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Innovative Technique Combining Laser Irradiation Effect and Electronic Nose for Determination of Olive Oil Organoleptic Characteristics

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1. Introduction

1.1 Olive oil

Olive oil has a characteristic flavor that distinguishes it from other edible vegetable oils. Its quality depends on the aroma, taste and colour, which in turn depend on many variables including location.

The International Olive Oil Council (IOOC, 2001) Standards and European Commission regulations have defined the quality of olive oil based on parameters derived from spectrophotometric studies that include free fatty acid content, but these methods only give information about the samples' oxidation level. A specific vocabulary has been developed for virgin oil sensory descriptors (IOOC, 1987). The positive attributes are classified as fruity, bitter and pungent and negative attributes as fusty, musty-humid, muddy-sediment, winey-vinegary, metallic and rancid.

Odour is an important parameter determining the sensory quality of olive oils and it is therefore of interest to investigate if volatile compounds contributing to the characteristic odour can be measured.

In the last decades many efforts have been made to study the aromatic fraction of olive oils based mainly on chromatographic determinations (S. de Koning et al 2008, S. Mildner-Szkudlarz, H. H. Jeleń 2008, C. M. Kalua 2007). The presence or absence of particular volatile compounds is a good indicator of olive oil quality.

The aroma of olive oil is attributed to aldehydes, alcohols, esters, hydrocarbons, ketones, furans and probably, other volatile compounds, not yet identified. More than 120 volatile compounds that contribute both positively and negatively to the sensory properties of olive oil have been identified (Aparicio, R., Morales, M. T. & Luna, G. 2006). Table 1 lists some volatile compounds associated with negative attributes determined by Morales et al. in 2005.

Descriptor	Volatile compounds
Mustiness-humidity	1-octen-3-ol
Fusty	Ethyl butanoate, propanoic and butanoic acid
Winey-vinegary	Acetic acid, 3-methyl butanol and ethyl acetate
Rancid	Several saturated and unsaturated aldehydes and acids

Table 1. Volatile compounds associated with negative attributes of olive oils

Odour activity is a measure of the importance of a specific compound for the odour of a sample. It is calculated as the ratio between the concentration of an individual substance in a sample and the threshold concentration of this substance. The minimum concentration of a compound able to give rise to an olfactory response is the compound's odour threshold value. For this reason, high concentration of volatile compounds is not necessarily the main contribution to odour. For example, Reiners and Grosch reported a concentration of 6770 µg/g of trans-2-hexenal with an odour activity value of 16 whereas 1-penten-3-one with a much lower concentration of 26 µg/g had a higher odour activity value of 36 (C. M. Kalua, 2007).

According to the European Community Regulations (ECR 640/2008, ECR 1989/2008) olive oil can be classified in extra-virgin (high quality), virgin (medium quality) and lampante (lower quality). The first two categories can be bottled and consumed.

The quality and uniqueness of specific extra virgin olive oils is the result of different factors such as cultivar, environment and cultivation practices. The European Community (ECR 2081/1992) allows the Protective Denomination of Origin (PDO) labeling of some European EVOO with the names of the areas where they are produced.

1.2 Analytical techniques

The methods used and / or proposed to evaluate the oxidative deterioration of olive oil based on the determination of volatile compounds are HPLC / GC-MS, analytical methods associated with some headspace extractive techniques. The volatile profile of VOO closely depends on the extraction used (S. Vichi, 2010).

Some of the traditional distillation methods applied in the analysis of plant materials as steam distillation (SD), simultaneous distillation/extraction (SDE) and microwave-assisted extraction (MAE) were used for this purpose (Marriott, Shellie, & Cornwell, 2001).

Among these distillation techniques, SDE appeared to provide the most favourable uptake for mono- and sesquiterpenes, as well as for their oxygenated analogues (Marriott et al., 2001). Hydro distillation (HD) has been applied for the analysis of leaf, fruit and virgin oil volatiles of an Italian olive cultivar (Flamini, Cioni, & Morelli, 2003). With hydro distillation, the volatiles in the steam distillate are strongly diluted in water when collected in cold traps. This can be overcome in simultaneous distillation/extraction (SDE) via solvent extraction of the distillate.

Dynamic headspace techniques have been used to correlate the composition of the olive oil headspace to sensory attributes (Angerosa et al., 1996; Angerosa et al., 2000; Morales et al., 1995; Servili et al., 1995) and or flavors "defects" (Angerosa, Di Giacinto, & Solinas, 1992; Morales, Rios, & Aparicio, 1997).

More recently, the solid-phase microextraction (SPME) technique has been introduced as a sample pre-concentration method prior to chromatographic analysis as an alternative to the dynamic headspace technique. Among other applications, SPME allowed the characterization of virgin olive oils from different olive varieties and geographical production areas (Temime et al., 2006; Vichi et al., 2003a), and the evaluation of varietal and processing effects (Dhifi et al., 2005; Tura et al., 2004). Since the SPME uptakes are strongly dependant on the distribution of analytes among the sample matrix, the gas phase and the fiber coating (Pawliszyn, 1999), some compounds present in virgin olive oil may remain undetected. In the case of other techniques such as SDE, the recovery of analytes is mainly related to their volatility.

