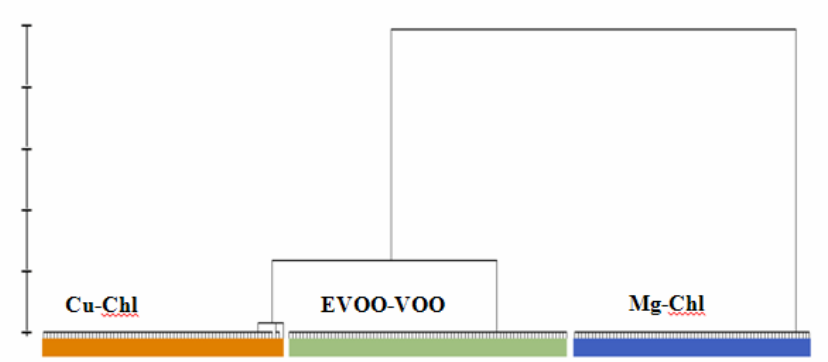

Fig X6. Dendrogram resulting from HCA for the set of VOO-EVOO and colored oils subject of this study. In y-axis is rescaled distance cluster combine.

In order to better visualize the differences among the three groups of samples obtained, a characterization was performed by linear discriminant analysis, LDA, obtaining a classification function to discriminate among EVOO- VOO and other oils (some HOVO, colored with Cu-Chl or Mg-Chl). For this purpose, one third of the data randomly selected was used as calibration set. The obtained results were also successful. The results are graphically shown in Fig.X7, where variables p1 are plotted versus p2. The different samples are classified in zones well separated.

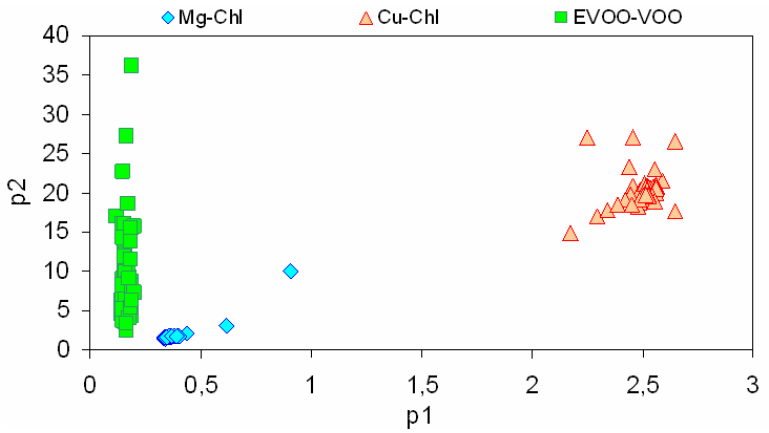

Fig. X7. Results of applying LDA to the oil samples using p1 and p2 as discriminant functions. These parameters allowed correct classification of 100%.

Parameters p1 and p2 were able to distinguish between counterfeit oils Cu-Chl or Mg-Chl. The good results obtained indicate that the methodology could be applied to other many cases of fake oil or even other type of food.

X3. Conclusions.

From a forgery possible point of a view, it has been shown how natural food colorants the same as those found in VOO, such as: β-carotene (E-160a), lutein (E-161b), coloring Cu-Chl (E-141i) and Mg-Chl are excellent to simulate VOO by comparing the global color space of colored seed oil with the global color space of the VOO, not only subjectively but also objectively. The selected colorants are the most difficult ones to detect since they are very similar to the natural pigments. It has been also demonstrated that not all the combinations of colorants are equally as good at imitating the VOO. The best simulations are made with Lutein+Mg-Chl and β-Carotene+Cu-Chl.

In order to achieve realistic colors, the spectra of colored oil and the one that is imitated must be as closely as possible. However, it has been found that such similarity has limitations.

Two ways to discern the colored seed oils from VOO were presented. The first way was achieved by using the determination coefficient, R^2. This value indicates the fitting spectra of mimic VOO with the spectra of the colored seed oil, which shows that, is possible to distinguish between them when seed oils are colored with Cu-Chl or Mg-Chl. The second way was done by studying the spectra of VOO and colored seed oils directly from where two spectroscopic parameters p_1 and p_2 were found to discriminate between VOO, colored seed oils with Cu-Chl and colored seed oils with Mg-Chl at the same time. The use of these parameters has led to an underlying classification.

Finally, the results from the discriminant function were able to discern clearly and precisely between the three colored HOVO oil and VOO by performing a simple UV-visible spectrum.

X4. *REFERENCES*.

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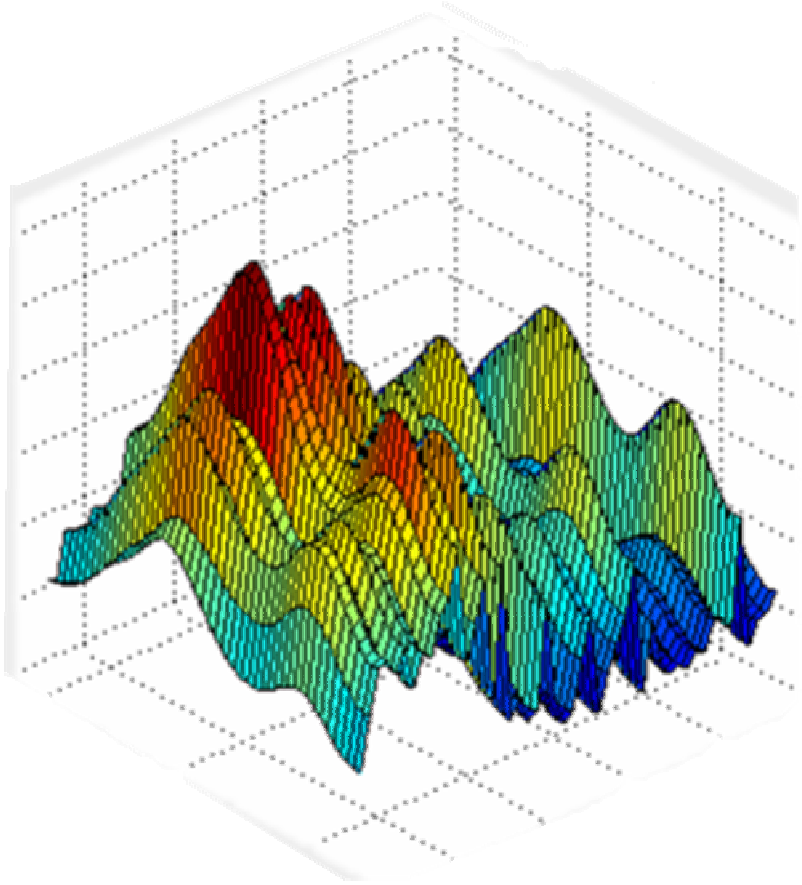
XI. Resolution of spectra study:

Novel method for the resolution of spectra by studying the standard deviations



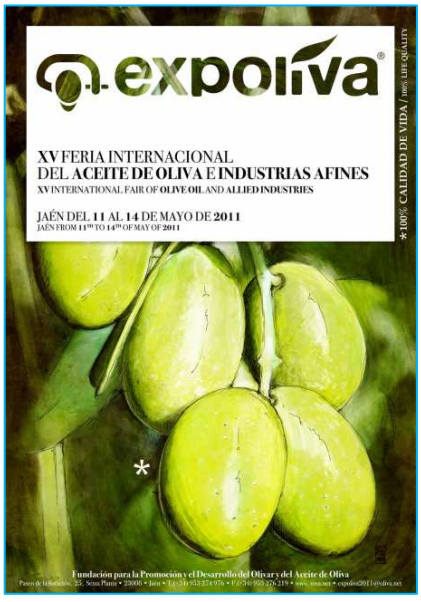
XI1. Introduction.

