Quality Assurance and Safety of Crops & Foods, 2014, 6 (3): 309-317
SPECIAL ISSUE*: Food chemistry and safety*

Novel oligopeptides based e-nose for food quality control: application to extra-virgin olive samples

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Received: 8 December 2013 / Accepted: 18 February 2014

RESEARCH ARTICLE

Abstract

The potential of an electronic nose to discriminate olive oil samples based on their sensory profiles is proposed. The e-nose was constituted by an array of seven quartz crystal microbalance sensors modified with Gold Nanoparticles (GNPs) conjugated to short peptides. Forty olive oil samples headspaces were characterised by headspace solid-phase microextraction gas chromatography-mass spectrometry analysis to evaluate chemical composition; in parallel, they were chemically and sensory evaluated according to European Regulation EEC 2568/91 and amendments and EU Regulation 640/2008. The steady state sensor responses obtained with the e-nose setup were used to evaluate the discrimination properties of the system by principal component analysis and partial least square method. The results of this study provided a promising perspectives for the use of the electronic nose as a low-cost, easy to use and rapid system for the quality control of extra virgin, virgin and lampante (non-edible) olive oil. This system will also be useful to quantify the prevalent defect level in virgin and lampante olive oil samples.

Keywords: analytical methods, EVOO, food quality, rapid methods

1. Introduction

Virgin olive oil (VOO) is one of the most used dressings and cooking fats in Mediterranean countries, and is a central component of the diet in this region (Bendini *et al.*, 2007). Its analytical characterisation by means of rapid methods of analysis has been investigated during the last years, in terms of phenolic content (Del Carlo *et al.*, 2012), fatty acid composition (Maggio *et al.*, 2009) and antioxidant capacity (Carrasco Pancorbo *et al.*, 2005; Del Carlo *et al.*, 2004; Gomez-Caravaca *et al.*, 2008) by our research group. It is well known that olive oils should undergo legal control involving sensory evaluation, and that a harmonised protocol is used for this purpose: EEC regulation 2568/91 (EC, 2008). The most important phase of olive oil sensory analysis is represented by identification of aromas. Food aroma in general is a very complex sensation, being thousands the volatile compounds in foods overall. Aroma perception is the complex result of the presence of each volatile compound depending upon its concentrations and sensory thresholds. The classification of virgin olive oils in different commercial categories (extra virgin, virgin and lampante; EVOO, VOO and LOO), is strictly dependent on the sensory analysis that evaluate the presence, and the level thereof, of sensory defects (García-González and Aparicio, 2010). The most frequent off-flavours of VOO are grouped into five main defects: fusty, muddy, mouldy, vinegary and rancid. As the sensory perception depends on the chemical composition of the olive oil sample, and particularly, on the head space composition, several works in the literature have focused on the correlation between defects perceived by a trained panel in VOOs and the presence of markers volatile compounds in the sample head-space. The use of dynamic headspace (HS) high-resolution gas chromatography coupled with mass spectrometry (GC-MS) detection and olfactometry has been reported to be a straightforward

approach for the understanding of the volatile compounds mainly responsible for the off-flavours (Morales *et al.*, 2005) In the latter work the authors concluded that the presence of C8 compounds produced by specific mould enzymes as 1-octen-3-one and 1-octen-3-ol were strictly related to the mouldy defect. Another important sensory defect, vinegary, which occurs upon sugars fermentation, has been associated to acetic acid and ethyl acetate. The fusty unpleasant odour was found to be dependent on 3-methyl-1-butanol as a consequence of an anomalous aminoacid degradation. Finally, saturated and unsaturated aldehydes, as nonanal and 2-heptenal were found the cause of rancid sensory defect. Many other GC-MS studies have been carried out in an attempt to characterise the molecules involved in the sensory perception of olive oil defects (Dierkes *et al.*, 2012; Esposto *et al.*, 2009; Lopez-Feria *et al.*, 2008; Procida *et al.*, 2005; Tena *et al.*, 2007).

Because the aroma perception is dependent not only on one single molecule, but it is strongly influenced by the environment in which the molecule is present, gas sensor systems such as electronic nose appears suitable to analyse food headspace for a number of purposes including classification, authentication, appreciation of sensory features (positive and negative attributes). Metal oxide semiconductor (MOS) sensors have been largely applied in VOO aroma control to detect a variety of sensory defects and to authenticate VOOs according to varietal or geographical origin of olives (Aparicio *et al.*, 2000; Cimato *et al.*, 2006; García-González and Aparicio, 2002; Lerma-Garciá *et al.*, 2010). Some of these studies were directed to investigate single defect such as rancid (Aparicio *et al.*, 2000) or vinegary or individual single defects in artificially prepared defected oils (Lerma-Garciá *et al.*, 2010), rather than, as in the aim of the present work, to classify the olive oil samples according to their sensory class (EVOO, VOO or LOO).