These last techniques are complex, expensive and time-consuming. They generally highlight only one or few aspects of the oxidation process, providing only partial information. On the other hand, the olive oil industry needs a rapid assessment of the level of oil oxidation in order to predict its shelf-life. Consumers usually expect manufacturers and retailers to provide products of high quality and seek for quality seals and brands. Therefore, the development of innovative analytical tools for quick and reliable quality checks on extra virgin olive oil is required.

1.3 Electronic nose

Gardner and Barlett (1993) defined the electronic nose as an instrument which comprises an array of electronic chemical sensors of partial specificity and an appropriate pattern-recognition system, capable of recognizing simple or complex odours.

The sensors used in the array of an electronic nose should have the following characteristics: high sensitivity to chemical compounds, low sensitivity to humidity and temperature, medium selectivity, high stability, high reproducibility and reliability; short reaction and recovery time; robustness and durability; easy calibration and data processing and small dimensions (Schaller et al., 1998).

The chemical interaction between the odour compounds and the gas sensors alters the state of the sensors giving rise to electrical signals which are registered by the instrument. Since each sensor is sensitive to all odour components, the signals from the individual sensors determine a pattern which is unique for the gas mixture measured and that is then interpreted by multivariate pattern recognition techniques.

Nowadays, there are different gas sensor technologies available, but only four of them are currently used in commercialized electronic noses: metal oxide semiconductors (MOS); metal oxide semiconductor field effect transistors (MOSFET); conducting organic polymers (CP); piezoelectric crystals (Bulk Acoustic (Wave-BAW), Surface Acoustic (Wave SAW)). Others, such as fiber-optic, electrochemical and bi-metal sensors, are still in the developmental stage.

The processing of the multivariate output data generated by the gas sensor array signals represents another essential part of the electronic nose concept. The statistical techniques used are based on commercial or specially designed software using pattern recognition routines like principal component analysis (PCA), cluster analysis (CA), partial least squares (PLS), linear discriminator analysis (LDA) and artificial neural network (ANN).

The use of an electronic nose for quality evaluation as a means of olfactory sensing is becoming widespread due to its advantages of low cost, reliability and high portability. Electronic noses based on different sensor technologies and using different recognition schemes have been employed for this task.

When samples of olive oil are analyzed with an electronic nose, the standard procedure is to put a fixed quantity in a vial and sense the headspace. The main drawback of this method is that the concentration of some compounds in the headspace may be quite different from their concentration in the liquid phase. For example, the concentration of methanol and ethanol is usually much higher in the vapor phase than in the liquid. However, these substances have been found to be irrelevant in the definition of the olive oil characteristics (S. de Koning, 2008). On the other hand, substances such as hexanal and trans-2-hexanal which are responsible for the organoleptic properties, are more abundant than methanol and ethanol (Cosio et al, 2006, C. Di Natale et al, 2001) in the oil, but are scarcely present in the headspace. It is well known that the odour activity of hexanal and trans-2-hexanal is higher than that of those alcohols because of their low odour thresholds (Morales et al., 2005, J. Reiners, W. Grosch 1998, A. Runcio et al., 2008). Despite the abovementioned drawback, several efforts have been made to use the electronic nose for olive oil quality control (Guadarrama et al., 2000). The combination of electronic nose fingerprinting with multivariate analysis enabled the study of the profile of olive oil in relation to its geographical origin (Ballabio et al., 2006; Cosio et al., 2006). García Gonzalez and Aparicio, 2002, detected the vinegary defect in Spanish VOO with the use of metal oxide sensors. They used an Alpha MOS e-nose equipped with 18 MOS sensors distributed in three chambers, and heated the samples to 34 °C during 10 minutes before testing the headspace. Servili et al., 2009, reported the first study of the use of an Electronic Olfactory System (EOS 835) on-line to control the formation and evolution of the volatile compounds that characterize the most important sensory notes of VOO during the malaxation process.

In 2010, M. J. Lerma-García et al. compared the response of an electronic nose (EOS 507) to classify oils containing the five typical virgin olive oil sensory defects with that of a sensory panel. They demonstrated the usefulness of this tool when combined with panels to perform a fast screening of a large set of samples with the aim of discriminating defective oils. Each sample was incubated at 37°C for 7 minutes before injection.

In the same year, Massacane et al. proposed a method to improve the electronic nose performance for discriminating among different olive oils without changing the properties of the original oil sample. This task is carried out by IR laser vaporization (IRLV) which seems to be a promising technique that modifies only slightly the headspace by volatilizing certain organic compounds or by cracking them. Thus IRLV improves the selectivity of the overall response of the electronic nose. Due to the extremely low sample vaporization that it produces this method can be considered non-destructive as most ablation laser Analytical methods (C. A. Rinaldi and J. C. Ferrero, 2001).

1.4 Laser irradiation effect

Normal vaporization occurs when the vapour pressure in the ambient gas is lower than the saturation pressure of the liquid at the liquid temperature (Xu, X., and D. A. Willis, 2002). As the liquid's temperature increases, so do the saturation pressure and the rate of vaporization.

