

Sensorial analysis and electronic aroma detection to compare olive oils produced by different extraction methods

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RESUMEN

Análisis sensorial y detección electrónica de aromas para comparar aceites obtenidos por diferentes métodos de extracción.

El análisis sensorial y el análisis de aromas por medio de sistemas sensoriales electrónicos han sido utilizados para comparar aceites de oliva producidos a través de dos sistemas de extracción diferentes.

Los métodos de extracción comparados han sido el sistema de prensas y el decantador de dos fases. Las muestras fueron producidas durante las cosechas del periodo 2002-2004, y las aceitunas eran todas de la misma variedad portuguesa *Galega sp.* Las aceitunas fueron seleccionadas y tratadas tecnológicamente bajo condiciones predeterminadas y supervisadas. Los aceites producidos resultaron mejor clasificados cuando fue aplicado el análisis sensorial por panel que cuando se utilizó el análisis con detección electrónica de aromas, incluso después de la optimización de los sensores. Esta observación está de acuerdo con el hecho de que los aceites son una matriz poco volátil y que es el "flavour", más que el aroma, el que junto con el gusto puede proporcionar una caracterización mejor que la detección electrónica, en la que el aroma es la principal característica evaluada.

PALABRAS CLAVE: Aceite de oliva – Análisis Sensorial – Aroma – Detección electrónica de aromas – Sistema de extracción.

SUMMARY

Sensorial analysis and electronic aroma detection to compare olive oils produced by different extraction methods.

A sensorial analysis and an aroma analysis by electronic sensory devices were used to compare olive oils produced according to two different extraction methods.

The extraction methods compared were the press system and two phase decanter. Samples were taken from the harvests of 2002-2004 and the olives were all from the same variety. The variety used was the Portuguese *Galega sp.* Olives were picked and technologically handled under predetermined and supervised conditions. Olive oils produced were better classified when the sensory analysis by a panel was applied than when an electronic sensory analysis was performed, even after sensor optimization. This observation is in accordance with the fact that olive oil has a

low volatility matrix and "flavor", rather than aroma, can give a clearer characterization than electronic sensory analysis alone, where aroma is the main characteristic evaluated.

KEY-WORDS: Aroma – Electronic nose – Extraction technology – Olive oil – Sensorial analysis.

1. INTRODUCTION

Food consumption is intrinsically connected to the stimulation of the human senses, namely taste and smell. The sense of smell is stimulated by the reception of a complex mixture of compounds, usually hydrophobic, from quite a large spectra of molecules which might be present under different concentrations, some of them even extremely low (Blake, 2007). The persistent and resident time of the aroma is usually related to the volatility of the compounds responsible for the smell. The aroma perceived by the nose is sent to the brain for identification (Smith *et al.*, 2001).

In order to "feel" the taste the mixed matrix has to be solved. The term usually used, "flavor", combines taste and aroma sensations.

So far, several compounds belonging to different chemical families have been identified in olive oil. Most of the volatile compounds in olive oil are formed after the oxidation of free fatty acids either by auto-oxidation, enzymatic oxidation or photo-oxidation (Gouveia 1995; Cavalli *et al.*, 2004). Enzymatic oxidation, catalyzed by lipoxygenase, is the most common pathway. These enzymes are able to catalyze oxidation from polyunsaturated fatty acids namely linoleic and linolenic acids. The enzyme becomes active as soon as the fruit is destroyed by crushing during extraction. This pathway is usually referred to whenever a fruit is exposed to stress conditions such as picking, crushing, warming, etc (Morales *et al.*, 1997; Morales *et al.*, 2000; Ridolfi *et al.*, 2002; Vichi *et al.*, 2003; Angerosa *et al.*, 2004; Cavalli *et al.*, 2004; Marques *et al.*, 2007).

This oxidation mechanism is responsible for the production of hexanal, *cis*-3-hexenal (with a

greeny/grassy sensorial note) as well as the *cis*-3-nonenal, *cis*, *cis*-3,6-nonadienal (with olfactory perceptions of "cucumber" and "apple"). These aldehydes are respectively transformed into C₆ and C₉ alcohols and esters (Morales *et al.*, 1997; Morales *et al.*, 2000; Ridolfi *et al.*, 2002; Vichi *et al.*, 2003; Angerosa *et al.*, 2004; Cavalli *et al.*, 2004; Marques *et al.*, 2007).

