THE ELECTRONIC NOSE APPLIED TO FOOD ANALYSIS

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Abstract

There is currently a great interest in developing new techniques for food quality assessment. Electronic nose is considered an attractive technique for evaluating food aroma. In this work we present an overview of application examples of an electronic nose based on sol-gel metal oxide gas sensor array for the assessment of some foodstuffs (milk, olive oil and peach fruit). The responses of the sensor array to the flavours of these foodstuffs have been correlated with the results obtained by other standard techniques.

Resumen

Existe actualmente un gran interés en desarrollar nuevas técnicas para la valoración de la calidad de alimentos. La nariz electrónica se considera una técnica atractiva para evaluar el aroma de alimentos. En este artículo presentamos una descripción de los ejemplos del uso de una nariz electrónica basada en un arreglo de sensores de gas del tipo óxido de metal (obtenido por el método de sol-gel) aplicados a algunos comestibles (leche, aceite de oliva y duraznos). La respuesta de la matriz de sensores a los aromas de los alimentos ha sido correlacionada con los resultados obtenidos por otras técnicas estándares.

1. Introduction

Food quality can be defined as being “a combination of attributes or characteristic data of a product, which have a significance in determining the degree of acceptability of the product by the user”. At present there are great efforts in developing new techniques for assessing food quality. Quality control of product fragrance and flavor is based on the comparison of sensory, instrumental, analytical, and if necessary microbiological data, with standards and specifications. Chromatographic and spectroscopic analytical techniques (and their combination such as GC-MS) and sensory analysis are commonly used. The “aroma” of a foodstuff is a complex mixture consisting of thousands of different chemical volatile species (*chemical patterns*). Analytical techniques allow chemical patterns to be separated and the individual components identified and quantified. However, these techniques are complex, expensive, time consuming and they require a well-equipped analytical laboratory and a skilled staff. On the other hand, sensory analysis provides the evaluation of foodstuff as a whole as deriving from the impact of its odors/flavors on human senses. Again, it's very costly to maintain a skilled sensory panel, and there is a limit to the number of replicate samples which can be evaluated due to olfactory adaptation to odors.

Some new trends are occurring in food quality assessment techniques, based on the use of non-destructive spectroscopic techniques and electronic noses [1-5]. The latter are based on arrays of solid state gas sensors, mainly chemoresistive metal oxide gas sensors (MOX) or quartz microbalance gas sensors (e.g. QMB). The gas sensor array, exposed to the complex "chemical pattern" of a foodstuff, analyze it as a whole, without decomposing it as occur for chromatographic techniques, giving a simply pattern of sensor responses as read-output. Electronic noses can find applications in food industry for quality control, process monitoring, freshness evaluation, shelf-life investigation and authenticity assessment. Considerable work has already been carried out on milk and dairy products [6-8], coffee [9], oils [10-12], wine [13,14], tomato [15], meat and salami [16,17], fruit [18-21], fish [22,23], and on many other foodstuffs and beverages, as well as on the odor quality evaluation of food packaging [24].

Historically, the concept of an *electronic nose* arose from the need to overcome the poor selectivity of chemoresistive gas sensors by linking a number of different non-selective chemoresistive sensors in array configuration and exploiting the unavoidable cross-sensitivity of the sensors. The term 'array' is not to intend to mean a particular spatial arrangement of the sensors in the device, on the contrary, it refers to a vectorial organization of the output of the single sensors under exposure to the same environment, so that the contribution of each array element is a component of a multidimensional general problem. The signals of such a sensor array can be processed by means of methods of Pattern Recognition (PARC) which, exploiting the cross-correlations between the sensor responses, extract information contained in the sensor-output ensemble [1]. In particular a PARC procedure performs a qualitative analysis of the environment; it evaluates the multidimensional data-set from gas sensor array seeking the underlying main relationships in the data-set itself just in order to analyze the data structure and discriminate between different data classes (*clusters*) belonging to different 'chemical patterns' (*classification*). Moreover, a PARC procedure has to assign a sensor array output from an unknown gas/odor to a class recognizing in such a way the occurrence of a particular chemical pattern (*identification*). Of course, such multisensor systems need calibration; by presenting many different chemicals to the sensor array, a database of patterns is built up. This database of labeled patterns is used to train the pattern recognition system. The goal of this training process is to configure the recognition system in order to classify and eventually quantify each chemical compounds in a gas mixture.

