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ELECTRONIC OLFACTORY SYSTEM TO EVALUATE THE FRUIT QUALITY

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Preface

Fruits and vegetables are currently receiving considerable attention because the consumers are nowadays more health conscious and pretend high quality products. Moreover, the contemporary dietary recommendations encourage a larger consumption of fruits and vegetables.

Fragrance, appearance, and hardness are the major factors in product evaluation by the consumers.

Various analytical and sensory methods have been proposed to evaluate both external and internal fruit quality, but many of them are time-consuming, very expensive, destructive, and also require trained person and equipped laboratories.

Aroma is one of the most important quality parameter perceived by consumers.

Gas Chromatography technique is usually employed to identify the fruit aroma, while sensory analysis is used to assess the intensity of aroma descriptors. The chromatographic techniques require special equipment and dedicated staff, as sensory analysis presents some drawbacks, such as the duration of panel training and, sometimes, dubious objectivity of the results.

Expanding markets of high quality fruits is a key factor for the fruit industry success. Therefore, the improvement of flavor properties of fresh fruit reaching the consumer would add value, increase consumption and create new markets. But the small fruit companies, which are the first step of the whole chain, cannot afford long waiting times for response or spend exorbitant costs for quality analysis.

For this reason it is taking steps to market the Electronic Olfactory Systems, commonly known as *Electronic Noses*, which allow obtaining, in a quick and economic way, quality information both close to the analytical determinations and well correlated with the responses of sensory analysis.

1. Introduction

The term “quality” implies the degree of excellence of a product or its suitability for a particular use. To investigate and control the quality, it is necessary to be able to measure quality parameters like sensory characteristics, nutritive values, chemical constituents, mechanical and functional properties and defects.

Moreover, the quality is always connected to product and consumer orientation. But one must always remember that there is more than one customer in the marketing chain, for example: grower, packer, distributor, retailer, produce manager, shelf stocker, shopper, and finally the ultimate consumer who actually eats the product. So the component attributes of quality vary with the context.

The person or institution requiring the measurement determines the choice of the quantities to be measured, the measurements techniques and the values of acceptance intervals.

Instrumental measurements are often preferred to sensory evaluations in research and commercial situations because they reduce variations in judgment among individuals and can provide a common language among researchers, industry and consumers.

The fruit quality is not a single, well-defined attribute but comprises many properties or characteristics. In many cases, traditional indicators of fruit maturity, such as color, diameter, total soluble solids and tritatable acids, may not be sufficient to determine optimal sensory quality (Mehinagic *et al.* 2006).

Appearance is one of the major factors that the consumer adopts to evaluate the fruits quality. In particular,he color is the first thing that attracts a customer; the concentration of pigments gives the better quality index becauseof its correlation with fruit ripening and other quality parameters such as sweetness and juiciness. Fruit color is measured by analytical and sensorial methods. The colorimeter, one of the available analytical instruments describes the colour by several color coordinate systems (Minolta 1994). Some of the most popular systems are RGB (red, green and blue) and CIE (Commission Internationale de l’Eclairage) parameters (L* a* b*). According to CIE concepts, the human eye has receptors for three colors (red, green and blue) and all the colors are obtained by their combinations. While the color is more directly related to consumer pleasure, the pigment concentration may be strictly linked to maturity and, definitely, is an indicative index of the flavor.

Texture is another important quality factor not only to the final consumer, as an indication of hardness, crispness and juiciness, but especially to the food processors and distributors, as an indication of freshness. Penetrometer testers, such as the Magness–Taylor (MT) Fruit Firmness Tester, often improperly called a ‘pressure tester’, and the similar U.C. Firmness Tester is widely used for firm-to-hard fruits and vegetables (Abbott 1999). Penetrometer measurements are moderately well correlated with human perception of firmness and with storage life, and

consequently this technique has received common acceptance for a number of horticultural products, such as apple, cucumber, kiwi, pear and peach.

The quality of fresh fruit is dependent on other aspects, different from the only appearance, for example flavor and nutritional characteristics.

Sugars, organic acids, phenolic compounds, vitamins and odor-active volatiles influence flavor and nutritional characteristics.

Sweetness is determined by the concentrations of the predominant sugars, such as fructose, sucrose and glucose. Sourness or acidity is determined by the concentrations of the organic acids as citric, malic, tartaric and amino acids, such as aspartic and glutamic acids (Ezura and Hiwasa-Tanase 2010).

Moreover, fresh fruits play a significant role in human nutrition because they are sources of constituents such as flavonoids, carotenoids, vitamin C, vitamin A, vitamin B_6 that may lower the risk of cancer and other diseases (heart disease and so on).

Anyway, continuous improvement in fruit flavor is required to satisfy consumer demands (Kader 2004), with particular concern to the aroma, considered to play a dominant role in overall flavor.

Fruits produce distinct volatile compounds during ripening that affect the characteristic fresh fruit flavor, required by the consumers.

Most fruits produce many volatile compounds as indicators of fruit ripening. For example, more than 300 volatile compounds are produced by apple, strawberry and melon fruit, which are comprised of diverse classes of chemicals, including esters, alcohols, aldehydes, ketones, and terpenes (low-molecular-weight compounds) (Dirinck *et al.* 1989, Latrasse 1991, Beaulieu 2006). Many of these volatile compounds are produced in trace amounts, below the thresholds of most analytical instruments, but detectable by human olfaction (Goff and Klee 2006), because considered odor-active.

Esters, for example, are the predominant class of compounds giving fruity flavor characteristics. Alcohols and aldehydes contribute to flavor and aroma of the fruit as well as serve as precursors for ester synthesis, their composition reflect the esters present in the fruit.

Large numbers of volatile compounds have been identified in many species of fruits, but more research is necessary to identify the compounds contributing to a desirable aroma, their threshold concentrations, potency, and interactions with other compounds.

Thus, to improve the quality of fruit through the selection of the best-tasting genotypes to be produced, research on flavor is necessary including both non-volatile and volatile constituents that contribute to the fruit taste and aroma.

The importance of aroma

Man possesses various sensory organs, which provide five different senses: taste, smell, vision, touch and hearing. These senses are our link between the external world and our consciousness.

From the evolutionary point of view, smell is one of the oldest senses, allowing identifying food, recognizing danger and so on. Consequently, the olfactory sense has become a key element in the development of many commercial industries that manipulate the aroma properties in order to improve product appeal and quality, thus consumers identify the individual commodities that have unique scents.

The aroma characteristics have contributed immensely to the value and appeal of many commercial products. So, research and quality control of aroma characteristics during product manufacturing has become of paramount importance in industrial production operations because product consistency and assurance of standard characteristic are essentials for consumer satisfaction.

The olfactory sense has long been intimately linked with human emotions, and, as a consequence, consumers are willing to pay for good-aroma products.

Aroma characteristics

Aromas are mixtures of volatile compounds present in the air at concentration that may be detected through the sense of olfaction. In some cases, the aroma is composed of a single chemical compound, which is “odor-active”. In others cases only few compounds are present, being only one the dominant or the principal component. However, an aroma derived from organic sources in most cases may be composed of hundreds of different compounds all contributing to its unique quality and characteristic.

Aromatic compounds usually have relatively low molecular masses (between 30 and 300 Da). The volatility of molecules is determined by the strength of bonds between them, being non-polar molecules more volatile than the polar ones. In fact, most aromatic molecules have no more than one or two polar functional groups infact molecules with more polar functional groups generally are not volatile. Volatile compounds frequently contain an oxygen molecule, although nitrogen and sulphur may also be present (Strike *et al.* 1999).

Aromatic compounds are mainly characterized by their chemical structural and functional groups, such as heterocyclic or aromatic rings and double bonds that contribute to the overall shape of the molecule and produce a particular aroma or flavor sensation (Gardner & Bartlett 1999). Hydrocarbons usually do not exhibit odors of interest or of a well defined character, although certain unsaturated hydrocarbons such as cyclic alkenes have been identified and associated with typical and pleasant notes, such as fruity, green, and floral odors (Anselmi *et al.* 2000).

Four quantifiable qualitative dimensions in general characterize aromas: *threshold, intensity, quality, and hedonic assessment*.

The *detection threshold* value is defined as the lowest concentration of aromatic compounds detectable by human subjects as an aroma (Yoshii *et al.* 2002). The detection threshold is determined by diluting the aroma to the point where 50% of the test population or human panel cannot longer detect the aroma (Yuwono *et al.* 2004). The threshold value varies with aroma species and between individuals, according to level of training.

Intensity refers to the perceived strength of the aroma sensation, and increases as a function of concentration.

Quality is the third dimension usually expressed through the use of descriptors, or common-use words, that associate the aroma to the aroma qualities of known substances. McGinley and McGinley (1998) proposed eight aroma groups with examples of descriptor types, representative of each group, as follows:

(1) *earthy aromas* (musty, mouldy, musk, stale, grassy, herbal, woody); (2) *floral aromas* (fragrant, flowery, perfume, eucalyptus, lavender); (3) *fruity aromas* (citrus, orange, lemon, apple, pear, pineapple, strawberry); (4) *spicy aromas* (cinnamon, mint, peppermint, onion, dill, garlic, pepper, cloves, vanilla, almond, pine); (5) *fishy aromas* (fishy, prawns, amine); (6) *sewage aromas* (septic, putrid, rancid, sulphurous, rotten, decayed, cadaverous, foul, sour, pungent, burnt, swampy); (7) *medicinal aromas* (disinfectant, phenol, camphor, soapy, ammonia, alcohol, ether, anesthetic, menthol); (8) *chemical aromas* (solvent, aromatic, varnish, turpentine, petroleum, creosote, tar, oily, plastic).

Really Linneaus was the first who proposed an aroma classification. He defined seven primary aromas: aromatic, fragrant, musky, garlicky, goaty, repulsive and nauseating (Linnaeus 1756, Wise *et al.* 2000).

The food, beverage and perfume industries that manage and manipulate product aromas, have consistently tried to name and classify aromas using the American Society for Testing and Materials (ASTM) classification. ASTM has classified 830 aroma descriptors (Ohloff 1990). Nevertheless, human panel tests have indicated that human subjects after good training can correctly identify an average about 100 aromas (Desor & Beauchamp 1974).

The last aroma's dimension is the *hedonic assessment*. It is associated with the relative pleasantness or unpleasantness of the aroma. Hedonic assessment may be quantified using values ranging from 1 (completely dislike) to 10 (very good, pleasant, and agreeable) or through objective judgments (excellent to terrible) using descriptor terms indicating relative satisfaction or agreeableness of the aroma (from very pleasant to completely unpleasant).

The chemical volatile compounds, transported by the inhaled air, are trapped and dissolved into the olfactory epithelium; the latter is a special membrane, in a particular nasal region, where the olfactory sensory neurons are located (Bozza & Mombaerts 2001). The olfactory signal is thus transmitted to the brain, where the final perceived odor results from a

series of neural computations. Odors are recognized thanks to the memory effect of previous experienced smells, thus accounting for the high subjectivity of the odor perception (Pearce 1997, Freeman 1991) (fig. 1).

Sensitivity to aromas can be improved and varies considerably from person to person.

Gilbert and Wysosky (1987) tested the sensitivity of about 1,500,000 people to selected aromas. They discovered that sensitivity varies widely with the nature of the aroma, sex, age, physiological moment and health of the people tested. Some psychophysical studies have clearly demonstrated the existence of specific anosmias (lack of olfaction or absence of ability to smell), hyposmia (decrease of ability to smell), and parosmia (distorted sense of olfaction, resulting in phantom, non-existent and mostly unpleasant smells) (Hutton *et al.* 2007, Doty *et al.* 1989, Royet *et al.* 2001).

The relatively low sensitivity and discrimination capabilities of the human nose, coupled with the common occurrence of olfactory fatigue, has led to the need for electronic instruments with sensors capable of performing repeated discriminations with high precision. For these reasons, there is great interest in the development of electrochemical sensors capable of precisely quantify and express the aroma characteristics.

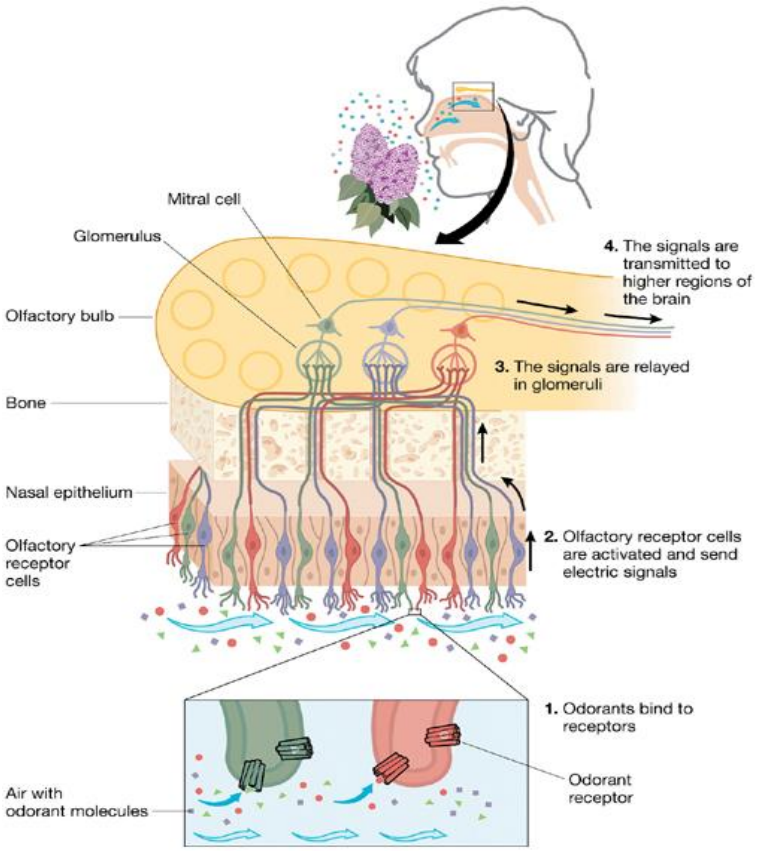


Fig.1. Description of the human sense of smell. (Rinaldi 2007)

Analysis of flavor and aroma volatiles

Many factors can influence the quality and quantity of volatile compounds in fruit including cultivar, cultural practices, ripeness and postharvest handling (Fellman *et al.* 2003, Dixon and Hewett 2000, Forney *et al.* 2000, Lester 2006). Among Of these factors, maturity plays a critical role in fruit volatile development. Ideally, fruit should be harvested at optimal eating quality to optimize volatile content for flavor. However, immature fruits are often harvested in order to increase storage and market life span and minimize physical damage and disorder expression. Although immature fruits are more successfully stored and transported, flavor is often lacking due to the close relationship between maturity and volatile biosynthesis (Kader 2004).

Fruit volatiles molecules can be classified as ‘‘primary’’ or ‘‘secondary’’ compounds, indicating whether they are present in intact fruit tissue or produced as a result of tissue disruption. Volatile molecules collected from intact fruits reflect the consumer smelling and perceiving ripening signals of the fruit. Volatiles generated after tissue disruption may better represent the flavor (taste and aroma) perception during eating (Song & Forney 2008).

Concentrations of volatile compounds in fruit are often below the detection limit of many analytical instruments and, therefore, analytical techniques have been developedincrease their concentration.

Volatiles from intact, cut or macerated fruits can be collected using headspace techniques and analyzed directly (or after fruit concentration) using various trapping technologies. In addition, volatile compounds can be extracted from homogenized fruits using various distillation and solvent extraction techniques.

Extracts of homogenized fruits usually represent the composition of the whole volatile compounds present in the fruit, whereas headspace isolates represent the composition of the volatiles present in the air above the fruit.

Prior to collection, fruits are enclosed in inert vessels and volatiles are collected after equilibration using either ‘‘static’’ or “purge-and-trap” methods. Purge and trap or dynamic headspace techniques have been widely applied by many researchers to isolate volatile fruit compounds. A popular method commonly used involves the collection of the volatile compounds from the headspace onto adsorbent materials over fruits or fruit tissues. Solid phase micro-extraction (SPME) is a relatively new technology that has been used in food flavor analysis (Yang & Peppard 1994, Pawliszn 1997). Due to its speed, sensitivity (ppbv, parts per billion by volume) and lack of extraction solvents, SPME has become one of the most widely used aroma/volatile sampling techniques for fruit volatile analysis. However, due to the matrix effects, site competition on the adsorbent fiber, poor repeatability and challenges with standard curve calibration, SPME can only offer a semi-quantitative analysis of volatiles.

Extracted volatiles are typically analyzed using Gas Chromatography (GC) techniques. Upon the separation step (chromatographic phase), the molecule may be identify with different detector types: Flam Ionization Detector (FID), Thermal Conductivity Detector (TCD), and Electron Capture Detector (ECD; where only the first one is a destructive method.

At the end of a chromatographic column, a Mass Spectrometer (MS) detector is used to identify the structure of each one molecule. This technique (GC/MS) offers good sensitivity and a wide dynamic range for almost all fruit volatiles. Depending on the method of sample collection, a variety of methods of sample introduction can be used. These include liquid injection, cold on-column injection of headspace samples, thermal desorption of SPME fibers in temperature programmable injection ports, and thermal desorption of collection tubes. The latter requires specialized equipment, but the use of collection tubes provides many advantages, flexibility in collection volumes and storage of collected samples.

Another techniquenamed Gas Crhomatography-Olfactometry (GCO) is also used to identify and describe compounds contributing to aroma of fruits. The use of human nose as sensitive detector in GC was proposed by Fuller and co-workers as early as 1964. In this technique the relationship between odor activity of single compounds and their behavior in an aroma mixture is put in evidence (van Ruth 2001). Human perception of volatile compounds is determined by two primary factors: the fruit volatile concentration and the human aroma perception threshold.

The importance of Sensory Analysis to determine fruit quality has recently received increasing attention. Both descriptive and consumer panels are used to evaluate and screen quality of new genotypes selected by breeding (Hampson *et al.* 2000).

The combination of sensory analysis and instrumental provides deep insights into the impact of volatile compounds on fruit flavor than either alone.

Moreover, new technologies have been tested on fruits aiming for fast and, in some cases, non-destructive volatile detection. The electronic nose (E-nose) and mass spectrometry-based electronic nose (MS E-nose) are new technologies that can be used to predict fruit quality. These technologies may offer alternatives to the classical approach of fruit volatile measurement by means of GC/MS.

2. The Electronic Nose

The first studies reporting measurements on whole aroma were done in the 1920s by Zwaardemaker and Hogewind who measured the electricity of a fine spray of water containing volatile substances. They noted that the spray-electricity increased when different volatiles substances were added to water. The first real instrument for measuring aromas was developed by John Hartman 30 years later. The sensitive element of the apparatus was a microelectrode measuring the flow of current by a millivoltmeter. Hartman was also the first to propose the idea that an aroma-detecting device could operate with several different coated sensitive elements so that different electrode-coating substances could be capable of giving differential responses with different compounds (Hartman 1954).

In the early 80's, the idea of an electronic-nose instrument with a chemical array sensor system for aroma classification was suggested by the studies of Persaud and Dodd (1982) and Ikegami and Kaneyasu (1985). By that time, the development of computers and electronic sensors made conceptually possible to obtain an electronic device capable of imitating the mammalian olfactory system.

The term "Electronic Nose" was used for the first time in 1988 by Gardner and Bartlett, who later defined it as *"an instrument which comprises an array of electronic chemical sensors with partial specificity and appropriate pattern recognition system, capable of recognizing simple or complex odors"* (Gardner & Bartlett 1994).

According to Mielle *et al.* (1995), this type of system is '*obviously electronic but not nose*'. In fact the only aspect in common with human odor sensing organ is its function. Like the mammalian nose, it detects gases through sensors that send signals to a recognition organ (the brain or a computer), but the operating steps and the number of sensors, as well as the sensitivity and selectivity, are very different. This is the reason why some scientists prefer to call this instrument in other ways, for example, '*flavor sensor*', '*aroma sensor*', '*odoursensing system*' or '*multi-sensor array technology*'.

As analytical instruments, these systems must be designed to obtain high repeatability (the ability to obtain the same pattern for a sample on the same array over short intervals of time) and reproducibility (the ability of different sensor array to produce the same pattern for the same sample).

The optimal characteristics of an ideal sensor must be: high sensitivity to chemical compounds comparable to that of the human nose, low sensitivity to humidity and temperature, medium selectivity, capacity of responding to different compounds present in the headspace of the sample, high stability, high reproducibility and reliability, short reaction and recovery time. Ideal sensors should be also robust and durable and of small dimensions. An easy calibration procedure and a simple processable data output are highly desired (Shaller *et al.* 1998).

In the recent years much work has been done to understand the principles of odorant receptors and the organization of the olfactory system (Firestein 2001, Buck 2005, Mombaerts 2004) with the aim to developing a device able to mimic the human olfactory system (fig. 2). In the latter, in each olfactory receptor cell it is located only one type of odorant receptor capable of detecting a limited number of substances. Several olfactory receptors are simultaneously activated by a complex odor, composed of multiple odorant molecules.

In comparison, the electronic nose is at the same time more and less powerful because it offers the possibility of detecting some important non-odorant gases, but has a relative small number of sensors. One of the main reasons why it has not been possible to make a one to one copy of the human nose is the high specificity of the human receptors.

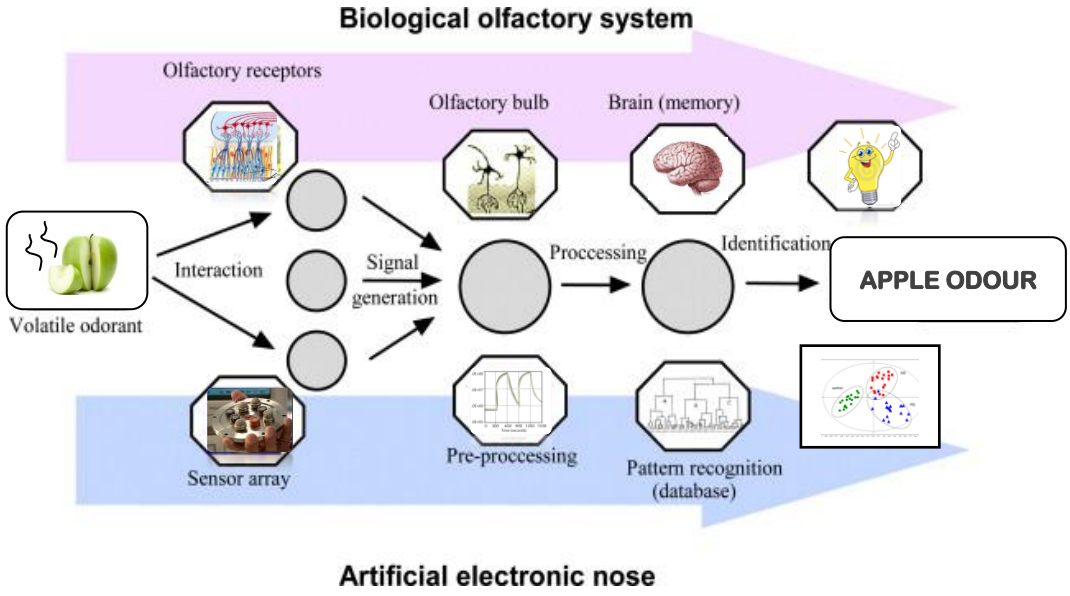


Fig. 2. Symbolic analogy between the biological and the electronic noses process.

After the initial euphoria produced by the prospect of replicating the biological olfaction, the limits of electronic nose technology were recognized and linked to the fundamental sensitive components (Stetter *et al.* 2002). The problem is that, in contrast to the nature, the information obtained by adding many sensors rapidly saturates. While it is possible, of course, to improve the individual sensors and by-pass the problem, the complexity of the electronic nose increases because sampling system, sensor array itself, reference data set, and the data evaluation algorithms require further attention and improvement.

For historical reasons, the main research fields for electronic nose technique are still related to those areas where the human olfaction system is relevant and where trace components are the subject of interest.

Even if the modern research has shown that the perception of human noses of some odorants is comparable to that of canines and rodents (Laska *et al.* 2000), the ability of the latter is superior in many fields. For instance, dogs are able to identify individuals by their scent, to track them, or to track down hidden narcotic drugs or explosives (Lorenzo *et al.* 2003, Göth *et al.* 2003). However, dogs show behavioral variation depending on changes condition, and all animals are subject to fatigue. To decrease the complexity of execution, it would be desirable to have an artificial system with the same performance and ability to detect dangerous compounds too.

The human perception is usually sensitive to odor compounds down to the parts per billion range (Cometto-Muñiz *et al.* 1998). However, the detection threshold of some substances is several orders of magnitude lower. An example is the case of 2,4,6-trichloroanisole shows (Prescott *et al.* 2005), the target compound for the cork taint in wine quality applications. This benchmark established by human perception is the target for an electronic nose detection (Ragazzo-Sanchez *et al.* 2006, Martí *et al.* 2003, Martí *et al.* 2005, Santos *et al.* 2004, Ragazzo-Sanchez *et al.* 2005). Additionally, it must show its ability when compared to analytical systems too (Buser *et al.* 1982, Rapp 1998).

2.1. Structure of an electronic nose

To be defined an electronic nose (e-nose); an apparatus should consist of the following components:

1. an aroma delivery system, transfering the volatile aromatic molecules from the source material to the sensor array (sampling system);
2. a measure chamber, where sensors are housed, usually at fixed temperature and humidity, otherwise affecting the aroma molecules adsorption;
3. an electronic transistor, converting the chemical signal into an electrical signal, and amplifying and conditioning it;
4. a digital converter, transfering the signal from analogical to digital;
5. a computer, reading the digital signal and displaying the output on which the statistical analysis for sample classification or recognition can be done.

It is important to underline that the sampling and the sensor array system are the most important parts of the electronic nose device because volatile compound adsorption or contact with the sensor surface is *conditio sine qua non* for sensing. The typical complete sampling time for e-nose analysis is a function of the sensor material, the elements to be analyzed, the operating temperature of the sensor, the ambient humidity, the statistical method used for results analysis, and the accuracy of the microprocessor (Gardner & Bartlett 1999)

Summarizing, the electronic nose instruments are composed of three elements: a sample handling system, a detection system and a data processing system.

2.1.1. Sample Handling

Sample preparation and sampling are error prone steps and have to be well considered to achieve reliable results. Sample handling begins with a representative selection of samples, continues with their appropriate pre-treatment including possible pre-concentration and separation steps, and ends with a reproducible sample delivery procedure to the sensor array.

Each step can cause statistical as well as systematic errors, but, at the same time, the sample preparation offers additional opportunities to improve the results. It has the potential to considerably increase the sensitivity of the whole system and, in addition, to remove the problems caused by background interferences.

The samples can be solid, liquid, or gaseous and their nature may differ a lot, so it is difficult to give a complete overview of the strategies that can be used. For instance, aqueous samples can be stirred, heated, or salted out, or the pH can be varied to increase the concentration of volatiles in the headspace (Friant *&* Suffet 1979). When the samples are gaseous, the sampling is made according to the vapor partition; a pre-concentration step can be useful to make the instrument more sensitive to the system studied, (Landy *et al.* 2002) and to increase the analyte amount in the head space (Baltussen *et al.* 2002). In this case, the analytes follow the concentration gradient according to Fick's first law of diffusion (capable to quantify the movement of molecules from a region of higher concentration to a region of lower concentration).

To introduce the volatile compounds present in the headspace (HS) into the e-noses detection system, several sampling techniques have been used in literature (Ampuero *et al.* 2003, Martí *et al.* 2005, Pèrés *et al.* 2003):

1. ***Static headspace*** (SHS) technique consists in putting the sample in a hermetically sealed vial and then, extablished the equilibrium between the matrix (in a liquid or solid state) and the gaseous phase, and then sampling the headspace (HS). Sample temperature, equilibration time, vial size and sample quantity are the main parameters to be optimized. An automatic HS sampler is recommended to improve the poor repeatability of manual HS injection. In some applications a vapor-flow system it has been used providing a control better than that obtained in the case of manual headspace injection of the operating temperature and of the amount of analyte introduced into the detector.

2. ***Purge and Trap*** (P&T) and ***Dynamic headspace*** (DHS) techniques are used in some applications to increase sensitivity, since they provide a pre-concentration of volatile compounds. In these systems, the volatile components are purged by a stream of inert gas and trapped onto an adsorbent matrix. In the case of P&T, the gas flow is injected through the sample, whereas, in the case of DHS, only the HS is purged by the gas. The trapped molecules are subsequently desorbed by heating and introduced into the detection system. Apart from the choice of trap, the

main parameters to be optimized are the temperature of the sample, the equilibration time, the flow rate of the extractor gas and the purge time of the HS.

3. ***Solid-phase microextraction*** (SPME) is a user-friendly pre-concentration method. It consists in exposing a silica fiber covered with a thin layer of adsorbent material in the HS of the sample in order to trap the volatile components. The adsorbed compounds are then desorbed by heating and introduced into the detection system (fig. 3). Apart from the choices of the covered adsorbent, the main parameters to be optimized are the equilibration time, the sample temperature and the time exposition. This technique has a considerable concentration capacity and it is very simple because, unlike P&T or DHS, it does not require especial equipment to desorbs the absorbed molecules.

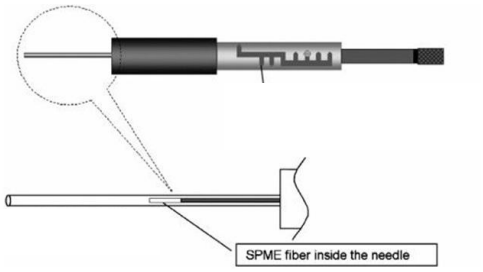


Fig. 3. Schematic design of a commercial SPME fiber. The fiber is extended through the needle and exposed to the target analytes. After the volatiles have reached equilibrium between the fiber coating and the gaseous phase (or after a strongly defined time), the fiber is withdrawn into the needle. Desorption will take place in a heated inlet under a similar procedure. (Supelco)

4. ***Stir bar sorptive extraction*** (SBSE) makes use of a magnetic bar coated with polymers. Its loading capacity is much higher than that of SPME. Even though it has been developed only recently, SBSE is a promising extraction technique when very high sensitivity is required.

5. ***Membrane introduction mass spectrometry*** (MIMS) is based on a sample handling system used in mass spectrometry (MS) based e-noses. This technique allows the direct introduction of specific compounds of a liquid or gas sample into a mass spectrometer. A thin membrane is installed between the sample and the ion source of a mass spectrometer. The volatile compounds dissolve in the membrane, diffuse through it and, finally, evaporate directly into the ion source (Pérez Pavón *et al.* 2006).

Sample handling is a critical point of e-nose analysis whose importance is very often ignored. Each of the above described methods can be advantageous and disadvantageous, and the choice of one of them depends on the particular application (Batterman *et al.* 2002).