Laser vaporization (LV) produces a local heating of the irradiated liquid surface and, in consequence, some molecules are driven to the gas phase. This phenomenon can be produced by the use of either pulsed or continuous wave (cw) lasers. For a fixed wavelength, the main difference lies in the amount of energy emitted per unit time, or power. Pulsed lasers produce an increase of the liquid surface temperature without producing a significant change of the bulk volume temperature (Christensen, B., and M. S. Tillack, 2003). These lasers produce only a local heating of the surface allowing a large amount of vapour to be generated in a short time period.

Due to the intrinsic nature of the LV this surface effect is produced immediately after the irradiation time lapse. Therefore, it is common to speak of a "thermal spike" rather than simply "thermal heating", because of the transient nature of the high temperature. The characteristics of this spike are determined by the laser fluence and its pulse length (Taglauer, E, A. W. Czanderna and D. M. Hercules, 1991).

The cw lasers used to vaporize liquids can cause an increase of the bulk sample temperature and can induce chemical reactions, thus, modifying the sample's properties. However, the appropriate choice of the irradiation time lapse and the laser power make them suitable for this application.

In the present work the results of experiments carried out to illustrate the use of the combined techniques of electronic nose and pulsed or continuous wave laser irradiation for olive oil quality determination are reported.

2. E-nose + laser vaporization technique

2.1 Pulsed laser irradiation

In this experiment the Infrared Laser Vaporization, IRLV, properties to improve the e-nose selectivity are investigated. The role of the laser wavelength is additionally analyzed. This is due to the fact that the quality and the quantity of the chemical compounds incorporated the headspace depend on the laser parameters, particularly, the fluence and the pulse length, as it was mentioned in Section 1.4.

Two extra virgin olive oils produced in the same geographical region of Argentina (San Juan) classified as A and B, were tested. Three samples of 15 ml of each oil were subjected to three different analytical methods in order to compare the effects of the laser vaporization.

All analytical methods were carried out under the same temperature and humidity conditions, of 25 °C and 43%, respectively. The samples were introduced in 100 ml T-shaped Pyrex test tubes with screw-caps in air inlet and outlet channels and a CaF₂ window in order to allow the laser beam admission, referred to as vials.

The following analytical methods were undertaken:

Method I: Vial with oil sample A is kept closed during 2 minutes. Immediately afterwards the vial headspace is subjected to 35 seconds sampling with a Cyranose® 320. This procedure is repeated 5 times. The same operation is performed with oil sample B.

Method II: Vial with oil sample A is kept closed for 1min. The sample is subsequently irradiated with Nd:YAG laser pulses of 1064 nm at a repetition rate of 10 Hz during 1

minute. The laser is turned off and the vial headspace is immediately subjected to 35 seconds sampling with a Cyranose® 320. This procedure is repeated 5 times. The same operation is then performed with oil sample B.

The Nd:YAG laser (Continuum, Surelite I) has a pulse length of 5 ns and an output energy of 80 mJ. The laser beam is focused in order to obtain a spot size of 0.037 cm² at the surface of the sample so that a fluence of 2.14 J/cm² is therefore achieved.

Method III: The vial with oil sample A is kept closed for 1 minute. The sample is subsequently irradiated with a homemade TEA CO₂ laser (D. Petillo, J. Codnia, M. L. Azcárate, 1996) operating at 10.59 μm with a repetition rate of 1 Hz during 1 minute. The laser is turned off and the vial headspace is immediately subjected to 35 seconds sampling with a Cyranose® 320. This procedure is repeated 5 times. The same operation is made with oil sample B.

The TEA CO₂ laser has a pulse length of 100 ns and output energy of 1.45 ± 0.04 J/pulse. The beam is focused in order to obtain a spot size of 0.68 cm² at the surface of the sample; a fluence of 2.14 J/cm² is therefore achieved. The software provided by the Cyranose® 320TM electronic nose allowed the processing of the raw data given by the 32 sensors responses.

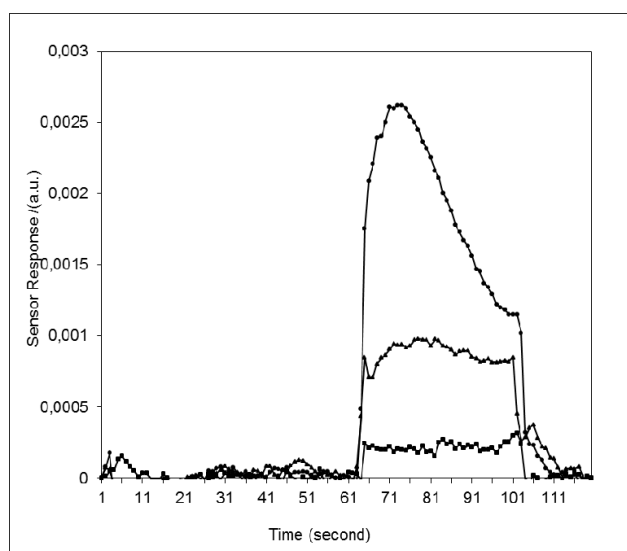


Fig. 1. Sensor response time dependence. (▪) Without laser, (▲) Nd:YAG laser, and (•) CO₂ laser. (Massacane et al., 2010) Permission?