In this work a brief overview of the applications of an electronic nose based on a metal oxide gas sensor array to the evaluation of some foodstuffs as milk, olive oil and peach fruit is shown. The responses of the sensor array to the flavors of these foodstuffs are correlated with the results obtained by other standard techniques. In particular, we used microbiological and SHS/GC/MS analysis for the assessment of dry salami, visible/near-infrared spectroscopy and destructive techniques as refractometer and penetrometer for the evaluation of the total soluble solid content (SSC) and firmness in peach fruit respectively, and finally SHS/GC/MS analysis and pH measurements by standard pHmeter for the evaluation of milk used for the production of mozzarella cheese. The aim of the work was to demonstrate that the Electronic Nose can be a useful complementary tool, not totally alternative, to conventional techniques in specific applications of food industry. Indeed, this type of electronic device gives comparative rather than quantitative information, hence, it is ideally suited for quick quality control/quality analysis (QC/QA) checking.

2.1 Experimental

Figure 1: *Image of the portable Electronic Nose developed at IMM-CNR - Lecce (Italy).*

A portable Electronic Nose has been used for the headspace analysis of the foodstuffs considered in this work. The device has been entirely designed and realized at the IMM-CNR - Lecce for application in food industry and environmental monitoring. It is small, compact and completely automatic. The developed Electronic Nose instrument (12x15x30 cm sized) consists of an electronic subsystem composed of an embedded PC (5 stack modules in PC/104 format) and 2 electronic boards for the control of sensors (an array of 8 metal oxide-based gas sensors and 1 digital temp/humidity sensor) hosted in a stainless steel cell. The sensing elements are metal oxide thin films prepared by the sol-gel technique, that is a wet chemical method known to be suitable for the preparation of nanoscaled systems. In particular the sensing materials are undoped and Ni, Os, Pt, Pd, Rh-doped SnO_2 and undoped and Fe-doped In_2O_3 thin films. Each sensor is deposited on an alumina substrate 2 x 2 mm^2 sized and provided with a Pt-resistive type heater on the backside and Au-interdigitated electrodes (50 μm spaced fingers) on the front side. The sensors are bounded on rectangular standard sockets and introduced into the test cell of the device. Internet and wireless connections are also available. A flow of ambient air, previously dried and cleaned by suitable filters, is extracted by a micropump and two electrovalves designed to deviate the air flow into the sample vial for the collection and the transfer of the sample volatile compounds into the sensor array cell. In Figure 1 an image of the instrument is reported.

3. Results and discussion

3.1 Milk

One of the most important technological tasks in the production of dairy products is the quality control of starting milk. The chemical analysis of flavors in dairy products is complicated by the heterogeneous nature of milk. In fact, significant levels of lipids, proteins and carbohydrates in milk make difficult to separate flavor-active chemicals based on general properties like polarity or volatility. It is also very difficult to separate smell-active compounds from the multiple phases found in milk, especially if the aim is to obtain an extract, fraction or aliquot that is both enriched and representative. It is reported in literature [25] that a lot of volatile organic compounds (VOCs) are present in the headspace of milk (acetone, hexanal, 2-pentanone, 2-butanone, toluene, limonene, heptanal, etc.) and their concentration depends on the nature and thermal treatment of milk (UHT, pasteurized, sterilized, etc.). Furthermore, a high quantity of water is also present, so the headspace turns to be represented by a complex mixture of volatile organic compounds in an atmosphere containing a high percentage of relative humidity. Analysis of milk volatile compounds by electronic noses has been shown to be a promising tool to get information on milk quality and to distinguish among different types of milks mainly in relation to the thermal treatment [6, 26].