A different type of electronic nose is based on and array of quartz crystal microbalance (QCM) sensors; the output, is, in this case, due to the mass change adsorbed onto the surface that leads to a frequency shift. The modification of sensors can be achieved with different materials, typically polymers and solid state molecular materials. In this regards porphyrins coated QCM have been used to this purpose giving excellent results also in food aromas detection (Santonico *et al.*, 2008).

Recently, a QCM based electronic nose was evaluated as an olfactory tool to classify olive oil samples according EVOO, VOO and LOO classification. The QCM sensors were modified with typical GC stationary phases obtaining a clear separation between edible (EVOO and VOO) and non-edible (LOO) samples, though 6 non-edible samples out of 48 were classified as edible (Escuderos *et al.*, 2010).

On the other hand few papers have been proposed on the use of amino acids or oligopeptides as sensing modifier for gas phase analysis. Efforts have been directed in the immobilisation of purified olphactory receptor proteins (Escuderos *et al.*, 2010) and in the immobilization of different peptides for the detection of volatile organic carbons (García-González and Aparicio, 2002). The use of designed peptides obtained with molecular modelling and docking experiments was used by Sankaran *et al.* in 2 papers (Sankaran *et al.*, 2011a,b) to obtain gas sensing of alcohols (3-methyl-1-butanol and 1-hexanol) associated with the presence of *Salmonella* contamination in meat. Using the structural info on the intracellular aryl hydrocarbon receptor and molecular modelling our group developed eptapeptide sensors for dioxins (Mascini *et al.*, 2004). Some of these sensors exhibited selectivity for dioxins vs. PCBs. These are still, up to date, the only reported gas sensors based on peptides used in real food samples analysis even though after an extraction/pre-concentration step necessary to get the required sensitivity (Mascini *et al.*, 2005).

In the present work we used oligopeptides modified quartz crystal microbalance for headspace analysis of olive oil consisting of different chemical classes (alcohols, aldehyde, ketones, organic acids, esters, hydrocarbons) with variable C backbone ranging from C1 (methanol) to C8 (e.g. 1-octen-3-one) in an attempt to classify olive oil samples according to their sensory quality. Peptides were firstly immobilised on gold nanoparticles and then deposited on the surface of the quartz crystal microbalances. In the present work we compare the headspace analyses of 40 samples of olive oil, characterised by a 'panel test', using both GC-MS and a peptide based electronic nose. The aim was to develop a quali-quantitative predictive model, based on electronic nose measurements, able to discriminate among EVOO, VOO and LOO via principal component analysis and to quantify the prevalent defect level by partial least square analysis.

2. Materials and methods

Chemicals

HCl, HNO_3, $HAuCl_4$, $NaBH_4$, cysteine, cysteinyl-glycine (Cys-Gly), γ-glutamylcystein (γ-Glu-Cys), L-reduced gluthatione (GSH), thioglycolic acid were purchased by Sigma-Aldrich (Milan, Italy). Nitrogen was purchased by Rivoira (Milan, Italy). 20 MHz QCM sensors were from Elbatech (Isola d'Elba, Italy).

Olive oils

Forty olive oil samples were from Adriatic Regions, Abruzzo and Marche, Italy. The oil samples were stored at -20 °C until use. Olive oils were characterised for the principal chemical quality indices as free acidity (FA), peroxide

value (PV) and spectrophotometric index (SI). These parameters were determined according to the official methods described in European Regulation EEC 2568/91 and amendments; all the analyses were done in triplicate. FA was expressed as percentage of oleic acid and PV was expressed as meqO_2/kg of oil.

Sensory analyses

Sensory evaluations were performed by 9 panellists. All oils were subjected to an extended panel test as reported in the annex of EU Regulation 640/2008, as well as the instructions for the objective assessment of olive oils (COI/T.20/Doc. No.15/Rev.3) (EC, 2008).

HS-SPME-GC-MS analysis

In order to obtain a semi-quantitative description of the chemical composition of the olive oil samples head space, a solid-phase microextraction (SPME) fibre (length 1 cm) coated with 50/30 µm polydimethylsiloxane/divinylbenzene/carboxen phase (Supelco Ltd., Bellefonte, PA, USA) was used. Before use, the fibre was conditioned by introducing it into the injector of the gas chromatography (GC) system set at 260 °C for 2 h in a stream of helium.

A 10 g sample spiked was placed in a 100 ml headspace vial fitted with a silicone septum. After an equilibration time of at least 10 min, SPME sampling was performed by exposing the fibre for 30 min in the headspace of the sampling at 40 °C under magnetic stirring. The fibre was then desorbed in an Perkin Elmer programmable temperature injector (Perkin Elmer, Monza, Italy). The injector temperature at the beginning was 250 °C. After the GC run, for reconditioning, the SPME fibre was left for 20 min in the hot injector at 270 °C. An Autosystem XL gas chromatograph coupled with a Turbomass quadrupole mass spectrometer (Perkin Elmer) was used for GC-MS determination. The chromatograph was equipped with a Restek HP-5MS capillary column (5% diphenyl; 95% dimethylpolysiloxane; 30 m long, 0.25 mm internal diameter, 0.25 µm film thickness; Restek Superchrom, Milan, Italy). Helium (99.998%, Rivoira, Milan, Italy) was used as carrier gas at flow rate of 1.0 ml/min.