SHS is the most common technique due to its simplicity. Here the aromatic material is stored in a closed volume and allowed to build headspace, after that the volatile compounds are removed from the sample vessel using a syringe and injected into the sensor chamber

maintained at a constant temperature and purged with a clean reference gas (usually conditioned or filtered environmental air) after sensor readings. An automatic headspace delivery system can significantly reduce the sampling time and standardize the aroma concentration. A drawback of SHS technique, in some applications, is the low sensitivity because the volatile compounds are not pre-concentrated. The pre-concentration systems improve the sensitivity making the detection easier. They also allow to extract semi-volatiles compounds which otherwise would be undetectable. However, the introduction of a supplementary step in the method increases the time of analysis. Moreover, analytical artifacts (memory effects, bleeding or irreversible adsorption) are generated in some cases. In this respect, the pre-concentration is a good opportunity to increase the measure sensitivity, but it must be carefully chosen.

2.1.2. Detection System

The sensor array in an electronic nose performs functions very similar to those of the olfactory receptors in the human olfactory system. Thus, the sensor array may be considered the heart and most important component of an electronic nose.

The classical e-nose, consisting of an array of chemical gas sensors as detection system, is the most common approach, although new technologies such as Mass Spectrometry (MS) and ion mobility spectrometry (IMS) have recently entered new field of application.

A chemical sensor is a device capable of converting a chemical quantity into an electrical signal that can be related to the concentration of specific particles such as atoms, molecules, or ions in gases or liquids (Pearce *et al.* 2003).

Many types of sensors can be used in an e-nose need to respond to molecules in the gas phase, typically formed by volatile organic compounds (VOCs) with different relative molar masses. Many categories of sensor arrays have been involved in the development of e-noses.

A summary of the types and mechanisms present in gas sensor technologies is reported below:

1. ***Piezoelectric sensors*** (also called gravimetric or acoustic sensors), based on the propagation of acoustic waves produced by piezoelectric materials (i.e. quartz or $LiNbO_3$) in a multilayer structure. Surface acoustic wave (SAW), bulk acoustic wave (BAW) and quartz microbalance (QMB) are the most common acoustic sensors used in e-nose sensory arrays.

2. ***Electrochemical sensors***, including amperometric, potentiometric and chemiresistive or conductimetric sensors. Among them, chemiresistive sensors such as Metal Oxide Semiconductors (MOS) and Conducting Polymer (CP) are widely used to built arrays for gas and odor measurements. Several potentiometric gas sensors using Metal Oxide Semiconductor Field Effect Transistors (MOSFET) have been also developed and used in e-noses technology.

The basis of electrochemical gas sensor operation involves the interaction between gaseous molecules and sensor-coating materials.

This interaction modulates the electrical current passing through the sensor and detected by a transducer that converts the modulation into a recordable electronic signal.

3. ***Optical sensors***, such as optical fibers, as well as the more traditional devices used in absorbance, reflectance, luminescence and surface plasmon resonance (SPR) techniques.

4. ***Calorimetric or thermal sensors***, in which the heat of a chemical reaction involving the analyte is monitored by a transducer such as a thermistor.

For all these types of sensors it is important to take into consideration the parameters listed below:

- S*ensitivity*: the magnitude of the output signal in response to a given input (perturbation/stimulus).

- *Response time*: the time that the sensor signal takes to pass from 10% to 90% of its excursion in reaching a new steady state, during the response dynamics.

- *Recovery time*: the time that the sensor signal takes to pass from 90% to 10% of its excursion in reaching a new steady state, during the recovery dynamics.

- *Resolution*: the minimal value of the input magnitude to which the sensor is able to response for a given signal-to-noise ratio, at a fixed working point.

- *Limit of Detection* (LOD): the minimum gas concentration that a sensor is able to detect for a given signal-to-noise ratio.

- *Selectivity*: the capability of the sensor to distinguish a given aromatic input from another one belonging to a different class.

- *Drift*: the progressive change of the sensor output signal caused not by an external input but by intrinsic reasons (sensor material, electronics) of the sensor.

- *Stability*: the attitude of the sensor to keep constant in the time its metrological characteristics; in other words, its response during the measurements' time.

- *Repeatabilit*y: the attitude of the sensor output signal to give an equal response to a given fixed input in different repeated measurements (Brattoli *et al.* 2011).

The first types of e-noses tended to be based on an array of gas sensors of the same type, but practical experience showed that often their response did not produce enough information in many real applications. The growing tendency is that of combining different types of gas sensors to produce hybrid systems. However, this involves more complex electronic systems and adds a new step necessary to normalize or standardize the different sensor outputs (Martì *et al.* 2005).

The most widely used class of gas sensors is that of metal-oxide gas sensors (fig. 4).

They were first commercially used in the 1960s in Japan as household gas alarms (Schaller *et al.* 1998). More recent uses include applications in many different industrial processes. Basically, a metal-oxide sensor consists of a ceramic support tube containing a heater spiral, usually made of platinum. The most widely used coating material is tin-dioxide (SnO_2), doped

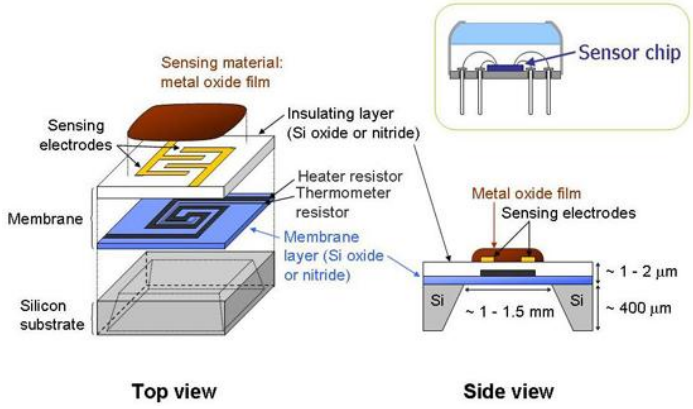


Fig. 4. Example of an MOS technology sensor. The typical dimensions are shown (Simon *et al.* 2001).

with small amounts of catalytic metal additives (also called Taguchi sensors, from the name of its inventor).

Metal-oxide sensors are very high sensitive (sub-ppm levels for some gases) and respond to oxidizing compounds (zinc-oxide, tin-dioxide, titanium-dioxide, iron oxide) and some reducing compounds, mainly nickel-oxide or cobalt-oxide (Mielle 1996). From a chemical point of view, the sensing reaction is based on the oxygen exchange between the volatile gas molecules and the metal coating material. The attraction of electrons to the loaded oxygen results in a decrease of the sensor conductivity (Demarne *et al.* 1992). The sorption of gas molecules produces changes in conductivity.

Metal oxide semiconductor (MOS) sensors consist of three layers: a silicon semiconductor, a silicon oxide insulator and a catalytic metal through which the applied voltage creates an electric field. When polar compounds interact with the metal, the electric field is modulated and recorded by the transistor (Schaller *et al.* 1998). The doping metal (or gate) can be a thick (100-200 nm) or thin (6-20 nm) film. In the first case, the sensor can only respond to dissociated hydrogen. Thus, sensor sensitivity to hydrogen non-releasing molecules such as ammonia or carbon monoxide is very low. A thinner layer of metal on the sensor improves the catalytic activity towards these kinds of molecules (Winquist *et al.* 1985, Åbom *et al.* 2002a, Åbom *et al.* 2002b). These sensors by necessity operate at high temperatures ranging from about 300 °C to 550 °C, since at lower temperatures, the rate of the reactions on the oxide surface is too low. At temperatures below 100 °C, the low vapor pressure of water molecules inhibits the oxidative chemical reactions (Yamazoe *et al.* 1983).

The functioning of gas sensors-based e-noses is affected by several problems, such as sensor poisoning, profile masking by some major constituents of the sample (ethanol for example), strong influence of moisture and non-linearity of signals (Pérez Pavón *et al.* 2006). To

overcome some of these problems, the Mass Spectrometry detection technique has been used for food aroma profiling (Martí *et al.* 2005, Pérez Pavón *et al.* 2006).

MS-based e-noses are referred to as mass sensors or, sometimes, new-generation electronic noses. The volatile compounds are introduced into the ionization chamber of a MS instrument (usually a quadrupole mass spectrometer) without prior chromatographic separation. Each obtained ion fragment (m/z ratio) of the mass spectrum acts as a "sensor" and its abundance is equivalent to the sensor signal. Therefore, the number of sensors in MS-based e-noses is variable, readily modifiable and high in most cases. Moreover, these "sensors" provide chemical information about the sample. It must be mentioned that MS-based e-noses are promising instruments for the analysis of alcoholic beverages because ethanol does not cause saturation problems in the MS, unlikely than in gas sensors. However, the ethanol fragment ions are much more abundant than the other volatile compound fragment. As a consequence, when the chemometric analysis is performed, samples may be differentiated by their ethanol content alone. This problem can easily be solved if the fragment ions corresponding to ethanol are not included in the fragment-ion range selected for the MS analysis (Martí *et al.* 2005). Thus it is possible to say that the alcoholic beverages are one of the examples where MS-based e-noses have shown better performance than the classical gas sensor based e-noses.

However, gas sensors have some advantages such as: larger easiness of use compared to that of complex MS devices;cheap cost and easy maintenance; possibility of easy utilization due to their portability. Therefore a good approach could be to consider gas sensor based e-noses for screening purposes and MS-based sensors for confirmatory purposes.

In fact, more information on the smell or identity of a sample can only be obtained by comparison of the signals of several sensors or receptors. It can be shown that by using sensors with different transducer principles the gain in useful information correlates with the increase of the sensor set, which, in turn, can be further extended (Ulmer *et al.* 1997, Pardo et *al.* 2005).

There is a variety of advantages and disadvantages by using various e-nose sensors depending on their response and recovery times, sensitivities, detection range, operating limitations, physical size, inactivation by certain poisoning agents, and other limitations that are specific to individual sensor types. The types and categories of advantages and limitations associated with individual types of e-nose sensors are closely linked to the nature of the technology that determines the principle for detection and the types of gaseous analytes detectable with each sensor type. A list of some of the major advantages and disadvantages associated with each type of e-nose sensor is reported in table 1.

Finally, it is important to note that even by combining all types of available sensors there are limits to the useful dimensions of the array; indeed, the increase of the array size may amplify the noise instead of provide new information about the gaseous composition.

The best method to arrange a sensor-based electronic nose is not to use as many different sensors as available, but rather to be careful to select the desired application and the knowledge

of the analytical data. This is the only way to be sure that the recorded signals truly corresponds to the substances to be detected.

The ideal all-purpose electronic nose does not exist: however, systems that can be applied to more than one application field are available (Röck *et al.* 2008).

Thus, the unique combination of advantages and disadvantages related to individual sensor types largely determines the range of capabilities and potential applications of each sensor type to the analysis of various gaseous analytes in specific operating situations. Some other important considerations for sensor selection include operational, maintenance and training costs, and easiness of use by the operator.

Tab. 1. Summary of advantages and disadvantages of e-nose sensor types (Wilson & Baietto 2009).

Sensor type	Advantages	Disadvantages
Calorimetric or catalytic bead (CB)	Fast response and recovery time, high specificity for oxidized compounds	High temperature operation, only sensitive to oxygen-containing compounds
Catalytic field-effect sensors (MOSFET)	Small sensor size, inexpensive operating costs	Requires environmental control, baseline drift, low sensitivity to ammonia and carbon dioxide
Conducting polymer Sensors	Ambient temperature operation, sensitive to many VOCs, short response time, diverse sensor coatings, inexpensive, resistance to sensor poisoning	Sensitive to humidity and temperature, sensors can be overloaded by certain analytes, sensor life is limited
Electrochemical sensors (EC)	Ambient temperature operation, low power consumption, very sensitive to diverse VOCs	Bulky size, limited sensitivity to simple or low mol. wt. gases
Metal Oxides Semiconducting (MOS)	Very high sensitivity, limited sensing range, rapid response and recovery times for low mol. wt. compounds (not high)	High temperature operation, high power consumption, sulfur & weak acid poisoning, limited sensor coatings, sensitivity to humidity, poor precision
Optical sensors	Very high sensitivity, capability of identify individual compounds in mixtures, multi-parameter detection capabilities	Complex sensor-array systems, more expensive to operate, low portability due to delicate optics and electrical components
Quartz crystal Microbalance (QMB)	Good precision, diverse range of sensor coatings, high sensitivity	Complex circuitry, poor signal-to-noise ratio, sensitivity to humidity and temperature
Surface Acoustic Wave (SAW)	High sensitivity, good response time, diverse sensor coatings, small dimension, low cost, virtual sensitivity to all gases	Complex circuitry, temperature sensitive, specificity for analyte groups affected by polymeric-film sensor coating

2.1.3. Data processing system

The digital outputs generated by e-nose sensors have to be analyzed and interpreted in order to provide useful information to the operator.

The electronic nose often consists of non-selective sensors that interact with volatile molecules. The physical or chemical changes generate a signal sent to a computer that makes a classification based on a calibration and training process leading to pattern recognition.

Pattern recognition (PR) techniques are used for data processing of responses generated by the sensors of e-nose. The PR methods may be divided into supervised and no supervised methods although a combination of both can be used.

The interpretation of the data sets from e-nose is carried out by use of multivariate statistics, no supervised algorithms, such as principal component analyses (PCA), linear discriminant analysis (LDA), discriminant function analysis (DFA), hierarchical cluster analysis (HCA), soft independent modeling of class analogy (SIMCA) and partial least squares (PLS). Also artificial neural networks (NAA), a supervised method, can be used for modeling the data.

Principal Component Analysis (PCA) is the major used unsupervised technique, while artificial neural network (ANN) is the best-known supervised technique.

The success of PR techniques can be enhanced or simplified by a suitable previous treatment of the data such as feature selection and feature extraction. Persaud and Dodd, 25 years ago, used the value of the steady-state sensor responses for data evaluation. Data obtained by modern e-nose are often so complex that they cannot be manually evaluated. Thus PR analysis must be preceded by a pre-processing of the data (Adams 1995). The main aims of this stage are:

1. The reducting of the amount of data irrelevant to the study.
2. The individualization of the significative data to achieve the desired goal.
3. The extraction of the information in (or transform the data to) a form suitable for further analysis.

Probably the most common method of pre-processing data is their normalization. More complex approaches involve the calculation of a covariance matrix between variables and the extraction of the eigenvectors and eigenvalues or a correlation matrix (Appendix A). Eigen analysis will provide a set of variables, which are linear combinations of the original ones. This has the effect of reducing the dimensionality of the data and making the analysis simpler.

However, in handling large amounts of data, it is important to consider redundancy. As the new techniques increase the dimensions of the data set, the number of theoretical features becomes large, and hence, the selection of the right features becomes a challenge (Hajek *&* Havranek 1978).

Therefore, a lot of work has been recently performed to select the best features (Perera *et al.* 2001, Pardo *et al.* 2007, Gualdron *et al,* 2004, Gualdron *et al.* 2006, Llobet *et al.* 2005, Llobet *et al.* 2007) or even the most appropriate sensors (Gardner *et al.* 2005).

The choice of the method utilized depends on the type of available input data acquired from the sensors and the type of information that is sought. The simplest form of data reduction is graphical analysis useful for comparing samples or aroma identification elements of unknown analytes with those contained in reference libraries. Multivariate data analysis (MDA) comprises a set of techniques for the analysis of data sets with more than one variable. MDA reduces the high dimensionality in a multivariate problem when variables are partly correlated, so that they can be displayed in two or three dimensions. For electronic-nose data analysis, MDA is very useful when sensors have partial-coverage sensitivities to individual compounds present in the sample mixture. Multivariate analysis can be divided into untrained or trained techniques. Untrained techniques are used when a database of known samples has not been previously built. Therefore it is neither necessary nor intended for recognizing the sample itself, but for making comparisons between different unknown samples in order to discriminate them (Shaller *et al.* 1998).

The simplest and most widely used unsupervised MDA technique is Principal Component Analysis (PCA). PCA is more useful when no known sample is available, or when hidden relationships between samples or variables are suspected. On the contrary, trained or supervised learning techniques classify unknown samples on the basis of characteristics of known samples or sets of samples with known properties contained in a reference library that is accessed during the analysis. Briefly, PCA is a linear feature extraction technique, which reduces the dimensionality of data with a minimum loss of information. This is achieved by projecting the data onto fewer dimensions that are chosen to exploit the relationships between the variables, so that the maximum amount of information is retained in the smallest number of dimensions. This technique allows to better assess the similarities and differences between samples.

If we use 20 sensors (one measurement can thus be represented as a point in a 20-dimensional space) for our measurements, some of them probably will respond in a similar (but not identical way). This means that the number of dimensions in the data set can be reduced without any loss of information. If we use, for example, only three sensors, the co-variance between the sensors can be represented in a three-dimensional graph (one sensor for each axis). Let us suppose to observe the data spreading out along a line, as shown in figure 5.

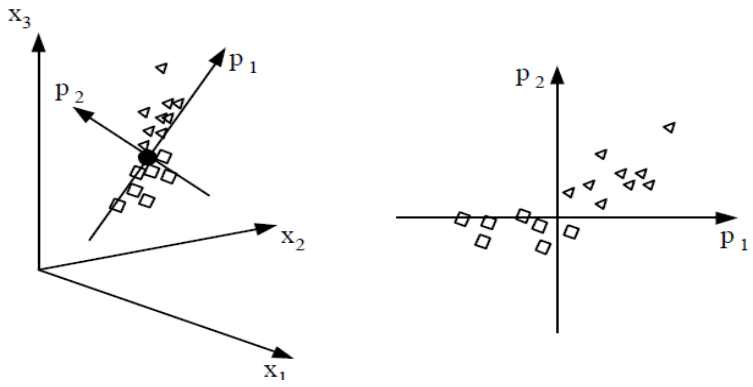


Fig. 5. The low-dimensional projection of the data can be used as a simple, but good, approximation of the data set. The direction with most of the variance in the data set is the first principal component (P1) (Davide *et al.* 2001).

If we project all data on the line drawn in the figure, the three-dimensional problem can be reduced to two dimensions with a very little loss of information. The projection corresponds to the first principal component in a PCA, defined as the direction along which the data have the highest variance. The second principal component, containing most of the remaining variance, is directed in a direction orthogonal to the first component. In the case of several sensors, the procedure can be repeated up to a total number of principal components equal to the number of sensors. Most variation in the sensor signals will be detected in the first few principal components that can be conveniently used for visualizing the data. If we plot the first principal component as a function of the second one, we will be able to study variations in the data set. This type of plot is usually called a principal component analysis score plot (PCA score plot), and if desired can also be made by using other principal components. A loading plot of a PCA shows to what extent the different sensors contribute to the principal components. In this plot, sensors with similar contributions (i.e. containing similar information) will be close together. Sensors that are close to the origin have comparably small variance, and therefore probably contain little information (see figure 6).

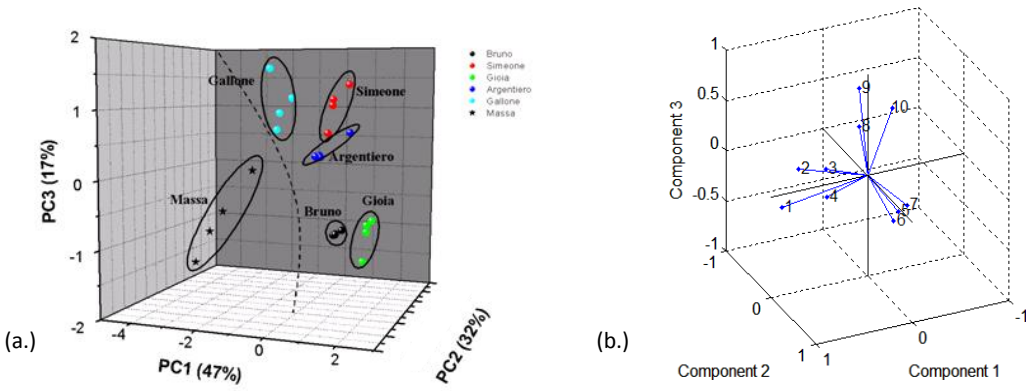


Fig. 6. (a.) Principal Component Analysis (PCA) score plot; (b.) PCA loading plot.

One of the most popular supervised methods to handle electronic nose data is the artificial neural network (ANN), which bears a certain resemblance to the function of the human brain. In principle, an ANN is constituted of many (in the order of 50-100) artificial neurons. The artificial neurons are organized in different layers (see figure 7), often three, forming a network. An artificial neuron is a simple processing element that, similarly to biological neurons, uses signals from several inputs to produce one output.

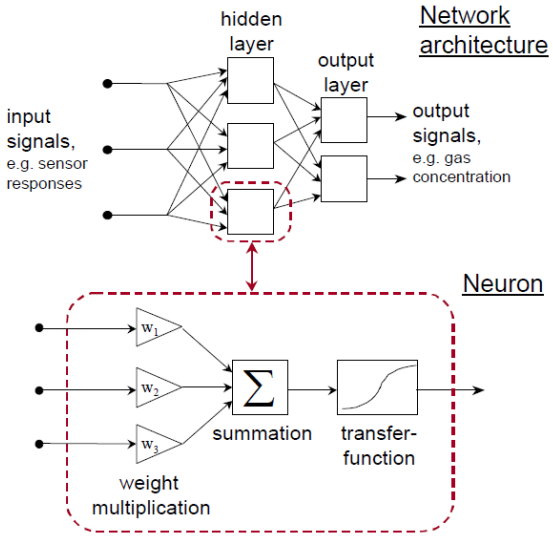


Fig. 7. Schematic network architecture applied on electronic olfactory system sensor responses (Davide *et al.* 2001).

A linear combination of all the inputs is taken to give a single value. This value is then used in a transfer function, which could have an arbitrary shape. One example is the step function, which, like the biological neuron, gives a non-zero value out when the calculated value from the linear combination is above a certain threshold, and zero otherwise. More common, however, is the use of a smooth function, e.g. a sigmoid. The learning in an ANN is performed by changing the parameters in the linear combination, and possibly even the shape of the sigmoid. By feeding data from known odors into the network, the parameters can be adapted to recognize the sensor signals from these odors. In order to adjust the parameters, the training data has to be used many times. This is very similar to the humans training for odor recognition. Once being exposed to an odor , we seldom remember it very well, while we are able to recognize odors we have experienced in youth even after very long time. It is important to note that an ANN, just like the human nose, can not identify odors never experienced before. In this case the ANN can only say or it does not recognize the odor. When confronted with the sensor signals from a new odor, the ANN can only say which of the known odors the signal are most similar to, or (even better) that it does not recognize the odor. A human can easily say if an unknown odor is pleasant or not, while an electronic nose cannot make any subjective judgment of that type (Davide *et al.* 2001).

2.2. Commercial devices

Nowadays, many electronic noses are commercially available with a wide range of applications in various markets and industries ranging from food processing, industrial manufacturing, quality control, environmental protection, security, safety and military applications to various pharmaceutical, medical, microbiological and diagnostic uses.

Some of the most widely used electronic noses including manufacturers, models available and technological basis are listed in table 2.

The list includes instruments with single-technology sensor arrays and instruments with a combined-technology of e-noses working in tandem with classical analytical systems. The additional need to identify individual chemical species or components within sample mixtures has prompted the generation of new instruments whose borders between electronic noses and conventional analytical instruments are confused. These new hybrid devices are not considered e-noses in the strictest sense because they do not provide a collective data output from a sensor array and are designed to detect and identify individual components of a gaseous mixture.

The new trends in the odor detection are strongly driven by nanotechnologies and nanomaterials (Cheng *et al.* 2009, Penza *et al.* 2010, Fryxell *et al.* 2007, Patolsky & Lieber 2005). Nanotechnology has recently attracted a lot of attention, particularly in the research and industrial communities. The ability to design, synthesize and manipulate specific materials at nanoscale level lies at the very heart of the future promises of nanotechnology (nanotubes, nanowires, nanocrystals, nanoparticles, etc.).

Several studies concerning the use of nanomaterials as gas sensor have been reported in literature. Penza *et al.* (2010) studied an array of four sensors based on carbon nanotube layers functionalized with metal catalysts for landfill gas monitoring applications. Patoslky and Lieber (2005) developed an individual silicon-nanowire to implement a field effect transistor (FET) functionalized with DNA and proteins for detection of biological and chemical species in the area of healthcare and life sciences. This device may be called a nanosensor. However, the nanosensors based on individual nanowires have been integrated by Cheng *et al.* (2009) in an array of multiple sensing elements to implement a nanoelectronic nose based on hybrid nanowire/nanotubes and micromachining technology for sensitive gas discrimination.

This nanoelectronic nose has a great potential for detecting and discriminating a wide variety of gases, including explosives and nerve agents.

Tab. 2. Some commercially electronic noses, models and technologies (Wilson & Baietto 2009).

Instrument type	Manufacturer	Models produced	Technology basis
Single-technology (e-nose sensors only)	Air sense Analytics	i-Pen, PEN2, PEN3	MOS sensors
	Alpha MOS	FOX 2000, 3000, 4000	MOS sensors
	Applied Sensor	Air quality module	MOS sensors
	Chemsensing	ChemSensing Sensor array	Colorimetric optical
	CogniScent Inc.	ScenTrak	Dye polymer sensors
	CSIRO	Cybernose	Receptor-based array
	Dr. Födisch AG	OMD 98, 1.10	MOS sensors
	Forschungszentrum Karlsruhe	SAGAS	SAW sensors
	Gerstel GmbH Co.	QSC	MOS sensors
	GSG Mess- und Analysengeräte	MOSES II	Modular gas sensors
	Illumina Inc.	oNose	Fluorescence optical
	Microsensor	Hazmatcad, Fuel Sniffer,	SAW sensors
	Systems Inc.	SAW MiniCAD mk II	
	Osmetech Plc	Aromascan A32S	Conducting polymers
	Sacmi	EOS ^{835} , Ambiente	Gas sensor array
	Scensive Technol.	Bloodhound ST214	Conducting polymers
	Smiths Group plc	Cyranose 320	Carbon black-polymers
	Sysca AG	Artinose	MOS sensors
	Technobiochip	LibraNose 2.1	QMB sensors
Combined-technology (e-nose + other types)	Airsense Analytics	GDA 2	MOS, EC, IMS, PID
	Alpha MOS	RQ Box, Prometheus	MOS, EC, PID, MS
	Electronic Sensor Technology	ZNose 4200, 4300, 7100	SAW, GC
	Microsensor Syst.	Hazmatcad Plus CW Sentry 3G	SAW, EC SAW, EC
	Rae Systems	Area RAE monitor IAQRAE	CB, O2, EC, PID Thermistor, EC, PID, CO2, humidity
	RST Rostock	FF2, GFD1	MOS, QMB, SAW

2.3. Electronic Nose applications in food analysis

In the past two decades, the applicability of electronic noses has been tested in every imaginable field where odors or odorless volatiles and gases are thought to play a role (Ampuero & Bosset 2003, Strike *et al.* 1999, Gardner & Bartlett 1994, Casalinuovo *et al.* 2006). Today, electronic noses are used in military, security, and safety and medical applications, food processing, and pharmaceutical industry. The electronic nose is also used in quality assurance-quality control (QA-QC)

Process control is really a promising application field. Independently on the character of the product, it is important to ensure that it has always the same characteristics. Therefore, e-nose application area ranges from control of industrial production lines as in the pharmaceutical industry and in the manufacture of food packaging, to the control of composting processes. Besides the control of temperature, humidity, optical appearance, viscosity, etc., the electronic nose adds another dimension in the observation and can help to minimize the variability between different batches.

Recently, many efforts have been made in the field of foodstuff and beverages with particular concerns for the study of time-dependent processes (Casalinuovo *et al.* 2006, Capone *et al.* 2005, Deisingh *et al.* 2004). These include unwanted processes such as changes during storage or spoilage, as well as the ripening or fermentation of particular products.

A very promising application field for the electronic nose is its use in spoilage detection of foodstuffs. The fight against the autolysis and growth of microorganisms is the main objective for food preservation that and can be pursued in different ways. The most popular approaches are pasteurization, refrigeration, removal of water, change in pH, the use of packaging under vacuum, the use of food additives, or a combination of them. In all cases, food deterioration cannot be prevented but only postponed. Therefore, the challenge is to detect spoilage at earlier stages or, alternatively, to predict it (Dainty 1996). The field is quite complex as both the nature and origin of the foodstuff and the preservation technique employed influence the species of bacteria, fungi, or enzymes responsible for spoilage. Due to the variety of different substances that can be produced during spoilage, the biologically evolved human perception is still the best detection method for most applications of off-odor and off-taste detection. To use an instrumental analysis, one has to be aware of the substances relevant for each sample type, but despite our knowledge of the formation of free radicals, influence of enzymes, different bacteria produced, yeast and mold strains, and their metabolism products, the experience in their detection by an electronic noses is still at the beginning.

The critical point is the generalization and, closely connected to it, the question of the usability of the electronic nose results without a previous thorough exploration of all applications' variables (different samples, different batches, long-term behavior, etc). Because

foodstuff is very heterogeneous, there is no warranty that the results will be reproducible for a sample set with the varying of an unconsidered parameter.

In addition to the assessment of food, the human nose gives us further important information. For example, it warns us about dangers such as fire, air pollutants, and so on. For this reason, electronic noses are being investigated in the security field for the detection of hazardous substances and explosives.

The question from the electronic nose point of view is which additional information can be obtained through its use and in which fields it can replace the established techniques. To get a feeling on what is feasible, one has to acquire knowledge about the substances detected by the human nose, classical analytical detectors and the electronic noses. For this reason, gas chromatography experiments are very helpful because they reduce the problem of a whole bouquet to single substances. In aroma and odor analysis GC-olfactometry (GC-O) helps to identify which volatiles are responsible for the respective odor impression. Direct comparison of GC-O results with GC/FID or GC/MS results gives information about which marker substances are detectable without a sensory test panel and which are reliably detected only from the human nose (fig. 8). This knowledgment is important because measurements on foodstuff such as daedal peel oil (Song *et al.* 2000), green Mexican coffee (Cantergiani *et al.* 2001), grapefruit oil (Lin *et al.* 2001), cooked asparagus (Ulrich *et al.* 2001), cashew apple nectar (Valim *et al.* 2003), or Croatian Rhine Riesling wine (Komes *et al.* 2006) have shown that sometimes there is a big discrepancy between the substances detectable by the human nose and those detected with commercial detectors and vice versa.