Here, since milk freshness is of primary importance for the milk processing industry, we tested the aptitude of our EN to distinguish two different types of milk (commercial UHT and pasteurized milk) as a function of their storage days when its rancidity becomes relevant. For both types of milk, different gas-sensing tests at different ageing days have been carried out by measuring the sensor response to the headspace of 10 ml milk in 20 ml vial on a daily basis. The data acquired by the EN-measurements were collected for a period of 8 days for UHT and 3 days for pasteurized milk. Principal Component Analysis (PCA) was then performed on the normalized data responses of the array for each kind of milk. In both cases the clusters related to subsequent days of ageing are well separated, so that it is possible to follow the evolution of rancidity of milk during time. As an example, in Figure 2 the PCA score plot for the pasteurized milk sample is reported. It can be easily observed that the clusters evolve along preferential directions according to the different degree of rancidity. The obtained results suggest an interesting application of the electronic nose in dairy farming for the milk quality control measured on milk ageing and rancidity.

In next stage, we evaluated the application of the Electronic Nose in the qualitative analysis of the aroma of a mozzarella cheese produced in a local dairy (at Brindisi, Italy) [27]. Since the quality of mozzarella depends on the composition and types of basic milk as well as on the production process, we exposed the sensor array to the volatile compounds of both, mozzarella samples and dairy parcels of basic milk. In this dairy farming, milk parcels, coming from different stockbreeders, are daily mixed in a single tank to form the basic milk (milk "massa") that is used for the production of mozzarella cheese. In particular, we analyzed by EN stocks of milk of different stockbreeders –labelled as "*Gallone*, *Bruno*, *Argentiero, Gioia, Simeone* and *Massa*". PCA performed on sensor array response gave satisfactory results concerning to the discrimination among the different milk parcels (Figure 3). Moreover, the responses of the sensor array to the volatile compounds of milk samples have been coupled with the value of pH measured with a pHmeter. Multiple regression methods (MLR, PCR, PLS, Surface Response Modeling) have been used to correlate the sensor signals to the pH value that has to be predicted. Surface Response Modeling, that is a polynomial regression method, applying the MLR method and a quadratic ANOVA, gave the best prediction results since it is more suitable to account for the non-linear behavior of sensors (Figure 4).

After the analysis by EN, a SHS\GC\MS analysis was carried out on the same milk samples kept at room temperature. This kind of approach allowed us to optimize an analytical method for the headspace analysis of milk samples by SHS\GC\MS which is comparable with the headspace sampling method used for the analysis with the electronic nose. In this way the array response to milk flavors can be correlated to the results of SHS\GC\MS analysis. As expected, the gas chromatograms of milk samples showed not many peaks due to the particular HS conditions (T_{vial}=46 °C for 15 min); the choice of these conditions was imposed by the need to correlate the GC-MS results to those of Electronic Nose. However, since the gas-chromatogram pattern constituted a fingerprint of the headspace milk sample, the separation between the data clusters related to the different milk parcels in the PC-space can be justified by the composition in percentage of the milk sample headspace.

3.2 Olive oil

Olive oil has a fundamental importance in the 'Mediterranean diet' and in the market, therefore there is a great interest towards all the techniques able to provide an analysis of olive oil quality. Olive oil was the first foodstuff to be classified by both chemical and sensorial analysis according to the EU Normative. It means that an olive oil can be labelled as extra-virgin only if both, chemical and sensorial characteristics, are within certain standards established by law. Electronic Nose can be a promising tools for olive oil industry that requires urgently reproducible, reliable, inexpensive, easy to train and to use, objective 'sniffing' electronic device dedicated to olive oil for different applications, the main being classification and degradation studies. Authenticity is an issue of major concern across the oil industry. Olive oil is marketed based on acidity grade in 'olive-husk oil, olive-oil, virgin olive oil, extra-virgin olive'. Olive oil is also marketed based upon country and region of production and upon olive type. There is a very large difference in price among, for example, Italian and Spanish or Greek olive-oil.

