

Short Communication

Monitoring odour of heated extra-virgin olive oils from Arbequina and Manzanilla cultivars using an electronic nose

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It is known that fats and oils, when submitted to prolonged heat, are subjected to a series of chemical–physical modifications, generating changes in sensory and nutritional characteristics. The aim of the present research was to develop an accurate and repeatable method to predict appearance of undesired odour when extra-virgin olive oils from Arbequina and Manzanilla are submitted to heating processes, using an electronic nose. Among the metal oxide sensor response (electronic nose) an odour index was performed. Emissions of several volatile organic compounds released during the process were analysed by SPME-Gas chromatography. Linear discriminant analysis was applied on the sensor response; it was found that among eight sensors, four sensors were more specific for detecting release of volatile organic compounds during the heating process. The Mahalanobis distance method was adopted to create an odour index and was correlated with emissions of *n*-pentanal, *n*-hexanal, *n*-heptanal and *n*-nonanal. Data showed that the methodology accurately and rapidly predicted changes in odour pattern between cultivars.

Practical applications: Changes in odour pattern of different cultivars of extra-virgin olive oil subjected to heating processes can be performed quickly and easily by applying electronic nose. Appearance of undesired odour due to thermo-oxidation can be detected applying an odour index based on the metal oxide sensors response. High correlations can be observed when linear regressions are applied to odour index and volatile compounds (VOCs) content. New markers of oxidation can be obtained applying this methodology. Electronic nose instruments are attractive for a number of significant features: the relatively fast assessment of headspace, a quantitative representation or signature of a gas and cheap sensors which can be easily integrated to quality control. The motivation of the present research was to incorporate metal oxide sensors in electronic nose as a useful tool to analyse the odour pattern and quality of different products.

Keywords: Metal oxide sensors / Quality / Scalar index / Volatile compounds

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1 Introduction

Frying is the most commonly used method worldwide due to its simplicity and because it generates pleasurable aromas, appearances and textures in the cooked food. The manner of

employing olive oils varies within culture, but it is principally their use at high temperatures in the presence of atmospheric oxygen that causes the alteration in their physical characteristics such as changes in the flavor stability, quality, colour, texture, viscosity, decomposition of nutritional quality and chemical characteristics, leading to the production of toxic compounds [1].

The hydrolysis, oxidation and polymerization of oil are common chemical reactions in heating oil's and produce volatile or nonvolatile compounds. Most of volatile compounds (VOCs) evaporate in the atmosphere with steam and the remaining VOCs in oil undergo further chemical reactions or are absorbed in fried foods [2]. On the other

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Abbreviations: **ARB**, Arbequina; **EVOO**, extra virgin olive oil; **MAN**, Manzanilla; **MOX**, metal oxide sensors

hand, deep-fat frying decreases the unsaturated fatty acids of oil and increases foaming, colour, viscosity, density, specific heat and contents of free fatty acids, polar materials and polymeric compounds [3]. Determination of VOCs is considered one of the best methods to evaluate the extent of oxidation in edible oils and fats and hence the customers acceptability of the food product. A study of the evolution of VOCs during the course of thermo-oxidation is therefore required to establish what compounds are to be taken as index of oxidation [4]. Due to these characteristics it is necessary to monitor changes in the pattern of odour within time among the emissions of volatile aldehydes generated in the process.

Flavor in food products are determined through biochemical assays and sensory panels which are time consuming, less reliable and of low sensitivity. Among the years electronic nose device has been introduced in the market due that it is able to recognize and compare individual or complex odours of substances. Several authors have reported the application of metal oxide gas sensors for process monitoring [5], shelf-life [6], freshness [7], authenticity assessment [8] and quality control for cooking and fermentation process [9].

The aim of this research was to develop an accurate and repeatable method to predict the appearance of undesired odour when olive oils from Arbequina and Manzanilla are submitted to heating processes, using an electronic nose composed of metal oxide sensors.

2 Materials and methods

2.1 Extra-virgin olive oil samples

Samples of extra-virgin olive oil cultivars Arbequina (ARB) and Manzanilla (MAN) (being the original Spanish names given preserved) were properly obtained from fresh, mature fruits of good quality provided by an olive oil mill (Argentina). Table 1 shows physicochemical parameters of the cultivars used in the present research.