Figure 1 shows the signals measured with one sensor corresponding to the samples of oil A subjected to the three analysis methods. We observe that the signal-to-noise ratio (S/R), is considerably increased by the laser vaporization. The highest S/N ratio is obtained when vaporization is performed with the CO₂ laser.

As it is well known, the fluence and the pulse length determine the laser radiation absorption mechanisms and these parameters modify the laser power. In this work the same power was used in both IRLV methods although different total energies were delivered to the sample in each analysis method: 47.5 and 87 J. in Methods II and III, respectively. This energy range produces a negligible temperature increment. Even assuming an ideal oil absorption of 100% of laser energy the sample temperature increment would be about 3°C,

as may be calculated from the volume of the sample and its average heat capacity. Therefore, the temperature of the sample remains constant throughout the experiment.

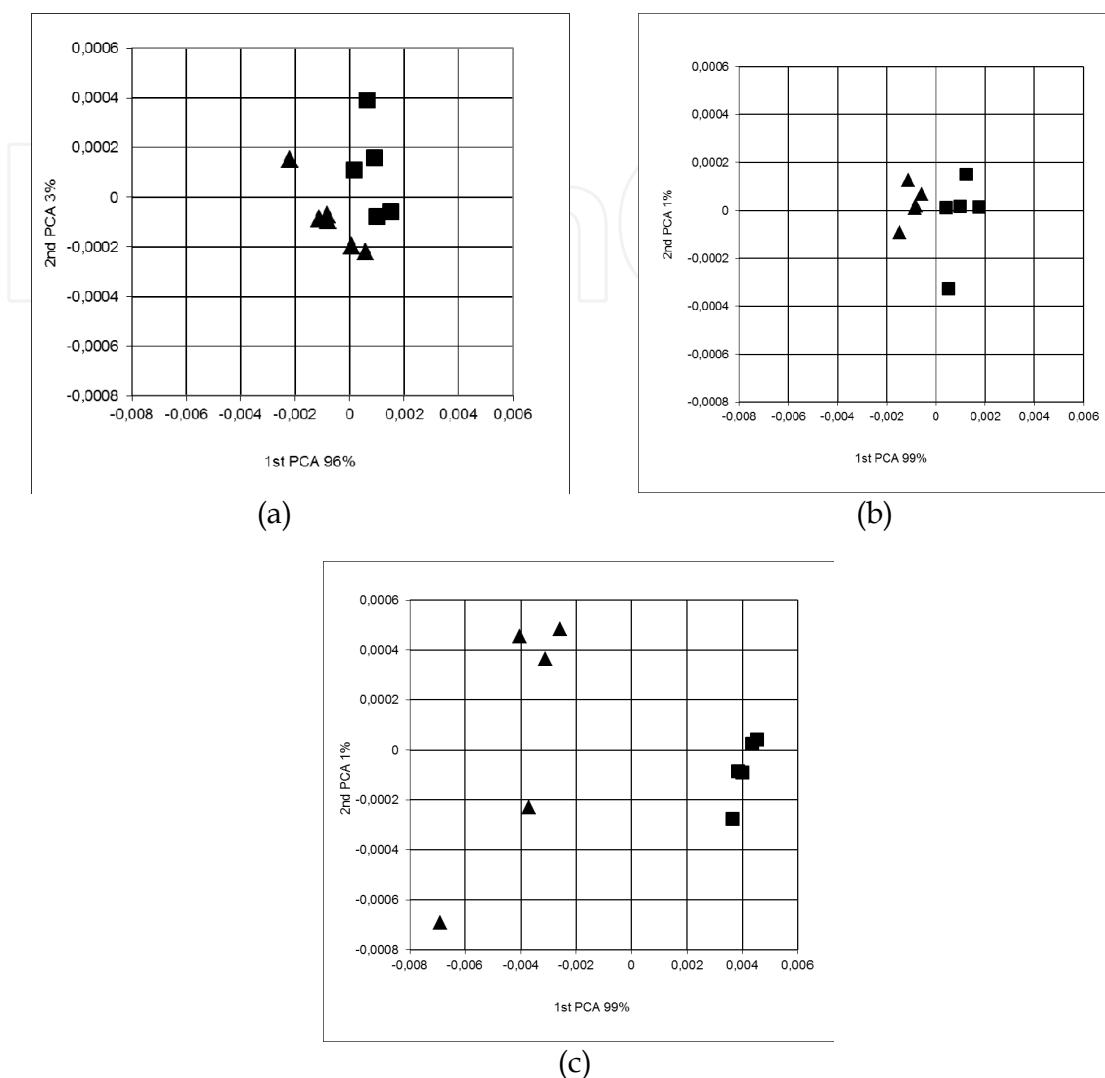


Fig. 2. PCA for olive oils (■:A; ▲: B) performed for experiments (a) without laser, (b) with Nd:YAG laser, and (c) with CO₂ laser. (Massacane et al., 2010) Permission

Not only the S/N ratio is increased by IRLV but also the discrimination of samples corresponding to different oils is dramatically increased. This fact can be verified by the results shown in Figures 2(a), 2(b) and 2(c). Thus the modification of the headspace is made evident by this result.