However, it should be considered that the assessment of olive oil by an electronic nose is not a simple task due to the large difference between the compositions of the oil and its headspace. Indeed, those substances, on which fragrance and quality are based, are poorly volatile, while other compounds (such as ethanol and methanol) which are present

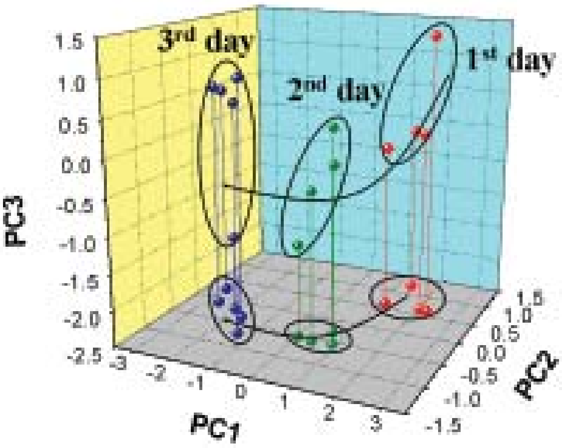

Figure 2: *PCA score plot related to the sensor array response to the volatiles of the pasteurized milk sample in different aging days.*

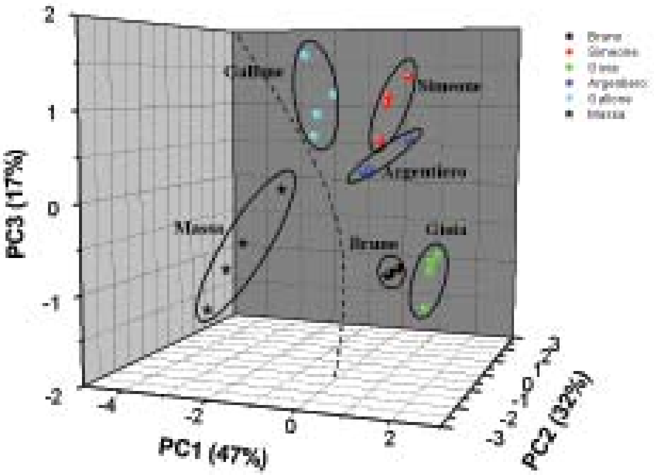

Figure 3: *PCA score plot related to the sensor array response to the volatiles of different stockbreeders milk samples – labeled as "Gallone, Bruno, Argentiero, Gioia, Simeone and Massa" as provided to an Italian dairy industry.*

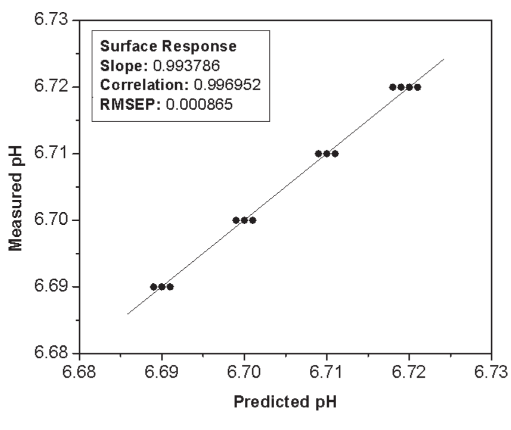

Figure 4: *Measured versus predicted pH values of milk samples based on a quadratic regression method (Surface Response Modeling).*

at low concentration in the liquid phase, due to their low boiling point, are present at high concentration in the headspace.

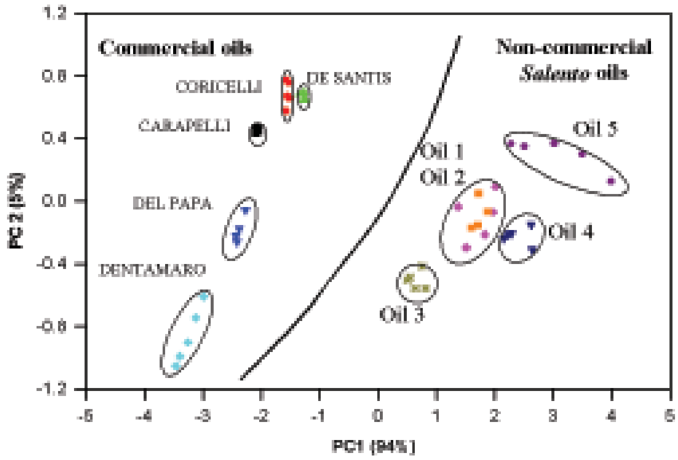

Figure 5: *PCA score plot related to the sensor array response to the volatiles of some commercial and non-commercial local olive oils from Salento province (Italy).*