Table 1. Physicochemical parameters of Manzanilla and Arbequina extra-virgin olive oil

Physicochemical parameters	Manzanilla	Arbequina	EVOO ^a
Free fatty acids ^b	0.21 ± 0.02	0.46 ± 0.03	≤0.80
Peroxide value ^c	9.20 ± 1.02	11.90 ± 1.16	≤20.0
K270	0.13 ± 0.01	0.15 ± 0.01	≤0.22
K232	1.48 ± 0.01	1.56 ± 0.01	≤2.50

^aExtra-virgin olive oil, IOOC (2011).

^b%C18:1.

^ceq O₂kg⁻¹.

2.2 Heating process of extra virgin olive oil

Frying pans (11 × 9.4 cm²) were filled with 600 mL of ARB and MAN and heated at 180°C. Oil samples were obtained at the beginning (*t*₀) of the experiment and then, four times at intervals of 15 min (*t*₁₅, *t*₃₀ and *t*₄₅) during 60 min (*t*₆₀) and one time at interval of 60 min (*t*₁₂₀). Samples were cooled at room temperature and stored under nitrogen atmosphere at -5 ± 1°C until analysis. Heating procedure was repeated thrice.

2.3 Electronic nose analysis

The electronic nose system was equipped with an array of eight pure metal and doped semi-conductive SnO₂ gas sensors (S1, S2, S3, S4, S5, S6, S7 and S8; MOSES II, Modular Sensor System). Doping with different elements increases SnO₂ selectivity for different gases. In the presence of a reducing gas there is absorption with an electronic exchange of gas towards the sensors, the conductance of the *n*-type increase. The adopted configuration results very flexible for general purposes and convenient for a wide range of applications. The response of each sensor was determined from $\Delta R/R$ value, which is the change in the resistance of metal oxide sensor due to the VOCs of extra-virgin olive oil with the respect to the base value [10]. The base value is the resistance shown by the sensors due to VOCs of freshly (non spoiled) extra-virgin olive oil.

Samples of 3 ± 0.005 g of each EVOO oil (ARB and MAN) were placed in five 10 mL glass vial equipped with a screw cap and silicon septum. Optimisation of electronic nose process parameters were fixed through preliminary trials. The protocol of selected analysis was defined as followed: during the acquisition process, samples were kept at 50°C for 10 min and shaken at 500 rpm in order to obtain equilibrium in the headspace. An aliquot of 1 mL per vial was taken using a syringe, which was pre-heated at 50°C to avoid condensation. Then, 100 µL was injected into the device. The system was continuously purged with synthetic air (synthetic air, Air Liquid) set at 150 min⁻¹. The acquisition time was set at 120 s and the delay time (time elapsed between subsequent analyses) was 18 min. These experimental conditions ensured that each step during data acquisition was enough to establish a correct baseline to collect VOCs and to allow the recovery up of sensors between sample analyses. The maximum amplitude in the sensor response curve was considered for analysis.

2.4 Sensor response and scalar index

In order to analyse metal oxide gas sensors signal that are most sensitive towards VOCs emissions from heating process, the signal responses $\Delta R/R$ of eight sensors were subjected to a discriminant analysis. A scalar index was generated from the electronic nose data by computing the

Mahalanobis distance, which is proportional to the electronic nose sensor signal response between each sample M_i and the baseline N (freshly EVOO) [11]. For each interval (i), vector (M_i) was prepared from the data obtained from the selective sensor and Mahalanobis distance was calculated between M_i and N and a scalar value called 'odour index' was performed. The aim of a scalar index was to evaluate changes in odour pattern due to the emissions of VOCs generated in the heating process. On the other hand, this scalar index can predict the end of the induction period or appearance of undesired odour in the samples.

2.5 Determination of volatile compounds

The following VOCs were analysed according to references [3, 12–16]: *n*-pentanal, *n*-hexanal, *n*-heptanal and *n*-nonanal. The references reported that these aldehydes can be possible markers to predict the end of the induction period.

Solid-phase micro-extraction gas chromatography (SPME) was conducted to evaluate emissions of VOCs content in EVOO during frying process. Aliquots of 5 ± 0.05 g of ARB and MAN were placed into 10 mL headspace vials adding 100 μ L of internal standard (4-methyl-2-pentanone, Aldrich[®]). Volatile compounds were extracted with Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μ m fiber [17]. SPME extractions were carried out placing the sample vial in a water bath at 50°C for 40 min. SPME holder assembly was changed to 4.4 scale units prior to injection into the gas chromatography (GC) injector port, SPME fiber remained in the injector for 5 min.