The aim of this study is to present an alternative method for the resolution of multicomponent system where neither initial estimation, nor constraints are needed. It is an easy and rapid method based on the study of the standard deviation of the spectral data after normalizing procedures.

For a better understanding the method presented in this study was applied and successfully validated into 3 systems. The first one was a set of VOO spectra which is a two-component system. The second system was a set of three components: Ni^{2+}, Co^{2+} and Cu^{2+} solutions, which is a typical example very useful for academic purpose. [1] The last system was also a set of 3 components containing olive oil from different qualities.

In VOO, it is a well-known fact that the spectra can be explained by two groups of pigments: carotenoids and chlorophylls. These two groups are the only ones identified in VOO. In fact, two spectra (of chlorophylls and carotenoids respectively) were already determined in virgin olive oil in another study [2]. It is possible to mimic all spectra for VOO with only these spectra. Therefore, VOO was selected as an illustration purpose for our method. A set of 79 visible spectra from virgin olive oils was used for the validation of the method.

As consequence of the success of the method, the resolution of spectra obtained from the third set was used for a characterization study of oils regarding their quality. This study will be also presented *in the technical – scientific symposiums Expoliva, XV interantional fair of olive oil and allied industries* that will takes place in Jaén, Andalusia.



XI2. Data analysis.

Principal component analysis (PCA) is generally used to determine the number of relevant spectral species present in the data. Very often this is the number of pure components, but intermediates may add to this number. It is also used to reduce the number of data and eliminate irrelevant random variables. We implemented PCA using commercially available software. Other statistical parameters were calculated using a spreadsheet.

The variables used were the visible spectra for all samples and the global spectra matrix was gathered into a matrix of 79 spectra recorded at 196 different wavelengths (D_{196x79}) for the case VOO, and 3 spectra recorded at 569 different wavelengths (D_{569x3}) for the metal ions solutions. The usage of a larger number of absorbance values did not result in any appreciable improvement.

Table XI1 summarizes the results of PCA. As shown in the case of VOO, the first two factors account for more than 99.9% of the information contained in the body of spectra for the samples, whereas the third factor accounts for less than 0.05 of it. Such a low percentage might have been the result of noise in the spectra or, rather, of the truncation of absorbance data, but not of the presence of additional components. Therefore the visible spectrum for VOO can be expressed as the combination of the spectra for 2 of its principal components. On the contrary, in the case of Ni, Co and Cu ions set, the first 3 factors account for 100% of the information contained in the body of spectra. This is because only three ionic solutions were prepared at the laboratory with similar concentrations of the 3 compounds, but the most commonly is having a situation such as that of virgin olive oil.

Component	**Accumulative % of the variance**		
1	99.105	76.631	97.298
2	99.932	95.992	98.860
3	99.962	100.000	99.960
	Case1	Case 2	Case3

Table XI1 Statistical results of PCA. The first column lists the number of potential components; the second shows the accumulative percentage of variance accounted for the factors for VOO set (case 1); the third gives the accumulative percentage of variance accounted for the factors for Ni, Co and Cu ions set (case 2); and the fourth and last gives the accumulative percentage of variance accounted for the

XI3. Theory.

In this work we propose a novel method for curve resolution named "deviation method" that is based on the study of the standard deviations of the data set from the body of spectra. This is detailed in the following sequence. Some steps of the explanation are illustrated with the VOO case. Before describing this method, the global spectral data obtained from experimental must be gathered into a matrix of "n" spectra recorded with "m" values of absorbances at different wavelengths, $D_{n,m}$.

1. ***Determination of components potentially present in the sample, rank.*** If we do not know beforehand we compute a PCA to the whole spectra from the matrix, D. As initial criterion it is selected the number of factors that account for more than 99.9 % of the information contained in the data matrix and that number is the rank of the matrix (k). Based on the bibliography, we know that the rank of VOO is equal to 2. Nevertheless, it is advisable to apply a PCA not only to confirm the rank, but also because in most cases a refinement of the spectra is needed, and to do so we need the PCA functions, or factors, as it is shown in step 5.

2. ***Detection of significant wavelengths***. The spectra are explored in order to detect significant wavelengths where the contribution of one component is distinctive to the others. If there is not too much overlapping, these significant wavelengths can be the peaks of the spectra. As can be seen in fig. X11, λ_1= 670 nm and λ_2= 480 nm are the significant wavelengths in VOO set.

3. ***Normalization of the spectra at a selected wavelength***, λ_i, where" i" varies from 1 to k. With the normalizations of each spectrum at λ_i , the same importance one of the compound is given in all set of spectra. To do so, each spectrum of every sample is divided by its absorbance value at the selected wavelength (λ_i). A new data matrix with the same dimension is obtained where all samples have been scaled and have a value of absorbance of the unity at the selected wavelength (λ_i) due to the normalization process at that point. The figs. X12a and X12b show the spectra after normalizing at λ_1 and λ_2.

4. ***Calculation of the standards deviation of the normalized spectra at the different wavelengths***. The standard deviation, SD, is calculated at each wavelength in whole body of spectrum, in order to annul the spectral profile of the chosen compound,

because where all spectra have the same values, or very similar, the SD is null or nearly. By this way, we eliminate the effect of one of the compounds and emphasize only the bands or profiles due to the rest. Plotting the SD at each wavelength is obtained a new spectrum of SD, denoted deviation-spectrum, where the number of compounds (rank) is k-1, due to the annulled compound. In the case of VOO the initial rank is 2, therefore when we plot the deviation spectra, the rank becomes 1. Basically, the curve resolution for this case is just completed, and the resulting deviation-spectra are shown in figs. X1 3a and X13b.

5. ***Elimination of artifacts***. The procedure described above of transformation of spectra by SD of normalized spectra produce some numerical artifacts. At selected wavelengths where all spectra are normalized, λ_1 and λ_2 , the absorbance values become zeros after applying SD. Therefore, the initial solutions of curve resolution, S_1 and S_2 , undergo this handicap. In order to remove the artifacts caused by the calculations we can refine our deviation-spectra S_i. This refinement is a very easy process. We propose to express the refined deviation-spectra, S^*_i, by linear combination of the principal factors from the PCA previous applied in the 1st step above. Just a simple multilinear fitting of deviation-spectra S_i to the k principal factor is required.

$$\mathbf{S_i(\lambda) = a_i \cdot PC_1(\lambda) + b_i \cdot PC_2(\lambda) + c_i \cdot PC_3(\lambda) + \ldots + z_i \cdot PC_K(\lambda)}$$

where $PC_1(\lambda)$,… $PC_k(\lambda)$, are the spectral profile of a principal factors from PCA and a,b,c…,z are linear coefficients, x.

Once we have obtained the linear coefficients, x, the resulting spectra as refined deviation spectra, $S^*(\lambda)$, can be reproduced by the same equation in (1):

$$\mathbf{(1)} \qquad \mathbf{S_i^*(\lambda) = \Sigma\, x_i\, PC_i(\lambda)}$$

For the case of VOO, just we need to refine the deviation spectrum of the fig X13b, where there is a clear artifact at λ_2 = 480 nm. The refined deviation-spectrum is superimposed in the same fig.