Olive oil sensory characteristics are the main consideration to determine consumer acceptance. High quality olive oils have a volatile profile whose balance in the oil allows for the designation of the positive attributes "fruity" or "green". These positive attributes can be diminished by the presence of negative sensorial notes that will depreciate the initial pleasant ones. Defects are identified as belonging to four main groups "mouldy", "winey", "buttery" and "fusty". Each of these defects presents a perfectly distinct volatile profile (Angerosa *et al.*, 2004; Morales *et al.*, 2004; Vaz-Freire *et al.*, 2008).

"Mouldy", "winey" and "fusty" are usually associated with defective storing conditions before extraction, while "buttery" is associated with a defective olive oil storage (Morales *et al.*, 2004).

The degree of consumer acceptance depends on the degree of intensity of each defect. CE regulation n°1989 from 2003 classifies the olive oils as "lampantes" whenever a defect's intensity is too high. These olive oils are not acceptable for human consumption.

A sensorial analysis can be defined as a discipline used to measure, analyze and interpret the reactions to food product characteristics by our senses (Ardeshtir, 1993).

A sensorial analysis uses the human senses as a measuring tool, although as a more precise tool, it depends on previous stimulus as well as the physiological, psychological and social condition of the tasters (Blake, 2007). The food Industry uses these parameters in a very precise, but rather expensive way.

The sensorial analysis can be classified as discriminative when it indicates the presence or absence of differences (Poste *et al.*, 1986; Lyon 1987) or it can be descriptive whenever the intensity of the differences is to be evaluated (Poste *et al.*, 1986; Lyon 1987; Verrelli 2008).

For a sensorial analysis concerning taste and aroma COI (international olive council) is normalizing the analytical methodology going as far as the normalization of glasses, rooms and environmental conditions as well as selection, training and recycling of tasters besides vocabulary and final classification of the different attributes (COI/T.20/Doc. no. 5, 1987; COI/T.20/Doc. no. 6, 1987; COI/T.20/Doc. no. 13/Rev.1, 1996; COI/T.20/Doc. no. 15/Rev.1, 1996; IOOC - Resolution no. RES-3/75-IV/96, 1996).

Since 1991, the sensorial analysis coupled with a fixed and extensive group of chemical and physical analyses are necessary, according to the CEE regulation N°2568/91 (European Commission, 1991), with the corrections introduced by the CEE

regulation N°796/2002 (European Commission, 1992) to correctly classify virgin olive oil according to the COI's established conditions.

The main issue of some of these recommendations and regulations is probably the fact that although the sensorial analysis is well established and considered, it is rather difficult and expensive to train a sensorial panel, besides the fact that not everyone can be trained as a panellist.

Bearing this in mind and, in order to try to obtain more routine and "unspecialized" results, we used electronic sensory analysis (sometimes called "electronic nose"). The basis of an "electronic nose" is the use of a matrix of non-selective chemical sensors, (usually polymeric or metallic oxides) along with complex statistical treatment, to mime the human olfactory system and the human brain. The sensors will receive the chemical information, carried by the vapour above the sample and after the generation of some mathematic algorithms, the processed information is translated into a two dimensional plot similar to a "fingerprint" information of the aroma (Brezmes *et al.*, 2007; Sousa *et al.*, 2007). Currently a specialized instrument, specific to olive oils, is being developed (Brezmes *et al.*, 2007). The main difference from the "normal" instruments is the specificity of the sensors adjusted to the matrix.

2. MATERIALS AND METHODS

2.1. Sample preparation

Experiments were carried out by mechanically processing picked olives of the Portuguese cultivar *Galega Vulgar*. All olives were picked according to proper controlled sanitary conditions. Olives were picked during the harvests of 2001, 2002, 2003 and 2004.

From each cultivar a 120Kg sample was collected. Fruits were stored in open boxes at ambient temperature (5-15°C) with reasonable air flow and without direct light incidence. Extraction was done during the next 24h. Before extraction, leaves and dirt were removed by washing under cold running water.

2.2. Extraction Technology

An homogeneous 20 Kg sample was processed for each of the technologies under study: a hammer-mill press line (Vieirinox, Portugal) and a hammer-mill integral decanter line (Oliomio, Italy) were used. No water was added to the olive paste in both systems and malaxation time, about 1 h, was the same for both methods. Three replicates were made for each extraction /season.

2.3. Chemical analysis: acidity , UV spectroscopy, peroxide index

Acidity, UV spectroscopy and peroxide index were determined according to CE regulation nr. 2568/91. Results refer to the mean value of the replicates.