A Shimadzu series 14B Gas/liquid chromatography, equipped with a flame ionization detector was used. The injector was operated in the splitless mode at 260°C and the detector at 280°C. Nitrogen was used as carrier gas at a flow of 1 mL/min. A 30 m \times 0.32 mm id \times 1.00 μ m ATM5 capillary column was used. Column temperature-programming sequence used: an initial temperature of 40°C was maintained for 5 min before being increased to 80°C at 2°C/min then, it was raised from 80 to 150°C at 4°C/min and, finally, from 150 to 280°C at a rate of 10°C/min and held for 10 min. Peak identifications were based on comparison of retention time of unknowns with authentic standards. The relative concentrations of individual compounds were determined by comparing the peak area of the compound in each chromatogram with internal standard and considering the relative response factors of each studied compound. The internal standard quantification method was based on that described in reference [17] with the following modifications: standard mixtures with concentration in the range of 0.2–1.0 μ g/g for 3 *n*-pentanal (Aldrich[®]), *n*-hexanal (Aldrich[®]), *n*-heptanal (Aldrich[®]) and *n*-nonanal (Aldrich[®]) and were analysed under the conditions described earlier; the absolute response factors for each compound were then obtained from the slope of the linear

regression of the total peak area as a function of the concentration and the relative response factors were calculated as the ratio between the absolute response factors of each compound to that of the internal standard. The relative concentrations are the average of three separate SPME extractions collected from each sample.

2.6 Statistical analysis

In the current investigation, statistical analyses were carried out to study the redundancy in the data sets obtained from sensor responses of different EVOO cultivars using an electronic nose. One way ANOVA was carried out to study the emissions of VOCs content. Regression equations were generated to predict changes in odour pattern by emissions of VOCs contents as a function of odour index (electronic nose). Significant differences between means were determinate by Tukey test. A P value of 0.05 was used to verify the significance of all tests. All statistical tests of this experiment were conducted using SPSS-Advanced Statistics 12 software (SPSS Inc., Chicago, IL).

3 Results and discussion

3.1 Volatile compounds determined by conventional assays

Volatile compounds are represented in Table 2 for MAN and ARB EVOO. ANOVA study shows that in both cultivars, volatile compounds release increased significantly ($P < 0.0001$) in content during heating process.

Different studies on extra-virgin olive oil showed that volatile compounds such as *n*-pentanal and *n*-hexanal derived from linoleic acid (C18:2). On the other hand, *n*-nonanal is emitted after degradation of oleic acid (C18:1), precisely from the haemolytic fission of the R-O bound of the fatty acid [13, 16].

Several authors have reported that *n*-hexanal in extra-virgin olive oil can be used as an oxidative deterioration parameter [14, 15]. Van Durme [16] reported that measuring secondary volatile lipid oxidation products in olive oils, such as C5 aldehydes (*n*-pentanal), hydrocarbons, etc. can also be used as relevant marker of molecules for evaluating the degree of chemical lipid oxidation.

Morales [3] reported that when thermo-oxidation process was applied to extra-virgin olive oil, the initial volatiles (a total of 60), many of them responsible for the pleasant sensory characteristics of the oil and produced mainly through biochemical pathways, disappeared in the first hours, and the formation of off-flavors, produced through oxidative pathways, gradually increased.

Rao-Katragadda [15] reported that when extra-virgin olive oil is heated at 210°C, the emissions of *n*-pentanal, *n*-heptanal and *n*-nonanal are increased during heating time, showing the highest content for *n*-nonanal.

Table 2. Volatile compounds content of extra-virgin olive oil of Manzanilla and Arbequina obtained during heat among time (15, 30, 45, 60 and 120 min)

Cultivar-Heating period (min)	Volatile compounds ($\mu\text{g/g}$)			
	<i>n</i> -pentanal	<i>n</i> -hexanal	<i>n</i> -heptanal	<i>n</i> -nonanal
<i>Manzanilla</i>				
0	0.12 ^a	0.29 ^a	0.10 ^a	0.34 ^a
15	0.15 ^a	0.31 ^a	0.16 ^a	0.49 ^a
30	0.25 ^b	0.58 ^b	0.56 ^b	2.53 ^b
45	0.38 ^c	0.94 ^c	0.72 ^c	3.50 ^c
60	0.44 ^c	1.00 ^c	0.73 ^c	3.59 ^d
120	0.41 ^c	1.21 ^d	0.86 ^d	4.49 ^d
<i>P</i> -value	0.0001	0.0001	0.0001	0.0001
RSME	0.03	0.04	0.04	0.23
<i>Arbequina</i>				
0	0.33 ^a	1.10 ^a	0.19 ^a	0.68 ^a
15	0.45 ^a	1.24 ^a	0.31 ^a	0.81 ^a
30	0.62 ^b	1.96 ^b	0.95 ^b	3.68 ^b
45	0.63 ^b	2.70 ^c	0.97 ^b	4.63 ^c
60	0.87 ^c	2.93 ^c	1.14 ^c	5.61 ^c
120	1.06 ^d	3.49 ^d	1.18 ^c	6.36 ^d
<i>P</i> -value	0.0001	0.0001	0.0001	0.0001
RSME	0.06	0.16	0.06	0.19