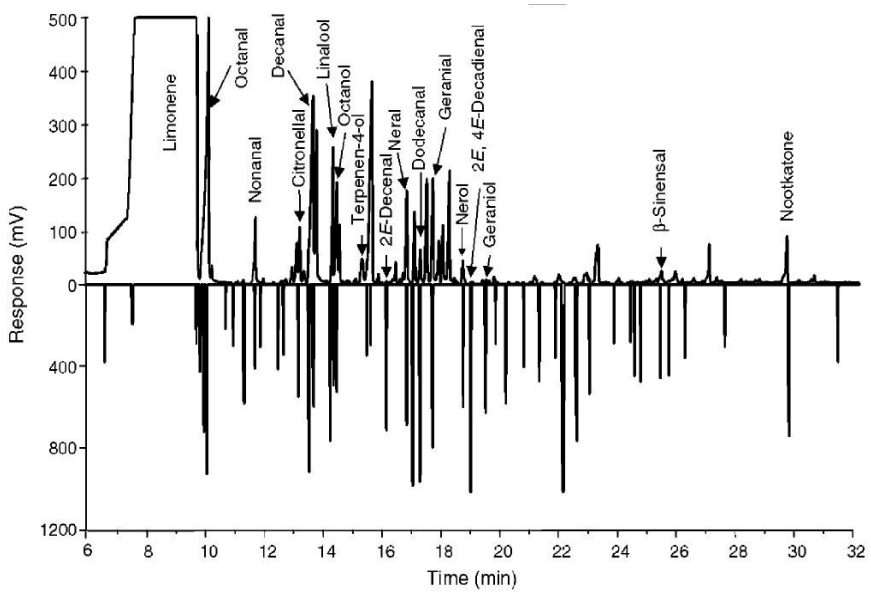


Fig. 8. Comparison of a GC/FID chromatogram (top) with a time-intensity aromagram (GCO) of grapefruit oil. It is obvious that the human nose is sensitive to substances the flame ionization detector is not able to detect and vice versa (Röck et al. 2008).

A search through the recent and relevant literature shows that there are five major categories of use for electronic noses in food control. These are (i) process monitoring, (ii) shelf-life investigation, (iii) freshness evaluation, (iv) authenticity assessment and (v) other quality control studies.

- ***Process monitoring***

Several successful applications of the electronic noses to the monitoring of flavor and/or aroma components along a food production process have been published.

García *et al.* (2005) have made use of an e-nose to identify spoiled Iberian hams during the curing process. The sensors used were tin-oxide semiconductor thin films, some of which were doped with metal catalysts such as Cr and In. A good discrimination (success rate of 100%) between two types of Iberian hams (spoiled and unspoiled) was obtained through the statistical methods of PCA and probabilistic ANN (PNN).

Pani *et al.* (2008) used a MOS based e-nose (AirSense, Alpha MOS's technology) for monitoring the changes in aroma profile of tomato slices during air dehydration processes. Two kinds of samples (untreated and osmodehydrated in corn syrup) were studied. E-nose data analysis by means of PCA was able to characterize the process aromatic fingerprint, which could be helpful to understand and parameterize the degradative events caused by dehydration.

Lebrun *et al.* (2008) undertakook a study to discriminate between mango fruit maturity by volatiles using a Fox 4000 e-nose (Alpha MOS's technology) (with 18 metallic oxide sensors) and Gas Chromatography (GC). Three different mango fruit varieties (*Mangifera indica L.*) were harvested at different maturities and with different sizes. Immediately after harvest (green) or after 1 week of ripening at room temperature (ripe), fruit were homogenized or left intact and evaluated by the e-nose or by GC for aroma and other volatiles as well as for soluble solids and acids. Volatile data from the different harvest maturities and ripening stages were discriminated by using discriminant factor analysis (DFA). Both the e-nose and GC were able, in most cases, to separate fruit from different harvest maturities (at both the green and ripe stages) as well as to discriminate green from ripe fruit, and fruit from the different varieties within a maturity stage. Solids and acids data indicated that later harvest maturities resulted in sweeter fruit and later-harvested fruit had a different volatile profile from earlier harvested fruit. These results demonstrate the benefit that could be obtained from the development of a hand-held e-nose device capable of determining the optimal harvest maturity for mangoes on the tree by the volatiles emitted, or an e-nose device that could be used as a screening tool on fruit after harvest.

During black tea manufacturing, tealeaves pass through a fermentation in which the grassy smell is transformed into a floral smell. The optimal fermentation is extremely crucial in deciding the final quality of finished tea and it is very important to terminate the fermentation

process at the right time. Bhattacharya *et al.* (2008) presented a study on real-time smell monitoring of black tea during the fermentation process using an e-nose (Figaro's, Japan, sensors: TGS-832, TGS-823, TGS-831, TGS-816, TGS-2600,TGS-2610, TGS-2611 and TGS-2620) as well as prediction of the correct fermentation time. Different time-delay neural networks (TDNNs) and self-organizing map (SOM) methods for the prediction of optimum fermentation were used and both the methods appeared to be suitable for the purpose. According to the authors, the results showed excellent promise for the instrument to be used by the industry.

A French Alpha MOS e-nose and headspace volatile analysis using SPME were used to group 76 commercial and 120 self-prepared citrus juices according to fruit type, cultivar and treatment using LDA. In one case, a commercial orange juice grouped close to grapefruit and two declared grapefruit juices ended up being miss-assigned to grapefruit and were in fact pummelo, exposing wrong or misleading supplier information and human error (Reinhard *et al.* 2008).

- ***Shelf-life investigation***

Many e-nose applications in the literature are devoted to monitor the ripening process of fruits and other vegetables during their shelf-life period (from harvest until consumption) (Brezmes *et al.* 2001, Herrmann *et al.* 2002, Berna *et al.* 2004, Saevels *et al.* 2004, Hernández-Gómez *et al.* 2007, Benedetti *et al.* 2008). Monitoring and controlling ripeness is a very important issue in fruit and vegetables management since it is a very important quality indicator for consumers. Many methods to monitor fruit ripeness have already been proposed but they are not useful for packinghouses and most of them require the destruction of samples used for analysis. Therefore, predictions of shelf-life ripeness state are mainly based on practical experience. Leaving these critical decisions to subjective interpretation implies that large quantities of fruit and vegetables reach consumers markers in poor condition. In this framework, e-noses have proved to be promising tools for fruit ripeness assessment.

Brezmes *et al.* (2001) used an e-nose, consisting of 21 MOS sensors array, to assess the ripeness state of 'Pinklady' apples through their shelf life. In order to evaluate the e-nose performance, fruit quality indicators, such as firmness, starch index and acidity, were also obtained, and results from both techniques were compared. Pinklady apples were harvested at their optimal date so that e-nose and fruit quality measurements could be performed on the fruit samples during their ripening process. A PCA analysis did not show any clustering behavior that might be attributed to ripening. On the other hand, fuzzy art, an unsupervised ANN classification algorithm, showed a tendency to classify measurements regarding to their self-life period. Finally, good correlation coefficients were obtained between e-nose signals and classical fruit quality parameters (firmness and acidity) by means of PLS modeling, thus indicating that e-nose signals were related to the ripening process of apples.

In the paper by Herrmann *et al.* (2002), an e-nose based on arrays of differently coated QMB was used to discriminate between VOCs formed during the post-harvest ripening of apples. The compounds monitored were aldehydes and esters. The relative ratios of these compounds change during post-harvest ripening and this allows them to be analyzed by PR methods. This is due to the formation of characteristic patterns of sensor responses. During the ripening of apples, trans-2-*hexenal* can serve as an indicator compound because its concentration increases significantly. It was found that the detection limit of trans-2-*hexenal* was 20 ml/m^3. Both qualitative (type of apple) and quantitative identification were possible. However, the correlation between the vapor concentration and sensor response was linear within a limited concentration range. It was found that the shape of the curve is similar to the Langmuir adsorption model, thereby limiting the range, which could be used.

The performance of LibraNose (a QMB coated by modified metallo-porphyrins and related compounds based e-nose) and a MS-based e-nose for tomato aroma profiling was evaluated by Berna *et al.* (2004). SPME headspace sampling combined with GC was used as a reference method. In the first experiment, the changes in tomato aroma profiles of two different cultivars were monitored during the shelf life (days 1, 8, 12 and 19). The score plot of PCA for the e-nose measurements showed a slight shift along the first principal component corresponding to an increasing number of days of shelf life. However, the tomato aroma profiles measured on days 1 and 8 could not be discriminated by the e-nose. In contrast, MS nose score plots indicated an evident change in aroma profile with the shelf life.

The potential of a LibraNose e-nose and a MS-based e-nose to monitor changes in apple fruit volatiles during shelf life has been studied by Saevels *et al.* (2004). These techniques were compared with a traditional technique to measure volatiles, GC–MS. Apples were stored for 8 months under three different storage conditions and the volatile profile changes were followed subsequently over a period of 15 days. Analysis of the PCA score plot for the e-nose measurements showed no storage history effect and only very little shelf-life effect. In contrast, the MSE-nose and GC–MS score plots clearly indicated the presence of both shelf life and storage history trend. Moreover, the volatile profile changed during shelf life and it was depending on the storage history. The loading plots of the PCA of the GC–MS data revealed which volatiles are important to differentiate between storage conditions and which ones are important during ripening on the shelf. PLS models based on the three data sets to relate firmness and days of shelf life with the volatile production of apples were built based on the three data sets. It was found that the models based on the e-nose data had worse prediction performance than those based on the MSE-nose data.

The possibility of exploiting information on the aroma behavior to assess fruit ripening stage has been the cornerstone of the work of Hernández-Gómez *et al.* (2008). The objective of this study was to evaluate the capacity of e-noses to monitor the change in volatile production of ripeness states for tomato, using a specific e-nose device with 10 different metal oxide sensors

(portable e-nose, PEN 2). PCA and linear discriminant analysis (LDA) were used to investigate whether the e-nose was able to distinguish among different ripeness states (unripe, half-ripe, full-ripe and over-ripe). The loadings analysis was used to identify the sensors responsible for discrimination in the current pattern file. The results proved that the PEN 2 e-nose could differentiate among the ripeness states of tomato. The electronic nose was able to detect a clearer difference in volatile profile of tomato when using LDA analysis than when using PCA analysis. Using LDA analysis, it was possible to differentiate and to classify the different tomato maturity states, and the method was able to classify 100% of the total samples in each respective group.

Benedetti *et al.* (2008) evaluated the capability of a PEN 2 e-nose to classify four *Prunus persica L.* cultivars and to assess their ripening stage during shelf life. Firstly, PCA and LDA were carried out using the e-nose sensors responses on peach fruits at the first day after the harvest, and both analytical methods were able to distinguish the cultivars. Secondly, PCA applied on the e-nose data (collected during shelf-life from harvest until senescence) revealed that only one sensor (W5S) was relevant to differentiate peaches during ripeness on the basis of their shelf life, so allowing to classify them as unripe, ripe and over-ripe. The performance of the e-nose was compared with the results of classical and non-destructive techniques such as ethylene measurement and color evaluation, frequently used to assess the ripening stage of climacteric fruit.

In addition to the evaluation of fruit and vegetables ripeness states, other e-noses applications to shelf-life investigation have been performed in cheese, milk and oil samples (Benedetti *et al.* 2005, Riva & Mannino 2005, Labreche *et al.* 2005, Cosio *et al.* 2007, Mildner-Szkudlarz *et al.* 2008).

- ***Freshness evaluation***

Freshness is another important quality factor in the food industry. Since a number of different volatile compounds are generated during storage of foods, the electronic noses have shown their potential in detecting freshness or spoilage of different food raw material and products. Electronic noses appear particularly powerful when applied to foods where significant release of volatiles occurs during storage due to rapid degradation by bacterial processes, such as fish (Di Natale *et al*. 2001, Du *et al.* 2002, Olafsdottir *et al.* 2005, Chantarachoti *et al.* 2006, Korel *et al.* 2001), shrimps (Luzuriaga *et al.* 2007), eggs (Dutta *et al.* 2003) and meats (McElyea *et al.* 2003, El Barbri *et al.* 2008).

Most freshness investigations with electronic noses have involved studies with fish and fish products. In one of these, Di Natale et al. (2001) used the measurements of two e-noses, based on different sensor technologies and sampling methodologies, to detect freshness of codfish fillets. One of the e-noses consisted of an array of eight thickness shear mode resonators

coated with various kinds of metaloporphyirns (LibraNose), while the other one (FreshSense) was based on five electrochemical sensors each oriented towards a certain gas (CO, H_2S, NO, SO_2 and NH_3). E-noses data were analyzed by means of partial least square-discriminant analysis (PLS-DA). Over a period of 17 storage days, the two sensor systems showed different resolution, while the integration of both e-noses improved the performances allowing an almost complete evaluation of the freshness of samples. In comparison, the evaluation with conventional techniques (flow injection analysis-gas diffusion method) of Trimethylamine and total volatile basic nitrogen, showed a non-monotonic behavior, thus inducing the possibility of large errors in freshness estimation.

An e-nose with 12 CPs sensors was used to measure odors of raw shrimp treated with different chemicals (Luzuriaga *et al.* 2007). Headless shell-on pink shrimp (*Pandalus jordani*) were treated with different amounts of bleach, phosphates and sulfites and stored at 2°C for 48 h. Odors were evaluated by sensory panels and the e-nose; moreover aerobic plate counts were performed. DFA results showed that the e-nose could discriminate differences in odor due to chemicals present in shrimp. The correct classification rates for bleach, phosphate and sulfite treated shrimp were 92.7%, 95.8%, and 99.2%, respectively.

Mc Elyea *et al.* (2003) used a FOX 3000 e-nose to determine changes in lipid oxidation and microbial load of ground beef throughout simulated retail display. Aerobic, vacuum and CO_2 mixing treatments were used to determine their impact on e-nose responses, lipid oxidation and microbial characteristics. After grinding and mixing, ground beef was stored under simulated retail display, and analyzed at days 0, 1, 2, 3, 6, and 10. Analyses included thiobarbituric acid reactive substances (TBARS, a widely used procedure to estimate lipid oxidation of meat), aerobic plate count (APC) and e-nose characteristics. The e-nose detected changes in ground beef lipid and microbial stability as did conventional TBARS and APC measures. Therefore, the e-nose may hold promise for a rapid detection of meat freshness and safety.

El Barbri *et al.* (2008) used an e-nose system containing an array of 6 tin-oxide gas sensors for the quality control of red meat. E-nose and bacteriological measurements were performed to analyze samples of beef and sheep meat stored at 4°C for up to 15 days. First, PCA and Support Vector Machine (SVM) based classification techniques were used to investigate the performance of the e-nose system in the spoilage classification of red meats. PCA (a linear technique) could be used for spoilage classification of beef meat but not in the case of sheep meat. A very good success rates in the classification of spoiled or unspoiled beef and sheep meats (98.81% and 96.43%, respectively) were obtained when SVM (non-linear) were employed. On the other hand, PLS regression models showed good correlation coefficients between the e-nose signals and bacteriological content for beef and sheep meats. According to these results, an e-nose system can become a simple, fast and non-destructive alternative tool to bacterial analysis for shelf-life determination (i.e. quality assessment) and spoilage classification (safety assessment) of red meats.

- ***Authenticity assessment***

There is no doubt that, in terms of identification, alcoholic beverages provide the best-known example of electronic noses use.

In a study with the French Alpha MOS e-nose, not-from-concentrate (NFC) orange juice was separated from frozen concentrated orange juice (Goodner *et al.* 2002).

A study using Alpha MOS e-nose with 18 sensors, shown the ability of this device to separate orange juice from fresh squeezed oranges, orange juice from a simulated commercial process (including pasteurization), orange juice from fruit harvested from healthy trees and the same commercially processed juice made from fruit harvested from Huanglonbing (HLB) infected trees and fresh squeezed tangerine juice (fig. 9). Using PCA, the e-nose was able to separate all these juices, even the juice from HLB-infected trees, which were shown to have fruit with off-aroma and a bitter-metallic flavor (Baldwin *et al.* 2011, Plotto *et al.* 2010).

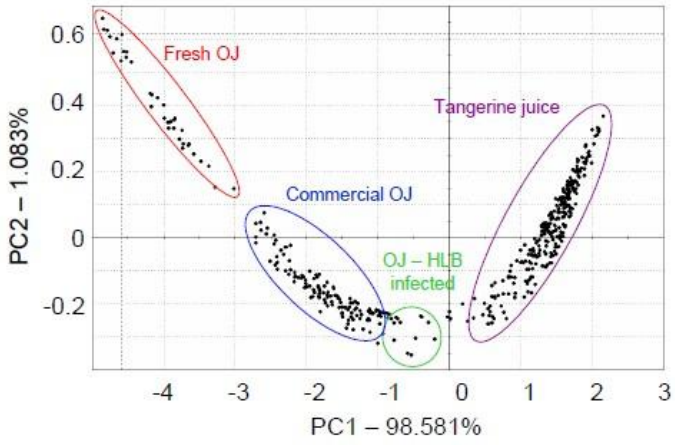


Fig. 9. PCA plot of citrus juices based on the electronic nose signals. The observations are grouped by juice type, fresh squeezed orange juice (OJ) with high peel oil, processed OJ, processed OJ from Huanglongbing (HLB) infected fruit, and fresh squeezed tangerine juice (Baldwin *et al.* 2011).

Successful applications to differentiation of wines on the basis of geographical origin and grape variety have been reported in the literature, as well as for the recognition of adulterations (Ragazzo *et al.* 2001, Penza & Cassano 2004, Lozano *et al*. 2007, Aleixandre *et al.* 2008, Lozano *et al.* 2006, Martí *et al.* 2004).

Penza and Cassano (2004) fabricated a set of 4 thin-film (WO_3) metal oxide sensors, surface-activated by Pt, Au, Pd, Bi metal catalysts, for the recognition of some adulterated Italian wines (two white, four red, two rosè from different denominations of origin and vintages) added with methanol, ethanol or another same-color primary wine. Multivariate analysis including PCA and BP-ANNs (Back-Propagation Artificial Neural Networks) were used to identify both the

adulteration of wines and to determine the added content of adulterant agent of methanol or ethanol up to 10% volume. The cross-validated ANNs provided the highest achieved percentage of correct classification of 93% and the highest achieved correlation coefficient of 0.997 and 0.921 for predicted vs. true concentration of methanol and ethanol adulterant agent, respectively.

Moreover, other foods were analyzed for identification purposes, include olive oil, cheese, honey, vegetable oil, fruit juices and vinegars (Reid *et al.* 2006). For example, authenticity studies with e-noses have been successfully carried out for the determination of the geographical origins of Valencia orange juices (Steine *et al.* 2001) and Emmental cheese (Pillonel *et al.* 2003), for the differentiation of unifloral honey samples (Ampuero *et al.* 2004) as well as for the discrimination of 'Aceto Balsamico Tradizionale di Modena' on age basis (Cocchi *et al.* 2007).

3. Aim of work

Several studies have demonstrated that the aroma emanating from fruits can indicate the maturity level and thus the quality and shelf life of the marketed product.

The volatile composition of fruits is extremely complex and the Gas Chromatography coupled to Mass Spectrometer detector is the major analytical technique used today to separate and identify, one by one, these volatiles. Many volatile components are flavor active, but the most difficult problem is to understand which combination of components and in what proportion are responsible for the perceived aroma. Sensory scientists studying this topic have become increasingly inclined to characterize flavors using trained human judges.

Because the analytical technique and the sensorial analysis are very time consuming to monitor fruit freshness and shelf life product, it could be advantageous to utilize e-noses to maximize corporate profits and optimize customer satisfaction, before and during the marketing.

In this research several experiments were carried out in three different countries (Italy, Argentina and Spain) with the aim to studying the application of different Electronic Olfactory Systems (EOSs) to evaluate the aroma of some fruits as quality parameter.

Three different EOSs, one made in Italy, one in Argentina and one in Spain, were used. These EOSs, with different sampling and sensors technology, detected the aroma of fresh fruit, fresh squeezed fruit juice and minimally transformed fruit, named "IV Gamma" or "ready-to-eat products".

Becouse different fruit cultivars can deliver very different flavor characters, the characterization and discrimination among differences of flavor volatile components has been conducted by using traditional analytical techniques, like gas chromatography technique, sensorial analysis, and EOSs.

We also used the EOS on the ready-to-eat products to evaluate, in a rapid way, the aroma's changes during the shelf life period.

4. Experiments in Italy *(EOS^{835}, Sacmi)*

A commercial Electronic Nose, *EOS*^{835} (Sacmi, Imola, Italy) available at the **Institute of Biophysics** (IBF), National Research Council (CNR), of Palermo (Italy) has been used for the experiments carried out in Italy. This device was used to discriminate the aroma of some fruits grown in Sicily. *EOS*^{835} was also used for monitoring and control of fresh-cut fruits (IV Gamma). In particular the experiments carried out in Italy were the following:

- **Discrimination of fruit aroma of 5 *Malus comunis* cultivars by electronic nose.**

- **Shelf life monitoring by electronic nose, chemical, physical and sensorial analysis, of ready-to-eat fresh-cut apples (cv. Fuji)** (samples were packaged with air and with nitrogen); the study was carried out in collaboration with Department Pr.I.M.E. University of Foggia (Italy).

- **Use of the electronic nose to discriminate 3 *Eriobotrya Japonica* Lindl. cultivars**; in this case Gas Chromatography analysis was also applied and results obtained by both techniques were compared.

- **Fruit quality evaluation of 4 loquat (*Eriabotrya japonica* Lindl.) cultivars grown in Sicily (Italy);** in this study the e-nose's results were compared with Sensory Analysis results obtained using Panel Test. The research was carried out in collaboration with the Department DOFATA, University of Catania (Italy).

- **Preliminary study on quality and healthy characteristic of 4 mango (*Mangifera indica* L.) cultivars grown in Sicily**; in this case the determination of classic parameters of quality and nutritional components (such as antioxidants with health benefits) was carried out through classical chemical analysis. This study was carried out in collaboration with the CRA-ACM, Consiglio per la Ricerca e la Sperimentazione in Agricoltura – Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Acireale (CT), (Italy).

At the beginning of XX century several studies were made by Italian University research centers operating in the innovative materials field; thanks to these studies Sacmi, an Italian company, identified a series of advanced technologies with the potential for industrial-scale development.

One of the major themes of Sacmi Company was the manufacturing technology to make metal oxide semi-conductor (MOS) gas sensors. This technology was developed by the Gas Sensors Lab of the Material Engineering Chemistry-Physics Department at the University of

Brescia, by the Prof. Sberveglieri's group. Thus a transfer of technology began from research center to company.

From this collaboration derived the belief that complex industrial problems (e.g. foodstuff quality control through odor monitoring) can be solved only through a total control of the measuring system, in this case by the sensors. This means be able to select and modify the sensors characteristics by acting on the materials they are made, the semi-conductor oxide (MOS) films. Equally important from an industrial reliability viewpoint is the need to ensure consistent, repeatable sensor response over time. So the *EOS*^{835} was built: an electronic olfactory systems made by Sacmi that can in-line sample and monitor odors , acquire and quickly process data by using a new software capable of analyzing complex situations and singling out the quality determinant factors.

Some papers in literature (report below)have been performed using this type of electronic olfactory system, *EOS*^{835}, for detecting the food aroma.

One of the first works was for the determination of the ripening level of a roasted coffee blend inside the production chain of an Italian company (Falasconi *et al.* 2005).

Bianchi *et al.* (2009) tested *EOS*^{835} capability to distinguish pure and microbiologically contaminated canned tomatoes.

Concina *et al.* (2009) investigated *EOS*^{835} ability to perform an early diagnosis of microbial contamination of canned peeled tomatoes with the aim to design an analytical protocol for an objective quality control at the end of the production chain. Processed tomatoes are a food category extremely exposed to safety risks related to the presence of both chemical residuals, like pesticides and herbicides, and microbial contaminants such as bacteria and fungi. Therefore, the improvement of tomato quality is of prior importance for customer's safety. To assure the commercial saleability of the end product, the presence of microbial contaminants in canned tomatoes is usually controlled through a stability test. It consists in incubating the cans at 30 °C for 2 weeks and at 55 °C for 1 week in order to favor, if present, the microbial growth; the possible consequent package swelling is used as indicator of the microbial presence. However such test is not completely reliable because not always the absence of swelling implies absence of spoilage; for this reason it is necessary to control product's pH value and sensorial parameters in order to ascertain the absence of non-gas producer microorganisms. These procedures force to a noteworthy delay between the end of production and the actual trading, with consequent economic losses and logistic problems for the producers. This motivates producer companies in demanding for tools that allow an early screening of microbial contamination, and provide a quick response in few hours, as an electronic nose.

EOS^{835} was also used by Concina *et al.* (2010), as a diagnostic tool of the presence of *Alicyclobacillus spp.* (ACB) in some commercial soft drinks. Contamination by ACB was firstly observed in apple juice (1982 in Germany), but since then a larger variety of fruit juices as well as herbal drinks and vegetable-derived products, such as canned tomatoes, have been involved. Soil

is considered to be the main source of *A. acidocterrestris* spores and it is believed that fruits in contact with the soil during harvest become contaminated. Fruit contamination is not the unique source of contamination of final products: beverage contamination can also result from waters and raw material contaminated used during the production of fruit beverages. It is manifested by off-flavors, due mainly to the production of 2-methoxyphenol (guaiacol), 2,6-bromophenol , 2,6-chlorophenol, and a light sediment. Recently, some strategies have emerged devoted to inhibit the ACB metabolism, thus preventing the contamination appears a goal not yet accomplishable. Among the strategies used, the application of high hydrostatic pressure during the pasteurization step, the addition of preservatives such as sodium benzoate or potassium sorbate and nisin, the use of some plant extracts, such as rosemary, the use of active packaging, such as Ag-containing polymers seem to be promising at laboratory level, but no actual solution has been yet envisaged at industrial scale. Therefore producer companies are demanding tools capable of providing a more rapid and simple screening, and a yes/no response on contamination that could help to identify and isolate spoiled lots. Being based on unspecific chemical interaction between the sample volatile compounds and the sensor surfaces, the EN present some limits, such as low sensitivity and selectivity, and inability to identify specific compounds. Still EN can be very useful when the interest is addressed to a yes/no response rather than a specific analysis, as for a producer company looking for line monitoring tools. This study demonstrates the possibility of exploiting an electronic olfactory system to identify the presence of *Alicyclobacillus spp.* (ACB) at very low levels in selected soft drinks. Tests have been performed on naturally contaminated matrices, ready for the market, without any previous treatment. The *EOS*^{835} system demonstrated the ability to perform an excellent identification of contaminated samples, providing almost 100% of correct classification rate. These results open the way to the possibility of performing an early diagnosis of ACB.

Gobbi *et al.* (2010) used *EOS*^{835} for the detection of *A. acidoterris* and *A. acidocaldarius* artificially inoculated in peach, orange and apple fruit juices. The system was able to detect the presence of *Alicyclobacillus spp.* in all the tested fruit juices at 24 h from inoculation. The EOS^{835} could detect bacterial concentration as low as <102 colony forming unit/ml and it was also able to classify bacterial contamination independently of the *Alicyclobacillus* species.

Device description

The *EOS*^{835} (fig. 4.a) system employs gas sensors of the metallic oxide semiconductor (MOS) resistive type installed inside a patented measuring cell (sensor chamber). The interaction with odors molecules causes variation in their electrical conductivity. The EOS^{835} system features 6 sensors with different metallic oxides (tab. 4.A) that react differently to the same odorous molecules, thus generating a set of signal (*olfactory imprint or aromatic fingerprint*) characteristic of the sample analyzed.

Tab. 4.A. MOS Sensor array configuration of the EOS^{835} (Sacmi Imola, Italy)

Sensor No.	Sensor Code	Sensing layer	Operating Temperature
1	CJ1316	SnO_2 cat SiO_2	450 °C
2	SB0225	SnO_2 cat Ag	400 °C
3	SD0515	SnO_2 cat Mo	400 °C
4	SH0612	WO_3	375 °C
5	SJ0717	SnO_2	450 °C
6	WHT19	WO_3	400 °C

The EOS^{835} hardware and software consist of:

- an HT200H autosampler (HTA s.r.l., Brescia, Italy) with a forty position try and six position oven that allow optimizing the preparation time. The sample is heated and simultaneously shaken to facilitate the state change of the volatile compounds. The extraction is made inside the oven to assure the sample thermal stability and avoid sample condensation in the case of long extraction times.

- a pneumatic section, designed to aspirate the vapors of the sample being analyzed and regulate the carrier gas flow;

- a sensor chamber (thermally controlled) with 35cc of internal volume

- an electronic section to control and to start and stop the sample measurement

- a Windows-compatible software (NosePatternEditor) that controls the experimental setting and processes the data using specific algorithms as Principal Component Analysis.

Fig. 4.a. *EOS^{835}* (Sacmi, Imola, Italy) at IBF-CNR Palermo (Italy).

4.1. Discrimination of fruit aroma of 5 *Malus comunis* cultivars by electronic nose.

Apple (*Malus domestica*) is one of the most frequently consumed fruit. The fruit quality in the shelf market is determined not only by its appearance (color, surface damage), firmness and texture, but also by its flavor. Fruit aroma is a complex mixture of a large number of volatile compounds all contributing to the wholel sensory quality of fruit, specific to species and cultivar (Sanz *et al.* 1997). Over 300 volatile compounds have been measured in the apple aroma profile. These compounds include alcohols, aldehydes, carboxylic esters, ketones, and ethers (Dimick & Hoskin 1983). About 20 of these chemicals are "character impact" compounds. Although they are present in very low concentrations and potently contribute to the characteristics of apple aroma/flavor (e.g., ethyl-2-methyl butanoate) (Flath *et al.* 1967). Others contribute to the aroma intensity (e.g., *trans* -2-hexenal) or are related to aroma quality (e.g., ethanol) (Dürr & Schobinger 1981).

Typical apple flavor develops during ripening. Maximum endogenous volatile concentration occurs at the climacteric peak but it is not known whether the volatile biosynthetic enzymes are constitutive or induced during the climacteric.

Volatile production is greater at higher temperatures in the range from 0 to 30°C but exposure to low temperatures (< 3°C) for more than 3 months decreases production.

Until the late 1970s most research on aroma and flavours of apple fruit focused on identifying the volatiles produced by ripening (Tressl *et al.* 1975). Recent reviews have discussed the biochemical origin of aroma volatiles and the improvements of methods for separation and identification of volatile compounds, often in amount of few parts per million (Dimick & Hoskin 1983; Yahia 1994; Sanz *et al.* 1997).

Traditionally, the aroma of horticultural products is measured by means of sensory panels. Alternatively, gas chromatography, which separates volatiles into their individual components, is used to quantify the different volatiles constituting the fruit aroma (Pathange *et al.* 2006).

Some studies used the electronic nose technique as potential maturity indicator for determining the ripeness stage (Hines *et al.* 1999, Young *et al.* 1999), and monitoring changes in apple fruit volatiles during shelf life (Saevels *et al.* 2004). In our knowledge, there are no studies on the differentiation of apple's cultivar aroma by electronic nose.