From these refined deviation spectra, the concentrations of each compound in the mixture can be easily calculated. According to the Lambert–Beer Law, the D matrix can be expressed as a product of two matrices:

$$\mathbf{(2)} \qquad \mathbf{D = CS^{*T}}$$

And the estimator of the unknown concentration matrix can be represented as follows:

(3) $\mathbf{C} = (\mathbf{DS^*})(\mathbf{S^{*T}}\,\mathbf{S^*})^{-1}$

Where $D_{n,m}$ is the initial matrix of spectral data body (n spectra with m values of absorbance); $\mathbf{S}_{k,m}^*$ is the matrix containing the k spectra of each chemical component obtained by the process of curve resolution (the refined deviation-spectra); and $\mathbf{C}_{n,k}$ is the matrix of concentrations of the chemical components at each spectrum.

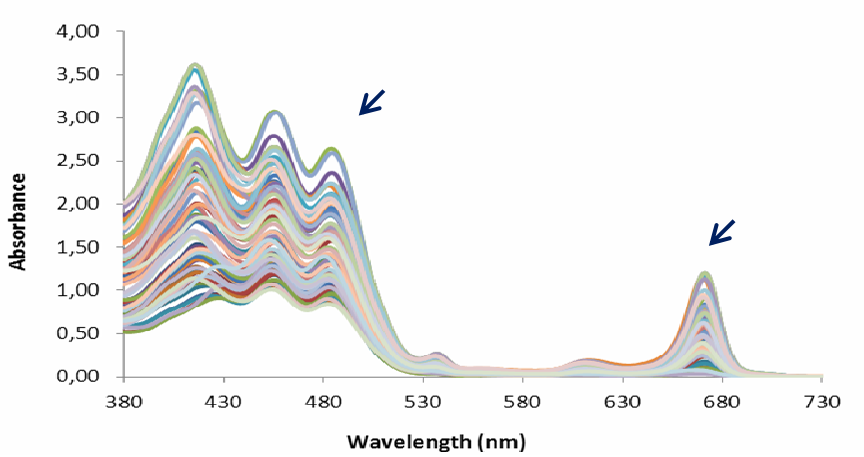


Fig. XI1. Body of spectra of 79 samples of virgin olive oil that were used for the validation set. The arrows indicate the two selected wavelengths: λ_1=670nm (maximum of the band of largest wavelength of chlorophylls in olive oils) and λ_2 = 480 nm (typical band of carotenoids in olive oils and where chlorophylls almost show no signal)

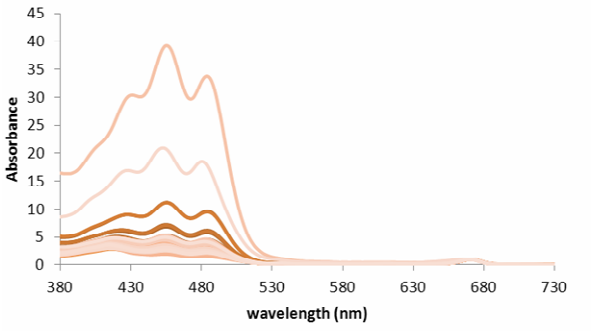


Fig. XI2a. Body of spectra after normalizing at λ_1. The contribution of chlorophyll is the same in all spectra. As a result the peak at 670 nm has disappeared.

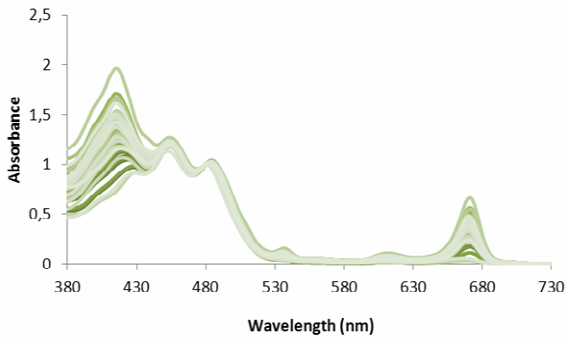


Fig. XI2b. Body of spectra after normalizing at λ_2. The contribution of carotenes is the same in all spectra

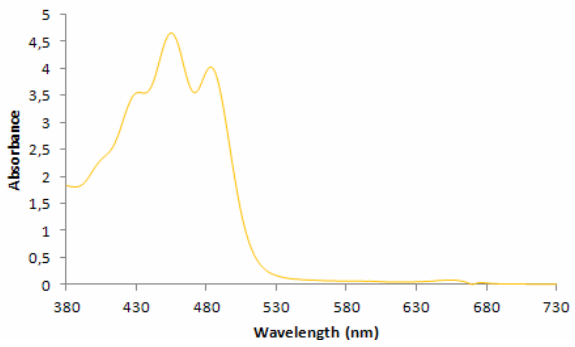


Fig. XI3a. Deviation-spectrum of body of spectra in the fig. XI2a. All peaks and bands due to chlorophylls have been removed.This spectrum is indistinguishable from the carotenoids spectrum with the typical peaks around 450 nm.

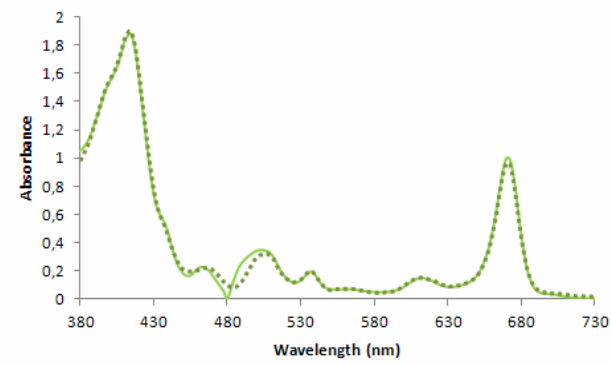


Fig. XI3b. Continue line: Deviation-spectrum of body of spectra in the fig. XI2b, with the typical peaks from 670nm to 420nm (the latter is overlapped with the carotenoids band in VOO). Broken line the refined deviation spectra after eliminating the artifact at 480nm.

XI4. Results and discussions:

XI4.1. Validation of the method.

Virgin olive oil is the organic phase extracted directly from crushed olive fruits and, hence, is a complex organic solution which composition also depends on the particular fruit variety and ripeness [2, 3-5]. However, its spectroscopic properties in visible depend only from two groups of pigments: carotenoids and chlorophylls. In such way, only two spectra can explain more than 99.5% of variance for the spectra of an extended number of samples [2]. Therefore, VOO was selected as a two component system for the validation of our method. In this case, is used a set of 79 virgin olive oils.

After applying the sequence given in theory, the two spectra resulting of curve resolution are plotted in figs. XI3a and XI3b. On the other hand, real spectra of chlorophylls and carotenoids were isolated by photodegradation of VOO in other study [2]. Therefore, once we obtained our refined deviation spectra, they were compared to those real spectra of pigments. Due to the differences of intensity bands, both deviation-spectra and both real spectra were normalized by assigning one absorbance unit to the maximum with respect to the peak at 670nm for chlorophylls (fig. XI3b), and to the peak at 455 nm for carotenoids (fig. XI3a). As can be seen in figs. XI 4a and XI4b the deviation-spectra for the two components obtained in VOO are very similar to the previous spectra published [2] at most of the wavelengths being particularly striking the case of carotenoids in which both spectra are completely overlapped.

The developed method was able to resolve the spectra by processing the data without any external treatment of the sample.