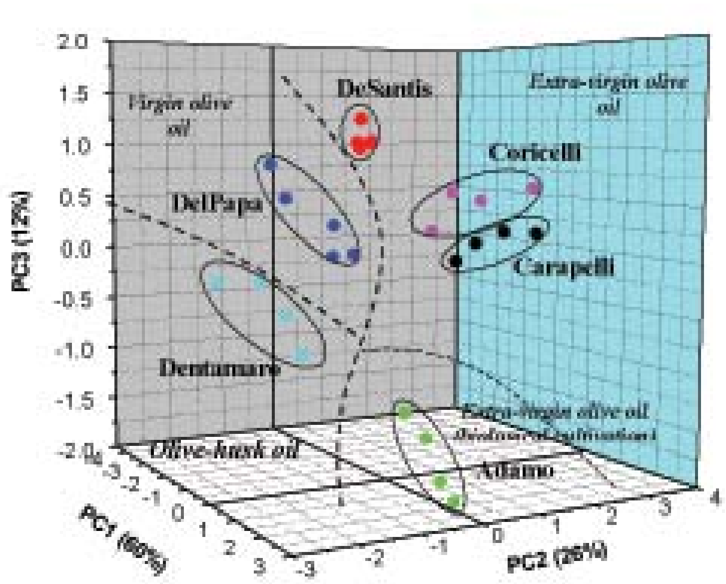

Figure 6: *PCA score plot related to some commercial olive oils including an extra virgin olive oil derived from a biological cultivation.*

The authors applied the developed Electronic Nose for testing a number of different commercial olive oils of various quality produced in different areas in Italy [10, 11]. Furthermore, the analyzed olive-oils were compared with local oils of the Salento region in Apulia (Italy). Indeed, one of the most important goals in the field of olive-oil characterization is their classification on the basis of their origin region. This is most important for the regions exhibiting the so called D.O.P. (Protect Origin Denomination) label which is an index of the quality of the product. A significant result was obtained analyzing the array response to the volatile compounds of some commercial and non-commercial local olive oils from Salento by PCA. In the score plot it can be clearly

distinguished all the different classes of olive oils and the discrimination between the commercial and non-commercial local olive oils (Figure 5). In Figure 6 the PCA score plot, related to some commercial olive oils including an extra virgin olive oil derived from a Salento biological cultivation, is reported. Also in this case, the PCA representation gave an idea of the good ability of the sensor array to distinguish among olive oils of different quality, so that in the future pattern recognition techniques could be applied.

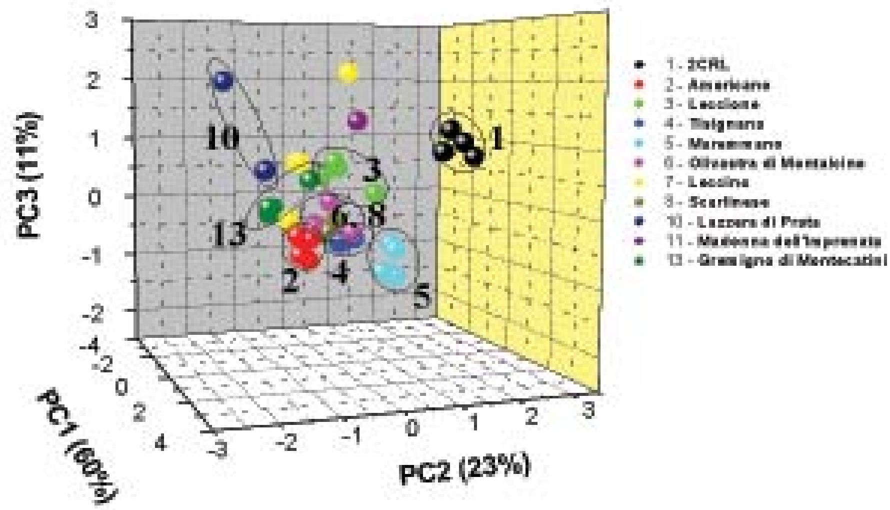

Figure 7: *3D-PCA score plot related to the sensor array response to the volatiles of different Tuscan single-cultivar EVOOs*