A preliminary trial has been carried out to test the *EOS*^{835}'s MOS sensors array on 5 cultivars of *Malus comunis* to evaluate if the sensors response, analysed with a multivariate statistical analysis (Principal Component Analysis), was able to discriminate and cluster the aromatic patterns of the different cultivars.

4.1.1. Materials and Methods

Apple fruits of five cultivars ('Granny Smith', 'Pinklady', 'Fuji', 'Royal Gala', 'Golden') were studied.

cv. GRANNY SMITH

cv. PINKLADY

cv. FUJI

cv. ROYAL GALA

cv. GOLDEN

Measurements on apple aroma were carried out using *EOS*^{835}. Instrument details had been reported above.

The samples were prepared in 20 ml glass vials. A small part of fruits (3g of pulp and peel) was sealed into vials with pierceable top. The vials were placed for 10 min at 40°C to obtain an homogeneous headspace. The autosampler (HTA s.r.l., Italy) automatically drew in 4 ml of headspace and injected it into the *EOS*^{835}'s injector. A chromatographic air flow (10 ml/min) carried the sample in the sensors chamber kept at a constant temperature of 55°C. When the aroma went through the sensors layer the initial resistance (R_0) fell down to a minimum value (R). The measurement duration was 15min. A set of six values {R1, …, R6} was obtained in each measurement and data pre-processing was carried out. The feature *Classica* = R/R_0 was extracted from the sensors response curve. Principal Component Analysis (PCA) was used as unsupervised statistic method to reduce the dimensional space by a correlation matrix of data (using the NosePatternEditor software) and plot the data.

The aroma determination was also performed by a gas chromatograph mass spectrometer with single quadrupole GCMS-QP2010S (Shimadzu) equipped with a capillary column SLB^{TM}-5ms (30 m x 0.25 mm x 0.25 µm) (Supelco). The *head-space solid-phase micro extraction* technique (HS-SPME) with Polidimetilsiloxano fibers (100µm x 1cm by Supelco) was used for sampling the volatile fraction on samples prepared as above reported (3 g of apple pulp and peel in sealed 20ml vials).

After 10 min of warming in a water bath at 40°C, the fiber was introduced in the vial and drawn out after 30 min of adsorption of volatile molecules. The desorption of all adsorbed molecules was allowed in the GC- injector at a temperature of 250°C. The GC-oven temperature was programmed as follows: an isothermal at 50°C for 2.5 min, then an increase to 200°C at a rate of 10°C/min, and again an isothermal at 200°C for 15 min. Other system settings were: 1 ml/min of speed of the mobile phase (He), 70eV of ionization energy, 33-500 m/z range.

4.1.2. Results and Discussion

The *EOS*^{835}'s MOS sensors array showed a good sensitivity. Its is defined as $S=R_0/R$, where R_0 and R are electric resistances in air and sample gas respectively. The figure 4.1.a shows a score plot in two-dimensional space for the five cultivars analyzed with PCA. PC1 and PC2 accounted for 87.54% of the score plot's total variance, thus evidencing a scarce capability of the instrument to discriminate between single cultivars. However the figure indicates a possible discrimination on PC2 axis.

According to gas chromatographic aroma analysis (with Mass Spectrometer detector) carried out on the same apple samples after electronic nose experiments, the identified volatile molecules were similar for 'Gala', 'Fuji', 'Granny Smith' and Pinklady', and different for 'Golden' (data no reported).

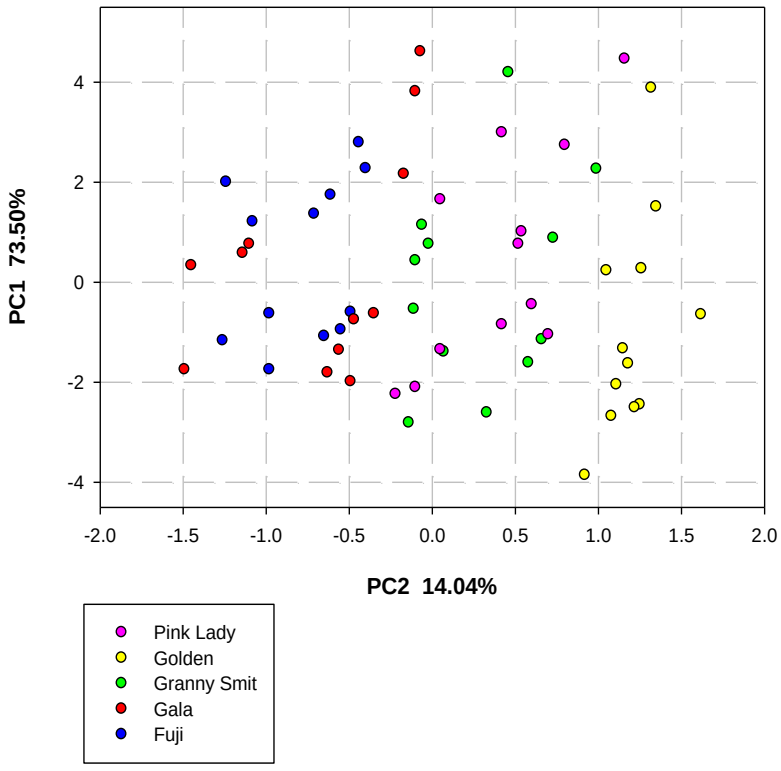


Fig. 4.1.a. PCA score plot by taking *EOS*^{835}'s data for five cultivars of apple: 'Gala', 'Fuji', 'Granny Smith', Pinklady' and 'Golden'.

4.1.3. Conclusions

In the present work the *EOS*^{835} showed a scarce capabilities to discriminate between the 5 cultivars: 'Gala', 'Fuji', 'Granny Smith', Pinklady' and 'Golden'. The cultivar 'Golden', according to the multivariate analysis seems to be different from the other cultivars and this was also confirmed by the GC-MS analysis.

The study suggests that *EOS*^{835} was sensitive to the apple aroma but low selective for differentiating the different cultivars.

4.2. Shelf life monitoring by electronic nose, chemical, physical and sensorial analysis, of ready-to-eat fresh-cut apples (cv. Fuji).

Recently the demand for minimally processed fruits and vegetables is greatly increasing because of the lifestyles, increasing purchasing power and health-consciousness of the consumers (Baldwin *et al.* 1995). Consumers request fresh-like processed products with quality attributes (such as appearance, texture, and flavor) similar to those of the raw products (Wong *et al.* 1994). Minimal processing has been defined as a combination of procedures, such as washing, sorting, trimming, peeling, and slicing or chopping, that do not affect the fresh-like quality of the food (Burns 1995). However, fresh-cut fruits are more difficult to produce than other minimally processed products, because the tissue integrity of fruits is more easily altered during processing (Rolle & Chism 1987). Fruit shelf life is affected by many factors, including cultivar, stage of ripeness at cutting, and storage atmosphere or temperature (Gorny *et al.* 1998).

Fresh-cut apples (slices, wedges, or cubes) are potential lightly-processed products and, although they are poorly commercially produced, fruit marketers have shown a great interest in their development. From a microbiological point of view, their shelf life has been estimated in the range of 2-3 weeks (Hoover 1997). But, in a few days they undergo biochemical deteriorations such as enzymatic browning, off-flavors, and texture breakdown (Varoquaux 1991). The Nicoli's group first proposed the use of a modified atmosphere (80% N_2/20% CO_2) to better preserve the apple slices from quality losses (Nicoli *et al.* 1994).

In this work, we studied by the electronic nose, the changes of *aromatic fingerprint* of apple slices, packaged in atmospheric air and in a modified atmosphere (100% N_2), stored at 4°C for 0, 4, 8 and 12 days. Moreover we determined classical quality parameters, such as total acidity, total soluble solids, firmness and sensorial profile by trained judges.

4.2.1. Materials and Methods

Fruits of the apple cultivar ('Fuji') were harvested in September (2009) in Caltavuturo, (Palermo, Italy), in a farm organically cultivated. 30 fruits were collected and selected for regular shape and uniform size. The apples were washed in distiller water and cut into slices of 1 cm thickness using a sharp knife. The slices were put, individually, in bags (10 cm x 10 cm) and sealed, by a thermal shutdown, in atmospheric air (100%Air) or in nitrogen saturated (N_2) ambient. The bags (Reber, Italy) presented a declared permeability to O_2 of 70.5 cm^3/m^2 24h atm (at 23°C and 0% RH), and of 10.5 cm^3/m^2 24h atm to N_2 (at 23°C and 0% RH). All the bags were stored in refrigerator at 4°C. The apples were analyzed just before packaging (zero time) and after 4, 8 and 12 days from storage at 4°C. At each storage time, 10 packages for trial (Air or N_2) were randomly taken and analyzed.

The Soluble Solids Content (SSC) was determined by an optical refractometer (Atago Co., Ltd., Japan); the total acidity (TA) and the pH were measured with an automatic titrator (Crison Instruments, Alella, Spain). Firmness measurement was performed by EFFEGI texture analyzer (fig. 4.2.a) by measuring the maximum force registered during penetration of a 6mm diameter stainless steel cylinder into the apple slice tissue for 6mm.

Fig. 4.2.a. EFFEGI texture analyzer.

A judge's panel was engaged. The judges were trained in some preliminary sessions, using different samples of apples, in order to develop a common vocabulary for the description of the sensory attributes. Eight descriptors were selected to describe the quality state of apple's slices: appearance, browning, flavor, consistency, juiciness, sweetness, acidity, and pleasantness. Samples were evaluated by assigning a score between 1 (absence of the sensation) and 5 (extremely intense), except for the descriptor 'browning' where a reverse evaluation (1= maximum; 5= minimum) was adopted. Water at room temperature was used to rinse the sample before tasting.

The *EOS*^{835}, above described, was used for monitoring the aromatic fingerprint at different storage time. Samples were prepared in triplicate, cutting 3g slices and placing them in sealed vials. Samples were conditioned in the oven at 40°C for 10 minutes. After reaching the equilibration state, the headspace of the vial (4 ml) was drawn in by using an automated sampling system (HTA s.r.l., Italy) and carried out by a continuous flow of 10 ml/min of chromatographic air to the sensors chamber kept at a constant temperature of 55°C. When the aroma went through the sensors layer, the initial resistance (R_0) fell down to a minimum value (R). The measure duration was 15min. A set of six values {R1, …, R6} was obtained in each measurement and data pre-processing was carried out. The feature *Delta* = R_0-R was extract from the sensors response curve. Principal Component Analysis (PCA) was used as unsupervised statistic method to reduce the dimensional space and plot the data, by a correlation matrix of data (using S-Plus software).

4.2.2. Results and Discussion

Results of some physical-chemical characteristics of apples samples processed in 100%Air and in 100%N_2 and cold stored for 0, 4, 8 and 12 days are reported in table 4.2.A. In both

conditions the soluble solids content values decreased and the total acidity increased linearly with the storage time. The behavior of firmness, measured by penetration test, showed little differences between slices storage in Air or in N_2. After 12 days, the texture of samples stored in Air was softer than that of the sample stored in N_2, thus indicating that a modified atmosphere (with N_2) was more able to preserve the sample's hardness.

Tab. 4.2.A. Comparison physical-chemical parameters of apple's slices stored in 100% Air and in 100% N_2

Storage time (at 4°C)	*Soluble Solid (%)*		*Tot. Acidity (g/L citric ac.)*		*Firmness (Kgf)*	
	100% Air	**100% N_2**	**100% Air**	**100% N_2**	**100% Air**	**100% N_2**
0 days	14.14 ± 1.29	12.97 ± 1.21	2.80 ± 0.65	2.59 ± 0.67	3.34 ± 0.34	3.23 ± 0.46
4 days	13.02 ± 1.03	12.40 ± 0.76	2.66 ± 0.55	2.52 ± 0.58	3.43 ± 0.41	3.35 ± 0.40
8 days	13.42 ± 0.98	12.55 ± 1.05	3.29 ± 0.47	3.19 ± 0.40	3.48 ± 0.39	3.55 ± 0.33
12 days	13.27 ± 1.12	12.50 ± 0.79	3.15 ± 0.59	3.05 ± 0.51	2.75 ± 0.55	3.30 ± 0.41

Results regarding sensory analysis are shown in the radar plot of the values for the 8 chosen descriptors (fig. 4.2.a). The global 'appearance' of samples stored in Air, at 8 and 12 days storage time, were the same. Samples stored in N_2 showed the lowest values of 'appearance' only at 12 days storing time. The perception of 'sweetness' was different for both types of storage atmosphere (Air and N_2); the values of 'sweetness' for storage time of 0 and 4 days were different from those of sample at 8 and 12 days storage time.

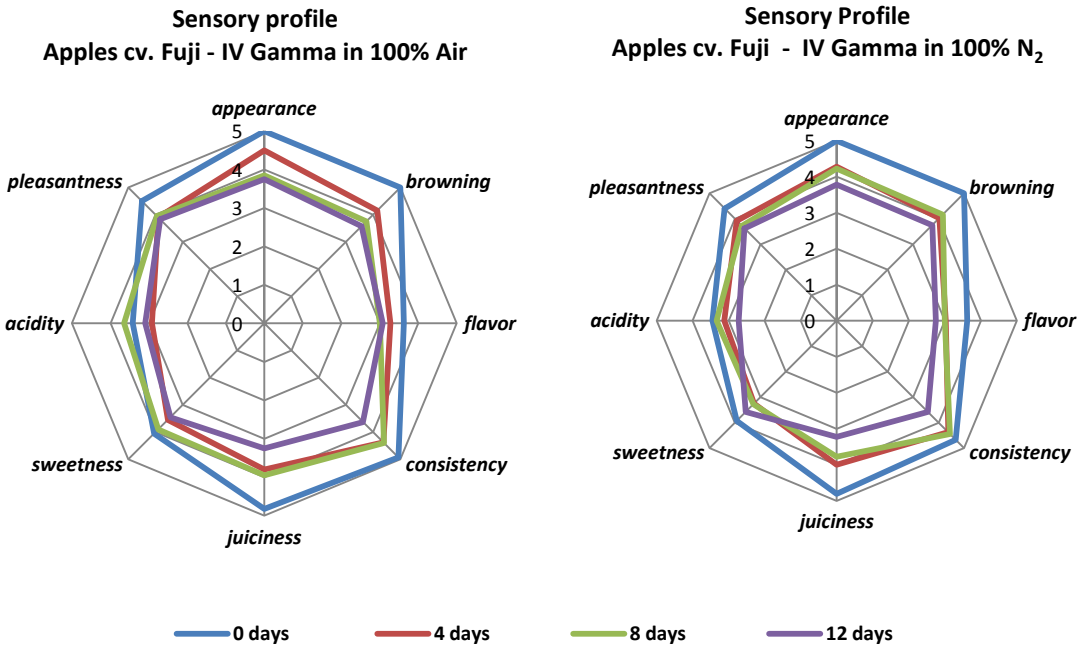


Fig. 4.2.b. Radar plot of sensorial profile for samples stored in 100% of atmospheric air and in 100% of nitrogen.

The pictures below help to better understand the values of descriptor as 'appearance' and 'browning' for both storage atmospheres (fig. 4.2.c).

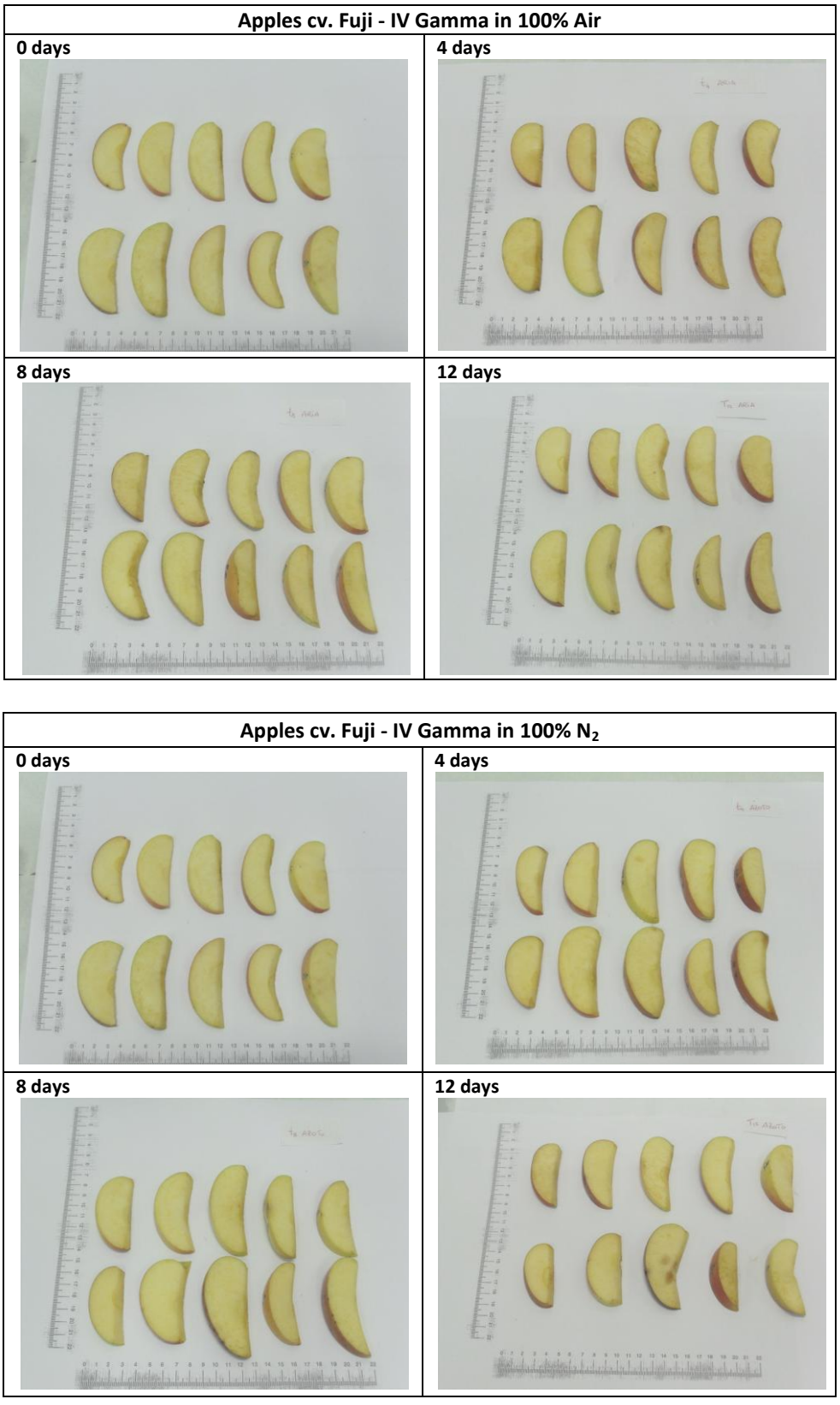


Fig. 4.2.c Pictures of apple's slices, stored at 4°C in sealed bags saturated with atmospheric air and nitrogen, taken at different storing time: 0, 4, 8, 12 days.

With respect to the EN analysis, by *EOS*^{835}, the first two principal components (PC1 and PC2), for the sample in 100% Air took into account 94.07% of the total variance in the score plot (fig. 4.2.d). The aroma fingerprint showed changes from 0 to 12 days of storage. At 0 and 4 days, as well as at 8 and 12 days, the *aromatic fingerprint* seems to be the same, or at least the MOS sensors responses of electronic nose EOS^{835} were very similar. The aromatic *fingerprint* behavior for samples packaged in atmospheric air was different from that of samples packaged in nitrogen saturated atmosphere. In the latter, in fact, the aroma seemed better preserved. The *aromatic fingerprint* of samples stored for 0, 4, and 8 days (in 100% N_2) was separated in a cluster independent from that relative to samples stored for 12 days. In this case the first two principal components (PC1 and PC2), for sample in 100% N_2, take into account 94.60% of the total variance in the score plot (fig. 4.2.e).

4.2.3. Conclusions

The electronic nose is able to discriminate changes in the aromatic pattern. Indeed, it showed a good sensitivity to changes in minimally processed fruit aroma, such as fresh-cut fruit (in this case fresh-cut apples slices) that resulted higher than the judges' human nose.

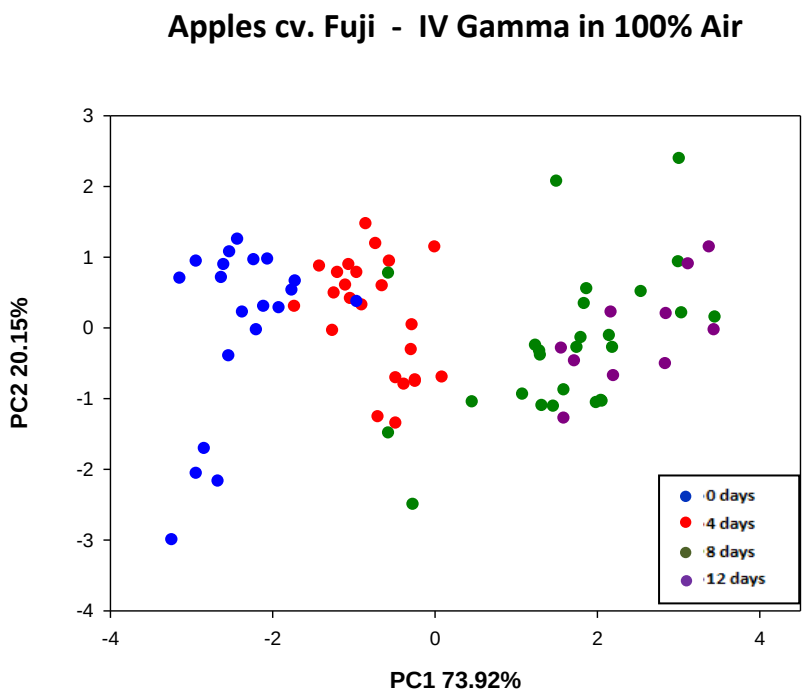


Fig. 4.2.d. Principal Component Analysis determined by the electronic nose system *EOS*^{835}, at different storage time (0, 4, 8, 12 days) for apple's slice stored at 100% of Air, using feature Delta (R_0-R) and a correlation matrix.

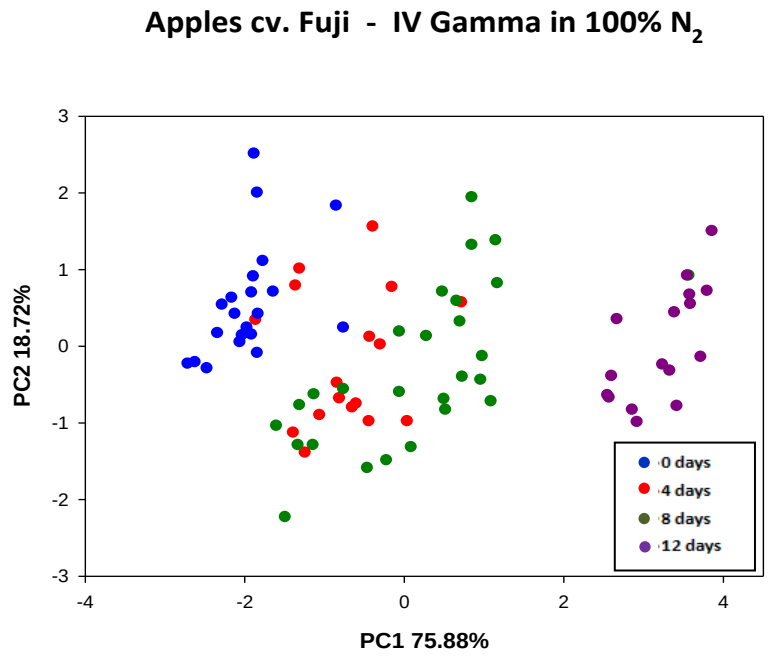


Fig. 4.2.e. Principal Component Analysis determined by the electronic nose system *EOS*^{835}, at different storage time (0, 4, 8, 12 days) for apple's slice stored at 100% of N_2, using feature Delta (R_0-R) and a correlation matrix.

4.3. Use of the electronic nose to discriminate 3 *Eriobotrya Japonica* Lindl. cultivars.

Loquat (*Eriobotrya japonica* Lind.) is a subtropical evergreen tree crop, probably originated in southeastern China and well adapted to mild-winter areas of the Mediterranean basin. It was introduced to Italy at the beginning of last century (Monastra et al. 1995) and its cultivation is located for 90% in Sicily, mainly in the province of Palermo (ISTAT 2008). The Italian consumers choose loquat fruits for their excellent quality. Shape, color, size and absence of skin defects are the main parameters determining the consumer's preference (Cañete et al. 2007). Weight, size, seed number and flesh thickness are relevant factors influencing the storage and the marketing of the fruits (Fu et al. 2009). Other characteristics playing an important role in loquat fruit quality are flavor and taste. The latter are linked to the concentration of sugars, acids and volatile compounds, and consequently, to the sensorial perception of sweetness, sourness and aroma (Baldwin 2004).

In Sicily, the fruit ripening period is concentrated in few months of the year: March, April and May (Calabrese *et al.* 2002), with a total loquat production of about 7.000 – 7.500 t (ISTAT 2008), destined to local and national markets.

Italian consumers for their excellent quality, sourness, and aroma appreciate loquat fruits.

In this study we used the *EOS*^{835} with an array of six non-specific Metal Oxide Semiconductors (MOS) sensors, to discriminate the aromatic pattern of fruits of three different loquat cultivars: 'Algerie', 'Claudia' and 'Nespolone di Trabia'. The *EOS*^{835} raw data were treated with an unsupervised exploratory analysis, the Principal Component Analysis (PCA). The differences in aromatic "*fingerprints*" were assigned to variation of the chemical class composition by using the Gas Chromatographic-Mass Spectrometry analysis.

4.3.1. Materials and Methods

The study was conducted in 2009, with fruits collected from loquat trees grown in Sicily. The field was located in the Palermo area at 150 m a.s.l.. The cultivars tested were 'Algerie', 'Claudia' and 'Nespolone di Trabia'. The trees were planted in 1994 at a 5 x 5 m distance and since 1998 the farm has been organically cultivated. For each cultivar, a sample of 80 fruits was randomly handpicked at the ripe stage (light orange), suitable for the fresh fruit market, and taken to the laboratory for analyses.

The Soluble Solids Content (SSC) was determined by an optical refractometer (Atago Co., Ltd., Japan); the titratable acidity (TA) and the pH were measured with an automatic titrator (Crison Instruments, Alella, Spain). The fruit weight was determined by an analytical balance while the longitudinal and transversal diameter and the flesh thickness were measured with a digital caliper. Other pomological variables such as seed number and peel rustiness (for the latter using a range from 5: none to 20: very much) were directly evaluated. The peel color was determined according to the method developed by Infantino and Lo Bianco (2004). The method uses an algorithm written on Matlab 6.1 (MathWorks Inc., Natick, MA, USA), which converts images from the original format (RGB) to the CIE 1976 (L*, a*, b*) colours space, separating the fruit from the background. The colors characteristics were calculated in terms of the distance of each pixel of a fruit color from an optimal reference. The color index can be between 1 (perfect color reference) and 0 (staining most distant from the reference). It provides an integrated data quality (color) and quantity (intensity) of the fruit exterior.

To identify the aromatic *fingerprint* of the different cultivars, the *EOS*^{835}, above described, was used.

Parts of the fruits (3g for each sample) were placed in triplicate in vials sealed in atmospheric air and satured with nitrogen (fig. 4.3.a). The headspace of the vial (4 ml) was drawn in by using an automated sampling system (HTA s.r.l., Italy) and carried out by a continuous flow of 10 ml/min of chromatographic air to the sensors chamber (constant temperature of 55°C). When the aroma went through the sensors layer, the initial resistance (R_0) fell down to a minimum value (R). The measurements were done on samples prepared either in ambient air and in saturated nitrogen atmosphere with the aim of testing the possible discriminatory effect of aromatic compounds derived from secondary oxidation processes (Colelli & Elia, 2009).

Fig. 4.3.a Electronic nose samples: vials sealed in atmosphere air (R) or satured of nitrogen (L).

For a better interpretation of EOS^{835}'s results, the response curve of sensors was transformed into a unique variable (Feature) taken as the difference between the electrical resistance of the sensor in the absence and presence of volatile substances (*Delta*=R_0-R). The Feature calculated for each sensor was processed using the Principal Component Analysis (PCA) based on a correlation matrix. The software used was provided by NosePatternEditor (Sacmi, Italy)

To better characterize the aroma of the three cultivars, the molecular profiles were determined by gas chromatography analysis.

The aroma analytical determination was performed by a gas chromatograph mass spectrometer with single quadrupole GCMS-QP2010S (Shimadzu) equipped with a capillary column SLB^{TM}-5ms (30 m x 0.25 mm x 0.25 µm) (Supelco). The *headspace solid-phase micro extraction* technique (HS-SPME) with Polidimetilsiloxano fibers (100µm x 1cm by Supelco) was used for sampling the volatile fraction. In the sample preparation, 3 gr of fruit, including pulp and peel, were put in 20 ml volume vials, sealed with pierceable caps. The analysis wasdone in triplicate.

After 10 min of warming in a water bath set at 40°C, the fiber was introduced in the vial, and drawn out after 30 min of adsorption of volatile molecules. The desorption of all adsorbed molecules was allowed in the GC- injector at a temperature of 250°C.

The GC-oven temperature was programmed as follows: an isothermal at 50°C for 2.5 min, then an increase to 200°C at a rate of 10°C/min, and again an isothermal at 200°C for 15 min. Other system settings were: 1 ml/min of speed of the mobile phase (helium), 70eV of ionization energy, 33-500 m/z range.

4.3.2. Results and Discussion

The 'Nespolone di Trabia' fruits showed highest size, highest color index of the skin and limited rusting. According to the qualitative characteristics (tab. 4.3.A), it also had a subacid pulp. 'Claudia' stood out for a balanced ratio of soluble solids content and acidity, big thick flesh and low number of seeds, thus resulting excellent as edible fruit. 'Algerie' fruits had good organoleptic characteristics but small size differently from literature data.

R_0-R was taken as feature for all array MOS sensors. The PCA score plot for air and nitrogen prepared samples, showed a good discrimination between the three cultivars.

The PCA score plot, PC1 vs. PC2, for the samples prepared in atmospheric air showed a good discrimination along the first principal component (PC1) accounting for 83.78% of total variance (fig. 4.3.b). Also the PCA of the samples prepared in nitrogen provided a good discrimination with a percentage value of total variance not very different from that in air (PC1 = 86.23%) (figure not reported). This indicated that the variations in the aroma due to oxidation were similar for air and nitrogen packaged samples.

For this reason, it was decided to conduct measurements with Gas Chromatography/Mass Spectrometer only on the samples prepared in air.