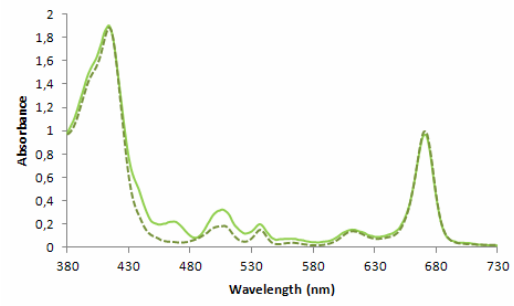


Fig. XI4a.Continuous line: normalized refined derivation spectrum of chlorophyll; broken line: normalized spectrum of chlorophyll as recorded in [2].

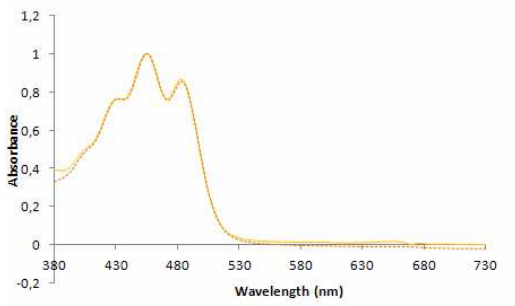


Fig. XI4b. Continuous line: normalized derivation spectrum of carotenoids; broken line: normalized spectrum of carotenoid as recorded in [2].

Once the method was able to resolve this two-component system, it was applied to a set of three components: Ni^{2+}, Co^{2+} and Cu^{2+} solutions.

XI4.2. Three Component system: Ni^{2+}, Co^{2+} and Cu^{2+} solutions.

This case was resolved to show how we can carry on with bigger system. In order to resolve the spectra of a three component system three spectra are needed. So, we employed the spectra of 3 colored solutions containing different amounts of Ni^{2+}, Co^{2+} and Cu^{2+}. Data was organized into a matrix of "3" spectra (I, II and III) recorded at "551" different wavelengths (from 350 nm to 900 nm), D_{3x569}, and we proceeded by following the steps described in the theory.

- ***Determination of number of compounds.*** In this case we knew the rank beforehand, k=3. The same information was obtained whit the PCA shown in table XI1.

- ***Detection of significant wavelengths.*** In the fig. XI5, is marked the three wavelengths over the plots of the 3 spectra λ_1= 390nm, λ_2= 515nm and λ_3=870nm. These are the wavelengths where Ni^{2+}, Co^{2+} and Cu^{2+} typically show a peak of absorption, respectively.

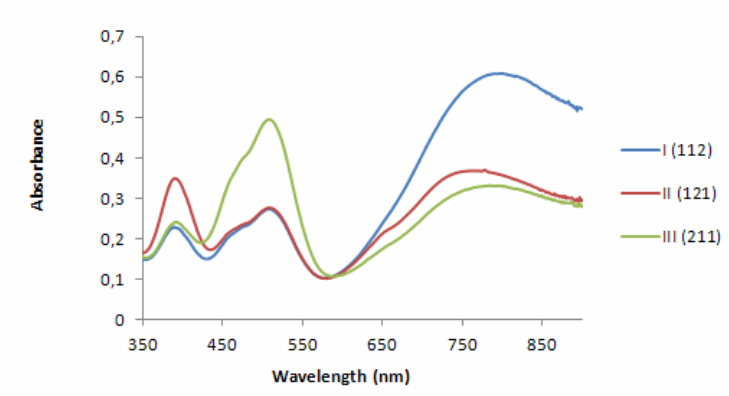


Fig. XI5. Body of spectra of the 3 solutions.

Normalization and standard deviation. For this case, this step has to split in several steps.

a) To start with, from the body of three spectra we made three subsets of "three minus one" spectra. In this case, these subsets were (I and II), (I and III) and (II and III).

b) For each of three subsets, we proceeded as usually doing normalization in each spectrum at a selected wavelength, i.e. at the maximum for Ni^{2+}, λ_1 = 390 nm. After normalization, a standard deviation was calculated for each subset. We got three deviation-spectra where the spectral signals of the Ni^{2+} were removed, see fig. XI6.

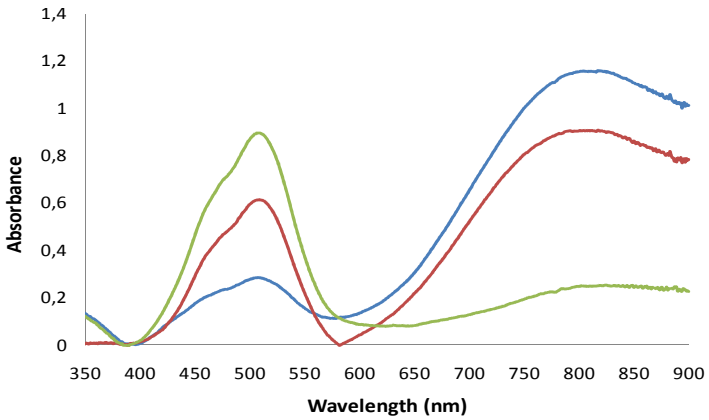


Fig. XI6. Three deviation-spectra without Ni^{2+} bands

c) After this, we got a new case of two-component, just Co^{2+} and Cu^{2+} as in the case of VOO. Therefore, from here, we continued in step 4 of the theory. Two normalizations at λ_2 = 515 nm and λ_3= 870 nm, and its respective deviation-spectra were needed to resolve the spectra of Co^{2+} and Cu^{2+}. In figs. X17a and X17b these two deviation-spectra (S_{Co}^{2+} and S_{Cu}^{2+}) are plotted.

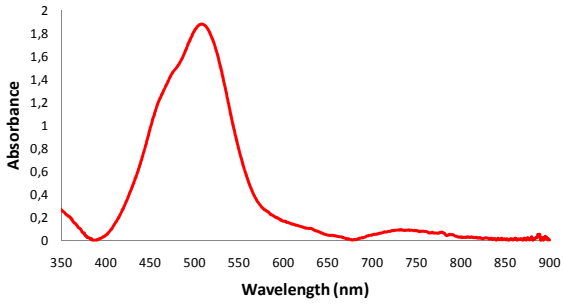


Fig. XI7a. Deviation-spectra of Co^{2+}

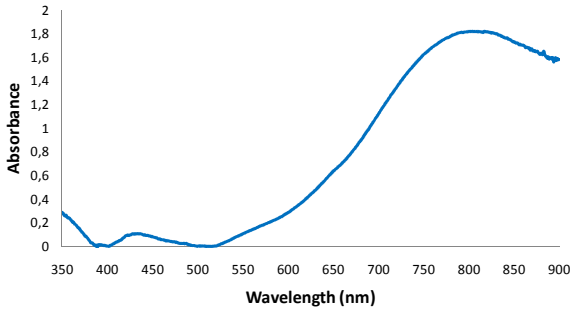


Fig. XI7b. Deviation-spectra of Cu^{2+}

d) In order to obtain the remaining spectrum, Ni^{2+}, we applied the same procedures performed in b) and c) with the three initial subsets, but in this case normalizing first at λ_2 = 515 nm (or λ_3 = 870 nm). After normalization, the respective three deviation-spectra without signals of Co^{2+} (or Cu^{2+}) are obtained. With a new normalization at λ_3 = 870 nm (or λ_2 = 515 nm) and subsequently the corresponding deviation-spectrum, we got the deviation-spectrum of Ni^{2+}($S_{Ni^{2+}}$) as fig. XI8 shows.