2.4. Sensorial Analysis

Pannel sensory analysis

A sensorial analysis was performed according to the rules established by the COI and CEE (IOOC - Resolution no. RES-3/75-IV/96, 1996; Regulation (CEE) nº2568/1991; Regulation (EC) nº 1989/2003). The panel had seven well trained tasters, trained to discriminate olive oil attributes. The sensorial analysis was performed in a sensory room. The room was isolated with an environmental temperature of 20-22°C and a relative humidity between 60-70%.

The samples were poured into adequate dark glasses and presented, anonymously, to each

taster, at a temperature of $28 \pm 2^\circ\text{C}$ covered by a watch glass. Apple slices as well as water glasses were present as palate cleansers. The three replicates of each extraction were considered as one batch and from each batch three samples were considered for the sensory analysis. Analyses were carried out on consecutive days.

Each taster smelled and tasted the olive oil and registered the intensity due to each positive and/or negative attribute perceived. The results were written on a normalized profile sheet adapted by Laboratório de Estudos Técnicos ISA/UTL, Lisbon in accordance to Regulation (EC) nº 1989/2003, (Table1).

Table 1

Smell-taste-touch notes							Punctuation Table		
Attribute	Intensity of perception ⁽²⁾						Defects	Characteristics	Global Evaluation
	0	1	2	3	4	5			
							None	Fruity olive or fruity from other fruits	9 8 7
Fruity Olive (mature or green) ⁽¹⁾							Light or almost unnoticed	Very slightly fruity	6
Apple							Perceptible	Fruity but with slight defects or other taste or smell abnormal	5
Other mature fruits							Perceptible but still acceptable	Fruity but with strong defects and unpleasant taste and smell	4
Green (leaves, grass)							Very strong and very perceptible	Taste and smells totally unacceptable for consumption	3 2 1
Bitter									
Pungent							Observations		
Sweet									
Other(s) acceptable attribute (s)									
Which?							Name		
Acid/sour/winey							Sample Code		
Muddy sediment									
Metallic									
Musty - humid							Date		
Fusty									
Rancid									
Other unacceptable attribute(s)									
Which?									

⁽¹⁾ Scratch the unnecessary preception

⁽²⁾ 0 = total absence ⁽³⁾

1 = almost unnoticed; 2 = slight; 3 = medium; 4 = strong; 5 = extreme

⁽³⁾ The total absence of sensorial preception is indicated by signing the corresponding space with an X

Electronic sensory analysis

The Electronic sensory analysis device used was an Alpha MOS FOX 3000, equipped with 12 metal oxide sensors: SYLG, SYG, SYAA, SYGH, SYGCTI, SYGCT, T301, P101, P102, P401, T702, and PA2 (Table 2). T and P are metal oxide with SnO_2 as semiconducting material, the difference between these two types of sensors is only related to the geometry of the sensors. On type T sensors the sensitive material is placed into an aluminium tube (T) while type P is a flat sensor. SY sensors are metal oxide sensors with a Titanium and Chromium oxide ($\text{Cr}_{2-x}\text{Ti}_x\text{O}_{3+y}$) and a Tungsten oxide (WO_3).

The sample headspace is carried through the sensors by a flow of continuous extra pure compressed air at a flow rate of 150 ml/min. For electronic sensorial analysis, the conditions used were: headspace development: incubation time 300 sec at 40°C; agitation speed 750 rpm; syringe temperature: 45°C; injection volume: 500 µl; injection speed 500 µl/sec; acquisition time, 120 sec with a period of 0.5 sec

2.5. Statistical analysis

A univariate statistical analysis as well as PCA ("Principal Component Analysis") were made using the Statistica 6.0 software (Stat Soft, Inc, USA).

3. RESULTS AND DISCUSSION

3.1. Sensorial analysis

The monovarietal olive oils from the Galega variety showed rather scattered sensorial values allowing the samples from the two extraction methods used to be classified according to CE regulation nr. 1989 (2003), from lampante (unacceptable for human consumption) to extra virgin olive oil. The analytical results associated to this classification were acidity, K and peroxide index. According to regulation CE 2568/91, based on the sensorial analysis alone, Galega olive oils never get the lampante classification which is attributed to a sensorial final score below 3.5 (Table 3).