Gas chromatographic profiles of the three cultivars are shown in figure 4.3.c : 'Nespolone di Trabia' presents the greatest number of molecules. After the recognition of individual chromatographic peaks by NIST library, the molecules identified were divided into chemical classes. The radial plot of chemical classes expressed as percentage area relative to single chromatographic run is shown in figure 4.3.d. The principal differences in the aroma of the different cultivars are the percentage of esters, acids and ketones. Figure 4.3.e shows the aroma *fingerprint,* determined by the signals of the individual MOS sensor. The sensors 1, 3 and 6 appear the most sensitive to the passage of headspace samples from the three cultivars.

Multivariate analysis on the EOS^{835}'s data is potentially discriminatory for the different cultivars 'Algerie', 'Claudia' and 'Nespolone di Trabia'.

Tab. 4.3.A Quality parameters of three loquat cultivars

Quality Parameters	***Cultivar***		
	'Algerie'	**'Claudia'**	**'Nespolone di Trabia'**
Weigh (g)	38.26 ± 1.73	37.07 ± 1.93	46.12 ± 2.05
LD (mm)	38.38 ± 1.31	43.96 ± 0.75	46.04 ± 0.70
TD (mm)	36.28 ± 1.41	36.41 ± 0.81	40.43 ± 0.70
FT (mm)	8.86 ± 0.77	13.79 ± 4.54	9.87 ± 0.25
Seed No.	2.60 ± 0.32	1.83 ± 0.17	2.11 ± 0.19
SS (Brix°)	13.33 ± 0.09	13.36 ± 0.22	12.40 ± 0.42
TOT. AC. (g/l)	19.77 ± 1.17	10.54 ± 0.74	25.92 ± 1.51
pH	3.28 ± 0.06	3.83 ± 0.10	3.19 ± 0.04
COL. IND.	0.97 ± 0.01	0.95 ± 0.01	0.97 ± 0.01
RUS. (%)	10.00 ± 1.58	12.67 ± 2.88	7.63 ± 1.35

Mean value of characteristics of loquat fruit ± Standard Error. Abbreviation: LD= longitudinal diameter; TD= transversal diameter; RUG= rustiness; COL.IND. = colors index; FT = thickness of the flesh; SS = soluble solids content; TOT. AC. = titratable acidity in equivalent of malic acid.

4.3.3. Conclusions

The electronic nose *EOS^{835}* equipped with MOS sensors array has showed to be sensitive and selective regarding the loquat aroma, allowing to discriminate among of three cultivars of *Eriobotrya japonica* Lindl.. The GC-MS analysis showed significant differences in the chromatographic profile for aroma of Algerie, Claudia and Nespolone of Trabia cultivars. The multivariate analysis of *EOS^{835}* data could be potentially discriminant for these different cultivars.

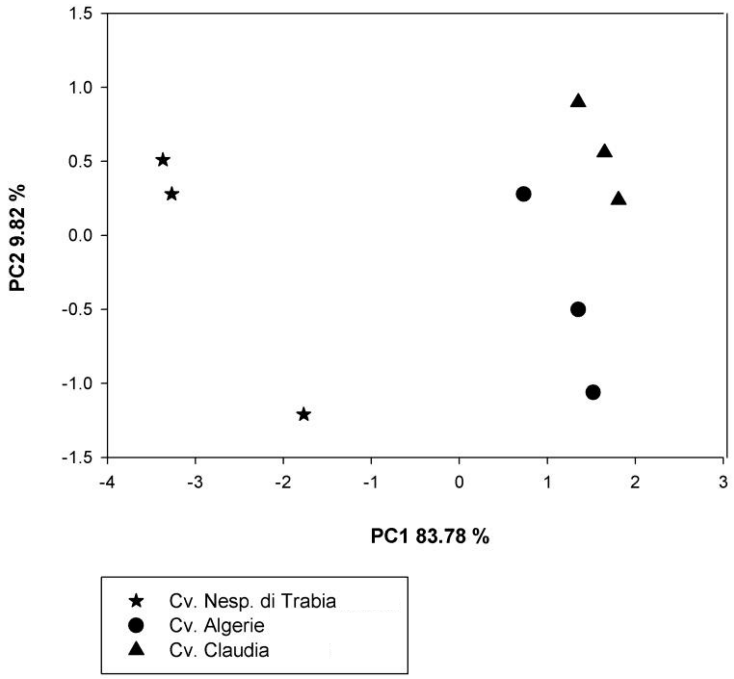


Fig. 4.3.b Discrimination of aromatic patterns of three *Eriobotrya japonica* Lindl. cultivars, with PCA of Electronic Nose (EOS^{835}). Data are relative to samples in atmospheric air.

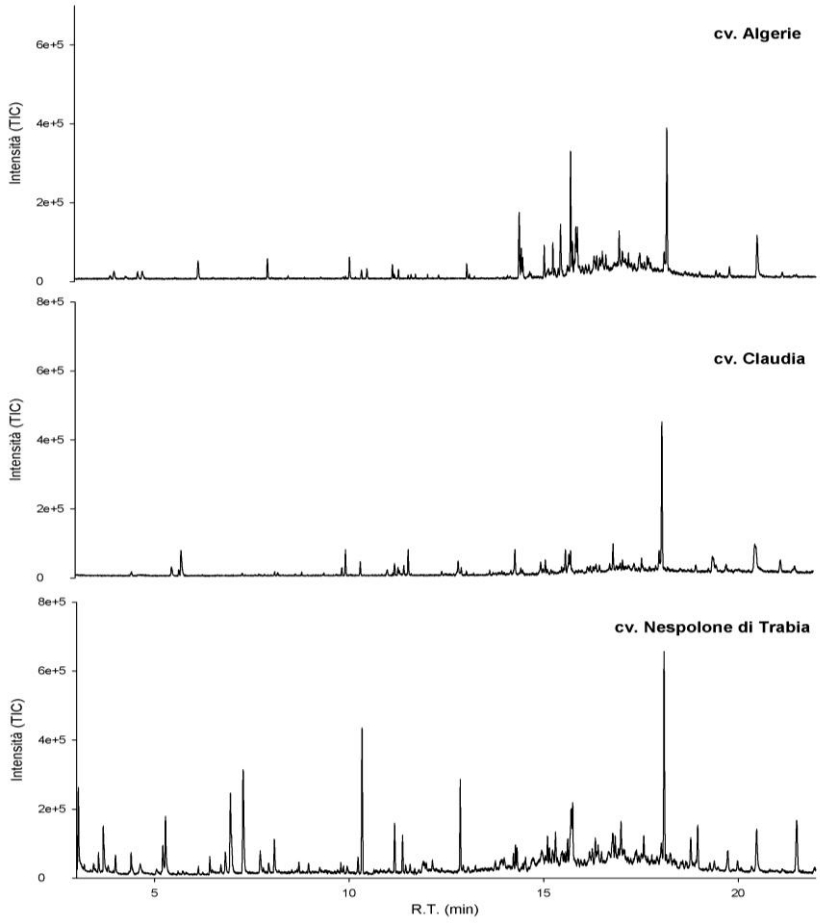


Fig.4.3.c GC-MS analysis of the three different cultivars studied ('Algerie', 'Claudia', 'Nespolone di Trabia').

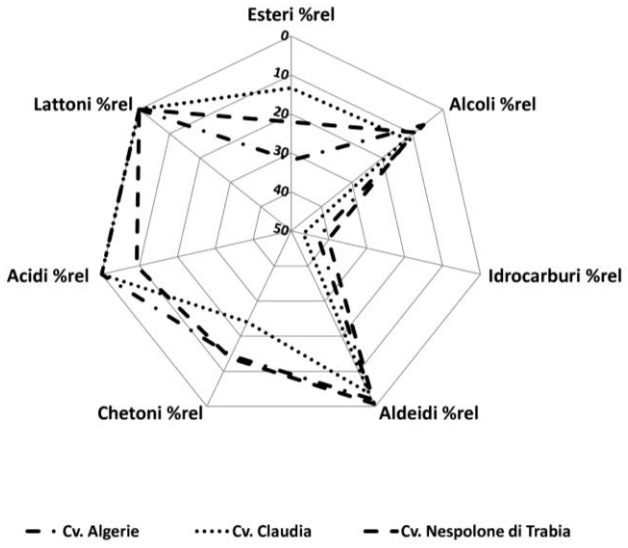


Fig. 4.3.d Radar plot of percentage content of chemical compound as determined by the qualitative analysis of the chromatographic peaks. The recognition of chemical compounds was performed by using GC-Solution Lib.Nist. Samples prepared in atmospheric air.

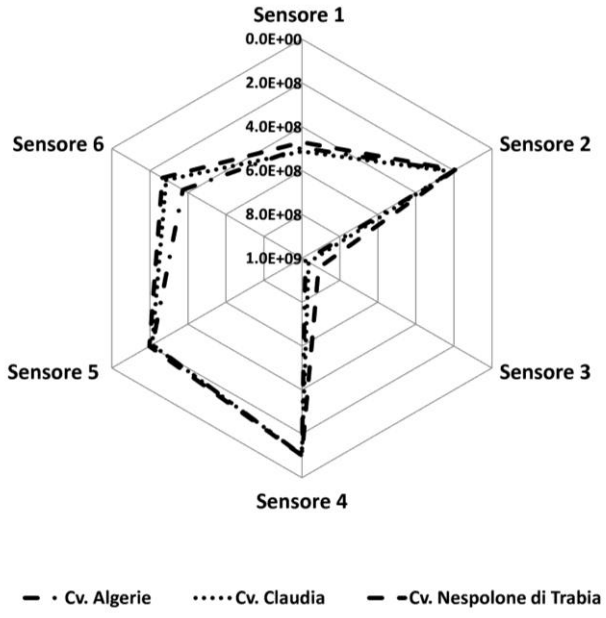


Fig. 4.3.e Radar plot of the features extracted from the signal of the MOS sensor array mounted on the sensor chamber of the EOS^{835}: Sensor 1= Delta-CJ1316*100; Sensor 2= Delta-SB0225*10; Sensor 3= Delta-SD0515*100; Sensor 4= Delta-SH0612; Sensor 5= Delta-SJ0717*10; Sensor 6= Delta-WHT19. Samples prepared in atmospheric air.

4.4. Fruit quality evaluation of four loquat (*Eriabotrya japonica* Lindl.) cultivars grown in Sicily (Italy).

In this work chemical, morphological, and sensorial characteristics of four loquat cultivars ('Algerie', 'Claudia', 'Nespolone di Trabia' and 'Sanfilippara') from mature, organically-grown trees in Sicily, were analyzed. The determinations were carried out by traditional instrumental techniques, by a panel test and by *EOS*^{835}. A panel of 10 trained judges was used to determine intensity of some attributes in the sensory profile of the different cultivars, while the e-nose was used to identify possible differences in the aromatic "fingerprint". The e-nose results were compared with sensory analysis results obtained using Panel Test.

4.4.1. Materials and Methods

This study was conducted in 2009 on loquat fruits collected. The field is the same above mentioned. The cultivars tested were 'Algeria', 'Claudia', 'Nespolone di Trabia' and 'Sanfillippara'. For each cultivar, a sample of 80 fruits was randomly handpicked at the ripe stage.

The Soluble Solids Content (SSC), the titratable acidity (TA), the pH, the weight, the longitudinal and transversal diameter, and the flesh thickness were measured as previously described (4.3.1 section). Other pomological variables such as seed number and peel rustiness (for the latter using an indicative scale with a range from 5: none to 20: very much) were directly evaluated. The peel color was determined according to the method developed by Infantino and Lo Bianco (2004).

A descriptive method (UNI 10957, 2003) was used to define the sensory profile of loquat samples. A panel of ten judges was employed. The judges were trained in some preliminary sessions, using different samples of loquat fruit, in order to develop a common vocabulary for the description of the sensory attributes characterizing loquat and to familiarize themselves with scales and procedures. On the basis of the frequency of citation (> 60%), fifteen descriptors were

selected to be inserted in the card: flesh color, easy peeling, easy stone, loquat, herbaceous and floral odor and flavor, sweet, sour, bitter and global preference. Random samples were evaluated by assigning a score ranging from 1 (absence of the sensation) to 9 (extremely intense) in individual booths under incandescent white lighting at the sensory laboratory of DOFATA Department (Catania, Sicily). Water at room temperature was used to rinse the sample before tasting. A computerized data collection program was used (FIZZ, Software Solutions for Sensory Analysis and Consumer Tests, Biosystemes, Couternon, France). The sensory data for each attribute were subjected to one-way Analysis of Variance (ANOVA), considering the cultivars as an independent variable and the sensory attribute as a dependentone. Significance was tested with the F test. The mean values were submitted to the multiple comparison tests using the LSD (Least Significant Difference) procedure. A correlation between the attributes, which differentiate the samples, was determined.

To identify the aromatic fingerprint of the cultivars, the *EOS*^{835} was used. The description of the instrument was above reported.

Parts of the fruit (3g for each sample with peel) were placed in triplicate in sealed vials. The headspace of the vial (4 ml) was drawn in by using an automated sampling system (HT200H, HTA s.r.l., Italy) and carried out by a continuous flow of 10 ml/min of chromatographic air to the sensors chamber kept at 55°C. When the aroma goes through the sensors layer the initial resistance (R_0) falls down to a minimum value (R). The first step of data pre-processing was to extract from the sensors response curve the R/R_0 features (named '*Classica*' according to the software NosePatternEditor). Principal Component Analysis (PCA) was used to reduce the multidimensional space and plot the data, by a correlation data matrix.

4.4.2. Results and Discussion

'Nespolone di Trabia' resulted the heaviest fruit, but its flesh was thinner than that of Claudia, which instead had the lowest weight. The largest chemical differences among the four cultivars were a highest acidity and a lowest fraction of soluble solids of 'Sanfilippara' and 'Nespolone di Trabia'; while the highest value of soluble solids was observed in 'Algerie' and, mainly, in 'Claudia' (tab. 4.4.A).

ANOVA results (tab. 4.4.B) showed significant differences in sour ($p \leq 0.001$), flesh color, firmness, sweet ($p \leq 0.01$) and global preference ($p \leq 0.05$). In particular, the cultivar 'Claudia' showed the lowest intensity of sour and the highest intensity of sweet and global preference (in fact 'Claudia' is also called "Vanilla") (fig. 4.4.a). The lowest flesh color score for 'Claudia' was normal, because it is a white-fleshed variety. All these results suggest that human senses are not able to discriminate loquat cultivars on the basis of their aroma (odor and flavor merger). On the contrary, the PCA score plot of EOS^{835} determinations provided a good discrimination between the aroma cultivars with 88.60% of total variance (fig. 4.4.b).

The results showed that although the human nose is a powerful tool in the perception of volatile compounds, allowing to evaluate the quality of loquat fruits, electronic nose is able to discriminate between different cultivars.

Tab. 4.4.A. Quality parameters of the four loquat cultivars

Quality parameters	***Cultivars*** [1]			
	'Algeria'	**'Claudia'**	**'Nesp. di Trabia'**	**'Sanfilippara'**
Weight (g)	34.49 ± 1.61	32.44 ± 1.58	43.95 ± 2.17	41.72 ± 1.89
TD (mm) [2]	34.93 ± 0.97	34.20 ± 0.79	39.51 ± 0.74	38.82 ± 0.74
LD (mm) [2]	39.30 ± 0.88	42.64 ± 0.60	45.29 ± 0.83	45.84 ± 0.80
FT (mm) [2]	8.86 ± 0.15	11.66 ± 2.73	9.73 ± 0.24	9.95 ± 0.16
Seed No.	2.60 ± 0.32	1.83 ± 0.17	2.11 ± 0.19	1.73 ± 0.21
SSC (Brix°) [2]	13.33 ± 0.09	13.36 ± 0.22	12.40 ± 0.42	12.13 ± 0.25
TA (g/l) [2]	19.77 ± 1.17	10.54 ± 0.74	25.92 ± 1.51	26.75 ± 0.98
pH	3.28 ± 0.06	3.83 ± 0.10	3.19 ± 0.04	3.06 ± 0.12
Color Index	0.966 ± 0.010	0.945 ± 0.010	0.969 ± 0.010	0.965 ± 0.010
Peel Rustines (%)	10.00 ± 1.58	12.67 ± 2.88	7.63 ± 1.35	8.42 ± 1.62

[1] Mean value of characteristics of loquat fruit ± Standard Error.
[2] LD = longitudinal diameter; TD = transversal diameter; FT = thickness of the flesh; SSC = soluble solids content; TA = titratable acidity in equivalent of malic acid.

Tab. 4.4.B. Attributes scored by sensory evaluation panel.

Attributes	***F values***	***Mean scores***			
		'Algeria'	**'Claudia'**	**'Nesp. di Trabia'**	**'Sanfilippara'**
Flesh color	5.09 **[1]	5.7b[2]	3.1a	5.0b	6.2b
Easy peeling	0.54 n.s.	4.9	5.7	6.0	5.7
Easy stone	0.29 n.s.	5.7	6.4	6.0	6.3
Loquat odor	0.57 n.s.	5.8	5.0	5.3	6.1
Herbaceous odor	0.49 n.s.	4.7	5.0	5.9	5.4
Floreal odor	0.47 n.s.	3.1	3.3	4.0	3.9
Firmness	6.32 **	7.6b	4.8a	7.5b	5.9a
Juiciness	1.25 n.s.	6.3	7.5	6.5	6.2
Sweet	5.36 **	4.7a	7.2b	3.8a	4.6a
Sour	7.64***	5.7b	2.2a	5.6b	5.1b
Bitter	1.34 n.s.	2.9	1.4	2.9	2.6
Loquat flavor	0.31 n.s.	5.8	5.7	5.1	5.8
Herbaceous flavor	0.66 n.s.	4.8	4.1	5.3	4.5
Floral flavor	0.18 n.s.	3.0	3.4	3.6	3.2
Global preference	2.86 *	4.7a	6.7b	4.5a	5.2ab

[1] F-test: *** significant difference for $p \leq 0.001$; ** significant difference for $p \leq 0.01$; * significant difference for $p \leq 0.05$; n.s. for no significant difference
[2] Mean comparison test using LSD procedure. Values within each row followed by different letters are significantly different.

Sensory profile

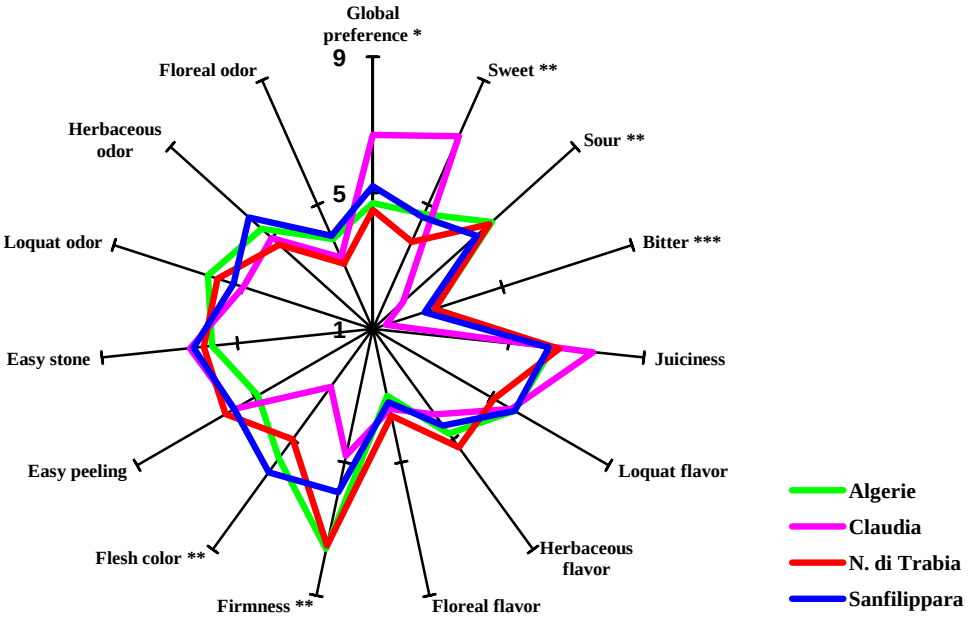


Fig. 4.4.a. Spider plot of sensory evaluation score; Oneway ANOVA: ***p≤0.001;

Electronic nose PCA plot

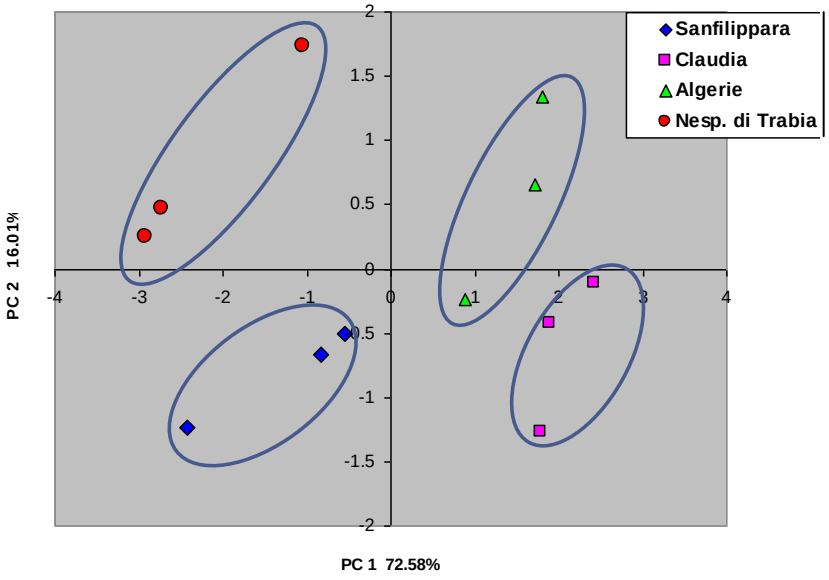


Fig. 4.4.b. PCA plot of EOS^{835} data, using as features Rm/ R0 ratio for each sensor.

4.4.3. Conclusions

Sensory evaluation demonstrated that the judges preferred ‘Claudia’ cultivar because of its sweetness and juiciness. This is in agreement with chemical determinations: ‘Claudia’ showed a higher SSC and a lower TA in comparison with the other tested cultivars. The good weight and size of the ‘Nespolone di Trabia’ resulted not determinant attributes for the judge’s global preference. The MOS sensors of EOS^{835} are able to detect the loquat aroma better than that of the humane nose. In fact the preliminary results about electronic olfactory system show that a discrimination between the aromatic fingerprint of ‘Algeria', 'Claudia', 'Nespolone di Trabia' and 'Sanfillippara' loquat cultivars exists, but more experiments are necessary to confirm these data.

4.5. Preliminary study on quality and healthy characteristic of 4 mango (*Mangifera indica* L.) cultivars grown in Sicily.

Mango (*Mangifera indica* L.) originating in India, is widely grown in many tropical areas of the world. In Sicily some localities characterized by favorable climatic conditions are particularly suitable for its growth. In the Catania, Palermo and Messina areas different cultivars of mango are grown and fruits are collected from August ('Glenn' is the earliest cultivar) up to late November ('Keitt' is the last one). The mango fruits, used fresh or processed, are especially appreciated for their juiciness and sweetness.

Many literature report the use of electronic nose as a useful experimental method to determinate the fruit ripening (Lebrun *et al.* 2008, Brezmes *et al.* 2000). The experiments reported in the present work has been carried out to verify the capability of this technique to discriminate between the aromatic patterns of 4 cultivars of mango grown in Sicily. Besides the volatile components, we also determined some classical quality parameters such as the presence of components with nutritional and health properties and the antioxidant activity. We thank Cupitur Company for kindly providing the fruits of the present research.

4.5.1. Materials and Methods

Experiments were conducted in 2009 on fruits collected from a field located in Furano (province of Messina, Italy). Four mango cultivars: 'Irwin', 'Glenn', 'Kensington Pride' and 'Maya' (fig. 4.5.a) were observed and for each cultivar 4 fruits were collected.

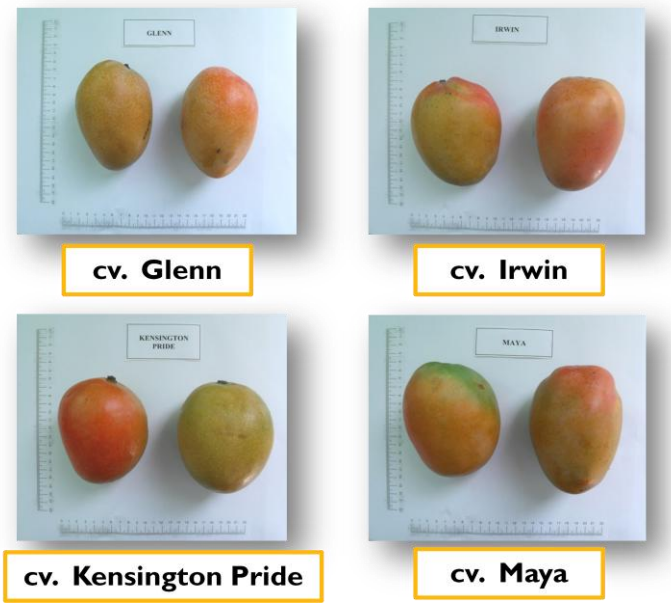


Fig. 4.5.a Four mango cultivars grown in Sicily: 'Glenn', 'Irwin', 'Kensington Pride' and 'Maya'.

Physical parameters such as weight, equatorial and longitudinal diameters were detected. Moreover, by standard methods, chemical parameters such as total acidity (TA) and total soluble solids (TSS) were determined. The determination of ascorbic acid content was performed by HPLC method (Rapisarda & Intelisano 1996), using a Waters (mod. 600E) liquid chromatography interfaced with a photodiode array detector (PDA) and managed by Waters Millennium Waters software. The column used was a Hypersil C18 250mm x 4.6mm x 5µm (Phenomenex, Torrence, CA), maintained at a temperature of 35°C and elution was performed with 0.02 M phosphoric acid at a flow rate of 1.0 ml/min. The wavelength was set at 260 nm.

Fruits, without peel, were reduced to a puree, 10 g of puree were treated with a solution of 3% metaphosphoric acid (100 ml). The sample was centrifuged at 5000 rpm for 20 min and filtered using a 0.45 µm filter befor HPLC analysis . The total carotenoids were determined spectrophotometrically at λ = 450 nm, after extraction of pigments from 10 g of puree with 20 ml of hexane: acetone: ethanol (50:25:25) and expressed as mg of β-carotene/100g flesh (De Ritter & Purcell 1981).

The total polyphenols in the juice were assessed by the rate of Folin-Ciocalteau (FC) (Singleton & Rossi 1965). A sample of juice (0.5 ml), obtained after centrifugation of the puree was diluted in 10 ml of water and then 1 ml of this solution was mixed with 5 ml of FC reagent (previously diluted with water 1:10 v/v) and 4 ml of 7.5% sodium carbonate solution. Total polyphenols amount was obtained by spectrophotometric measurements at λ= 740 nm and expressed as mg gallic acid/L juice. The antioxidant activity was measured using the ORAC assay (Ou *et al.* 2001), with slight modifications. The measurements were performed with a spectrofluorimeter Wallac 1420 Victor III.96, with a plate reader (EG & Wallac, Turku, Finland) with a fluorescence filter (excitation 485 nm, emission 535 nm). The reaction was conducted at 37 °C at pH 7.0, using as a standard Trolox (10 µM) and 75 mm phosphate buffer as a blank. The ORAC values were expressed as Trolox equivalenti/100g µmoli of pulp.

The statistical treatment of the results was performed by analysis of variance (ANOVA) using the software Statsoft 6.0.

The volatile component of the 4 considered cultivars were determined by molecular profiles with a system-QP2010S GCMS (Shimadzu, Tokyo, Japan), equipped with a capillary column SLBTM-5ms 30m x 0.25mm x 0.25µm (Supelco, Bellefonte, PA). For the sampling technique, adsorption on fiber headspace (HS-SPME), using fibers from Polidimetilsiloxano (PDMS) 100µm x 1cm (Supelco, Bellefonte, PA) was adopted.

The aroma of mango fruits was analyzed using the *EOS*^{835} described above (chapter 4). Samples were prepared as previously. After thermal conditioning, 4 ml of headspace were collected and then introduced into the measurement *EOS*^{835} chamber with an autosampler HT200H (HTA srl, Italy). The response curves of the sensors has been transformed into unique variables (feature), processed using Principal Component Analysis (PCA), based on correlation matrices. The feature, calculated for each sensor and used in the statistical analysis carried out by

the system software (NosePatternEditor), is the difference between the electrical resistance of the sensor in the absence of volatile substances and the same sensor resistance measured in the presence of volatile substances (*Delta*=R_0-R).

4.5.2. Results and Discussion

In table 4.5.A the quality, nutritional and health parameters of the analyzed cultivars are reported. The acidity (g/L malic ac. equivalent) level was found significantly lower in 'Glenn', when compared with to 'Kensington Pride'. The pH value has showsn a reverse trend. The SST was higher in 'Maya'. This cultivar also presented a content of ascorbic acid and carotenoids much higher than the other cultivars. The concentration of phenolic compounds is statistically higher in 'Maya' with a value of 367.74 mg/l eq. of gallic acid. This parameter, together with the high content of ascorbic acid and carotenoids, influences the antioxidant activity. In fact, ORAC (Oxygen Radical Absorbance Capacity) value in the 'Maya' cultivar is 1871µmolTE/100g, significantly higher than values observed in 'Irwin' and 'Kensington Pride', and similar to that one found in 'Glenn'. Probably the latter cultivar has other antioxidant molecules, different from ascorbic acid and carotenoids, not characterized (e.g., vitamin E).

Tab. 4.5.A. Quality parameters of four mango cultivars

Quality Parameter	***Cultivar***			
	'Glenn'	**'Irwin'**	**'Kensington Pride'**	**'Maya'**
Weight (g)	416.2 ± 82.4 ns	432.3 ± 72.1 ns	520.2 ± 36.1 ns	606.1 ± 70.5 ns
LD (mm)	11.1 ± 0.8 ns	10.8 ± 0.8 ns	11.3 ± 0.6 ns	12.2 ± 0.5 ns
TD (mm)	8.1 ± 0.5 ns	8.3 ± 0.6 ns	8.8 ± 0.3 ns	9.0 ± 0.3 ns
pH	6.1 ± 0.1 A	5.4 ± 0.1 B	5.2 ± 0.1 B	5.7 ± 0.1AB
Total acidity (g /L)	0.26 ± 0.01 B	0.30 ± 0.01 AB	0.32 ± 0.01 A	0.30 ± 0.01 AB
SST (%)	15.61 ± 0.06 CB	16.35 ± 0.02 B	15.30 ± 0.01 C	18.02 ± 0.26 A
Total Polyphenols (mg /L GAE)	294.88 B	216.90 C	304.76 B	367.74 A
Ascorbic acid (mg/100 g)	23.66 B	20.14 CB	17.67 C	41.05 A
Carotenoids (mg/100 g)	3.07C	4.09 B	2.95 C	6.11 A
ORAC units (µmol TE/100)	1864 A	1639 B	1152 C	1871 A

Mean value ± SD. The different significantly was carried out with ANOVA (p≤ 0.01).
Abbreviation: LD= longitudinal diameter, TD= transversal diameter, SST = total soluble solids.