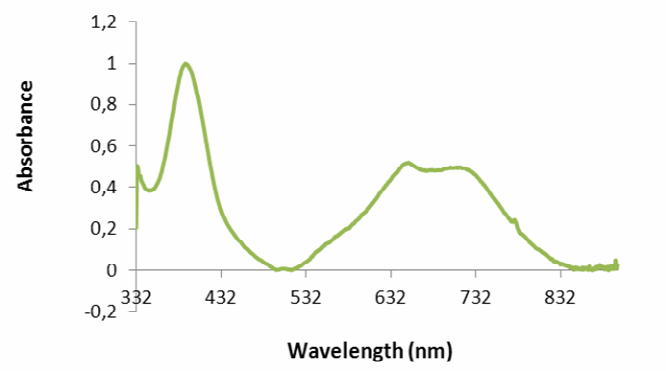


Fig . XI8. Deviation-spectra of Ni^{2+}

Elimination of artifacts. All spectra were refined in order to eliminate possible artifacts. According to the equation (1) it was obtained new spectra related to the original spectra by simple linear equation with the three factors from PCA. The resulting spectrum was called *refined deviation-spectrum of* Cu^{2+}, Co^{2+} *and* Ni^{2+}($S^*_{Cu^{2+}}$, $S^*_{Co^{2+}}$ *and* $S^*_{Ni^{2+}}$).

Fig. XI9.a shows the resulting refined deviation-spectra of Ni^{2+}, Co^{2+} and Cu^{2+}. In order to prove that our method was successful resolving the spectra of the solutions, they were compared to the reference spectra of Cu^{2+}, Co^{2+} and Ni^{2+}. As can be observed the resulting spectra are very close to the reference profiles display in Fig. XI9b. In order to compare, each spectrum was normalized by assigning one absorbance unit to the maximum. So the deviation methods proved also to be effective for bigger case than two component system.

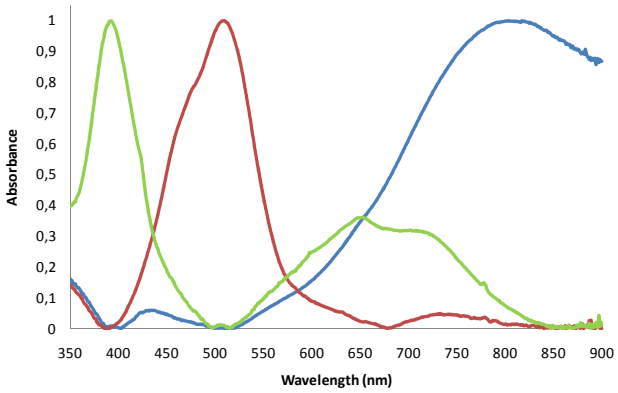


Fig. XI9a. Refined deviation-spectra Ni^{2+} (green), Co^{2+} (red) and Cu^{2+} (blue).

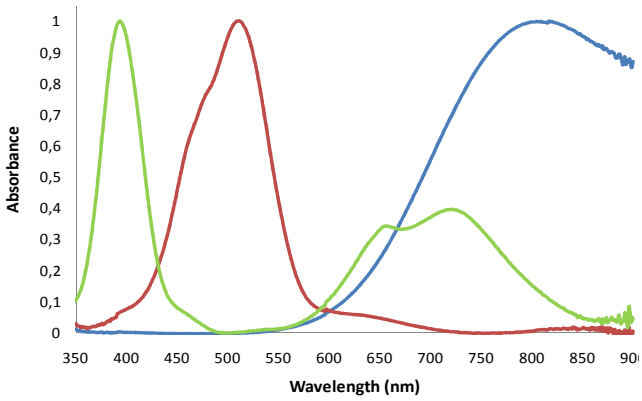


Fig.XI9b. Reference spectra Ni^{2+}, Co^{2+} and Cu^{2+} with the same colors as in 8a.

XI4.3. Three component system: VOO, refined oil and pomace oil.

Fig. XI10 displays the body of spectra of 108 samples of different qualities of olive oil: VOO-EVOO, olive oil and pomace olive oil.

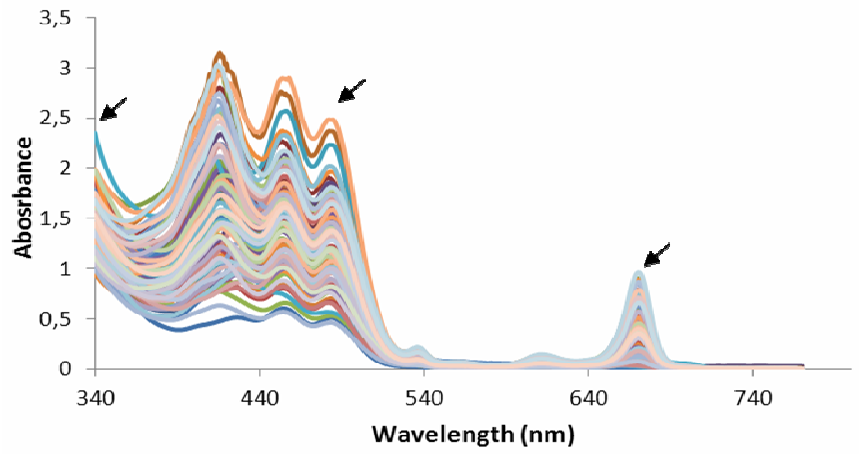


Fig. XI10. Body of spectra of 108 olive oil samples. The arrows indicate the three selected wavelengths: λ_1=670nm (maximum of the band of largest wavelength of chlorophylls in olive oils) and λ_2 = 480 nm (typical band of carotenoids in olive oils and where chlorophylls almost show no signal) and a new one at λ_3 = 340 nm.

Once again, it was proceed with the steps described in the theory. The results of the PCA applied to the body of absorbance values for this case are summarized in table XI1. Unlike the virgin olive oil validation set in which there were 2 factors accounted for more than 99.9%, this one requires a third factor . To the naked eye can be recognized the spectra corresponding

to chlorophyll and carotenoid pigments obtained in the validation set. The spectrum for the third factor is due to the olive oil and pomace olive oil samples (low quality olive oils). It was proceeded with the normalization process in every spectrum at the significant wavelengths (λ_1= 340nm, λ_2= 455nm and λ_3=670nm) and then with the calculation of the corresponding deviation spectra, for carotene, for chlorophyll and for the third component named as X component: *(S_{Chl} , S_{Carot} and S_x)*. The resulting spectra after the normalization and the elimination of possible artifacts (S^*_{Chl} , S^*_{Carot} *and* S_x^**)*, are represented in fig. XI11. This third component, X, can be used as an indicator of low quality in the case of virgin oil samples.

According to the results one more time the method has showed to be perfectly capable to resolve spectra.