Upon evaluation of the quality of extra-virgin olive oils (EVOOs) it should be considered that they are produced by mixing oils from different "cultivar", that is, different varieties of tree species coming from different geographical origin areas of cultivation. The organoleptic properties of olive oils also depend on the particular blend. In order to satisfy consumer requirements, oil from a certain producer must be easily distinguishable and identified by presenting the same smell as well as the same taste and color. On the other hand, for olives it is of great importance to evaluate and conserve their existing genetic diversity, still preserved in spite of the disturbance of the environments where they are cultivated. The origin and the geographical distribution of such high variability in the cultivated olive are still under investigation. Most studies agree that after a spreading of new varieties of olive along the Mediterranean basins, the majority of modern cultivars are derived from the crossing of these ancient cultivars between themselves or by their breeding with plants followed by local selection practices.

Hence, by considering this particular issue in olive oil industry, the Electronic Nose was used to analyze different "single-cultivar" EVOOs produced by Tuscan producers. In order to study a relation between the sensor responses and the real head-space of olive oil samples, analytical technique like Headspace Solid Phase Micro Extraction \ Gas Chromatography \ Mass Spectrometry (HS-SPME\GC\MS) analysis was applied to the analysis of volatiles compounds in EVOOs samples. The obtained GC-MS data were used to identify the particular compounds and characterize the chemical composition of the EVOOs samples. Statistical analyses were carried out on data obtained from Electronic Nose and GC-MS.

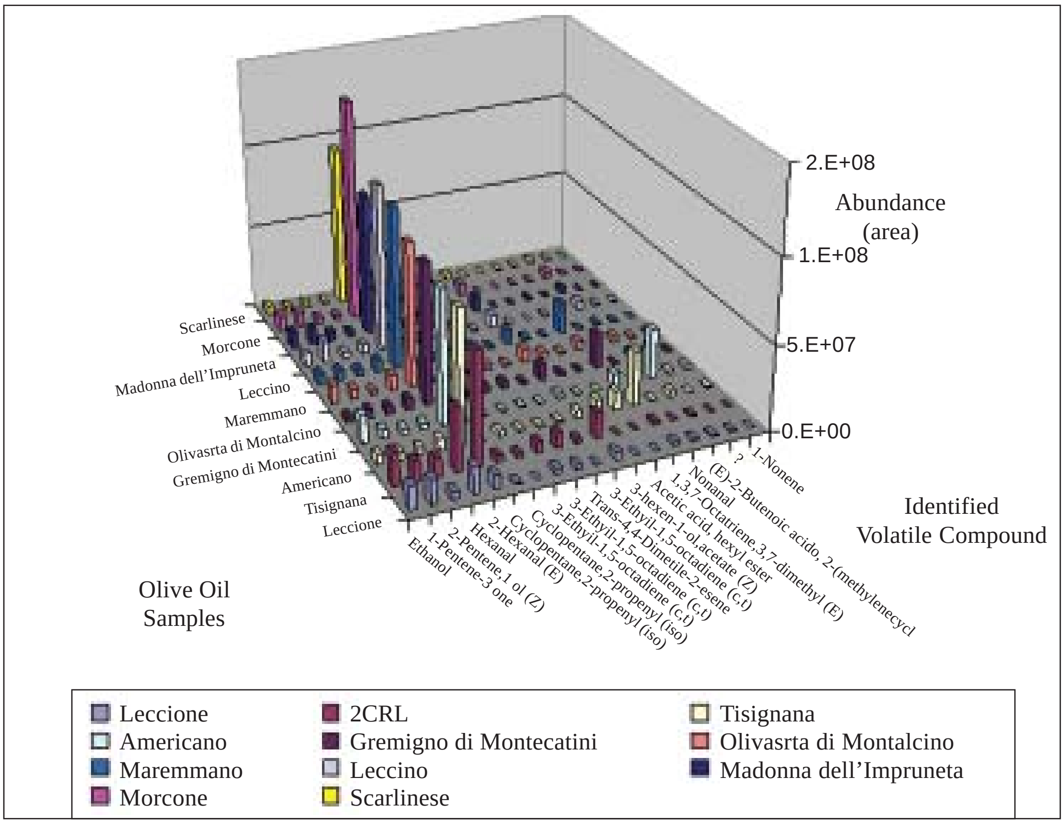

Figure 8: *Histogram showing the different chemical compounds identified by HS-SPME\GC\MS analysis in EVOOs aroma.*