Gas chromatography measurements revealed and identified 50, 34, 41 and 44 volatile compounds, for 'Glenn', 'Irwin', 'Kensington Pride' and 'Maya' respectively. In the identified aromatic pattern it is important to note that *δ-3-carene* is the major component in 'Glenn', 'Irwin' and 'Maya', but it shows a low olfactory activity (Pino *et al.* 2005), and t*erpinolene* was found to have high concentration in 'Kensington Pride' (data no reported). This molecule presenting specific olfactory smell of 'sweet', is relevant in determining the mango fruit flavour (Pino & Mesa 2006). Other monoterpenes and sesquiterpenes have been identified, such as *d-limonene, α-pinene, β-mircene, α-ocimene, β-phellandrene, β-cayophyllene, α-bergamotene, α-gurjunene, d-germacrene, and β-selinene*. Many of them are responsible for olfactory smell such as 'herbaceous', 'floral' 'spicy', 'forest' (Rezende 1999). The presence of *Ethyl acetate* and *ethyl butanoate* was also identified. The first one was found in the aroma of 'Glenn', 'Kensington pride' and 'Maya', while the second one was present in 'Irwin' only.

Figure 4.5.b shows the sum of peak areas of each chemical class (in percent of the total chromatogram area).

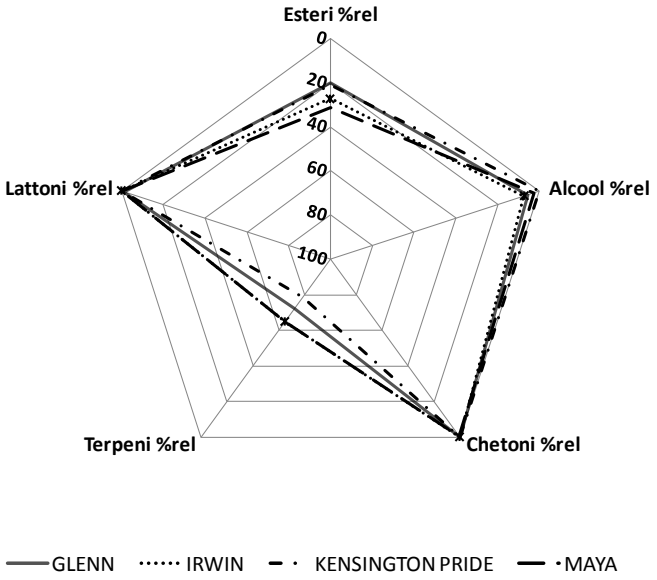


Fig. 4.5.b. Percentage contribution of each chemical family of compounds to the chromatographic area.

The applied multivariate statistical analysis, the Principal Component Analysis, shows a predominance of discrimination on the first principal component (PC1) that has a percentage of variance of 71.13% (fig. 4.5.c). This is probably due to different percentages of the major chemical classes present in the aroma of mango: terpens and ester compounds. Probably the non-specific sensors have perceived a difference in the flavor of four cultivars not for single molecules, but for the percentage value of chemical class. On PC1, in fact, it is possible to note a direct proportionality to the relative percentage of total terpenes (the results are show on fig. 4.5.d: 65.0% for 'Maya' and 'Irwin', 72.5% for 'Glenn', 78.6% for 'Kensington Pride'); on the contrary, the PC1 seems to present an indirect proportionality with regard to the percentages of esters (the results are show on fig. 4.5.d: 31.5% for 'Maya', 27.2% for 'Irwin', 21.1% for 'Glenn', 20.9% for 'Kensington Pride').

4.1.1. Conclusions

This work shows that the cultivars studied have a varied pattern aroma. The MOS sensor array, on *EOS*^{835}, used for the evaluation of the aroma is sensitive to the mango aroma and reports an appreciable discrimination between the cultivars. The study also underlines that the mango fruits produced in Sicily can be considered an excellent source of bioactive compounds, such as polyphenols, carotenoids and vitamin C, all substances with strong antioxidant properties and health benefits. In particular, the cv. 'Maya' is the richest variety of nutraceutical compounds.

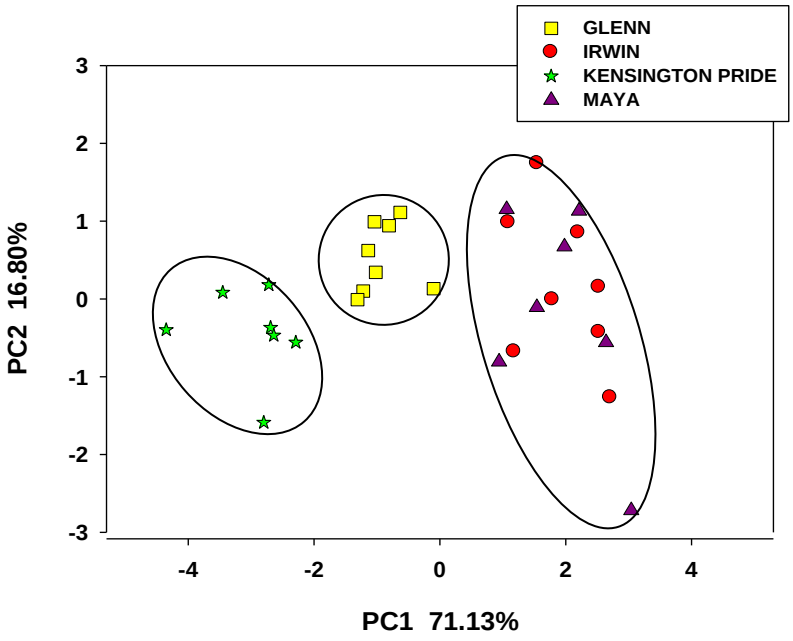


Fig. 4.5.c. PCA score plot of EOS[835] data relative to the aromatic pattern of *Mangifera indica* L. cultivars: 'Glenn', 'Irwin', 'Kensington pride', 'Maya'.

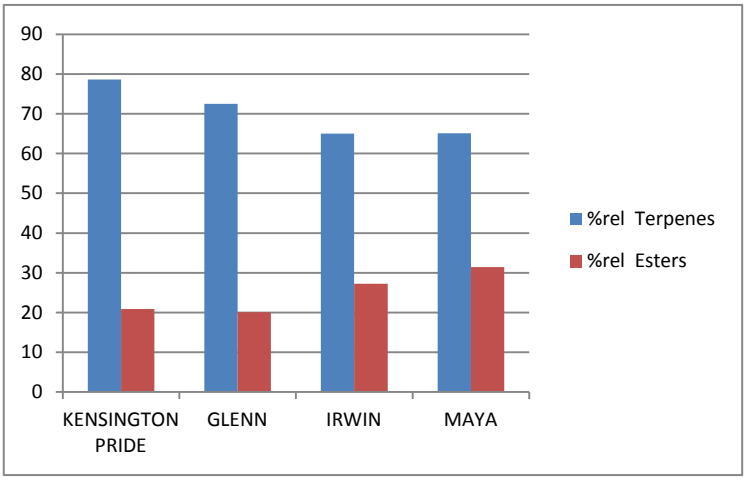


Fig. 4.5.d. Relative percentage of chemical classes ofterpens and esters in the four cultivars of mango grown in Sicily.

5. Experiments in Argentina *(E-nose INQUIMAE,* U.B.A.*)*

From March to June 2011, I carried out experiments with the group of **Prof. Martín Negri** and **Delia L. Bernik** at the Dep. INQUIMAE, **University of Buenos Aires** (UBA) (Argentina). During this period we studied the aroma characteristics of different cultivars of grapefruit (*Citrus paradisi*). Two different home-made electronic noses ("Nariz Estanca" and "Nariz por Aspiración"), with a static and a dynamic headspace sampling respectively, were used with the aim of identifying the best one in discriminating between the aroma of fresh squeezed and commercial juice of grapefruit. We also tested the "Flavormeter", patented by Profs. Negri and Bernik, which is able to simultaneously measure the soluble constituents and the volatile components responsible for the aroma (as a electronic nose and electronic tongue).

In the latter ten years, Martin Negri, adjunct Professor at the Department of Inorganic, Physical and Analytical Chemistry (INQUIMAE) University of Buenos Aires, and colleges developed different Electronic Nose (EN) models for the analysis of complex aroma composition systems in perfumery, food science, flavor release. EN was also used in the detection of volatiles compounds in water (Rodriguez *et al*. 2010, Monge *et al*. 2008, Negri & Bernik 2008, Diz *et al*. 2006, Monge *et al.* 2004a, Monge *et al*. 2004b, Branca *et al*. 2003).

In 2005 Prof. Martin Negri and Delia Bernik, Associate Professor in the Biotechnology and Food Technology Department, Engineering Faculty, Universidad Argentina de la Empresa, designed and implemented an electronic device for discriminating, identifying and analyzing liquid, semi-liquid and solid samples containing volatile components. The device has a closable and thermally stable chamber where the sample is placed. The presence of both gas sensors and electrodes allow performing a simultaneous evaluation of odor and taste of the samples. The authors patented this device that was called "Flavometer".

In the 2001, Negri's group carried out a work on the determination of fish freshness. They developed a portable electronic nose, with 11 Metal Oxide Semiconductor (MOS) sensors, purchased from Figaro, and applied it to Argentinean hake freshness determinations. They tested a new sample methodology, different from that based on the headspace method or glass syringe manipulation. The sample was directly introduced into the measuring chamber and the signals of the sensors, due to the fish emission, were recorded as function of time. The change of the sample's aroma was monitored after some days of storage. Two different pattern responses were obtained associated to rotten and non-rotten samples. This study showed that the experimental approach had an excellent potentiality for applications, such as in situ quality control of fish freshness at fish markets. The experimental procedure is simple and can be performed by intermediate technical staff (O'Connell et al. 2001).

Few years later, in 2004, the group studied the flavor release by the electronic nose. The release of volatile essences from gels is an issue of high relevance in food technology and food

colloids science, particularly in the case of a multi-component flavoring essence encapsulated in a biocompatible gel. The most popular methods for analyzing the flavor release are based on static headspace, followed by gas chromatography with flame ionization detection (GC/FID) or gas chromatography combined with mass spectrometry (GC/MS). Martin Negri and Delia Bernik, in collaboration with other colleges, used an electronic nose device consisting of an array of 10 non-specific Figaro's gas sensors and a sample chamber developed in their laboratory. The device was used to follow the flavor release kinetics of a multi-component essence encapsulated in gels made of commercial citrus hight-methoxylated pectin and "tutti-frutti" commercial essence. The whole data obtained from "tutti-frutti" release, detected with the e-nose, were analyzed by PCA. The results showed that PCA provided a good graphical description of the release behavior in different samples and that this type of analysis could be used to follow the flavor release over different days (Monge *et al.* 2004a)

Later, the group studied by the same tool the flavor release of other odorant molecules, such as limonene and multicomponent essential oils from flowers and leaves of *Tagetes minuta* L. and *Mentha pulegium* L. (*Poleo*). The electronic nose allowed discriminating samples according to a high or low limonene content. It is remarkable that the fingerprint of encapsulated complex mixtures differs from that obtained for the non-encapsulated oils, showing a preferential release of some encapsulated components (Monge *et al.* 2004b).

Moreover, they studied the influence of matrix viscoelastic properties on flavor release. The electronic nose technique and PCA were used for detecting changes in the fingerprint of the released vapor as a function of pectin concentration. The parallel study of the viscoelastic properties (by rheometry) and flavor release (by E-nose) provided a direct relation between the decrease of flavor release and the increased solid-like character of the matrix. This result showed the potentiality of E-nose in flavor release studies from encapsulation matrixes, a relevant issue in food technology, cosmetic chemistry and pharmacology (Monge *et al.* 2008).

Another electronic nose device, even developed at Negri's laboratory, was used for discriminating between samples of n-primary alcohols and phenol in water. Primary alcohols and phenol are some of the volatile organic compounds (VOCs) commonly found in wastewaters. Phenol is highly toxic for the microorganisms employed for the biological wastewater treatments. Traditional methodologies to detect the VOCs normally require collecting samples (with bag samplers, canisters or passive tube) and process them afterward in the laboratory by gas chromatography for separation and with mass spectroscopy for quantification and/or identification of the components. By using an electronic nose, an approximate indication of the dissolved VOC constitutes is obtained rather than an accurate determination that was usually determined only after concentrating one or many of the compounds present in the sample. In fact, EN tries to discriminate among different samples by associating a fingerprint to each one. The results show that the different alcohol can be discriminated by electronic nose methodologies responses. These results show the EN ability to establish the presence of primary

alcohols and phenol in wastewaters, including threshold limits for alarms in control of effluents (Diz *et al.* 2006).

In the 2008, Bernik and Negri used an electronic nose to detect and discriminate low amounts of codling moth pheromone (codlemone) immersed in a background odor of plants and fruit volatiles. The codlemone is a sex pheromone releases by female of *Cydia pomonella,* one of the most damaging pest insects in apple orchards worldwide. A fast and reliable methodology to detect changes in pheromone concentrations in the air would be desirable so that management decisions such as reinforcing with more dispensers and/or spraying prophylactic pesticide doses can be made rapidly. The authors trained an electronic nose to detect the cadlemone molecule in presence of other aromatic compound. The results of this work showed the feasibility of the use of an electronic nose to monitor low codlemone levels in the air of apple orchards with the presence of other potential masking agents, and opened a new way for the application of e-nose in pheromone control for pest management (Negri & Bernik 2008).

In the 2010 thegroup studied the change~~s~~ along days of the aroma released from a flavor ("tutti-frutti" essence) encapsulated in a polysaccharide gel matrix using the electronic nose methodology. The purpose is to explore the capacity of the sensor array to assign a pattern of aroma to the corresponding release over time within a total period of five days. Different procedures of data treatment and analysis were compared in order to achieve the maximum of information on the system under study in conditions of limited number of measurements. The electronic nose device allowed a highly satisfactory classification of the samples according to their aging (0, 1, 2, 3 and ≥4 days respectively) (Rodriguez *et al.* 2010).

Device description

The ENs used at the University of Buenos Aires are very simple home-made devices and consist of an array of non-specific commercial gas sensors and a sampling chamber.

The array, which is the heart of the instrument, consist of 12 gas sensor (tab. 5.A), purchased from Figaro company, based on tinoxide, whose electrical conductivity changes when exposed to the volatile compounds (thus, signal are indicated in volts). For each sensor, a voltage proportional to the respective electrical conductance is digitalized (12 bits resolution with voltages within 0–5 V).

Tab. 5.A. MOS Sensor array configuration of the *E-nose INQUIMAE*

Sensor No.	Sensor Code	Specificity from Figaro Inc, Japan
S1	TGS 880	Organic Solvent Vapors
S2	TGS 816	Natural gas and LPG monitoring
S3	TGS 823	Organic Solvent Vapors
S4	TGS 826	Amines
S5	TGS 825	Sulfur volatiles
S6	TGS 882	Organic solvent (alcohol)
S7	TGS 842	Methane
S8	TGS 2602	Air Contaminants
S9	TGS 2600	Air Contaminants
S10	TGS 2620	Volatile Organic Compound
S11	TGS 2610	LP gas (e.g. propane and butane).
S12	TGS 2611	Methane

Sensors' signals were continuously recorded after closing the chamber, up to a steady state was reached by each sensor signal, and stored on a laptop (fig. 5.a).

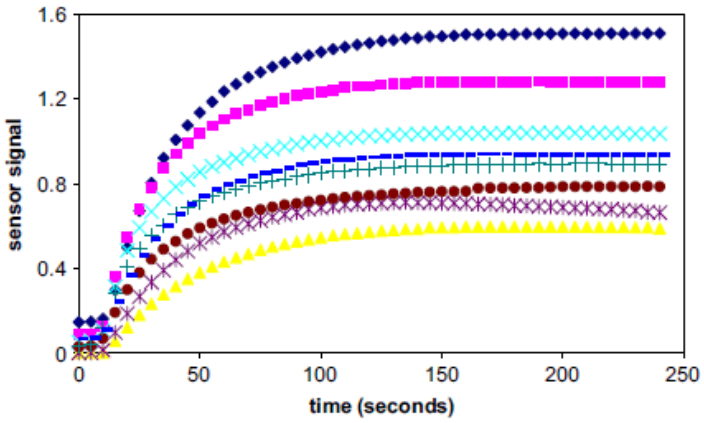


Fig. 5.a. A representative e-nose measurement. Each curve represents the signal of one individual sensor of the array. The vapors were aspirated since time zero and the signals increase up to reach a plateau. The value of signals at the plateau is the information used for the chemometric data analysis.

A home-made software allows to plot the responses of the sensors during the measurement. The same software is not able to perform a multivariate statistical analysis that is usually constructed with commercial software after determining which feature has to be used for representing the sensor's signal. Normally data normalization is the only pre-processing step.

With this type of EN, the discrimination of samples with differences in composition can be performed by simple visual check of the raw sensors data in the simplest cases (with radar plot of sensors' responses) or, when this not is possible, the multivariate data analysis methods can be

applied such as Principal Components Analysis (PCA) or Cluster Analysis (CA) among many others. Both PCA and CA are unsupervised methods, which group the input data according to similarities following user defined criteria for variance analysis.

The use of home-made e-nose provides many advantages similar to other commercial devices such as:

- Low operational costs
- Selection of sensors according to the analytical problem (non-specific chemical sensors find a large field of application when the analytical problem is that of comparing different samples, searching for similarities and differences)
- No destruction of the sample
- Transportability of device.

In our studies we used two different INQUIMAE's EN, with the same gas sensor array, but with different volatile sampling methods. One of them is a static sampling of volatile compound while the other one is a dynamic sampling by an aspiration pump of volatiles.

- ***Static sampling***

In this system the sample chamber is cylindrical (10cm *i.d.* x 8.5cm). The samples are introduced directly into the sealed chamber where on the top the sensors are placed. The sensors clean, between a measure and the other was made manually with hot forced air.

This approach has the advantages of instrumental simplicity, and of low influence of external environmental factors during the measurement.

The name for this type of device is "*Nariz Estanca*"(fig. 5.b)

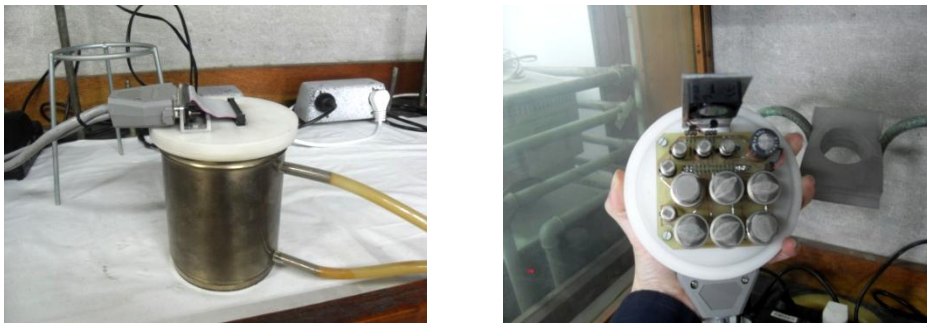


Fig. 5.b. *Nariz Estanca* (L); MOS sensor array of *Nariz Estanca* (R).

- ***Dynamic sampling***

The sample is putted in a sealed vials and an aspiration pump draw the headspace by a needle inserted into the silicon top. The aspiration flow was of 1 dm^3/min and it starts when the regulating valve is on switch-on. The sensors purge is made with pure air. The air is sufficient to completely remove odors from the sensors and sensor chamber.

The name for this type of device is "*Nariz por Aspiracion*" (fig. 5.c).

Fig. 5.c *Nariz por Aspiracion*.

5.1. Discrimination of hand fresh squeezed and commercial grapefruit juices.

Authentication of fruit flavors as natural is important in assuring compliance with labeling regulations. Methods of adulteration become sophisticated in response to the developing of new detection methodologies.

For example, significant research has been conducted to detect the adulteration of citrus essential oils. Methods of adulteration detection include the analysis of the ratio of key terpene components, enantiomeric compounds such as linalool, and non-volatile components such as coumarin. Testing the presence of flavor solvents is also a useful practice for detecting adulteration of citrus and other natural fruit essences, oils and extractives (Rouseff & Leahy 1995). However, all of these methodologies are time-consuming. In recent times, many researchers have employed EN for discriminating natural from commercial products.

We used two different types of EN for differentiating the hand fresh squeezed from commercial grapefruit juice.

5.1.1. Materials and Methods

A brick of 100% grapefruit juice was purchased at a local market ("Citric" juice). Fresh grapefruit (*Citrus paradisi Macf.*) fruits of the cultivars 'Star Ruby' (grown at Santiago del Estero), 'Rouge La Toma' (grown in Salta), 'Rio Red' (grown in Entre Rios) were purchased at Central Market of Buenos Aires (fig. 5.1.a).

cv. 'Rouge la Toma' (grown in Salta)

cv. 'Star Ruby' (grown in Santiago del Esteso)

cv. 'Rio Red' (grown in Entre Ríos)

"Citric" (commercial grapefruit juice)

Fig. 5.1.a Materials used: grapefruit's fruits of different cultivars samples cv. grown in Argentina and commercial grapefruit juice.

Chemical parameters (e.g., soluble solids content, titratable acidity, pH) and other fruit's characteristics (e.g., weight, longitudinal and transversal diameter) were analysed with classical methods. Fresh grapefruit juice was obtained by careful hand-squeezing of five fruits for each cultivar, in a home juicer. The juice percentage (w/w) was also detected.

Electronic nose (static): "Nariz Estanca"

An open Petri's dish with the sample (6ml of juice) was placed into the closed chamber of the e-nose for each measurement. On the top of the chamber an array of sensor was positioned, as above described, with a fan to homogenize the air inside the chamber. Sensors' signals were continuously recorded (a point of each 5 sec.) after closing the chamber, up to a steady-state situation for which the sensor signals reached a plateau (total time measurement: 10min). A set of 12 signals $\{S_1, ..., S_{12}\}$ was obtained for each experiment. The values of the sensor's signals at the plateau were used for the analysis after subtraction of the corresponding baseline (made with water as blank) ($F_e = R_{600s}-R_{LBA(600s)}$). Each sensors' signal (R_1.... R_{11}) is indicative of the electrical conductance increase in the respective sensor due to the presence of volatile aromatic compounds.

The Principal Component Analysis was calculated with covariance matrix of data (by S-Plus2000 software).

Electronic nose (dinamic): "Nariz por Aspiracion"

The sample (3ml of juice) was put in a sealed glass vial (10ml volume). Then it was attached to the sensor chamber pearcing the top with a needle linked to a tube non-reactive to flavor compounds. Sensors' signals were continuously recorded (a point of each 5 sec.) up to a steady-state situation for which the sensor signals reached a plateau (total time measurement: 5min). A set of 12 signals {S1 ,..., S12} was obtained for each experiment. The values of the sensor's signals at the plateau were used for the analysis after subtraction of the corresponding baseline (made with water as blank) ($F_a = R_{300s}-R_{LBA(300s)}$), in some cases different feature was used ($F4 =(R_{25s}-R_{LBA(25s)})(R_{50s}-R_{LBA(50s)})(R_{75s}-R_{LBA(75s)})(R_{300s}-R_{LBA(300s)})$).

Each sensors's signal (R_1.... R_{11}) is indicative of the electrical conductance increase in the respective sensor due to the presence of volatile aromatic compounds.

The Principal Component Analysis was calculated with covariance matrix of data. In some situation, Cluster Analysis was used to better understand the results (by S-Plus2000 Software).

5.1.2. Results and Discussion

The quality parameters studied ('Rouge la Toma', 'Star Ruby', 'Rio Red', "Citric" commercial juice) for the sample are shown in table 5.1.A. Comparing the fresh samples, the highest percentage of juice was found for 'Rio Red' with 47.00 ±0.94 % (w/w) value. Comparing parameters common to all samples, the lowest acidity was observed in "Citric" sample, while the highest soluble solid (SS) was found for 'Star Ruby'.

The value of conductivity, expressed in milli-Siemens (mS), that reflects the concentration of electrolytes like sodium and chloride ions in the samples and is correlated to acidity value, was lowest for "Citric".

Tab. 5.1.A Quality parameters of grapefruit fruits and their juices and commercial juice

Quality Parameters:	***Samples:***			
	'Rouge la Toma'	***'Star Ruby'***	***'Rio Red'***	***"Citric"***
Weight (g)	318.32 ± 18.25	208.22 ± 14.28	2.32 ± 25.19	/
DT (cm)	9.12 ± 0,29	7.81 ± 0.22	8.14 ± 0.35	/
DL (cm)	8.66 ± 0.33	7.44 ± 0.24	7.20 ± 0.37	/
% juice (w/w)	41.70 ± 0.72	38.71 ± 0.81	47.00 ± 0.94	/
pH	2.83 ± 0.1	2.73 ± 0.1	2.75 ± 0.1	2.70 ± 0.1
Acidity (g/L citric ac.)	5.51 ± 0.37	5.68 ± 0.20	6.73 ± 0.23	4.02 ± 0.30
SS (°Brix$_{20}$)	17.02 ± 0.11	19.1 ± 0.20	17.1 ± 0.25	17.6 ± 0.15
Conductivity (mS)	4.06	3.89	4.12	2.88

The aromatic patter was carried out using both type of INQUIMAE's EN above described.

Nariz Estanca results:

Figure 5.1.b shows the PCA score plot of data pre-processed by taking the feature F_e (R_{600s}-$R_{LBA(600s)}$). The two principal component (PC1 and PC2) account for about 99.0% of the total variance and it is possible to note a clusterization between the samples that appeared well distinct. The commercial sample ("Citric") is well distinct from the fresh hand squeezed samples on the PC2. Moreover the latter appeared well discriminate among them on PC1.

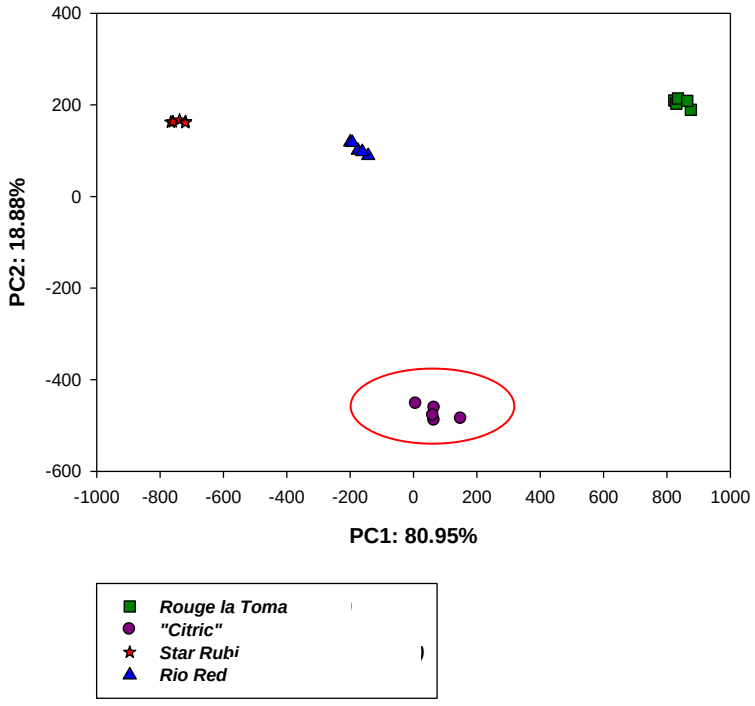


Fig. 5.1.b. PCA score plot of *Nariz Estanca*'s data (Feature F_e) for three fresh squeezed juices (from 'Rio Red', Rouge La Toma', Star Ruby' grapefruit cultivars) and a commercial grapefruit juice ("Citric")

Nariz por Aspiracion results:

Figure 5.1.c shows the PCA score plot of data pre-processed by taking the feature F_{ad} $(R_{300s}\text{-}R_{LBA\ (300s)})$. Even though the two principal components (PC1 and PC2) account for about 96.0% of the total variance, only the commercial sample ("Citric") appeared well distinct from the fresh hand squeezed ones and from the water, used as standard. In fact, the fresh squeezed sample can be discriminated, only when the third principal component is included, as shown in 3D sore plot PCA.

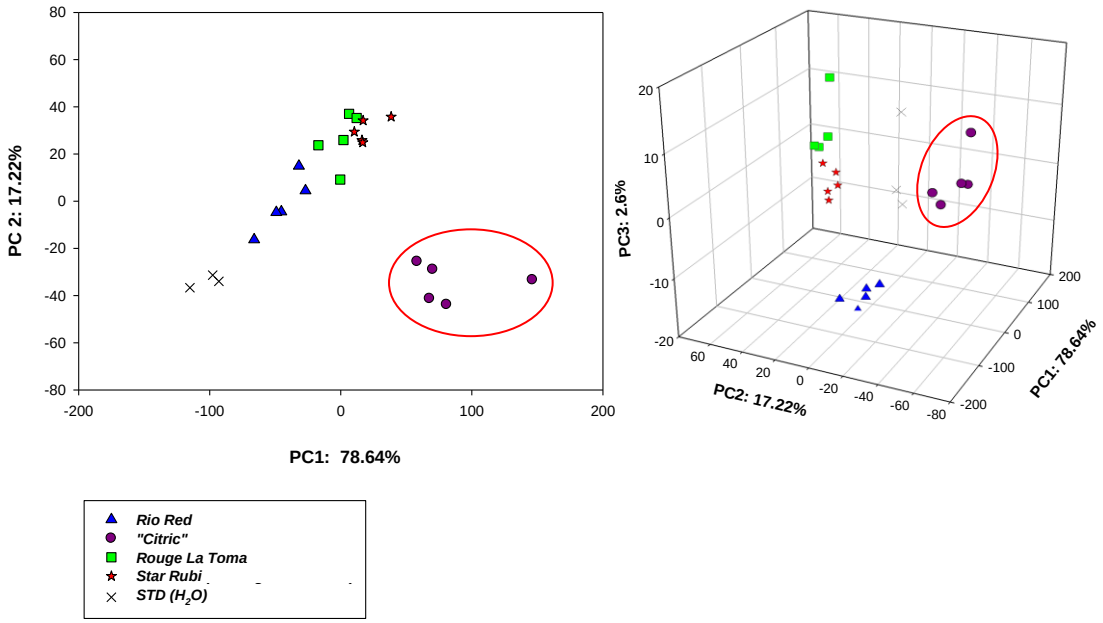


Fig. 5.1.c PCA score plot of *Nariz Aspiracion*'s data (Feature F_a) for three fresh squeezed juices (from 'Rio Red', Rouge La Toma', Star Ruby' grapefruit cultivars), commercial grapefruit juice ("Citric") and water, used as Standard. 2D (on L.) and 3D (on R.)