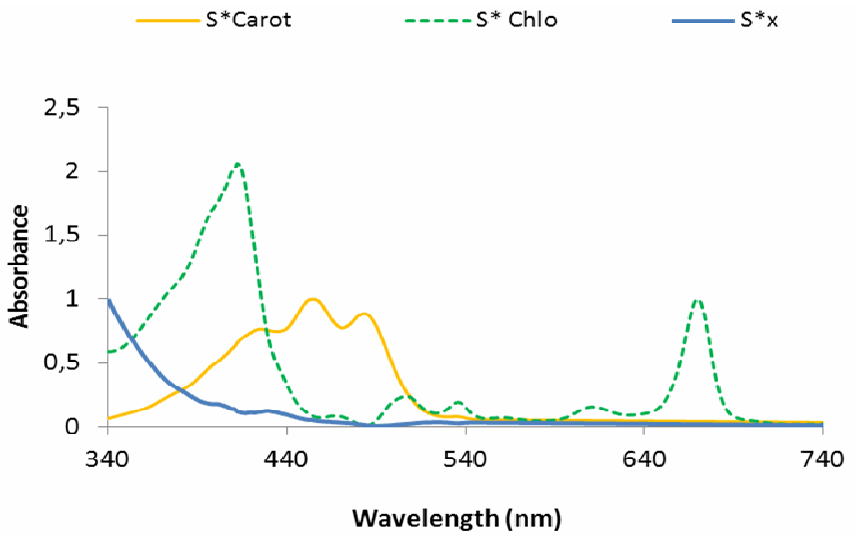


Fig. XI11 Normalized refined spectra for Carotene (orange), Chlorophyll (green) and Component X (blue)

XI5. Conclusions:

This experimental work has shown that the standard deviation of normalized spectra at adecuate wavelengths provides accurate resolution of spectra into individual components in a 2 components system and it can be used for multicomponent systems with bigger sizes. As such, the deviation-spectra method was applied to a system of three solutions containing Co^{2+}, Cu^{2+} and Ni^{2+} ions. The method was perfectly able to resolve the three spectra. Furthermore, the method was also applied to a third set of oil aiming at distinguishing among different qualities of olive oil samples, where the results were satisfactory too.

The deviation-spectra method is simple and rapid to apply. Unlike most of multivariate curve resolution methods, it does not require initial estimations, constraints and the

application of complex algorithms. Moreover, a simple spectroscopic technique, such as UV–Vis which is suitable to rapid data collection with minimal sample pre-treatment was used.

Based on the obtained results, the proposed method has demonstrated its potential in the resolution of spectra of 2 and 3 multicomponent systems, and it extension to a bigger size is very easy to implement. In addition, this method can be useful and applicable to further studies in other matrix.

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XII. Sherry wine vinegar study:

Characterization of Sherry wine vinegars

XII1. Introduction.

Several types of vinegar produced from different raw materials and by different production processes can be found in the market. Due to such diversity, it has been considered necessary to investigate reliable analytical methods to establish criteria for determining quality and origin. A large number of analytical methods have been developed for the individual estimation of compounds of interest in vinegar. However, most of these conventional methods are expensive, destructive, and time-consuming, which require skilled operators and have a high environmental impact.

The presence of color in vinegar is one of their distinctive characteristics. Sherry vinegar is available in a range of shades from the most crystalline of amber to darker mahogany. These tones are mixed in different varieties resulting in bright color compositions. One of the reasons of this color range is because the aging processes during which several process take place causing changes in the intensities.

This fact and the group experience in the study of color as classification parameter in food, has motivated us to study the presence of color in vinegar.

The initial idea was to apply the study of the presence of color in sherry wine vinegar samples with three classification purposes: First, the classification according to the used wine variety (Palomino Fino, Moscatel and Pedro Ximénez); second, the acetification process (traditional system in oak barrel or industrial process); third, the size oak and finally, the barrel low level.

XII2. Results and discussions.

First of all, the PCA was applied to the body of absorbance values in order to search for components potentially present in all vinegar samples. The first factor accounts for 99.3 % of the variance contained in the body of spectra, whereas the first two factors account for 99.97 % of the information. This minimal difference is due to noise and artifacts. This means that visible spectra for vinegars are qualitatively very similar and the only differences in the absorbance values were found. At a glance, one can easily see differences in the vinegar color, even giving a subjective classification of the vinegar samples; therefore, we thought that chromatic parameters could give us interesting information about the vinegar samples. CIE XYZ 1931(fig.XII1), CIE L a*b*(fig. XII2), CIE L C h (fig.XII3) spaces were calculated from the absorbance.

All vinegar samples were plotted according to different chromatic coordinates in the different chromatic systems.

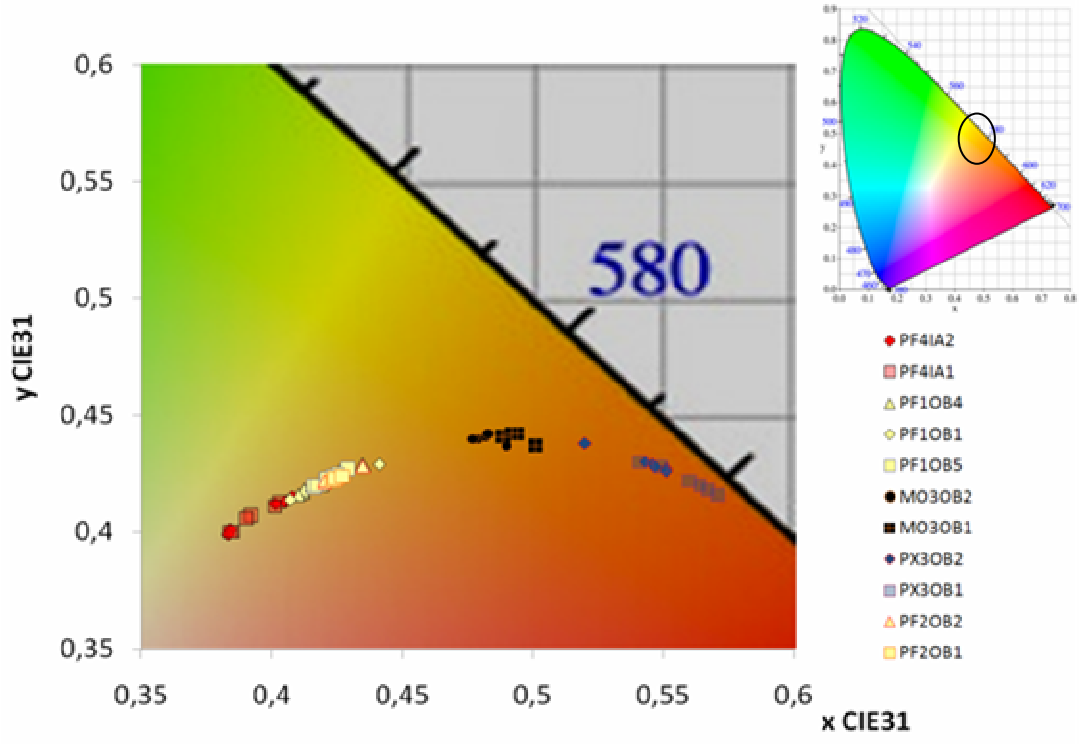

Fig. XII1 values of the *x* and *y* coordinates obtained by CIE 31 method and 86 absorbance values. PF samples (bottom-left), MO (center) and PX (bottom-right).