A 1 µl sample was injected into the split/splitless inlet in splitless mode (splitless for 1 min, with split flow 50 ml/min) at 250 °C. The temperature of the GC-MS interface was 200 °C. The oven temperature program started at 40 °C for 5 min, was increased of 8 °C/min to 240 °C which was maintained for 20 min. Full scan mode was used for mass spectra data acquisition.

Gold nanoparticles synthesis

All glassware was washed with aquaregia and rinsed with distilled water before the synthesis. Gold nanoparticles (GNP) were prepared by sodium borohydride reduction method (Mascini *et al.*, 2005). In a typical experiment, 100 ml acquous solution of tetrachloroauric acid (10^{-4} M) was reduced by 0.01 g of $NaBH_4$ at room temperature resulting in the formation of ruby-red gold hydrosol containing gold nanoparticles of 2 nm average diameter. GNPs, were then capped by self-assembly incubating with 10^{-4} M aqueous solution of thyolated compounds at room temperature for 2 h. GNPs were functionalised with Cys, GSH, γ-Glu-Cys, Cys-Gly, thioglycolic acid (TA) and an heptapeptide (N-Cys-Glu-His-Gly-Gly-Pro-Ser-C; HPT).

Gold nanoparticles deposition on quartz crystal microbalance

20 MHz QCM sensors were modified by drop casting of 50 µl of gold nanoparticles suspension on each side of the crystal and let dry at room temperature. QCM sensors were kept at room temperature in the dark when not in use. Sensors were as follows: GNPs-Cys (sensor 1), bare GNPs (sensor 2), GNPs-GSH (sensor 3), GNPs-γ-Glu-Cys (sensor 4), GNP-Cys-Gly (sensor 5), GNPs-TA (sensor 6) and GNPs-HPT (sensor 7).

Electronic nose

The electronic nose was developed in the Department of Electronic Engineering of the University of Tor Vergata. The system allowed allocation of up to 8 different sensors in the same measuring chamber. Head-space analysis of samples was carried out as follows: 10 ml sample were introduced in a 100 ml flask, the flask was then sealed and let to equilibrate for 30 min at 40 °C. The headspace was then fluxed to the sensor array by a constant flow of nitrogen at 12 l/h. The Δf (difference of frequency between the baseline and the stable signal frequency) was taken as the average of the last 20 measurements (1/s) before sending the sample and the average of the last 20 values before the cleaning procedure.

Statistical analysis

Principal component analysis (PCA) was performed using MatLab (Natick, MA, USA) and was used to obtain a classification of olive oil samples according to the electronic nose responses. Partial least square (PLS) was performed using the MatLaBb NIPLS algorithm. The 'leave one out' method was used as cross validation method. PLS was used to find the fundamental relations between chemical composition of olive oil headspace, measured by headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS), and sensory defects. It

was also used to find the relation between electronic nose response and the sensory defects.

3. Results

Free acidity, peroxide value, spectrophotometric index

Olive oil samples were analysed according to European Regulation EEC 2568/91 (EC, 2008) and amendments for FA, PV, SI. Results are reported in Table 1. FA ranged between 0.10 and 0.86% oleic acid (mean value 0.26), PV between 3.96 and 13.00 mEq O_2/kg(mean value 8.04), SI were between 1.700, 0.070, 0.000 and 2.670, 0,310, 0.008 with a mean value 1.990, 0.015, 0.002 for the absorbance at 232 nm (K_{232}), at 270 nm (K_{270}), and ΔK, respectively. Among the 40 analysed samples, 38 resulted to be classified as VOO according to the FA value and samples no. 4, 25, 30, 37, and 39 resulted to be classified as VOO because of the SI.

Sensory analyses

On the basis of sensory analyses (Table 1), 14 samples were classified as 'extra virgin' (defects = 0, fruitiness > 0). Among the remaining 31 samples, 22 were classified as 'virgin' (median defects ≤ 3.5) and 4 as 'lampante' (median defects > 3.5) according to annex of EU Regulation 640/2008 (EC, 2008). The oils had different off-flavours (25 fusty, 14 vinegary, 8 rancid, 1 other). The distribution of the olive oil samples within the three categories was interesting for the aim of the experimentation because there were samples classified as extra virgin according to classical chemical analysis (FA, PV, SI), that exhibited a sensory profile of virgin and/or lampante olive oil because of the volatile components.

HS-SPME-GC-MS analysis.