Different letters in a column indicate significant difference (Tukey Test, $P < 0.05$).

Results showed that in both cultivars, when heating process occurred, increase in release of VOCs started at 30 min. Relations among VOCs at t_0 and at t_{120} , revealed that MAN had higher release for all VOCs analysed when compared to ARB; being for *n*-nonanal: MAN (92.4%) and ARB (89.0%); *n*-hexanal: MAN (76.0%) and ARB (68.5%); *n*-heptanal: MAN (88.0%) and ARB (83.9%); *n*-pentanal: MAN (80.0%) and ARB (68.9%).

Morales [3] reported that the early measurement of some aldehydes such as *n*-nonanal could be an appropriate method to detect appearance of undesired odour or end of the induction period.

Morelló [18] reported that the amount released of volatile compounds depends on the content of protective molecules such as chlorophyll, carotenoids and total phenol among others in thermo-oxidation.

Release of VOCs can be due to difference in content of protective molecules in each cultivar among other factors.

3.2 Electronic nose analysis

For electronic nose data ($\Delta R/R$), an odour pattern was produced for fresh samples and heated samples. Sensors response signal was analysed applying linear discriminant analysis with Wilks' lambda stepwise method.

For MAN, three discriminant functions (DF) was obtained explaining 99.7% of the total variance respectively, with a success rate of correct classification of each sample in their respective group of 100 and 100% of the original cases and after cross validation (data non shown). Among eight metal oxide sensors, it was found that four sensors (S_2 , S_4 , S_5 and S_6) were more specific for detecting emissions of VOCs during heating process. For ARB, two DF was obtained explaining 95.7% of the total variance respectively, with a success rate of correct classification of each sample in their respective group of 94.4 and 88.9% of the original cases and after cross validation. Four sensors (S_1 , S_2 , S_6 and S_7) were more specific to evaluate changes in odour pattern among fresh and heated samples (data non shown).

In general with a large number of sensors, the discrimination indices in principal components analysis are generally poor and better discrimination indices are obtained when numbers of sensors are reduced to 4–6 [19–22].

3.3 Scalar index: Odour index

Electronic nose with metal oxide gas sensors could effectively detect changes in odour pattern of fresh and in heated samples. In order to monitor changes in odour, a scalar index called 'odour index' was performed using sensors signal for fresh and each heating intervals applying Mahalanobis distance method (Table 2). ANOVA was used to analyse the scalar index; it was found that the odour index increased significantly ($P < 0.0001$) in both cultivars. Greater distance from baseline indicated increases in release of VOCs due to heating process.

Nissiotis [23] reported in EVOO that hydroxytyrosol derivatives are the first antioxidants that are lost during thermal-oxidation (until a peroxide value of 20–30 meq/kg). The stated author also reported that other derivatives such as tyrosol in EVOO seem to be the most stable compounds, while α -tocopherol has an intermediate rate of loss at low peroxide values and is destroyed at peroxide values from 20 to 50 meq/kg. Losses in protective molecules in thermo-oxidation conduce to increase release in VOCs.

In both cultivars, the scalar index showed that among heating process odour changed; increasing after 30 min. In order to correlate odour index with release of *n*-pentanal, *n*-hexanal, *n*-heptanal and *n*-nonanal as possible markers [3, 15, 16], data were subjected to linear normalisation prior to PC analysis in order to efficiently suppress quantitative effects on the multivariate data. Two PC were obtained for each cultivar, explaining 92.3% (ARB) and 96.1% (MAN) of the total variance respectively.

Linear regression analysis was applied with the PCA scores of volatile compounds obtained from each PC of each cultivar with odour index (Figs. 1 and 2).