IR spectra of the liquid oil samples were registered both before and after methods II and III were performed. There were no significant differences between them indicating that IRLV does not produce changes in the liquid oil. On the other hand, IRLV does modify the vapour-liquid equilibrium conditions improving the selectivity of the electronic nose overall response. The only effect of IRLV is to increase the vapor concentration of the olive oil (Massacane et al., 2010)

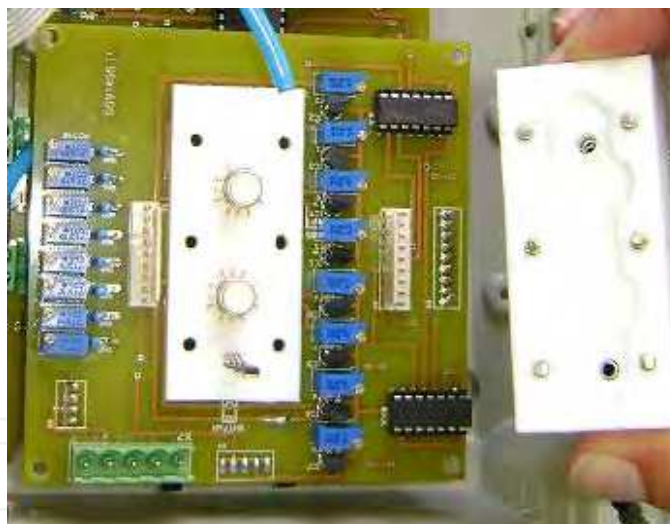
It has been shown that the electronic nose selectivity is dramatically increased by the use of IRLV and that it is rather insensitive to the recognition pattern employed.

2.2 Continuous wave laser irradiation

A homemade portable electronic nose, Patagonia nose, was used in this study. The instrument comprises three parts: the automatic sampling system, the sensors' chamber with the sensor array, and the software for the e-nose control, data recording and processing. The first two are integrated in the same device and the software can be installed on any notebook (Figure 3)



(a)



(b)

Fig. 3. a) Homemade e-nose (Patagonia) b) Sensor chamber

The chamber contains 2 MOS commercial sensors (Silsens MSGS 4000, sensor array). Each sensor has four thin SnO_2 films, one of them is doped with Pd. Each thin film is maintained at the temperature range between 300 and 500 °C during all the measurements.

Two EVOO produced in neighboring geographical regions of Argentina (San Juan) were classified as A and B. About 15 samples of both A and B EVOO were tested. Each sample was divided into three 15 ml samples so that three sets of the 15 samples were obtained to be subjected to three different analytical methods.

All the analytical methods were carried out under the same temperature and humidity conditions: 22 °C and 36 %, respectively. The samples were introduced in 100 ml T-shaped test tubes with screw caps, air inlet and outlet channels and BK7 upper windows to allow the laser beam admission referred to as vials.

The three analytical methods differ in whether the samples are irradiated or not and in the wavelength of the laser used for the irradiation. The vials were kept closed for about 90 seconds to allow the stabilization of the samples:

Method I: The vial headspace is subjected to a 15 seconds sampling.

Method II: After the headspace stabilization takes place, the closed vial is irradiated during one minute with a continuous wave diode laser emitting radiation of 98 mW at 450 nm. Immediately after the laser is turned off, the vial headspace is subjected to a 15 seconds sampling.

Method III: After the headspace stabilization takes place, the closed vial is irradiated during one minute with a continuous wave diode laser emitting radiation of 98 mW at 650 nm. Immediately after the laser is turned off, the vial headspace is subjected to a 15 seconds sampling.

Figure 4 shows a brief scheme of the experimental set up.