The HS-SPME-GC-MS analysis of the 40 olive oil samples was run to evaluate the trends of the volatiles in the headspace rather than to accurately determine single volatile compounds. 16 major peaks were found that were assigned, on the basis of the spectral data, to the corresponding structure (Table 2). Lampante olive oil samples resulted in a higher amount of ethyl acetate, which is related to the vinegary defect, with respect to VOOs and EVOO (P<0.005). The same trend was found for ethanol, 1-hexan-1-ol and 3-hexen-1-ol which are associated to various defects (Gomez-Caravaca *et al.*, 2008) such as fusty and mouldy (Figure 1). On the contrary, there were aromas that consistently decrease their concentration as the sensory quality decrease (Figure 2). The concentration ratio of 3-pentan-1-one in EVOO/LOO samples was exceptionally high (>74); in fact, this molecule is very rarely and scarcely retrieved in VOO and LOO and it is not associated to any defect as already reported in the literature (Gomez-Caravaca *et al.*, 2008). Lower, though significant EVOO/LOO hexanal (>4) and trans-2-hexenal (>7) ratios were found; both were associated to positive aroma such as green and green apple like, respectively (Dierkes *et al.*, 2012).

Electronic nose analysis

The final output of a device like an e-nose is strictly dependent of the variability of the binding functionalities on the sensor surface. Improvement of this variability has been attempted in using hybrid detecting approach. Practical experience has shown that, for classical MOS and metal-oxide-semiconductor field-effect transistor (MOSFET) based systems, this does not produce enough information to solve real problems because of the lack in selectivity of the different sensors. Variability in the response can be improved increasing the number and type (MOS and MOSFET) of sensors; however, this involves complex electronics and the normalisation of the different sensor outputs.

In our approach we evaluated that peptides can give enough variability to allow the use of just one detection system on the device. For this reason we selected a gas detection system operating at room temperature as QCMs, compatible with the use of peptides. The use of GNPs as substrate for aminoacidic structures bearing thiols has been taken into account because of 3 main reasons: the well-known formation of self-assembled monolayer onto gold substrates due to the thiolated gold affinity, the increase of the potentially binding sites for the volatile targets due to the very large surface of nanoparticles/volume ratio and the ease of preparation of modified GNPs. The QCMs sensors obtained exhibited increased sensitivity with respect to porphyrins (Compagnone *et al.*, 2013).

Seven different QCM sensors modified with GNPs bearing different functionalities have been used in the e-nose set-up for the analysis of olive oils. Four sensors were realised using GNPs modified with the commercially available aminoacids and dipeptides that constitute the well-known cysteine containing tripeptide glutathione; namely, GSH, Cys, Cys-Gly, γ-Glu-Cys. The variability of the sensor array was improved using three QCM sensors modified with GNPs, with GNPs derivatised with thioglicolyc acid and GNPs bearing a cysteine containing heptapeptide synthesised in our lab. In measuring conditions a typical sensorgram reporting the adsorption kinetics of the sensors during a sample measurement shows the steady state of the signal reached in 10 min (data not shown). Recovery of the signal was achieved in the same time.

Table 1. Quality and sensory analysis of the 40 olive oil samples.