In particular, twelve samples of different Tuscan single-cultivar EVOOs (labeled as *Leccino, Maremmano, Olivastra di Montalcino, Gremigno di Montecatini, Leccione, Madonna dell'Impruneta, Lazzero di Prata, Americano, Scarlinese, Morcone, 2CRL, Tisignana*) were analyzed by means of EN and HS-SPME\GC\MS [28]. At first, PCA analysis based on the sensor array data, gave a not full satisfactory discrimination among the different EVOO samples. Figure 7 reports the 3-dimensional score plot in which the drawn ellipses did not allow to identify clearly a complete separation among the clusters. By means of HS-SPME\GC\MS analysis, only eighteen different chemical compounds were identified in the EVOOs aroma. The histogram in Figure 8 summarizes these results. From a qualitative point of view, from HS-SPME\GC\MS analysis one can see that aldehydes, terpenes and alcohols are the main chemicals families present in the analyzed headspaces. Aldehydes, in particular hexanal and nonanal, coming from lipid oxidation, are the predominant chemical family present in the headspace.

3.3 Peach

Evaluating fruit ripeness has long been a fundamental issue in postharvest technology since ripeness is perceived by customers as the main quality indicator. Standard physical-chemical techniques enable to measure some quality indicators, as total soluble sugars content (SSC) and firmness, but they require the destruction of the fruit samples used for the analysis. This is why, nowadays, predictions of shelf-life ripeness state is mainly based on sensory analysis and practical experience, although they may be influenced by subjective evaluations.

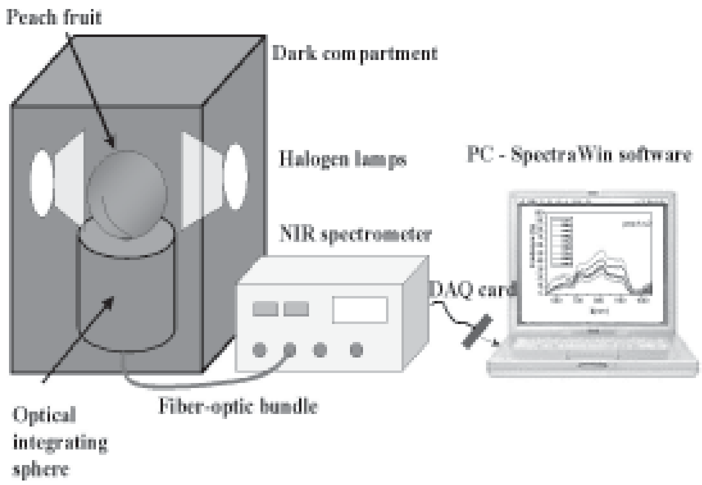

Figure 9: *Schematic diagram of the experimental setup for NIR testing of peach fruit.*