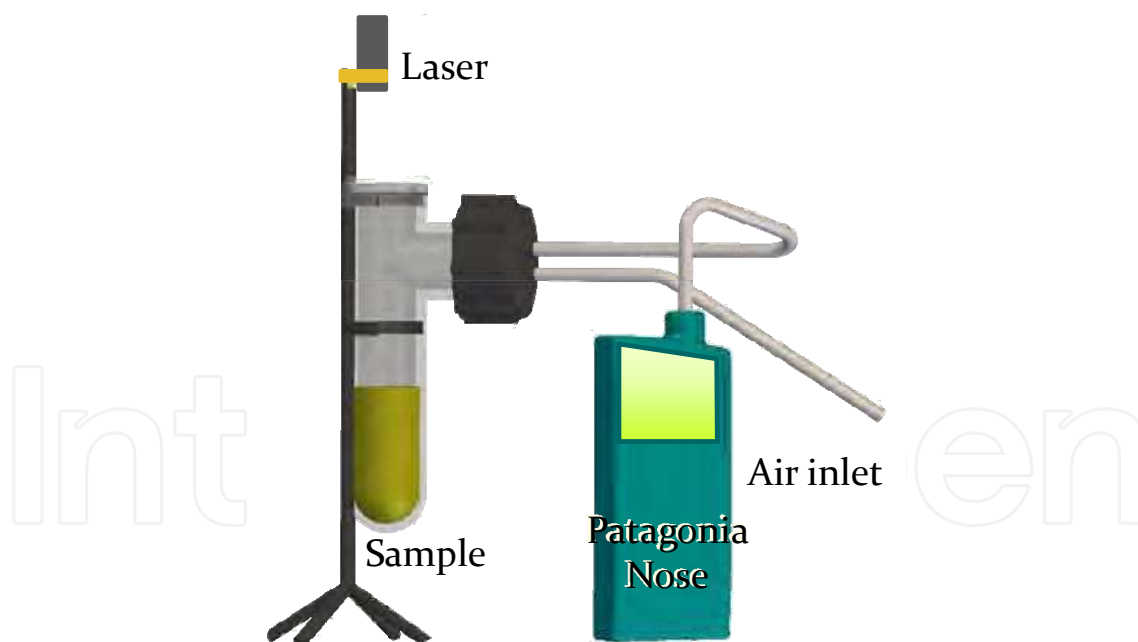


Fig. 4. Experimental set up

We have measured the V-UV spectra of the liquid oil samples before and after being irradiated during different time periods. We have noticed significant differences in the spectra of the liquid samples that had been irradiated during 5 minutes. Figure 5 shows the spectrum of each EVOO used before being irradiated.

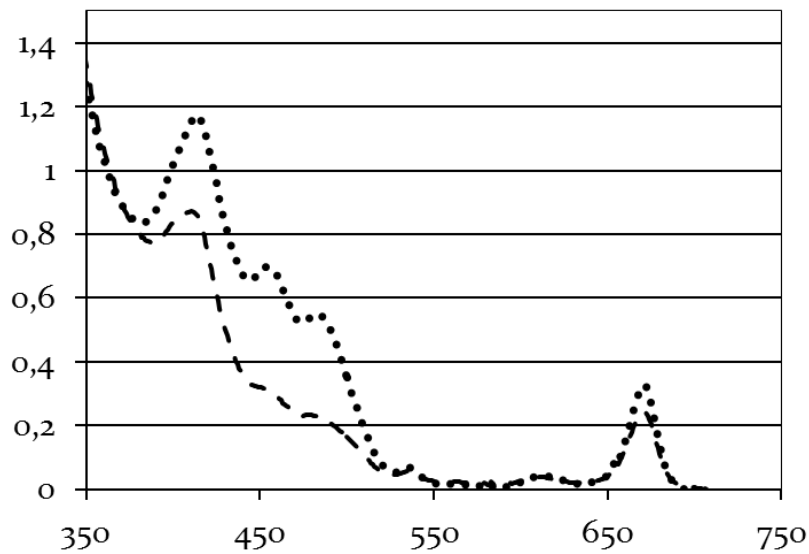


Fig. 5. V-UV Spectra of liquid EVOO A(- - -) and liquid EVOO B (. . .) before irradiation

Figure 6 shows the Principal Components Analysis (PCA) of the Patagonia Nose results obtained with Method I.