Sample id	FA (% of oleic acid)	PV (meqO_2/kg)	K_{232}	K_{270}	ΔK	Fruity	Bitter	Pungent	Fusty	Rancid	Vinegary
1	0.23	10.00	2.15	0.07	-0.001	0.8	0.1	0.2	2.3	1.2	0.0
2	0.11	8.26	2.01	0.14	-0.001	1.8	1.2	1.3	1.7	0.0	0.0
3	0.14	6.65	2.28	0.21	-0.001	2.9	1.7	1.5	0.0	0.0	0.0
4	0.17	9.03	1.97	0.31	0.002	3.1	1.5	0.8	1.5	0.0	0.0
5	0.25	10.14	2.20	0.11	0.000	1.7	1.2	0.3	1.8	0.1	0.0
6	0.17	8.33	1.97	0.12	0.000	2.4	1.1	0.7	0.5	0.0	0.0
7	0.14	5.41	1.77	0.13	-0.002	2.6	2.0	1.6	0.0	0.0	0.0
8	0.16	6.64	1.93	0.09	-0.001	3.0	1.8	1.7	0.0	0.0	0.0
9	0.10	4.57	1.82	0.11	-0.001	2.8	1.7	2.2	0.0	0.0	0.0
10	0.13	8.05	1.97	0.08	0.000	2.4	1.6	1.6	0.0	0.0	0.0
11	0.15	6.80	1.86	0.12	0.000	2.0	1.2	1.6	0.0	0.0	0.0
12	0.18	7.94	1.92	0.12	-0.001	2.5	1.6	1.8	1.9	0.0	0.4
13	0.19	8.08	1.83	0.12	0.000	1.6	1.3	0.6	2.5	0.0	0.0
14	0.19	5.80	1.71	0.14	-0.001	2.6	2.3	2.5	0.0	0.0	0.0
15	0.23	7.04	1.87	0.13	0.000	1.8	1.1	0.8	1.5	0.0	0.0
16	0.36	10.68	2.11	0.14	0.001	1.4	1.0	0.3	1.7	0.0	0.0
17	0.27	10.29	2.05	0.17	0.001	1.4	1.4	1.1	1.3	0.0	0.0
18	0.24	10.34	1.94	0.19	-0.001	3.7	2.3	2.5	0.0	0.0	0.0
19	0.25	6.14	1.84	0.13	0.000	2.1	2.0	1.3	1.2	0.0	2.7
20	0.29	7.35	1.95	0.17	0.000	1.7	1.0	0.6	1.9	0.0	0.7
21	0.30	8.78	2.01	0.20	0.001	1.4	0.8	0.6	2.1	0.0	0.7
22	0.31	8.80	2.20	0.17	0.000	2.5	1.8	1.9	2.1	0.0	0.7
23	0.34	5.87	1.70	0.18	0.001	2.6	2.1	1.9	1.2	0.0	0.0
24	0.34	10.12	2.27	0.18	0.001	0.9	0.7	0.6	0.0	2.1	3.9
25	0.30	11.03	2.51	0.18	0.000	0.7	1.1	0.8	3.0	2.5	2.7
26	0.29	5.24	1.74	0.17	0.000	0.8	0.6	0.1	4.6	1.3	0.0
27	0.29	4.96	1.83	0.17	-0.001	0.7	1.1	0.5	0.6	2.5	1.1
28	0.18	8.13	2.05	0.21	-0.002	1.1	1.0	0.9	3.8	0.0	2.1
29	0.18	5.90	1.79	0.17	-0.001	0.3	0.3	0.3	2.9	0.7	2.1
30	0.50	3.96	1.70	0.19	0.002	0.1	0.1	0.1	3.8	0.8	3.8
31	0.28	8.78	2.06	0.15	-0.003	4.0	3.8	2.9	0.0	0.0	0.0
32	0.22	6.86	2.07	0.14	-0.003	3.8	3.2	2.6	0.0	0.0	0.0
33	0.15	6.47	1.88	0.10	-0.002	2.4	1.8	2.0	0.4	0.0	0.0
34	0.17	7.35	2.06	0.12	-0.005	4.0	3.7	3.2	0.0	0.0	0.0
35	0.17	8.37	2.12	0.11	-0.002	2.7	2.2	2.0	0.0	0.0	0.0
36	0.41	11.39	1.98	0.11	0.000	1.0	0.7	0.1	0.5	0.0	2.3
37	0.52	13.00	1.83	0.18	0.008	0.5	0.0	0.0	0.0	0.0	3.5
38	0.86	9.50	2.09	0.13	0.001	0.9	0.5	0.4	3.2	0.9	0.0
39	0.28	12.50	2.67	0.13	0.000	0.5	0.0	0.0	2.6	0.0	1.9
40	0.19	6.97	1.88	0.13	-0.001	3.6	3.1	1.8	0.0	0.0	0.0
Mean	0.26	8.04	1.99	0.15	0.000	1.94	1.41	1.17	1.28	0.31	0.70
Min.	0.10	3.96	1.70	0.07	-0.005	0.1	0	0	0	0	0
Max.	0.86	13.00	2.67	0.31	0.008	4	3.8	3.15	4.6	2.5	3.9

FA = free acidity; PV = peroxide value; K_{232} = absorbance at 232 nm; K_{270} = absorbance at 270 nm; ΔK = difference of absorbance at the 270 nm region.

Table 2. HS-SPME-GC-MS analysis of the 40 olive oil samples. Data are reported as peak area percentage, identification was made based on retention time (given between brackets) and spectral data.