Table 2
Sensor types and volatile descriptors

Sensors	Description of volatiles analyses
P101, P102, SYGCT	Non-polar volatiles, methane, propane, hydrogen bonding compounds, aldehydes
PA2, SYAA, T301	Polar compounds, alcohol
T702	Alcohol, aromatic compounds
SYG, SYGH, SYGCTI	Ammonia and ammonia derivatives, sulfur, amines and amine containing compounds
P401, SYLG	Chlorinated compounds, aldehydes

Table 3
Sample classification according to the sensory panel results

Technology/year	Two phase 2001 (n=3)	Press 2001 (n=3)	Two phase 2002 (n=3)	Press 2002 (n=3)	Two phase 2003 (n=3)	Press 2003 (n=3)	Two phase 2004 (n=3)	Press 2004 (n=3)
(1)Attributes								
Fruited (mature and green)	1.5	1.3	1.2	1.8	1.5	2.7	2.2	0.7
Apple	0	0	0	0.3	0.3	0.8	0.5	0.0
Other mature fruits	0	0	0	0	0	0.3	0	0.5
Green	0	0	0	0	0	0.7	0.7	0.2
Bitter	0	0	0	0	0	0.8	0.2	0.3
Pungent	0	0	0	0.7	0.0	1.7	0.5	0.3
Sweet	0	0	0	1.0	0.3	1.2	0.7	0.5
Other acceptable attributes Which?	0	0	0	0	0	0	0	0
Winey/acid	0	0	0	0	0	0	0	0
Metallic	0	0	0	0.3	0	0	0	0
Musty-Humid	1.5	0.8	2.7	0	2.7	0.0	0.8	3.0
Fusty	0	0	0.0	0	0	0	0	0
Mouldy	0	0	0.5	0.3	0	0	0	0
Rancidity	0	0	0.0	0.0	0	0	0	0
Other unacceptable attributes	0.8	1.5	0.0	0.8	0	0.5	0	0
(2)Final punctuation	5.5	5.5	4.8	6.2	4.0	7.3	6.3	3.8

(1) 0= total absence; 1= almost undetected; 2=light; 3= medium; 4= intense; 5= extreme

(2) 9-7 = no defects; 6= defects light or almost undetected; 5= detected; 4= limit of accepted defects; 3-1= very strong defects

When an univariate test of significance (two-way ANOVA) was applied, no significant differences ($P > 0,05$) were found among the different olive oils when year or extraction methods were considered. However, when year/extraction interaction was considered a significant difference ($P < 0,05$) among olive oils was observed ($P = 0,014$). According to figure 1, olive oils produced in years 3 and 4 (2003, 2004) seem to be the ones mainly responsible for the significant differences observed. Results are not in agreement with others who noticed a clear impact on year over cultivar (Gracia *et al.* 2000) probably due to the fact that we were looking just to one cultivar and we considered only acidity, K and peroxide index. In fact, differences among cultivars are more evident when volatiles, fatty acids and sterol composition are considered (Vekiari *et al.* 2010).

When we analyzed the scores given to the samples in which the “musty-humid” attribute is perceived, we could detect a direct relation ($r^2 = 0,8638$) between this attribute and the mean score given by the panel, implying a direct impact, of this attribute, for olive oil classification. The “musty-humid” attribute is usually associated to very long filtration processes or excessive contact time with lees.

When PCA analysis was made using the different parameters (attributes in table 2) used for sensorial analysis we could verify a trend, showing that decanter extraction technology produces more consistent scores (over the years) than the press system (Figure 2).

3.2. Electronic sensory analysis

Data obtained from the electronic sensory analysis was also submitted to PCA analysis, using the

specific software of the Alpha Mos instrument. As in any usual PCA analysis the basic principle is to rewrite the original variables into new ones. These new variables are plotted in the two dimensional space by transforming the original coordinates (Ferreira *et al.*, 2002).

The discrimination index obtained gives a clear indication of the separation observed among groups. Negative discrimination indexes reveal overlapping samples/groups while a positive discrimination index shows a clear separation among sample/groups; when the index lies between 80-100, a perfect separation is obtained.

When the complete set of sensors are used (description associated to the sensors are shown in Table 2) no clear separation could be perceived and the discrimination index was -38. Since the instrument software allows an automatic selection of sensors, we used this feature to optimize the separation (Figure 3). As can be seen the two first PCA's (PC1 and PC2) account for a total variance of 100% (99,49% PC1 and 0,51% for PC2), however the discrimination index which is used to explore the data and to assess discrimination performance, giving an indication on the discrimination quality, is still negative thus a clear separation cannot yet be considered and an overlapping of samples/groups can be understood.