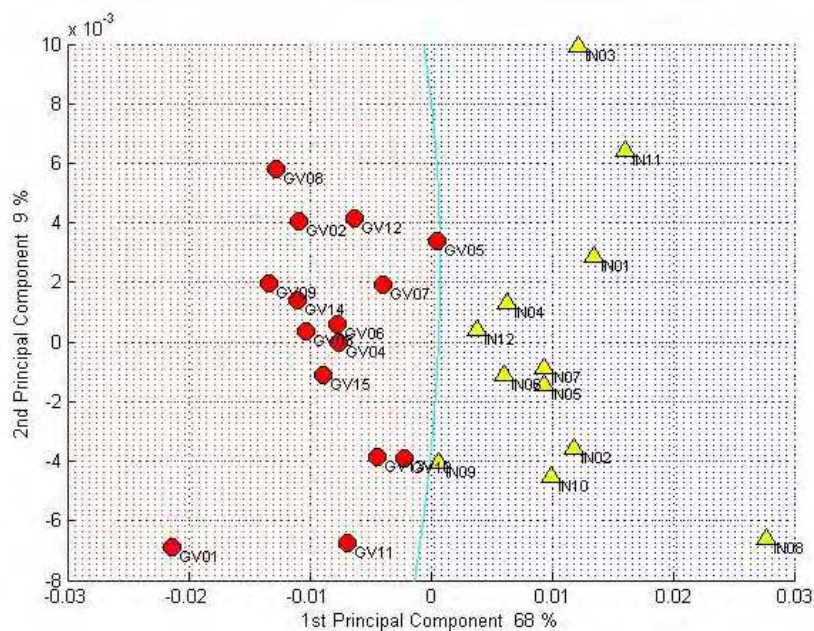
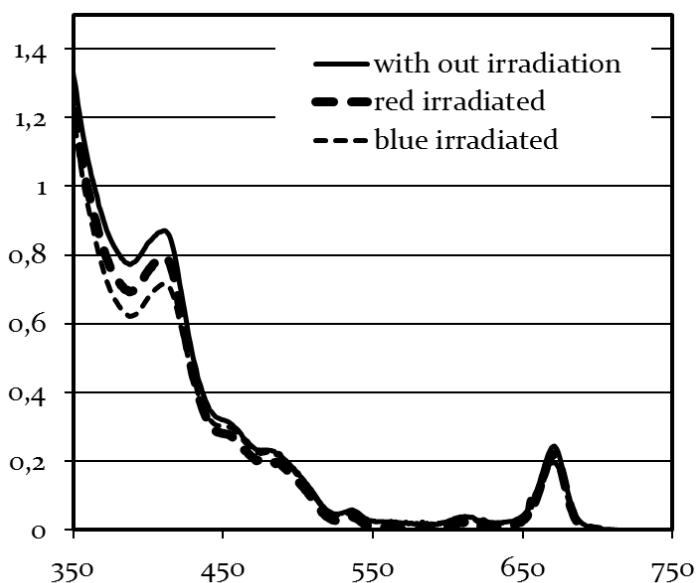
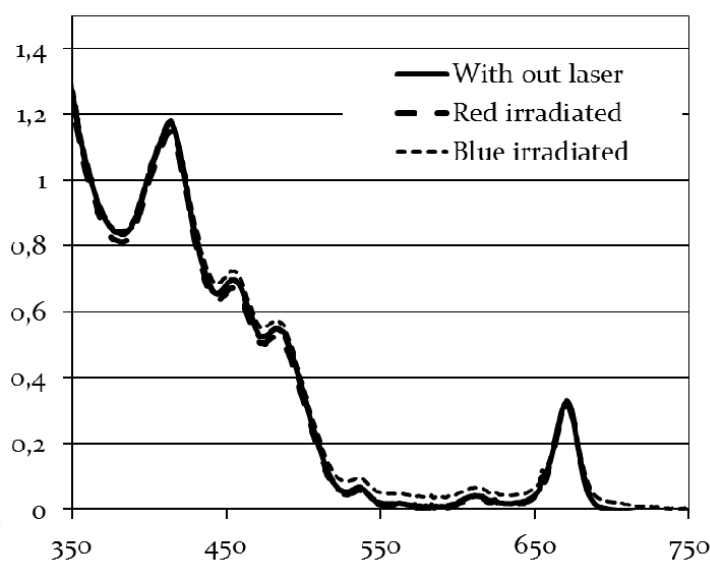


Fig. 6. PCA of EVOO A(GV) and B(IN) with Method I

Figure 7 shows the V-UV spectra registered for each liquid EVOO after the application of the three analytical methods. The experiment was repeated 5 times for each sample. It can be observed that the spectra of the irradiated samples of EVOO A exhibit significant changes with respect to the non-irradiated sample. The largest effect is produced by the irradiation with blue light (450 nm). This more energetic radiation affects the sample composition probably due to a photochemical effect. On the other hand, irradiation with the red light, gives rise to a thermal effect which causes the introduction of more molecules into the gas phase.



EVOO A



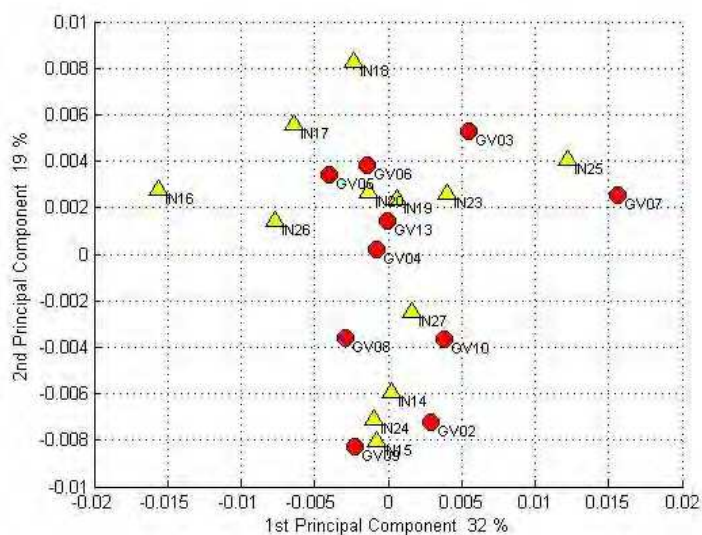
EVOO B

Fig. 7. V-UV spectra of EVOO (A) and (B)- Method I (____) - Method II (- - - -) - Method III (- - - -)