Sample id	ethyl acetate (11.47)	methanol (11.88)	ethanol (12.84)	hexane (14.16)	3-pentan-1-one (15.36)	3-ethyl 1.5-octadiene (16.18)	hexanal (16.85)	1-butanol (18.29)	1-penten-3-ol (18.46)	trans-2-hexenal (20.23)	2-butanal (21.56)	2-penten-1-ol (21.75)	1-hexan-1-ol (22.30)	3-hexen-1-ol (23.07)	2-hexen-1-ol (23.38)	acetic acid (24.12)
1	0.03	1.03	-	28.64	-	-	0.03	0.26	0.02	1.48	0.25	0.64	20.61	4.87	42.13	-
2	0.05	1.42	1.44	14.76	-	-	-	0.41	-	2.82	0.52	0.79	13.40	4.59	59.81	-
3	-	0.77	1.08	0.18	1.33	-	1.23	0.55	0.11	86.90	0.95	0.59	1.14	1.92	3.25	-
4	-	0.45	0.66	0.92	-	-	1.70	0.30	0.07	53.79	0.49	0.64	8.99	2.08	29.91	-
5	-	0.34	0.97	3.48	-	-	2.49	0.33	0.13	60.75	0.28	0.56	6.08	2.46	22.13	-
6	-	0.19	1.19	0.04	0.24	-	1.45	0.34	0.25	87.02	0.28	0.59	1.70	2.02	4.36	0.34
7	-	0.91	0.93	-	0.37	-	1.16	0.47	0.14	88.40	0.36	0.63	1.32	2.30	2.81	0.20
8	-	1.13	0.99	2.41	-	-	1.13	0.25	0.42	84.33	1.12	0.44	1.71	1.59	4.49	-
9	0.06	0.53	7.17	-	-	-	0.70	0.27	0.09	82.06	2.16	0.41	1.35	0.53	4.67	-
10	-	0.99	1.54	-	-	-	1.64	0.44	-	89.00	0.87	0.16	1.84	1.24	2.28	-
11	-	0.41	0.89	6.38	-	-	1.51	0.30	-	63.69	1.42	0.57	4.28	1.21	19.34	-
12	-	0.39	1.04	3.16	-	-	0.63	0.44	-	18.24	0.23	0.69	13.59	1.44	60.01	0.14
13	-	1.56	-	20.29	-	-	0.95	0.69	-	10.95	0.26	1.31	46.23	-	17.22	0.55
14	1.23	0.51	4.23	0.72	0.57	-	1.61	0.77	-	77.82	0.59	0.69	3.58	-	7.69	-
15	0.16	2.58	1.32	23.19	-	-	0.14	0.25	0.10	2.77	1.39	0.88	37.17	-	30.07	-
16	-	0.38	1.41	2.65	-	-	2.90	0.18	-	31.15	0.89	0.74	15.63	-	44.07	-
17	0.13	0.81	2.38	3.87	-	-	3.25	0.26	0.08	36.51	-	0.52	17.20	-	34.99	-
18	0.04	0.47	6.14	0.45	0.10	-	0.76	0.21	-	77.73	0.23	0.53	1.92	2.93	8.37	0.12
19	0.16	0.92	9.73	0.56	0.78	-	0.70	0.77	0.15	75.82	0.46	1.51	1.99	2.14	4.09	0.22
20	-	1.36	0.52	2.12	-	1.21	1.17	0.47	-	28.76	1.30	0.92	10.19	2.68	49.25	0.04
21	0.12	1.04	0.46	3.30	-	1.24	1.51	0.47	-	28.49	1.15	0.75	12.54	2.83	45.98	0.12
22	0.09	0.60	1.19	1.12	0.03	0.80	2.28	0.35	-	50.57	0.91	0.88	9.05	1.73	30.11	0.29
23	-	0.79	0.35	4.45	-	0.80	1.71	0.39	0.24	61.89	1.04	0.93	8.05	1.97	17.33	0.06
24	0.39	0.76	14.05	0.81	-	-	0.52	0.24	0.06	19.77	0.25	1.88	30.12	6.10	23.51	1.53
25	0.48	0.67	13.47	0.93	-	-	0.51	0.23	0.12	21.66	-	2.08	30.59	6.31	22.95	-
26	0.56	0.98	18.12	1.00	-	-	-	0.24	0.13	19.49	-	1.90	24.29	8.48	24.80	-
27	0.23	1.15	17.09	0.51	-	-	-	0.33	-	12.54	0.22	4.66	21.56	7.72	33.87	0.11
28	0.30	0.36	9.24	1.11	0.10	-	0.41	0.80	1.77	17.13	0.77	2.47	34.49	7.54	23.51	-
29	0.78	0.70	22.29	0.36	-	-	-	0.35	0.14	6.05	-	0.88	24.05	14.20	30.21	-
30	2.08	0.80	27.63	1.01	-	-	-	0.39	0.23	12.62	-	1.27	20.38	12.08	20.53	0.99
31	0.02	0.63	6.67	1.12	1.31	0.56	0.62	1.46	-	57.18	0.80	2.20	1.70	3.36	22.36	-
32	0.22	0.49	5.63	1.42	2.48	0.72	1.28	1.01	-	41.80	0.34	5.43	5.08	11.66	22.22	0.22
33	0.09	0.66	3.22	0.38	0.25	0.16	1.52	0.61	-	77.87	1.04	0.89	3.53	3.88	5.91	-
34	0.13	2.13	4.61	1.80	4.09	2.44	1.79	2.03	-	51.14	1.19	4.00	3.51	14.87	5.04	1.23
35	2.15	0.54	2.29	3.47	0.56	3.17	1.94	0.95	-	69.55	0.51	1.58	5.75	1.29	6.12	0.14
36	0.52	1.40	8.61	2.21	0.03	-	1.34	0.66	0.38	31.60	0.78	1.03	15.79	3.02	32.63	-
37	12.27	2.94	53.05	3.10	-	-	3.20	0.46	0.28	4.86	1.10	6.06	2.95	8.17	1.54	-
38	0.39	0.29	4.61	1.70	-	2.43	4.56	0.59	-	67.42	0.52	0.92	3.99	1.93	10.54	0.11
39	1.11	1.10	13.16	0.67	-	-	0.69	0.29	-	20.35	1.10	0.64	29.08	2.52	29.31	-
40	0.41	0.90	3.01	1.25	0.92	4.21	0.90	0.76	0.15	77.67	0.76	0.91	1.83	1.99	4.32	-

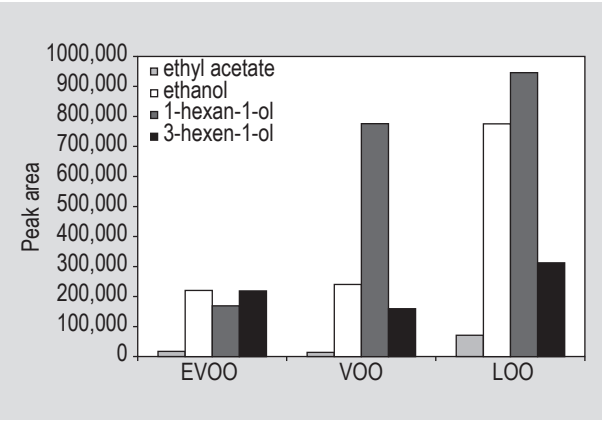

Figure 1. Molecules that increase with decreasing sensory quality. EVOO = extra virgin olive oil; VOO= virgin olive oil; LOO = lampante olive oil.