It has already been shown that the vulgar volatile composition of the Galega cultivar (Vaz Freire and al, 2009) is characterized by a reduced number of compounds which include an intense peak of hexen-2-al as well as other compounds with 6 carbon atoms, like Z-2-hexenol, E-3-hexenol, Z-3-hexenol when analysis is made by SPME/GCMS. This small number of compounds might

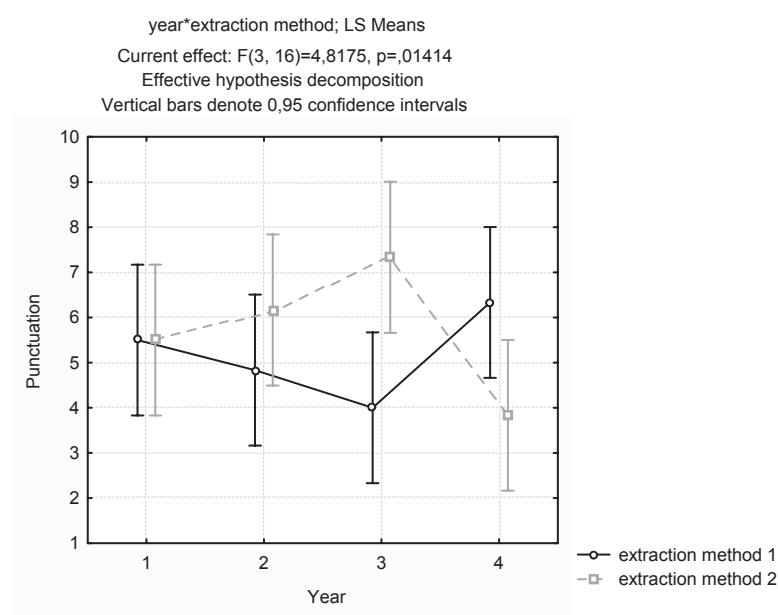


Figure 1
Effective hypothesis decomposition for Galega Vulgar, year and extraction method
(Vertical bars denote 0.95 confidence intervals).