To check if the exclusion of data relative to the standard may help for providing a better discrimination, PCA analysis was repeated without including water's data. The results are shows on figure 5.1.d , but this was not the case.

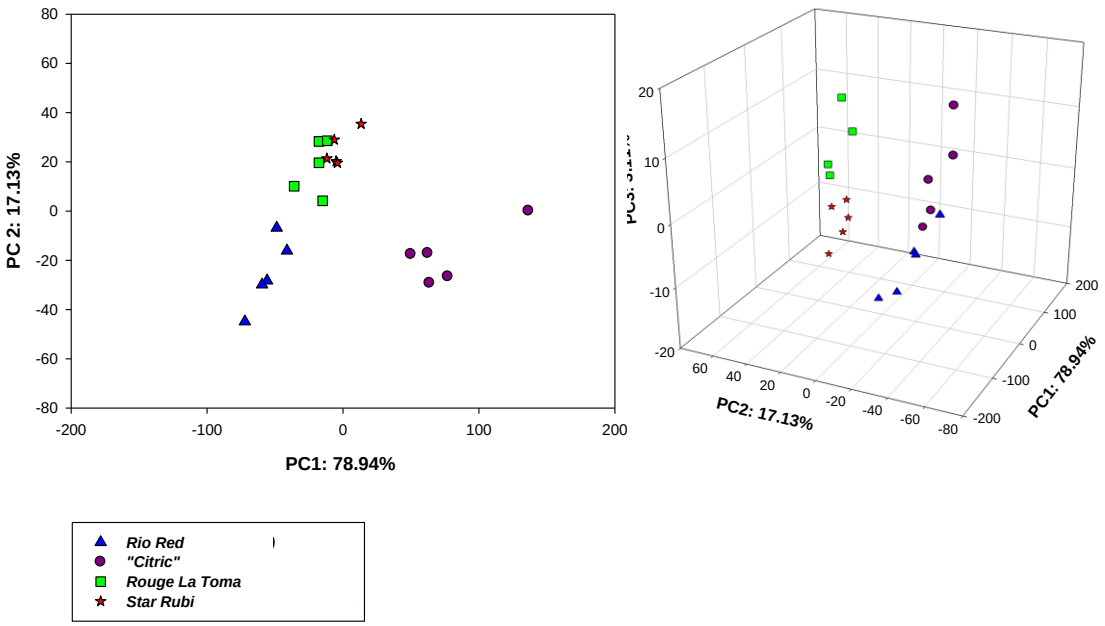
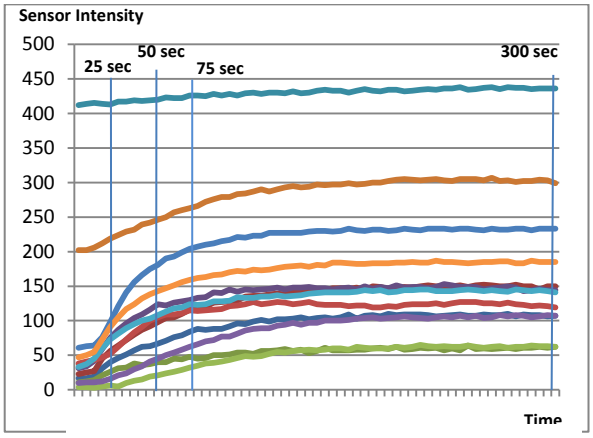


Fig. 5.1.d PCA score plot of *Nariz Aspiracion*'s data (Feature F_a) for three fresh squeezed juices ('Rio Red', 'Rouge La Toma', 'Star Ruby' grapefruit cultivars) and a commercial grapefruit juice ("Citric") without Standard. 2D (on L.) and 3D (on R.)

A further attempt to obtain a better discrimination was done by increasing the information amount of raw data. We extracted a new feature by taking the sensors response at different time (F4= $(R_{25s}\text{-}R_{LBA(25s)}),(R_{50s}\text{-}R_{LBA(50s)}),(R_{75s}\text{-}R_{LBA(75s)}),(R_{300s}\text{-}R_{LBA(300s)})$ as show here:



Than a PCA was calculated. The figure 5.1.e shows the PCA score plot with the two principal components (PC1 vs. PC2). The two principal components account for 86% of the total variance, so that the third component was also considered as show in figure e. Samples appear even worse discriminated, thus indicating that the new added information were poorly correlated.

Moreover a Cluster Analysis (with Partitioning Around Medoids, number of clusters: 4) was calculated by the PCA data provided from the feature F4, to find the optimal method for the discrimination. Even the Cluster Analysis fails to correctly distinguish the fresh squeezed samples from the commercial juice (figure 5.1.f).

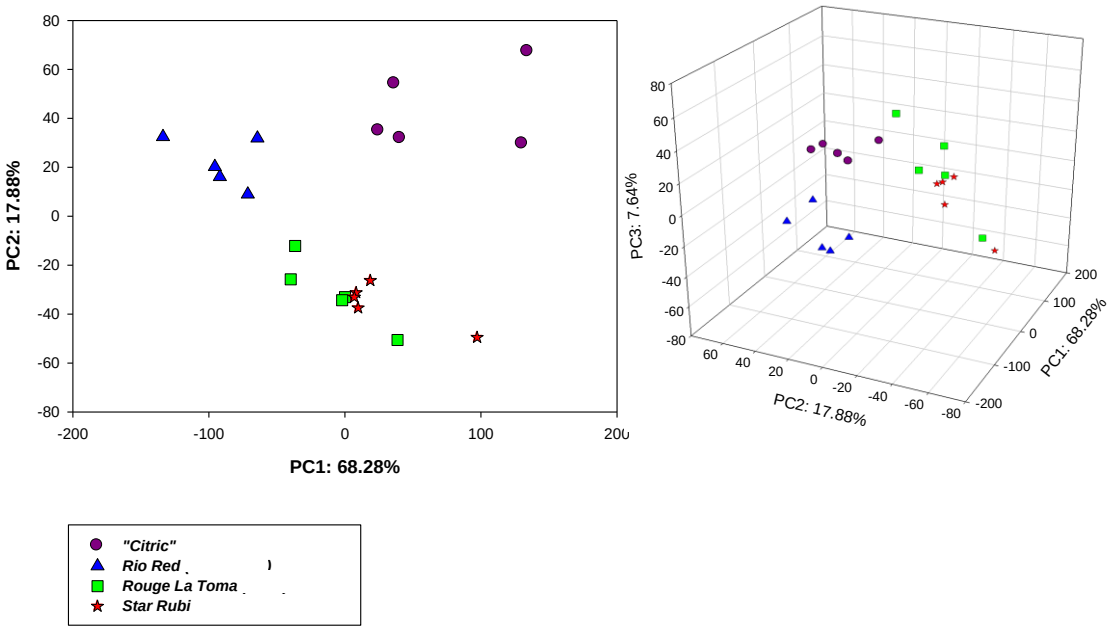


Fig. 5.1.e PCA score plot of *Nariz Aspiracion*'s data (Feature *F4*) for three fresh squeezed juices ('Rio Red', 'Rouge La Toma', 'Star Ruby' grapefruit cultivars) and a commercial grapefruit juice ("Citric") without Standard. 2D (on L.) and 3D (on R.)

#	Sample	Cluster id.
1	Citric 1	1
2	Citric 2	1
3	Citric 3	1
4	Citric 4	2
5	Citric 5	2
6	Rio Red 1	3
7	Rio Red 2	3
8	Rio Red 3	3
9	Rio Red 4	3
10	Rio Red 5	3
11	Rouge La T. 1	4
12	Rouge La T. 2	4
13	Rouge La T. 3	4
14	Rouge La T. 4	4
15	Rouge La T. 5	4
16	Star Ruby 1	4
17	Star Ruby 2	4
18	Star Ruby 3	2
19	Star Ruby 4	4
20	Star Ruby 5	4

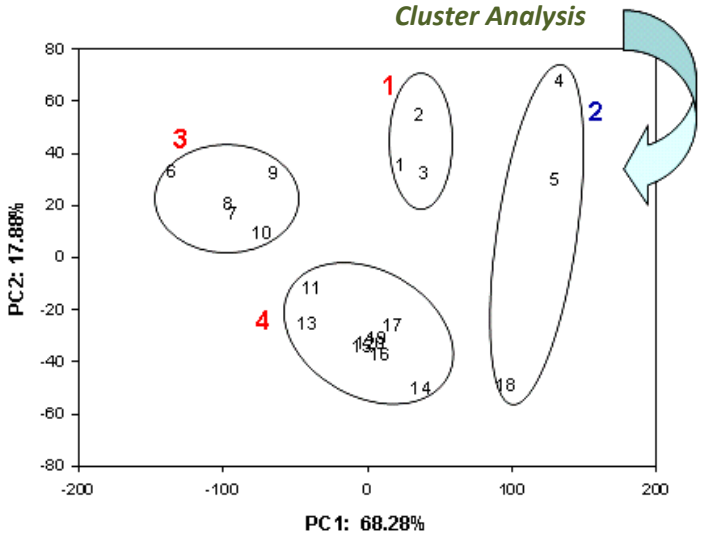


Fig. 5.1.f Results of cluster analysis (cluster id.) for the three fresh squeezed and commercial grapefruit juices PCA data (feature F4). Graphic representation of clusters from the cluster analysis.

5.1.3. Conclusions

Overall results suggest that although the *Nariz por Aspiracion* is able to discriminate between fresh squeezed juice and commercial juice, it is less powerful than *Nariz Estanca* in distinguish fresh squeezed juices from different cultivars, at least under the experimental conditions assayed.

5.2. Discrimination of 3 grapefruit (Citrus paradisi Macf.) cultivars, white and red pulp fruits, stored for 10 days at room temperature.

The characteristics of grapefruit juice as flavor and smell are extremely important in conferring value to the product.

We tested the capability of EN to distinguish between juices of different cultivars. Moreover we tested the capability of EN to discriminate samples of the cultivars that are stored at room temperature for 10 days.

5.2.1. Materials and Methods

Fresh grapefruit (*Citrus paradisi Macf.*) fruits of the cvs. 'Star Ruby' (Entre Ríos), 'Marsh Seedless' (know in Buenos Aires as 'Marshall') and 'Thompson' grown in Argentina were purchased at Central Market of Buenos Aires (fig. 5.2.a).

cv. 'Marshall' (grown in Corrientes)

cv. 'Thompson' (grown in Formosa)

cv. 'Star Ruby' (grown in Entre Ríos)

Fig. 5.2.a Sample: cultivar of grapefruit grown in Argentina.

5.2.2. Results and Discussion

The quality parameters for 'Marshall' and 'Thompson' (both white pulp fruits) and 'Star Ruby' (red pulp fruit) cultivars have been studied, when the fruit arrived in the laboratory (Time: 2nd June 2011). The results are shown in table 5.2.A.

The heaviest fruit was recorded in 'Star Ruby' (405.20 ± 30.00 g), but the major % of juice (weight/weight) was obtained from 'Thompson' (46.48 ± 1.2).

The lowest acidity (g/L citric ac.) and soluble solid (°$Brix_{20}$) was observed in 'Thompson' samples, while the highest soluble solid was found for 'Marshall' which has an acidity value comparable to 'Star Ruby's value.

The conductivity value, expressed in milli-Siemens (mS), that reflects the concentration of electrolytes like sodium and chloride ions in the samples and it is correlate to acidity value, was found lower for 'Marshall' and higher for 'Thompson'.

The Oxidation Reduction Potential (ORP), measured in milli-volts (mV) and indicating whether a solution is oxidizing or reducing, was also calculated. Any positive number indicates that the solution is oxidizing and of course, any negative number indicates a reducing or deoxidizing tendency. The highest ORP value was observed in 'Thompson'.

Tab. 5.2.A Quality parameters of three grapefruit cultivars

Quality Parameters:	***Samples:***		
	'Marshall'	***'Thompson'***	***'Star Ruby'***
Weight (g)	374.60 ± 15.00	282.13 ± 17.50	405.20 ± 30.00
DT (cm)	9.86 ± 0.24	8.79 ± 0.30	10.24 ± 0.25
DL (cm)	8.30 ± 0.16	8.44 ± 0.22	8.82 ± 0.28
% Juice (w/w)	42.40 ± 1.30	46.48 ± 1.20	44.14 ± 0.90
pH	3.00 ± 0.1	3.23 ± 0.1	3.10 ± 0.1
Acidity (g/L citric ac.)	7.18 ± 0.25	5.43 ± 0.35	7.26 ± 0.29
SS (°$Brix_{20}$)	19.46 ± 0.95	16.88 ± 0.55	18.19 ± 0.30
Conductivity (mS)	4.14	5.02	4.48
ORP (mV)	- 138.70	- 117.70	- 155.80

The aromatic patter was carried out using both type of INQUIMAE's EN above described to discriminate the differences between aroma cultivars.

The fruit was stored at room temperature for 10 days (from 2nd to 13th June 2011) and the aromatic patter was monitoring.

Nariz Estanca results:

Figure 5.2.b shows the PCA score plot of data pre-processed by taking the feature F_e (R_{600s}-$R_{LBA(600s)}$) when the fruits arrived in the laboratory (2nd June 2011). The two principal components (PC1 and PC2) account a total variance of 99.7%. An appreciable clusterization between the different cultivars was found.

Considering the intensity of each sensor's signal (data no reported), the PC1 showed a linear trend from water, with no volatile compounds, to 'Thompson', with, probably, most volatile compounds.

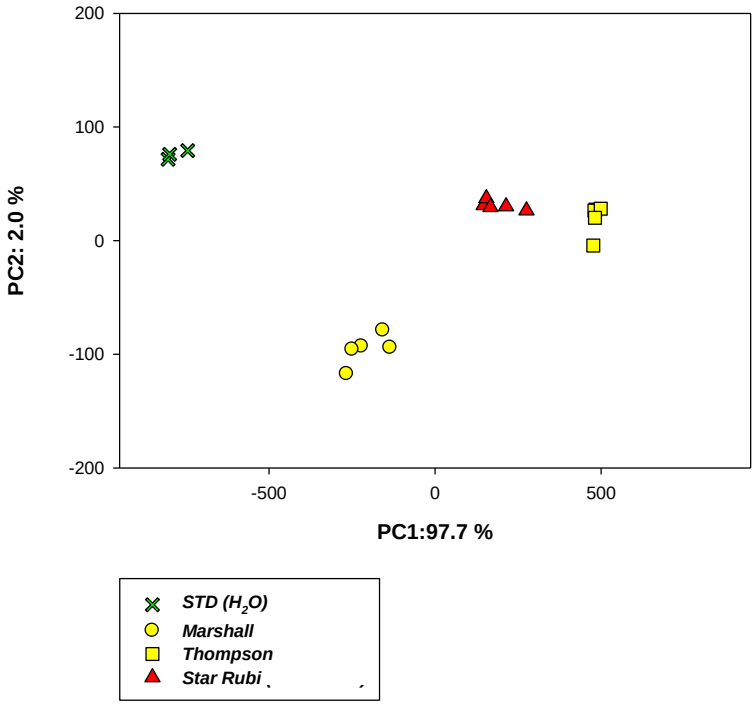


Fig. 5.2.b PCA score plot of *Nariz Estanca*'s data (Feature F_e) for three grapefruit cultivars and tap water as Standard (STD).

The grapefruit's cultivar aromatic pattern after 10 days was measured by *Nariz por Estanca*. The PCA score plot is shown in figure 5.2.c.

The total variance percentage values (PC1 and PC2) did not change.

The storage time, of the grapefruit samples, was mostly reflected on PC1, where the 'Thompson' and 'Star Ruby' samples at 10 days of storage present an appreciable shift. The 'Marshall' samples showed a little different behavior: they have an appreciable shift only on PC2.

An additional consideration, down reported, explains better the reason of these different behaviors.

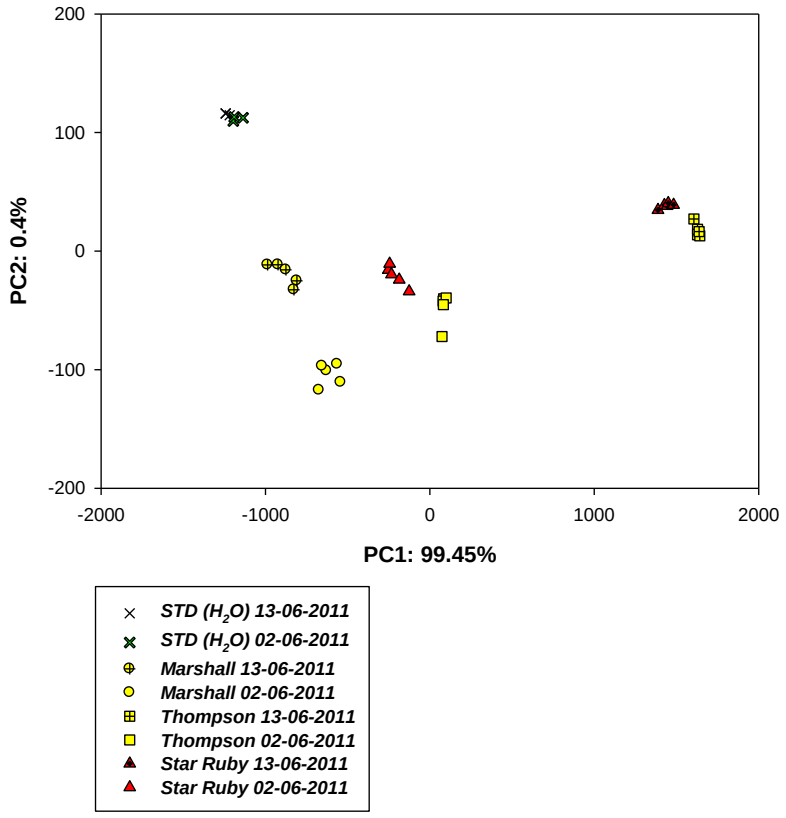


Fig. 5.2.c PCA score plot of *Nariz Estanca*'s data (Feature F_e) for samples of three grapefruit cultivars and tap water (as Standard - STD) at two different storage time: when the fruit arrived in the laboratory (02-06-2011) and after 10 days (13-06-2011).

Nariz por Aspiracion results:

Figure 5.2.d shows the PCA score plot of data pre-processed by taking the feature F_a (R_{300s}-$R_{LBA(300s)}$) when the fruit arrived in the laboratory at the first day (2nd June 2011). The two principal components (PC1 and PC2) account a total variance of 92.31%. A clustering is conceivable among the different cultivars.

The grapefruit's cultivar aromatic pattern after 10 days was measured by *Nariz por Aspiracion*. The PCA score plot is shown in figure 5.2.e.

It is possible to note a variance percentage values change on PC1 and on PC2: the PC1 value decreases and the PC2 increases. The third component was also considered to a clear data vision. The storage time, of the grapefruit samples, was most reflected on PC2, where the samples after 10 days of storage present a shift. According to this result explaining, the Marshall' samples doesn't change the aromatic pattern during the storage time.

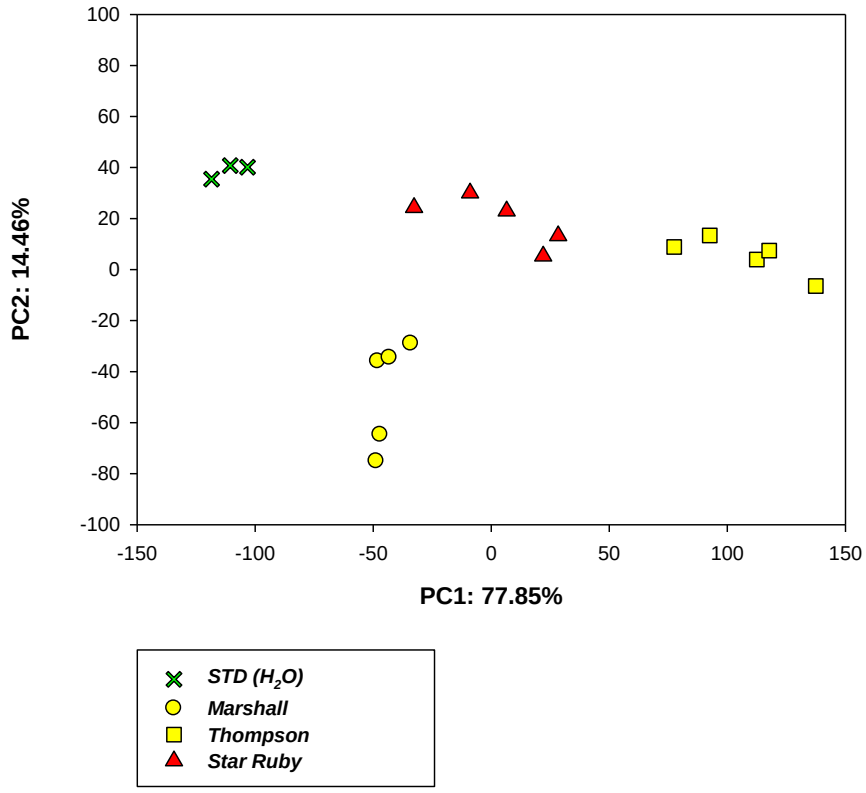


Fig. 5.2.d PCA score plot of *Nariz por Aspiracion*'s data (Feature F_a) for three grapefruit cultivars and tap water as Standard (STD).

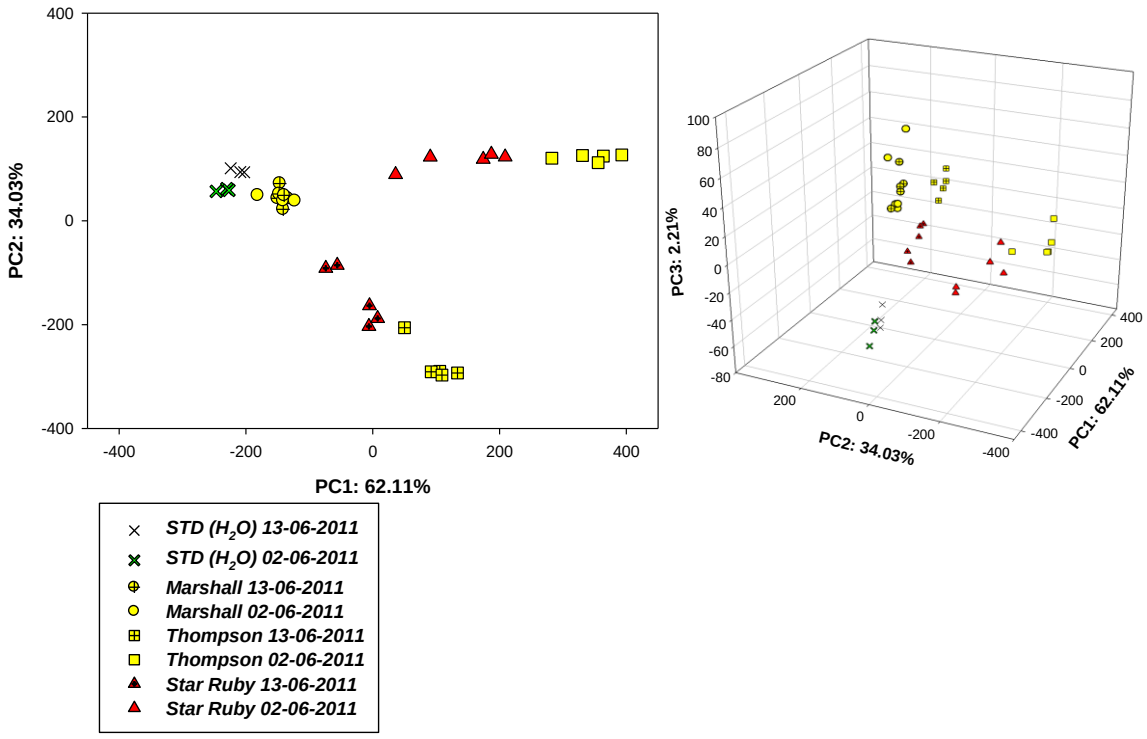


Fig. 5.2.e PCA score plot of *Nariz por Aspiracion*'s data (Feature F_a) for samples of three grapefruit cultivars and tap water (as Standard - STD) at two different storage time: when the fruits arrived in the laboratory (02-06-2011) and after 10 days (13-06-2011).

For both EN (*Nariz Estanca* and *Nariz por Aspiracion*) a closer comparison between the sensor's responses has been performed by radar plot. *Nariz Estanca* responses show that increases the intensity of the signal for each sensor maintaining a quite similar pattern (fig. 5.2.f). Instead *Nariz por Aspiracion* the increase of sensor's signal intensity is small and can be seen a little change in the pattern shape (fig. 5.2.g).

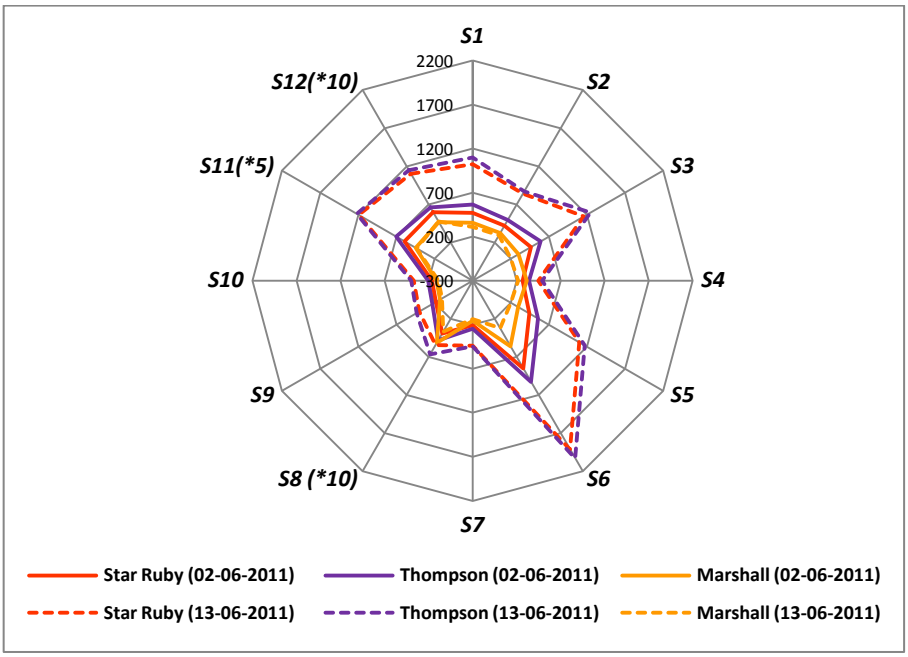


Fig. 5.2.f Sensors' responses at steady state for *Nariz Estanca* (600sec); the signal of S_8, S_{11}, S_{12} were multiplied by an arbitrary factor (respect 10, 5, 10) to better highlight the response on radial axis.

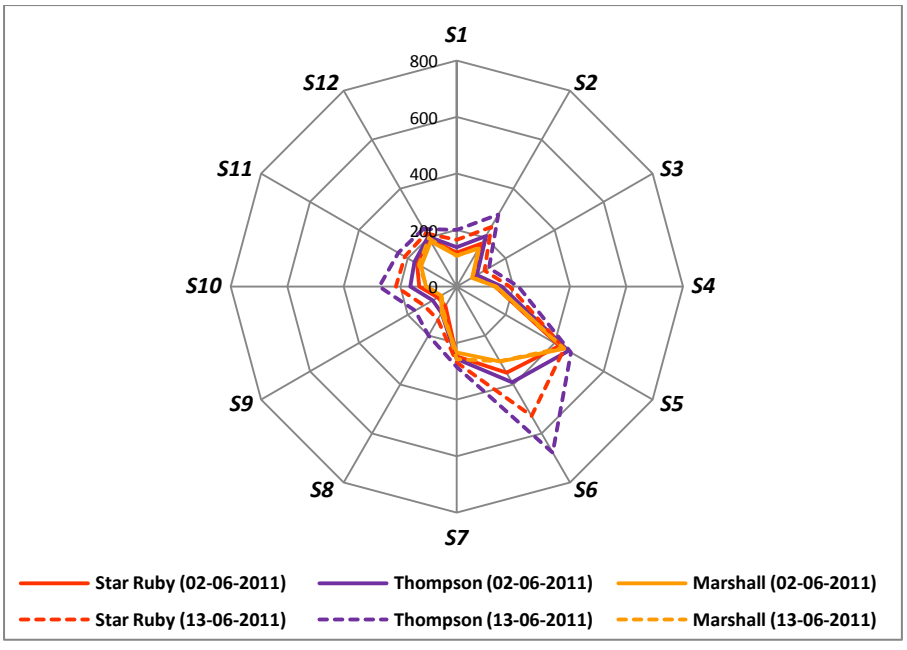


Fig. 5.2.g Sensors' responses at steady state for *Nariz por Aspiracion* (300sec).

For both EN (*Nariz Estanca* and *Nariz por Aspiracion*) the % change of each sensors response with time increasing was calculated for the three different cultivars ('Star Ruby', 'Thompson', 'Marshall'). The % change was calculated:

The results, plotted in figure 5.2.h and 5.2.i, evidence that the *Nariz Estanca* has sensitivity much higher than *Nariz por Aspiracion*, for fresh squeezed grapefruit juice.

Probably due to the sampling method, the results obtained with *Nariz por Aspiracion* are affected by the presence of environmental volatile compounds.

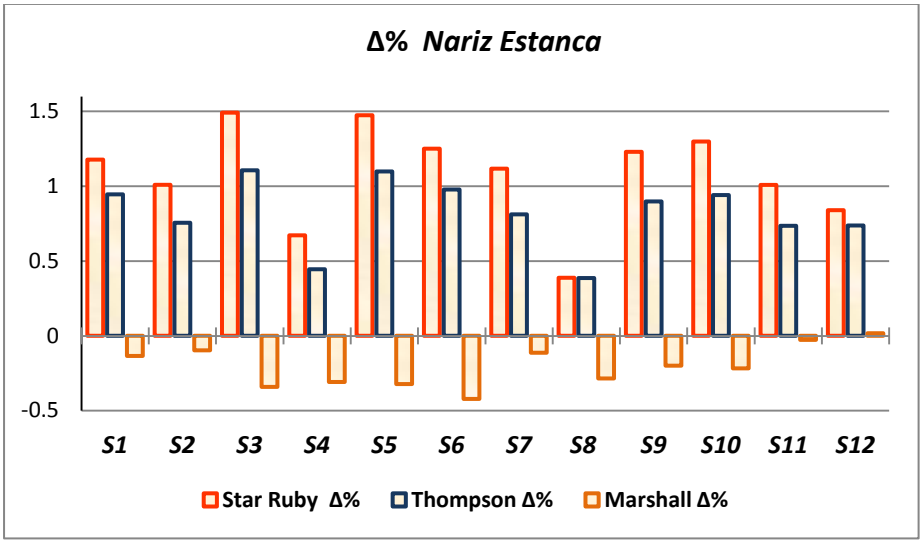


Fig. 5.2.h Percentage of intensity variation, for each *Nariz Estanca*'s sensor, measuring grapefruit fruit samples at two different storage time: when the fruit arrived in the laboratory (02-06-2011) and after 10 days (13-06-2011).