Results revealed a relationship between odour index and VOCs analysed. Therefore, linear regression equations were

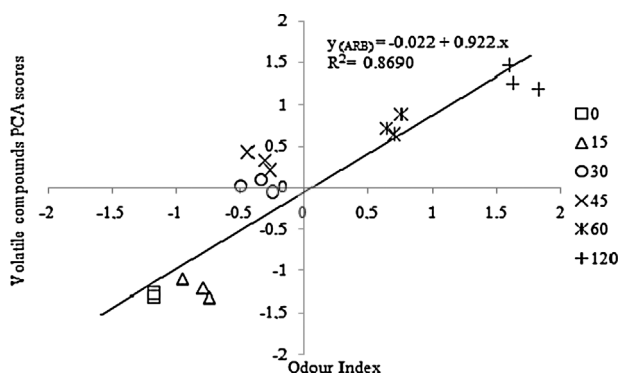


Figure 1. Linear regression of PCA scores of VOCs obtained by conventional assays and odour index of Arbequina extra-virgin olive oil submitted to heat among time: 0 (□), 15 (Δ), 30 (x̄), 45 (x), 60 (*) and 120 (+).

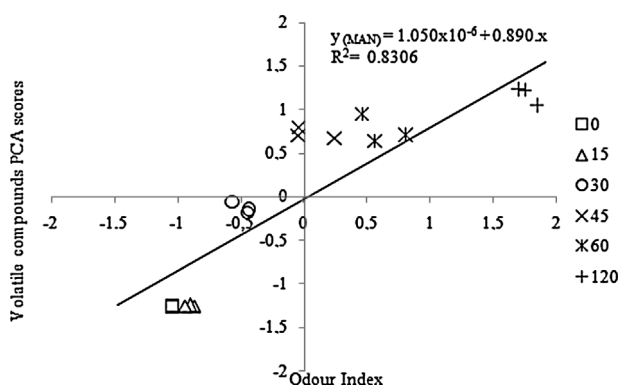


Figure 2. Linear regression of PCA scores of VOCs obtained by conventional assays and odour index of Manzanilla extra-virgin olive oil submitted to heat among time: 0 (□), 15 (Δ), 30 (x̄), 45 (x), 60 (*) and 120 (+).

Table 3. 'Odour index' of extra-virgin olive oil of Manzanilla and Arbequina obtained during heat among time (15, 30, 45, 60 and 120 min) using Mahalanobis distance methodology

Heating period (min)	Odour index	
	Manzanilla	Arbequina
0	0 ^a	0 ^a
15	0.31 ^a	0.47 ^a
30	1.26 ^b	1.98 ^b
45	2.47 ^c	3.61 ^c
60	3.73 ^d	4.63 ^d
120	6.30 ^e	7.20 ^e

Different letters in a column indicate significant difference (Tukey Test, $P < 0.05$).

Table 4. ANOVA data representing overall goodness of fit of the Eqs. (1) and (2) depicting volatile compounds data of each cultivar among time as a function of 'odour index'

Cultivar	Effect	Sum of squares	df	Mean squares	F	P-Level
Arbequina	Regression	137696	1	137696	77.47	0.00001
	Residual	28438	16	0.1777		
	Total	166134				
Manzanilla	Regression	134839	1	134839	61.36	0.00001
	Residual	35161	16	0.2198		
	Total	170000				

generated (Eqs. 1 and 2) which would denote volatile compounds PCA scores as a function of 'odour index'.

$$\text{Volatile compounds}_{(ARB)} = 1.05 \times 10^{-6} + 0.89 \text{ * odour index} \quad (1)$$

$$\text{Volatile compounds}_{(MAN)} = 0.02 + 0.90 \text{ * odour index} \quad (2)$$

Correlation coefficients of Eqs. (1) and (2) are 0.80 (MAN) and 0.83 (ARB) respectively. The overall goodness of fit of Eqs. (1) and (2) are reflected by their corresponding F -values of 61.36 (MAN) and 77.47 (ARB), obtained by ANOVA (Tables 3 and 4).

4 Conclusion

In the present research, changes in odour pattern of different cultivars of extra-virgin olive oil submitted to a heating process could be performed quickly and easy applying an odour index to the electronic nose metal oxide sensors response. High correlations were observed when linear regressions were applied to odour index and selected volatile compounds content analysed. In the future other VOCs must be analysed in order to improve the methodology. The development of electronic nose methodology with chemical sensory arrays constitutes a useful tool to analyse the odour pattern of different products. It will be useful to incorporate this methodology in the future since the quality control can be performed quickly with this approach.

The authors have declared no conflicts of interest.

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