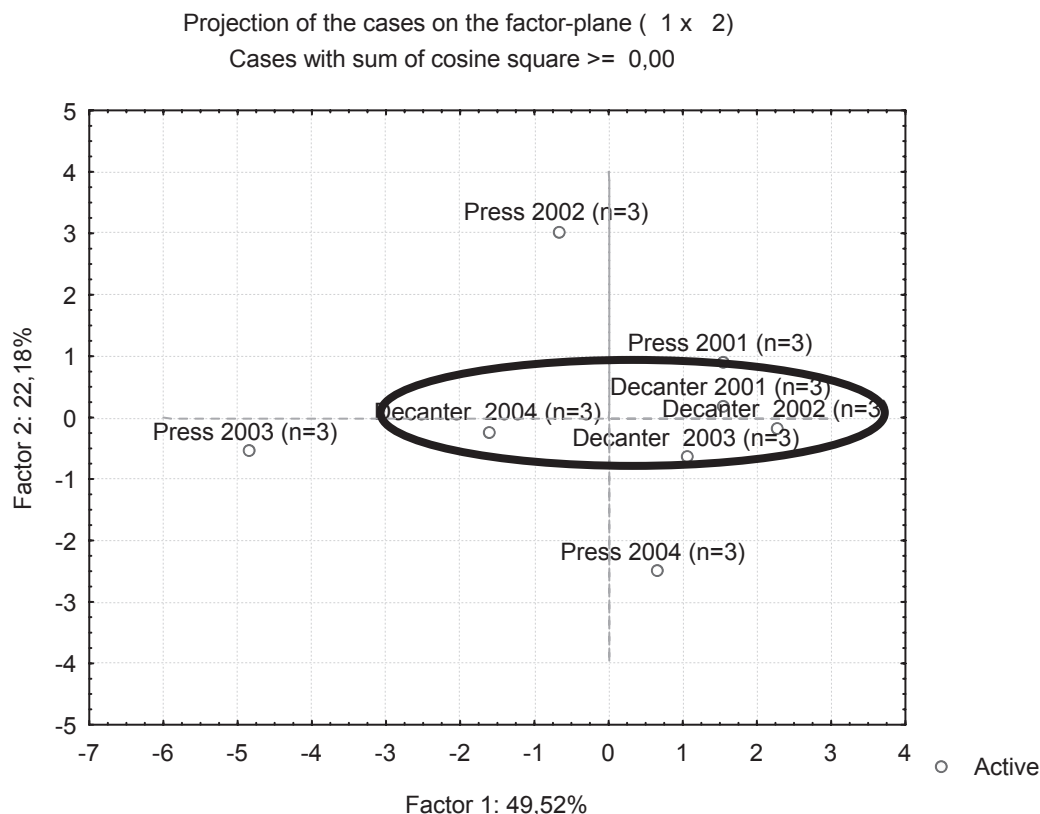


Figure 2
Projection of the cases on the factor-plane (1 x 2) for correlation matrix.

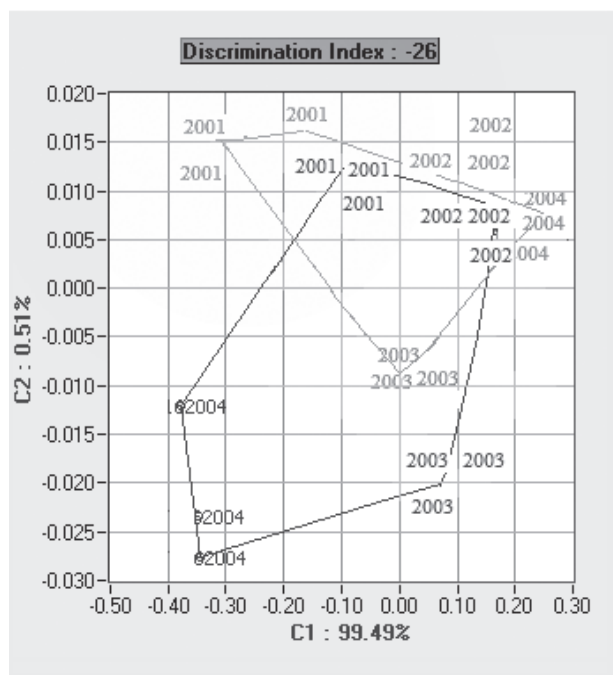


Figure 3
Plot of the PCA analysis of olive oil samples obtained during the studied years by two different extraction technologies using an optimized set of sensors of the Alpha Mos instrument; — - press system; - - two phase decanter.

explain the low discrimination obtained and can help understand which were the discarded sensors, SYG, SYGH, SYGCTI, which are mainly related to the detection of ammonia and ammonia derivatives, sulphur, amines and amine containing compounds. Moreover, the final result is also affected by the fact that the remaining sensors are all sensitive to similar compounds like alcohols and aldehydes (Table 1), which are exactly the dominant volatiles of the aroma fraction of the Galega olive oil.

Our results are not in accordance with previous ones that used an e-nose (zNoseTM 7100 vapor analysis system, EST, Newbury Park, CA, USA), consisting of a 1-m DB-5 column and a SAW detector with a parts per billion sensitivity (Kadirog'lu *et al.* 2010), in combination with chemometrics, and were able to separate extra virgin olive oils, according to the cultivar, geographical origin and harvest year. Also García -González *et al.* (2010) coupled the e-nose with GC, generating a so-called sensogram, and proved the sensor sensitivity towards alcohols, aldehydes and other compounds known as responsible for sensory defects in virgin olive oil. The use and comparison of the e-nose with the sensory analysis by a panel was not reported, however the need for a specialized e-nose for olive oil analysis with tailor made sensors was reported, namely for authenticity and detection of taints (Berna

A. 2010). These results support our conclusions that electronic detection in a non-volatile matrix such as olive oil, is not a straightforward analysis and results need careful interpretations.

4. CONCLUSIONS

A clear separation, among olive oils produced from the Galega olives during the harvests of 2001-2004 using different extraction procedures could not be detected using the electronic sensory device alone. Sample complexity, as well as low volatility and small concentration of important volatiles is probably responsible for these results (Drake *et al.*, 2003).

The sensorial analysis seems to be more effective in perceiving slight differences. These results might be explained by the complex set of compounds that account for the sensorial results (where samples are smelled and tasted) and not for the electronic sensing where just volatiles are perceived.

The results are in accordance with the fact that fraud in olive oil is usually detected by a complete set of analysis and sensorial analysis alone is not able to detect and confirm fraud.

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