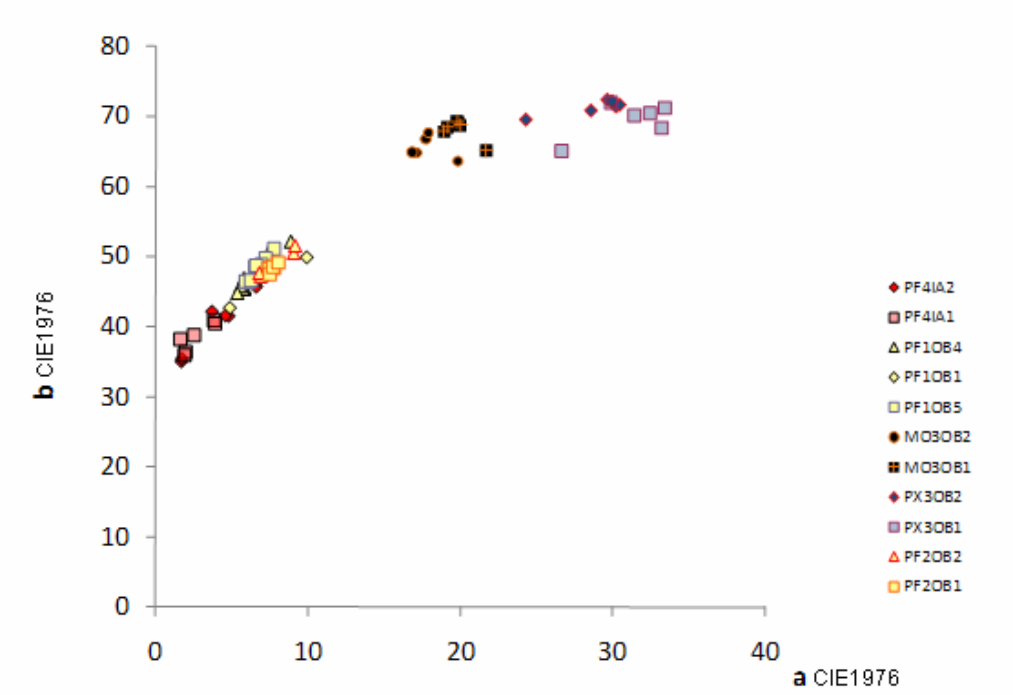

Fig. XII2 Displays the variation of color with the chromatic coordiantes of the vinegar samples, with coordinates a*(green-red) vs b* (yellow-blue) in CIE lab 1976 color space. PF samples (bottom-left), MO (upper-center) and PX (upper-right).

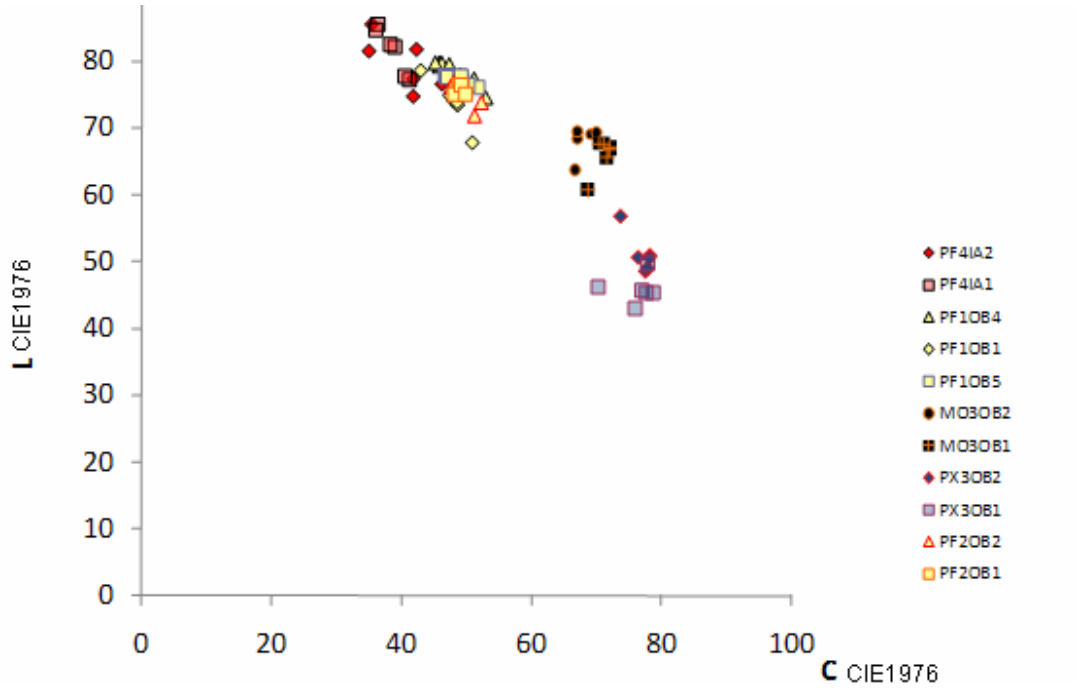

Fig. XII3 L(lightness) vs C(Chroma) parameters in CIE lab 1976 color space for vinegar samples. PF samples (upper left), MO (center right position) and PX (bottom left).

As can be seen, all samples fell preferentially in a specific zone of the color space giving rise to 3 well differentiated groups regardless of the color system that was used. The 3 groups correspond to the 3 varieties of origin wine used to elaborate the vinegar: MO, PX and PF. In fig. XII1, all of the samples are distributed in their corresponding ones except for the PX sample which fell close to MO samples. As fig. XII2 and XII3 display, the boundary of every group is also well defined. Any outlier was obtained and the zone of MO and PX was better bounded. As it was expected PX and MO samples have greater a*, b*, and C values, this is because these samples are perceived as dark mahogany in color (fig. XII2 and XII3). However, PF are the samples with higher L values, since they are the clearest samples.

Within the PF group, there were samples from the industrial acetification process and samples from traditional acetification process. Fig. XII1 shows a tendency to group within the group of PF samples. This tendency seems to be related to the acetification process since the industrial acetification samples fall on the left position and below those of the traditional acetification in oak barrels. This sub-classification appear when *x* and *y* parameters (chromatic coordinates) are used but not in the other 2 color systems, where all PF samples overlap making it impossible to distinguish between samples in relation with the acetification process. As a result, the *x* and *y* parameters being most ancient were the selected ones to use for further

studies. Due to the influence seems to be between color and acetification process, maybe the performing of a new study only taking PF samples would have been more proper. However, since the results were satisfactory in relation with the type of wine used, the whole set of samples were used for further studies.

The HCA (hierarchical cluster analysis) was applied by using the CIE 31 chromatic coordinates as a variables for forming groups. The results of the cluster analysis are shown by a dendrogram in fig. XII2 which lists all of the samples and indicates at what level of similarity (dissimilarity) any of the two clusters were joined. *Between-groups Linkage* method and *square Euclidean distance* were used as a distance for forming clusters. As can be seen, 4 outputs were obtained: the black one containing MO samples, the blue one that corresponds to PX samples and then PF samples were separated in 2 different groups according to the acetification process. The red cluster contains the samples obtained from industrial process and the yellow one those from oak barrel acetification. Less than 4% of the samples were misclassified in a different cluster: 2PF1IA were clustered as PF10B, and 1 PX was included in MO group.

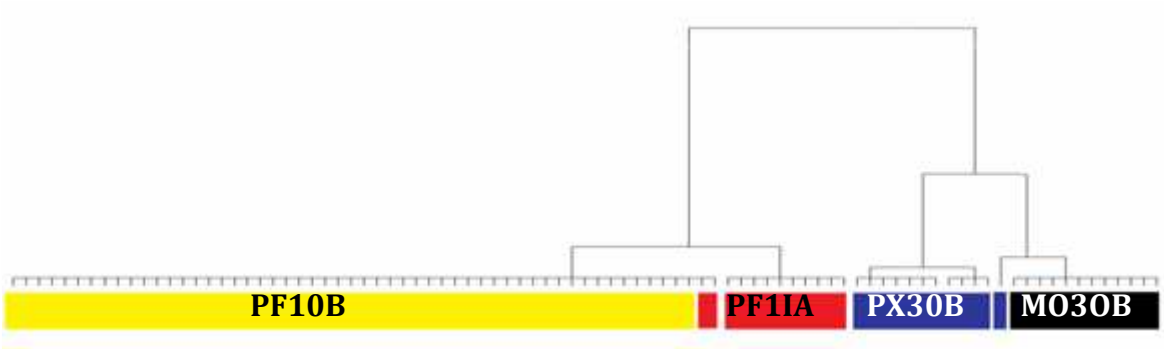

Fig. XII4. Cluster analysis of the CIE chromatic coordinates of the 86 vinegar samples.