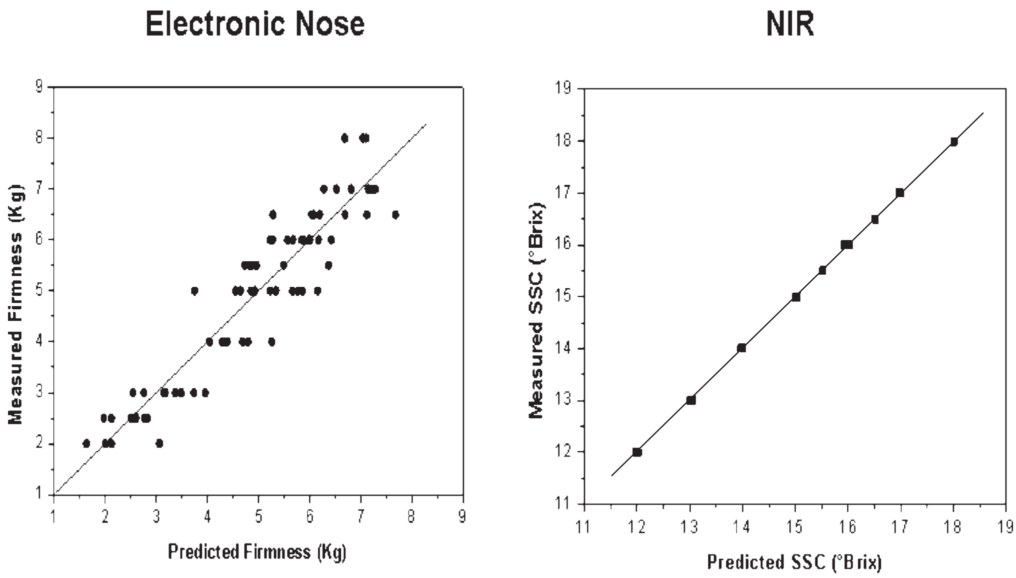

Figure 10: *Best prediction results for EN measurement (firmness assessment) and for NIR measurement (SSC assessment).*

Aroma analysis by Electronic Nose is to be considered an important index of fruit ripeness assessment. The formation of aroma compounds in fruits is a dynamical process, because volatile compounds are continuously synthesized and developed during fruit growth and maturation. Moreover, Near-Infrared Spectroscopy has been shown to provide rapid and non-destructive measurements of internal properties as SSC due the absorption bands of carbohydrates in this wavelength region.

We evaluated the performance of an Electronic Nose and Near-Infrared Spectroscopy as additional, non-destructive and objective techniques in the analysis of ripeness of post-harvested peaches [19, 20]. With this aim the electronic nose responses to the aroma of intact peach samples and the related NIR spectra have been coupled with the values of total Soluble Sugars Content (SSC) and firmness measured by a refractometer and a penetrometer respectively. Multivariate regression methods (MLR, PCR, PLS, Surface Response Modeling) have been used to correlate the sensor signals and the NIR spectra to the fruit quality indicator to be predicted.

A set of several peaches at different maturity degree was analyzed by Electronic Nose and NIR spectroscopy. Figure 9 shows the schematic diagram of the setup for NIR testing of peach fruit. After the analysis by the Electronic Nose and the NIR spectrometer the peach samples were destroyed to measure the SSC and the firmness. Total Soluble Sugars Content (SSC) is a major characteristic used for assessing peach fruit quality. Its measurement is normally performed destructively on juice. Two slides of flesh were cut from two opposite sides of the sample and the mixed juices were squeezed directly onto a refractometer which gives the medium value of SCC in °Brix. Firmness is one of the easiest, fastest and cheapest methods to assess ripeness and also one of the better correlated with ripeness parameters. It was measured with a penetrometer (8 mm tip) on the two opposite wider sides, at the center of each fruit. The instrument measures the force required for the mechanical probe to penetrate into the tissue of unpeeled fruit. The measured firmness values were averaged and expressed in Kg_{weight}.

By means of multivariate regression techniques, relationships were established between Electronic Nose response to aroma peach and fruit quality parameters (SSC and firmness). Firmness was more precisely predicted than SSC. The regression technique based on a quadratic regression (i.e. Surface Response Analysis) gave better prediction results respect to the linear regression techniques. Excellent prediction of the quality indicators were also obtained by NIR spectroscopy by simple linear regression techniques (i.e. PLS). As example, Figure 10 showed the best results for EN measurement (firmness assessment) and for NIR measurement (SSC assessment).

4. Conclusions

In this overview of possible applications of Electronic Nose in food industry, the responses of the sensor array to the flavors of some foodstuffs with the results obtained by other standard techniques were compared. It was demonstrated that the Electronic Nose, when properly used, can be a comparative and complementary, not totally alternative device to conventional techniques in specific applications for food industry. In the future, the authors´ aim will be to join together the information acquired by the electronic nose with other techniques (as Vis/NIR) data to take advantage of the synergic effect expected by the use of different techniques.

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