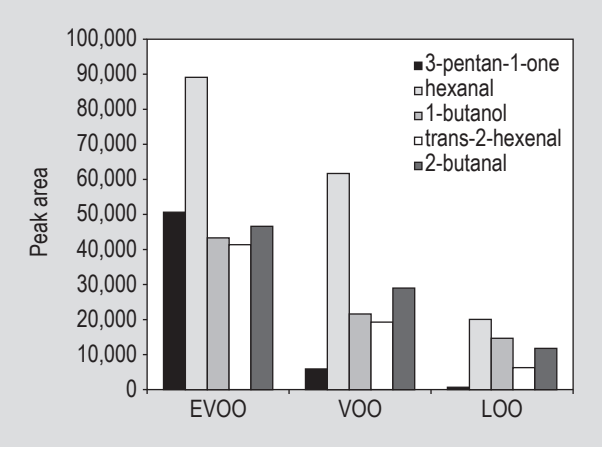

Figure 2. Molecules that decrease with decreasing sensory quality. EVOO = extra virgin olive oil; VOO= virgin olive oil; LOO = lampante olive oil.

Statistical analysis

A PLS analysis of the 40 samples was initially carried out to check the ability of the sensory panel to evaluate the chemical composition of the olive oil samples headspace. The HS-SPME-GC-MS data were then used for a semi-quantitative analysis of the volatile compounds with the purpose to correlate organoleptic results with QCM results. The PLS model was built using the entire HS-SPME-GC-MS data set, using all the peaks with over 1% of the total peak area. As expected, a close correlation between headspace chemical composition and panel test response was found (Figure 3). The classification in the three groups, EVOO, VOO and LOO, is strictly correlated to chemical analysis. This relationship is fundamental for the development of the electronic nose because it states a clear dependence between sensory classification and headspace chemical composition. The PLS model built on the HS-SPME-GC-MS data was able to predict the

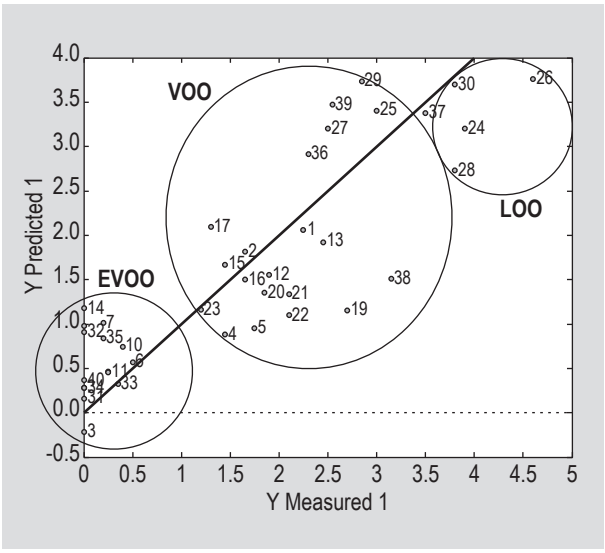

Figure 3. Partial least square analysis of gas chromatography data. EVOO = extra-virgin olive oil; VOO = virgin olive oil; LOO = lampante olive oil.

prevalent defect value with good correlation between the measured and predicted values (root mean square error of calibration; RMSEC = 0.686893; root mean square error of calibration in cross validation; RMSECV = 0.91558). This can be evidenced looking at the headspace chemical composition of different samples as, for example, sample no. 6 (EVOO), no. 13 (VOO) and no. 30 (LOO). In fact, sample no. 6 contains low amounts of the defects markers (ethyl acetate, ethanol, 1-hexan-1-ol and 3-hexen-1-ol) and high amounts of molecules related to positive attributes such as hexanal and trans-2-hexenal. The opposite was found for sample no. 37 (LOO) were high levels of ethyl acetate and ethanol were detected and hexanal and trans-2-hexenal were one order of magnitude lower than the average value of the EVOO group. An intermediate situation was found for the sample no. 13 (VOO) were low content of defected related molecules was found together with a high content of 3-hexen-1-ol. However, despite the good performance of the PLS model for the prediction of the defects, an overestimation of the chemical analysis vs. the sensory test was observed for EVOOs samples. This was somehow expected considering that the 'true value' to classify a sample as EVOO is zero defects (assigned by the panellists) while GC analysis is more selective (because of the separation) in detecting compounds at a concentration lower that the effective threshold of sensory analysis.