Based on these results, it was proceeded with the performance of a LDA (linear driscriminat analysis). 50% of the samples were randomly selected as a training set. From this training set, discriminant functions were obtained. Then, the other 50% of the samples were used as a validation set. The resulting linear discriminant functions were good enough as to acquiring a good classification of the samples. Fig. XII3 shows the results of the LDA.

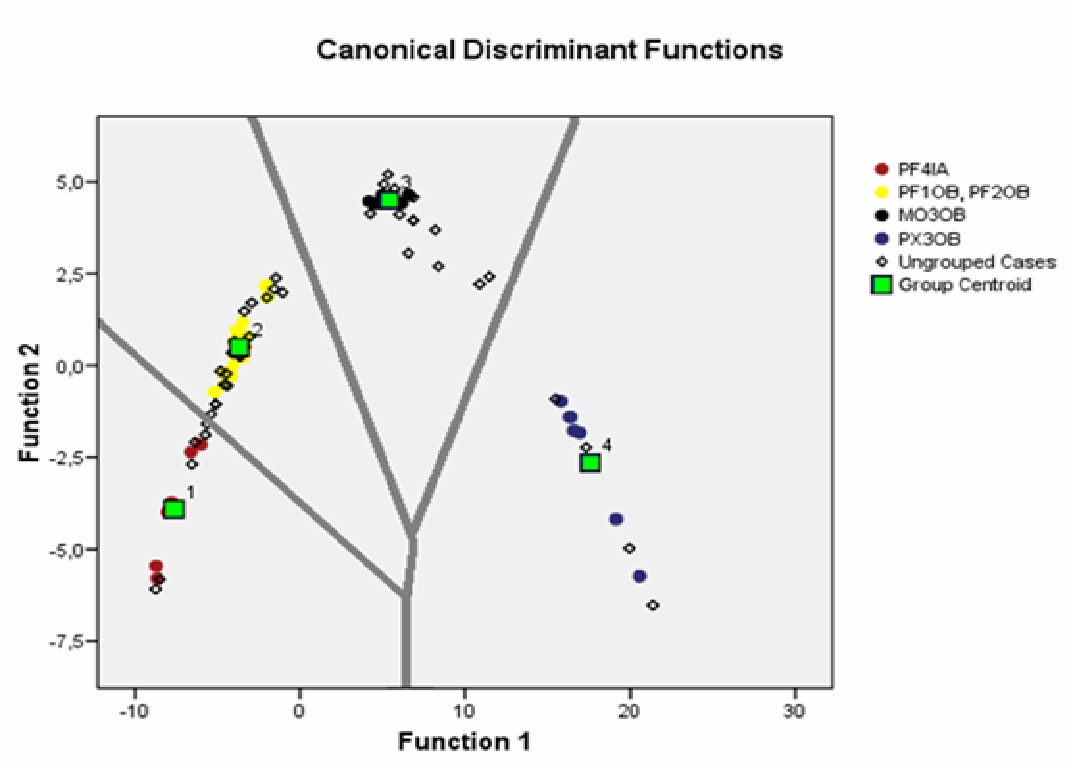

Fig. XII5. The results of applying CDA to the vinegar samples. The territorial map is overlapped. These canonical discriminant functions are similar to the CIE31 chromatic coordinates used previously as variables for classification.

Based on the results of the LDA, only 4 samples were misclassified: 2 PF4IA, 1 PF1OB and 1 PX3OB as follows: 2 PF samples from the industrial acidification were included in the group of PF from traditional acetidication , 1 sample from this latter group was misclassified as PF from industrial acetification and 1 PX sample was misclassified as MO.

Table XII1. Results of the HCA and LDA.

Samples	% of correct classification HCA	% of correct classification LDA
PF1 IA	83.33	66.66
PF10B	97.36	94.73
PF20B	100	100
MO3OB	100	100
PX	91.66	83.33

Table XII1 summarizes the results of the HCA and LDA. The first column lists the codes used for each vinegar group; the second shows the percentage of correct classification in the HCA and the third gives the percentage of correct classification in the LDA.

XII3. Conclusions.

According to the obtained results, it can be concluded that the chromatic coordiantes can be used as parameters to characterize sherry vinegars accordingly with the type of origin wine used to elaborate the vinegar.

The results were satisfactory regardless of the color coordinates used: *x* and *y in CIE 1931 system, a* and b* or L* and C* in CIE 1976.*

By using the CIE 31 chromatic coordinates as variables for forming groups an underlying classification of the vinegars was obtained being also possible to distinguish between acetification processes within the group of PF samples. Therefore, when HCA was performed using these x and y chromatic coordinates, 4 clusters were obtained: PF 4 IA, PF 1OB, MO and PX. The results were validated by applying a LDA, where more than 90 % of the samples were correct classified.

Regarding the size of the barrel and row barrel level, no differences in the color parameters were obtained.

Conclusions

UV-Vis spectroscopic has been demonstrated to be a versatile tool for a rapid and nondestructive analysis in oil and vinegar samples based on the study of the spectra. By treating the spectra with chemometric techniques adulteration and characterization studies without any cost were performed. According to the results, the conclusions are the following:

- From a forgery possible point of a view, it has been demonstrated how natural food colorants the same as those found in VOO, such as: β-carotene (E-160a), lutein (E-161b), coloring Cu-Chl (E-141i) and Mg-Chl are excellent at simulating VOO by comparing the global color space of colored seed oil with the global color space of the VOO.

- Direct UV-Vis spectroscopic characteristics can be applied through a fast, economic and simple procedure for detecting adulterations in low quality vegetable oils colored with two types of commercial chlorophyll colorants (Cu-Chl (E-141i) and Mg-Chl) in order to simulate VOO.
- VOO and colored seed oil with Cu-Chl or Mg-Chl can be distinguished by using the determination coefficient, R^2, of the simulated versus real spectra. R^2 values below 0.995 indicate that the oil is not VOO.
- VOO, colored seed oils with Cu-Chl and colored seed oils with Mg-Chl can be discriminated through the usage of two spectroscopic parameters p_1 and p_2.
- The standard deviation of normalized spectra at adequate wavelengths provides an accurate resolution of spectra into individual components in a two- components system and it can be used for multicomponent systems with bigger sizes.
- The standard deviation is simple and rapid to apply. Unlike most of multivariate curve resolution methods, it does not require initial estimations, constraints and the application of complex algorithms.
- Chromatic coordinates can be used as parameters to characterize sherry vinegars accordingly with the type of origin wine used to elaborate the vinegar and the acetification process.

As final conclusion, it should be brought to light that all of these methods mentioned above, are suitable for oil and vinegar production facilities for the rapid quality control of the products as spectra are acquired from samples as" received". Moreover, spectra are measured quickly and easily, without any pretreatment of the samples (free solvent), with no costs and no skilled operator.