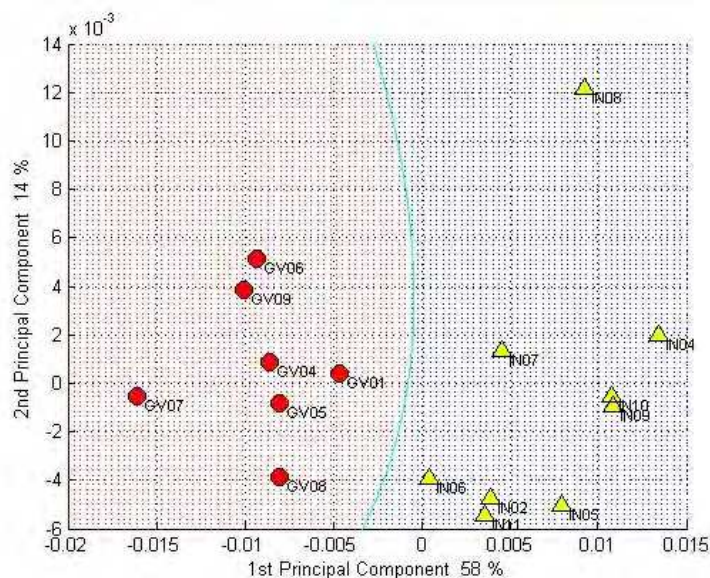
The discrimination ability of the three methods mentioned above was analyzed. For the data processing the absorption and desorption rates were taken into account in addition to the ratio of the peak value of each sensor response to the base-line value. Since different sensors have distinct desorption times, for each chemical compound, we have considered the integral of each time signal to the base line integral ratio.

The usual PCA of the data obtained with Methods II and III was performed. Figures 8 (a) and 8 (b) show the score plot obtained with both methods. It is evident that better discrimination is achieved with Method III.

A classification following the discrimination step was designed for Method III using about 70% of the available samples of both A and B oils.



(a)



(b)

Fig. 8. PCA (a) Method II (b) Method III (discriminating regions are shown).

For validation purposes, 30% of the measured samples were disregarded. Within the resultant restricted space, an unsupervised PCA was performed. It was then possible to establish multivariate confidence regions for the two identified classes: olive oil A and olive oil B. The assignment of a new multivariate measurement x to a given category k occurs when the quadratic discriminant $d_k(x)$ is maximized:

$$d_k(x) = -\frac{1}{2} \ln |\Sigma_k| - \frac{1}{2} m_k(x)$$

$$m_k(x) = (x - \mu_k)^T \Sigma_k^{-1} (x - \mu_k)$$

where $m_k(x)$ is the Mahalanobis distance, μ_k is the mean value of the corresponding class and Σ_k is the covariance matrix within each class sample.

3. Conclusions

An easy to implement method to improve electronic nose discrimination ability of a priori similar odours has been presented. This technique has been then applied to the case of virgin olive oils. The way laser vaporization of the samples improves this task has been additionally explored. The behaviour of the gas phase headspace following pulsed and cw laser irradiation with different wavelengths has been analyzed.

The use of pulsed IR lasers increases the sensitivity of the e-nose performance. Furthermore, the use of a CO₂ laser allows a better discrimination than the use of a Nd:YAG laser. When using the CO₂ laser, the signal-to-noise ratio (S/N) is increased by an order of magnitude with respect to the S/N without laser vaporization effect. The IR laser wavelength influences the discrimination capabilities of the method, probably due to the different IR absorption properties of the sample compounds. Further experiments in progress may shed some light on this question.

The use of continuous wave visible diode lasers (methods II and III) produces significant changes in the V-UV spectrum of one of the EVOO, (EVOO A). Irradiation with the diode laser at 450 nm produces larger changes than those produced by the 650 nm diode laser irradiation. The 450 nm laser induces chemical reactions in the liquid oil surface and as a result precludes the discrimination. On the other hand, Laser Vaporization at 650 nm modifies the vapour-liquid equilibrium conditions improving the selectivity of the electronic nose.

It is important to emphasize that although the discrimination obtained with IRLV is larger than that resulting from LV at 650 nm it is insensitive to the recognition pattern used. On the other hand, diode lasers are considerably cheaper than high power TEA CO₂ lasers and they produce very good results considering the benefit-cost ratio.

4. Acknowledgements

The authors acknowledge, Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and the Comisión Nacional de Energía Atómica (CNEA) and CONICET, for financial support

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Olive Oil - Constituents, Quality, Health Properties and Bioconversions

Edited by Dr. Dimitrios Boskou

ISBN 978-953-307-921-9

Hard cover, 510 pages

Publisher InTech

Published online 01, February, 2012

Published in print edition February, 2012

The health-promoting effects attributed to olive oil, and the development of the olive oil industry have intensified the quest for new information, stimulating wide areas of research. This book is a source of recently accumulated information. It covers a broad range of topics from chemistry, technology, and quality assessment, to bioavailability and function of important molecules, recovery of bioactive compounds, preparation of olive oil-based functional products, and identification of novel pharmacological targets for the prevention and treatment of certain diseases.

How to reference

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K. Pierpauli, C. Rinaldi, M. L. Azcarate and A. Lamagna (2012). Innovative Technique Combining Laser Irradiation Effect and Electronic Nose for Determination of Olive Oil Organoleptic Characteristics, Olive Oil - Constituents, Quality, Health Properties and Bioconversions, Dr. Dimitrios Boskou (Ed.), ISBN: 978-953-307-921-9, InTech, Available from: <http://www.intechopen.com/books/olive-oil-constituents-quality-health-properties-and-bioconversions/innovative-technique-combining-laser-irradiation-effect-and-electronic-nose-for-determination-of-org>

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