Having assessed the relationship between GC and sensory analysis the dataset coming from the e-nose was analysed by PCA. The 1st and the 2nd principal components (PC1 and PC2) explained 97% of the total variance and were enough to display the most interesting structures among the data. The scores plot for components 1 and 2 (Figure 4) shows a clear discrimination of the samples according to their

sensorial attributes. The VOO group, with positive values on PC1, is discriminated from EVOO and LOO which are plotted on the negative semi-plane. EVOO and LOO are separated along PC2, in particular LOO are plotted along the negative PC2 axis and EVOO in the opposite. Thus, using a simple PCA analysis a complete classification of the samples in their commercial categories, has been obtained. Commercial classification was recently proposed using 5 QCMs sensors modified with different stationary phases (Escuderos *et al.*, 2010). However, discrimination was not fully successful since a certain degree of overlapping among groups was observed. Considering the similar loadings obtained for all the sensor on PC1 and the overlapping of sensors 2 and 5 we think that the discriminating ability of the e-nose setup presented in this work could be further improved using different amino acidic functionalities (or other QCM sensors).

Finally, a PLS model was built, using electronic nose data, in an attempt to quantify the prevalent defect of olive oil samples, using the median of the prevalent defect, given by the panellists, as descriptor. For the reasons previously discussed (defect 0 by definition), the EVOO samples were not included in the model, Figure 5 reports the PLS plot obtained for VOO and LOO samples, the RMSEC was 0.48832, whereas the RMSECV was 0.682892. It is evident that the model is able to accurately predict the prevalent defect up to a median value of 3.0. For prevalent defect values >3 the systems exhibited an underestimation of the predicted values. The possibility to quantify the defect level in few min, without the presence of panellists appears very attractive both for olive oil producers and traders particularly for blending. This is a clear advantage of the present device in comparison to previously QCM based sensor array reported (Escuderos *et al.*, 2011). Similar results were obtained using a more sophisticated electronic nose based on semiconductor technology (Lerma-Garciá *et al.*, 2010) which results in a more expensive experimental set-up. Moreover, the modification of the sensors to get more variability does not appear straightforward.

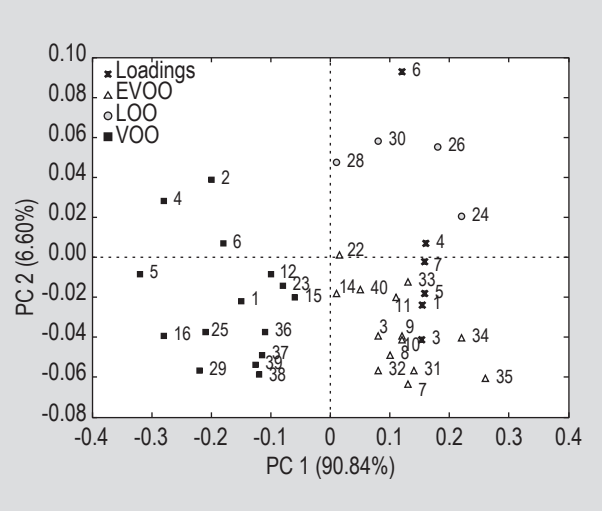

Figure 4. Principal component analysis scores plot of olive oil samples. EVOO = extra-virgin olive oil; VOO = virgin olive oil; LOO = lampante olive oil.

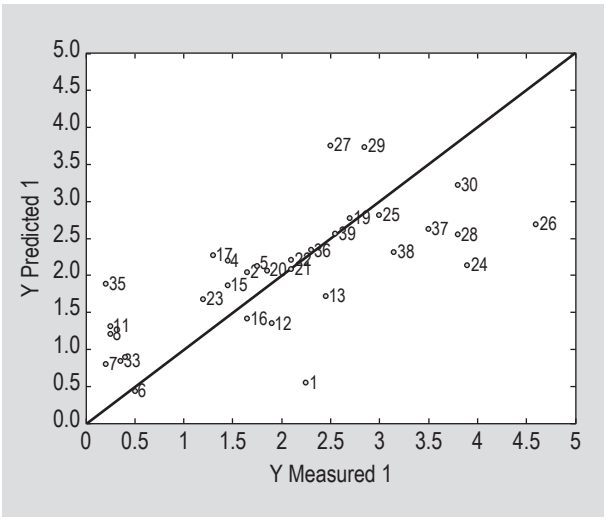

Figure 5. Partial least square analysis of electronic nose data obtained from virgin and lampante olive oil samples.

4. Conclusions

In conclusion, the developed GNPs-peptide sensors appears suitable for a rapid discrimination of EVOO, VOO and LOO using PCA. After this preliminary discrimination the prevalent defect value of VOO and LOO samples can be predicted using a reliable PLS model, with the significant advantages of ease of use of the instrumentation and non-invasive head space. This approach can represent an interesting tool for at-line monitoring during the production of olive oils.

Acknowledgements

Financial contribution of 'Fondazione Tercas', under the *'Progetti di Eccellenza'* scheme is acknowledged.

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