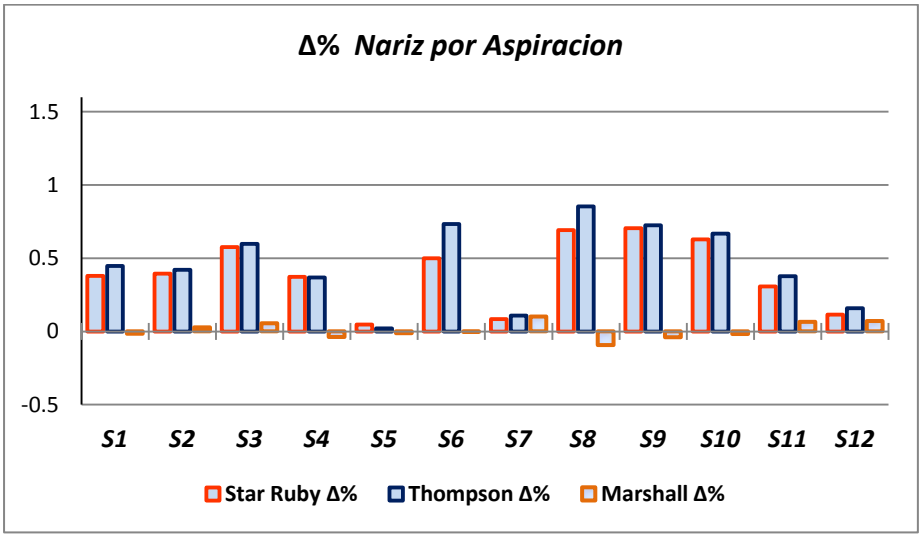


Fig. 5.2.i Percentage of intensity variation, for each *Nariz por Aspiracion*'s sensor, measuring grapefruit fruit samples at two different storage time: when the fruit arrived in the laboratory (02-06-2011) and after 10 days (13-06-2011).

5.2.3. Conclusions

The changes observed in the aroma pattern obtained with fresh fruits and the same lot of fruits measured after 10 days can be ascribed to different factors, such as light incidence, humidity changes, and the consequent natural oxidation and changes in hydration of the fruits. It seems to be a combination of factors, difficult to identify due to the large amount of components usually found in natural fruits. The study reflects the importance of the time of storage, even for non-climateric fruits.

6. Experiments in Spain *(E-nose IQMA,* U.P.V.*)*

From August to October 2011, I worked at the Instituto Agroforestal Mediterráneo (IAM), **Polytechnic University of Valencia** (U.P.V.) (Spain), with the group of **Prof. Manuel Agusti Fonfría**. We studied the volatile components of persimmon (*Diospyros kaki* L.) and quince (*Cydonia oblonga*) fruits, through the use of a homemade electronic nose and Gas Chromatography analysis with Mass Spectrometer detector.

Edoardo Garcia-Breijo and co-workers, from the Institute of Applied Molecular Chemistry (IQMA), Polytechnic University of Valencia, in 2009, designed and developed a homemade Electronic Nose System. They used commercial standard sensors to establish if some odors produced a characteristic response when the sensors worked in non-standard conditions.

After the initial test of the instrument, Garcia-Breijo's group applied E-nose IQMA to discriminate fish samples at different freshness levels.

At the moment Garcia-Breijo's group is working to improve the device performances so opening new fields of application. For example, they are planning to use this system for military application such as the monitoring of the presence of explosive and toxic gas.

Device description

The E-nose IQMA consists of 15 commercial sensors (FIGARO Engineering Inc., Japan), a homemade data acquisition system and a laptop in-line with the data acquisition system.

The sensors showed in table 6.A are of the MOS type and presents a commercial declared specificity for different gases (hydrogen, carbon, monoxide, butane, methane, etc.).

The measurement cycle for each sensor includes several steps: warming the heater, powering the sensor and measuring the sensor voltage.

The gas manager system includes two chambers: the concentration chamber (were the samples are introduced) and the measurement chamber (were the sensors are placed) (fig. 6.a).

The concentration chamber, where it is possible to put the whole fruit, has cylindrical shape (12cm i.d x 16cm h.) and it is connected to the measuring chamber with a pump. Once establishedAfter the equilibrium between the sample volatile molecules and the headspace has been established, the small pump draws the headspace from the chamber concentration and transfers it to the measuring chamber (12cm i.d x 14cm h.). A hard flow of air is used to purge the sensors.

To give flexibility to the equipment, the data acquisition system has been designed with a master-slave architecture (fig. 6.b and 6.c): each slave processor controls the measurement cycle of one sensor and the master processor collects the data from the 15 slaves and sends them to the laptop. The software used, Soft Nariz, homemade too, is able to record and plot the sensors

signal in a simple way (Masot *et al.* 2009). Really the device is not portable since it has been thought for laboratory measurements.

Tab. 6.A MOS Sensor array configuration of the *E-nose IQMA*

Sensor No.	Sensor Code	Specificity from Figaro Inc., Japan
S2	TGS 2201	Gasoline and Diesel Exhaust Gas
S3	TGS 823	Organic Solvent Vapors
S4	TGS 2106	Diesel Engine Exhaust Gas
S5	LM 35	Temperature
S7	TGS 2611	Methane
S8	TGS 3870	Methane and Carbon Monoxide
S9	TGS 2602	Air Contaminants
S10	TGS 826	Ammonia
S11	TGS 203	Carbon Monoxide
S12	TGS 2620	Volatile Organic Compound
S13	TGS 2610	LP gas (e.g. propane and butane)
S14	TGS 2180	Water Vapor Detection
S18	TGS 2442	Carbon Monoxide
S19	TGS 2612	Methane and LP Gas
S20	LM 35	Temperature

Fig. 6.a *E-nose IQMA* at the Institute of Applied Molecular Chemistry (U.P.V.) (Spain).

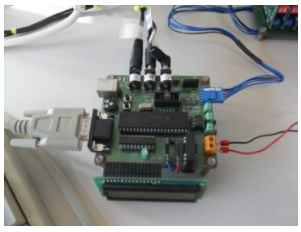


Fig. 6.b Acquisition system.

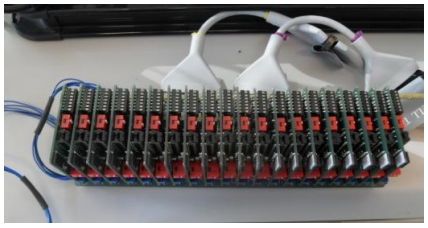


Fig 6.c Slave processors.

6.1. Discrimination of fruits aroma of 2 *Diospyros kaki* L. cultivars.

Persimmon (*Diospyros kaki* L.) is believed to be originated in China (Luo & Wang 2008), subsequently spread to Korea and Japan (Sugiura 1997), where it is a traditional crop, and then to other regions of the world. It gained popularity in Europe and in particular in the Mediterranean countries (Spain and Italy). In 2008 the main producers of persimmon are China (2,533,899 t), Korea (430,521 t), Japan (244,800 t), Brazil (169,000 t), Azerbaijan (132,179 t), Spain (70,000 t), Italy (50,000 t), Israel (30,089 t) and Uzbekistan (31,000 t) (FAOSTAT, 2010).

The persimmon color generally ranges from light yellow- orange to dark red-orange. Depending on the species, persimmons vary in size from 1.5 to 9 cm diameter, and may be spherical, acorn-, or pumpkin shaped. The calyx often remains attached to the fruit after harvesting, but becomes easier to be removed upon ripening. Persimmon fruits have high content of glucose, with a balanced protein profile, and present various medicinal and chemical uses (Ragazzini, 1985). These fruits are of great interest due to their content of biologically active important compounds (different types of carotenoids and vitamin C).

Persimmon cultivars are usually classified into two groups, astringent and non-astringent type, depending on the degree of astringency at the mature stage (Arnal & Del Rìo 2005, Matsuo 1998, Ragazzini 1985). When an astringent persimmon is eaten, the tannin cells in the flesh are crushed and soluble tannins are released, giving a strong astringent sensation (Taira 1996). The astringency disappears when soluble tannins become insoluble (Matsuo, 1998; Taira et al., 1997), at the mature stage. Persimmons are climacteric fruits whose ripening is regulated by ethylene (Wills et al., 1998). They are at their best quality at the end of the preclimacteric stage, when the sugar content reaches its maximum value and the required orange color of the fruit has developed just before the onset of the respiratory climacteric and the induction of ethylene. When astringent persimmons with firm texture are harvested, the fruit has not suffered the overripening that naturally removes their astringency. For this reason, astringent varieties of persimmon have a limited consumption as a fresh product and their features must be improved by technological processes (Agustì *et al.* 2006, Arnal & Del Rìo 2005, Hernàndiz 1999). Persimmon production is now increasing due to the application of techniques to remove astringency. This allows to commercialize and transport fruits still having a firm consistency (Arnal & Del Rìo 2003, Llàcer & Badenes 2002).

A few literature exists on the aroma characteristic of persimmon fruits, whereas the marketing of many products derived from persimmon flavor is continuously growing. We used gas chromatography with mass spectrometry technique to identify the aromatic pathway and the electronic nose, *IQMA,* to test its ability in discriminating the *odor fingerprint* of two different cultivar of *D. kaki* produced in Spain.

6.1.1. Materials and Methods

Two cultivars of persimmon (*D. kaki*) were used for the experiments. Samples of 'Rojo Brillante' (fig. 6.1.a) and 'Triumph' (fig. 6.1.b) were obtained from IAM's experimental orchard (near the Polytechnic University of Valencia). Twenty fruits for each cultivar were collected in October 2011. The fruits were ripened for commercial picking (Stage 8. 85 – BBCH scale; Garcia Carbonell et al. 2002). The fruits were selected with the aim of having high homogeneity in samples thus reducing the variability of the results.

Fig. 6.1.a *D. Kaki* cv. 'Rojo Brillante'.

Fig. 6.1.b *D. Kaki* cv. 'Triumph'.

Gas – chromatography

Purge and trap thermal desorption was used to extract the volatile compounds. Samples of each variety of kaki (30 g) were placed in a purging vessel flask and left in a water bath at 50 °C for 10 min. Purified nitrogen (100 mL/min) was forced through a porous frit placed at the bottom of the vessel. The stream of bubbles produced passed through the sample and collected the volatile compounds, which were trapped in 100 mg porous polymer (Tenax TA, 20-35mesh) packed into a glass tube placed at the end of the system. The volatile compounds were thermally desorbed using a direct thermal desorber (TurboMatrix TD, Perkin ElmerTM, CT-USA). Desorption was performed under a 10 mL/min helium flow at 220°C for 10 min. The volatiles were then cryofocused in a cold trap at −30°C and directly transferred onto the head of the capillary column by heating the cold trap to 250 °C (at a rate of 99 °C/s) (Escriche *et al*. 2011).

Finnigan TRACETMMS (TermoQuest, Austin, USA) was used to carry out the GC–MS analysis. Volatile compounds were separated using a DB-WAX capillary column (SGE, Australia) (60 m length, 0.32 mm i.d., 1.0 µm film thickness). Helium at a constant flow rate of 1 mL/min was used as a gas carrier. The temperature was programmed to increase from 40°C (2-minute hold time) to 190°C at 4°C/min (11-minute hold time) and finally to 220°C at 8 °C/min (8-minute hold time). The MS interface and source temperature was 250°C and 200°C, respectively. Electron impact mass spectra were recorded in impact ionization mode at 70eV and a mass range of m/z 33–433. A total of 3 extracts were obtained for each sample (Escriche et al, 2011). Identification of each molecule was carried out by comparing mass spectra with those contained in NIST and Wiley library databank.

Electronic Nose

The *E-nose IQMA*, whose detailed description is given above (Section 6.3.1 "Device description"), was used. A change of the apparatus was required for our experiments. In fact we directly connected the concentration and the measure chambers, bypassing the pump. This was done because in testing the method operations we noticed that the pump was not absolutely inert to the volatile molecules, but it absorbed part of them. Therefore a cross-contamination of aroma between subsequent measurements occurred. The new design of the system, with concentration and measurement chambers joint, is show on figure 6.1.c.

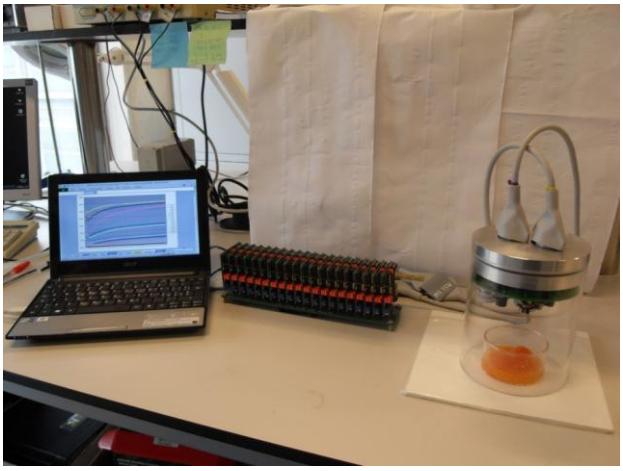


Fig. 6.1.c *E-nose IQMA* with joint concentration and measure chambers.

The measurement was carried out by putting inside the 'double chamber' half of cut persimmon fruit (40 g, pulp and skin), closing the top and starting to record data from sensors. Each measurement was stopped after 20min, when all sensors reached a steady-state (stable maximum value).

As a first step of EN's data pre-processing, the significant features were extracted from the sensors' response curves. In the present study, the feature considered was R, that is the maximum of sensor resistance during the exposure to sample headspace. Explorative data analysis was performed by Principal Component Analysis (PCA). The raw data matrix was a Covariance Matrix.

Colorimetric measurements

Changes in color were studied too. Color values were acquired by measuring the reflection spectrum in CIE-L*a*b* uniform colors space, with a spectrocolorimeter (Minolta, CM 3600D, Tokyo, Japan). In CIE-L*a*b*, L* indicates lightness while a* and b* indicate the chromaticity on axes ranging from green (–) to red (+) and from blue (–) to yellow (+), respectively.

6.1.2. Results and Discussion

GC analysis of the extracted volatiles showed relatively few compounds contributing to persimmons aroma. Table 6.1.A reports the integrated areas of all the molecules identified in the two cultivars, following their elution time from the GC column. The major present molecule is Ethanol. The aromatic pattern is qualitatively different for the two cultivars.

Tab. 6.1.A. Volatile compounds of Persimmon (*Diospyros kaki* L.)

R.T. (min)*	Identification (% Similarity)**	Chemical compounds	% Area ***	
			'Rojo Brillante'	*'Triumph'*
1.92	68.79	***Acetic acid. Anhydride***	-	0.27 ± 0.16
2.03	44.99	***Hidrogen azide***	0.23 ± 0.11	-
2.07	60.44	***Folic Acid***	-	0.24 ± 0.13
2.23	79.09	***Pentane***	0.21 ± 0.15	-
2.30	23.41	***1-propanol.2-methyl***	-	0.27 ±0.06
2.44	65.39	***Hexane***	0.16 ± 0.10	0.30 ± 0.17
2.55	53.76	***Ethyl Ether***	0.10 ± 0.07	-
2.72	17.14	***3-methyl Hexane***	tr.	-
2.89	64.96	***Heptane***	0.13 ± 0.10	-
3.03	70.05	***Propane + Acetaldeide***	37.00 ± 3.05	19.84 ± 5.19
3.96	53.61	***Propilene oxide***	0.22 ± 0.04	-
4.32	89.09	***Propanenitrile.2hydroxy.2 methyl***	-	0.90 ± 0.09
4.35	76.43	***Acetone***	1.38 ± 0.32	-
4.47	75.56	***1-propanol.2-methyl***	-	1.26 ± 0.43
4.50	75.49	***hydrazide-Acetic acid***	1.11 ± 0.31	-
5.50	98.74	***Ethyl Acetate***	1.11 ± 0.14	10.22 ± 4.06
5.68	61.81	***3-methyl Furan***	0.57 ± 0.12	-
5.89	33.94	***Ethoxy Ethene***	0.16 ± 0.01	-
6.20	32.68	***3-methyl Butanal***	0.13 ± 0.09	-
6.40	65.86	***Propil estere 2-hydroxy Propanoic Acid***	0.17 ± 0.01	-
6.61	90.6	***Ethanol***	52.19 ± 3.00	59.89 ± 7.76
7.72	24.78	***2-Pentanone***	0.17 ± 0.01	-
8.72	51.04	***trichloro-Acetaldehyde***	0.27 ± 0.16	-
9.27	95.06	***1-Propanol***	0.20 ± 0.03	0.33 ± 0.28
9.50	30.08	***2-propenilidene-Cyclobutene***	0.20 ± 0.03	-
10.41	15	***Acetyl bromide***	-	0.64 ± 0.57
10.59	73.71	***Ethanol. 2-2-(2 butoxyethoxy)ethoxy***	-	1.22 ± 0.96
10.87	51.3	***1-propanol.2-methyl***	-	0.34 ± 0.14
13.20	72.67	***1 butanol***	0.12 ± 0.02	0.40 ± 0.14
13.99	85.25	***Ciclohexene. 1 methyl 4 (1-methylethyldene)***	-	1.30 ± 1.20
14.35	90.34	***3-methyl-1-Butanol***	0.16 ± 0.06	0.76 ± 0.20
17.33	41.5	***Isopropyl alcohol***	-	0.85 ± 0.50
17.39	79.23	***3-hydroxy-2-Butanone***	0.48 ± 0.27	-
17.46	38.52	***Ethanol 2 methoxy***	-	0.63 ± 0.36

*** Mean of Retention Time; ** According to Nist and Wiley library; *** Mean value of relativity percentage of Area ± SD**

The PCA score plot (fig. 6.1.d) showed not only a distinct separation of fruit's aroma from atmospheric air (used as control), but also the distinguish ability of the two different cultivars of *D.kaki.* The first separation was on PC1 with 95.20% of variance and the second one on PC2 with 2.97% of variance. In this case it was not necessary to use more principal components.

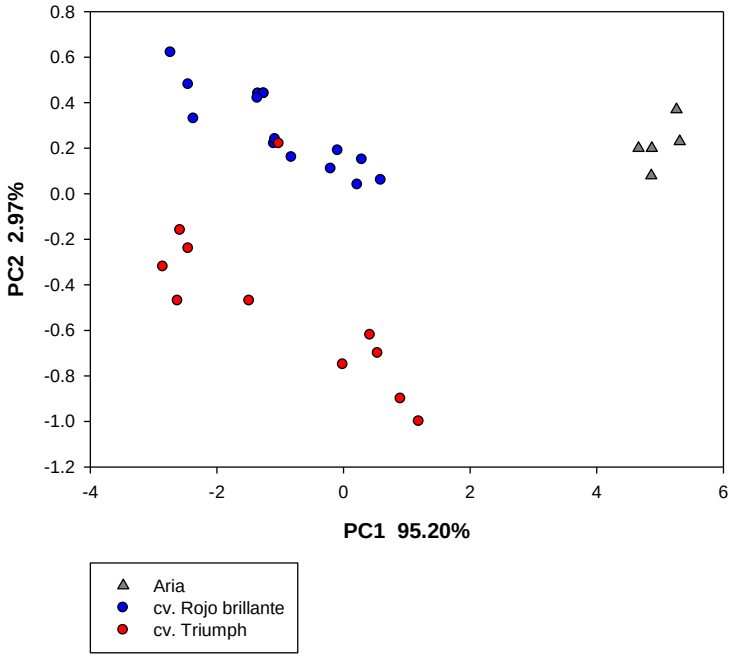


Fig. 6.1.d. PCA score plot of *E-nose IQMA* data for two cultivars *Diospyros kaki* L. : 'Rojo Brillante' and 'Triumph'.

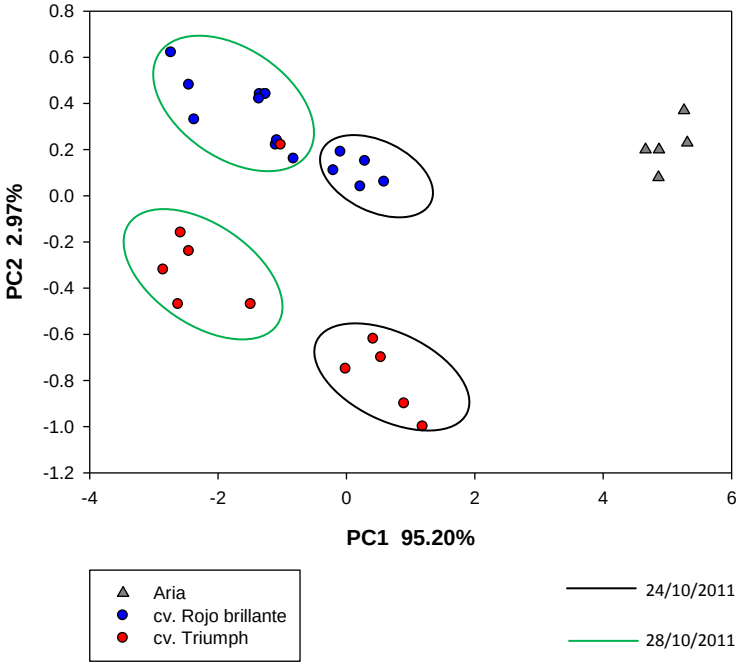


Fig. 6.1.e. PCA score plot of *E-nose IQMA* data for 'Rojo Brillante' and 'Triumph' cultivar according different ripe state.

Within cultivar's cluster it is possible to note two sub-clusters, according to the ripe state of fruit. The EN seems to be able to distinguish also the fruits according to the ripe state (fig. 6.1.e). The time evolution of color parameters and differences in samples of 'Rojo Brillante' and 'Triumph' persimmon is recorded in table 6.1.B. In both cases an increase of color parameters is observed over 4 days due to the ripening of these climacteric fruits.

Tab. 6.1.B Color parameters values of the two *Diospyros kaki* L. cultivars: time evolution.

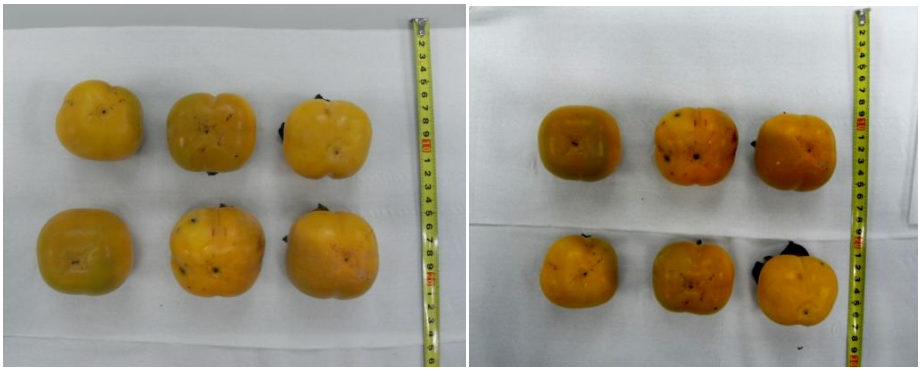
Cultivar:	**Data:**	**Color parameters[1]:**		
		*a**	*b**	*L**
'Rojo Brillante'	24/10/2011	15.65 ±0.68	33.84 ±0.77	57.53 ±1.05
	26/10/2011	19.16 ±0.34	33.43 ±0.17	57.12 ±0.28
	28/10/2011	22.90 ±0.97	32.80 ±0.70	56.43 ±0.77
'Triumph'	24/10/2011	8.55 ±1.07	29.17 ±0.52	52.75 ±0.35
	26/10/2011	10.17 ±1.34	31.10 ±2.53	54.57 ±3.01
	28/10/2011	10.47 ±1.73	30.15 ±0.17	53.68 ±0.33

[1]Mean value ± SD

Cv. 'Rojo Brillante' taken pictures at :

24/10/2011 28/10/2011

Cv. 'Triumph' taken pictures at :



24/10/2011 28/10/2011

6.1.3. Conclusion

The *E-nose IQMA* data show the ability of the instrument to distinguish two different cltivars of *D.kaki,* according to the GC-MS data. Moreover the instrument is capable to distinguish the fruits, inside the cultivar cluster, according to the ripe state after few days of storage.

6.2. Discrimination of fruits aroma of 2 *Cydonia oblonga M.* genotypes.

The study of particular flavor notes in uncommonly used vegetables and fruits receive the interest of the flavorist, who often searches and finds inspiration in natural sources not widely available. A rarely used flavor note is that of *Cydonia oblonga* Miller, fruit of the *Rosaceae* family, known in Italy as "mela-cotogna" ("quince" apple) and in Spain as "membrillo".

Quince fruit is a seasonal fruit. It has also recently received interest for its phenolic content (Silva *et al.* 2002, Silva *et al.* 2004) responsible for its antioxidant and free scavenging activities. A brief review of medical literature revealed that preparations from different parts of quince have been used as traditional remedies against cough, bronchitis, nausea, fever, diarrhea, cystitis, constipation, hemorrhoids, diabetes, hypertension. Their efficacy has been tested in several experimental or clinical studies.

Quince fruit is also widely used as a food, but being too astringent to be consumed fresh, it is frequently processed into a jam or jelly.

Few are the studies on the volatiles fraction of quince fruit. Some of them are focused on steam-distilled quince fruit oil (Tsuneya *et al.* 1983, Ishiara *et al.* 1986), in which a relevant number of C13 norisoprenoids were identified. But it is well know that the crushing process introduces flavor changes, presumably due mainly to the difference in the volatility of compounds such as esters and the monoterpene and sesquiterpene hydrocarbons. On the other hand, monoterpens and oxygenated compounds are vulnerable to steam distillation condition. Therefore, recent scientific literature has proposed alternative methods for the determination of volatile compounds, like HS-SPME (*head-space solid-phase microextraction*), that does not need particular treatments of fruits (Tateo *et al.* 2010). In more recent studies, more than 40 volatiles compounds were extracted, most of them was identified. Many compounds belonging to chemical classes as acetates, esters and sesquiterpens were found. Some of them, like *ethyl octanoate*, *ethyl hexanoate*, *ethyl decanoate*, *ethyl 2-octenoate*, *5-hesenyl acetate* and *ethyl acetate* increase with ripeness; instead, *3-hexenyl acetate*, *α-bergamotene* and *α-farnesene* decrease with ripeness.

A lot of interest has recently arisen from the possibility of revealing quince product adulteration by addition of apple and pear that have lower cost and similar texture. This type of adulterations can be monitored by detecting the presence of phloretin 2-xylosylosylglucoside and phloretin 2-glucoside, two dihydrochalcons found in apple, and arbutin found in pear and in another fruit from *Rosaceae* family (Andrade *et al.* 1998). It should be also possible to detect quince product adulteration by discriminating the volatiles compounds.

Solicited by the current adulteration problem and, more in general, by the request of modern technological approaches to be employed in the development of new flavors and fragrances, we checked the suitability of an electronic nose as rapid tool for identifying

adulteration agents or projecting new types of aroma. As preliminary step of this work we tested the EN sensibility to quince fruit's aroma.

6.2.1. Materials and Methods

Two genotypes of quince (*D. kaki*) were used for the experiments. Fruits of 'Vranja' and from a seedling tree (called in italian "Franco") (fig. 6.2.a), were obtained from IAM's experimental orchard (near the Polytechnic University of Valencia). Six fruits from the two genotypes were collected in October 2011. The fruits were harvested in a semi-ripening state, still turning color from green to yellow state.

Cv.Vranja

"Franco"

Fig. 6.2.a Materials used: quince fruits grown in Valencia.

Gas Chromatography

The volatile compounds were analyzed in the same way of persimmon fruits, as above described. Identification of the aromatic components was carried out by comparing the obtained mass spectra with those contained in NIST e Wiley library.

Electronic Nose

The instrument setting was the same used for the persimmon fruits analysis. The sample preparing was instead different: the mesocarp of quince was cut in small pieces and 40g were put inside the sealed 'concentration-measure chamber' (fig. 6.2.b). The analysis time was 20 min.

Explorative data analysis was performed by Principal Component Analysis (PCA), where the raw data matrix was a Covariance Matrix. The feature extracted from each sensors response curves was R, that is the maximum of sensor resistance during the exposure to sample headspace.

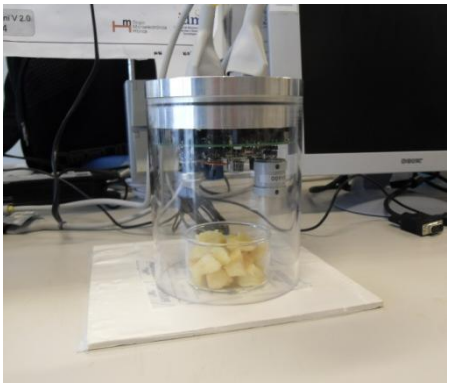


Fig. 6.2.b *E-nose IQMA* with joint concentration and measure chambers.

6.2.2. Results and Discussion

GC analysis, by purge and trap sampling method, was able to extract 42 molecules from aroma of quince fruits. The 'Vranja' contains all these molecules, more or less, whereas in "Franco" some are present only in trace. The major compound, in both genotypes, is *Hexanal*, an alkyl aldehyde used in the flavor industry to produce fruity flavours. The second molecule, with relative high percentage of area, is *1-Hexanol*, which is believed to be a component of the odor of freshly mown grass. Several identified compounds are present in trace (tab. 6.2.A).

The PCA score plot (PC1 vs. PC2) (fig. 6.2.c) shows that the electronic nose, *E-nose IQMA*, discriminates between the aroma of quince and the reference (atmospheric air). But while in the persimmons case *E-nose IQMA* was able to discriminate among the aromas of two different cultivars, this doesn't happen with quince fruits. To better verify the results, the third principal component was considered and the PC2 versus PC3 was plotted (fig. 6.2.d), but the discrimination was not improved.

4.1.1. Conclusion

We conclude that for the quince aroma, the *E-nose IQMA* has an appreciable sensitivity but a poor selectivity. In fact it is able to distinguish between air and quince aroma, but no differences between the two quince genotype aroma is observed, even if data from GC-MS show large differences between the volatile compounds inside the two quince genotype aroma.

Tab. 6.2.A Volatile compounds of Quince Fruits

R.T. (min)*	Identification (% Similarity)**	Chemical compounds	% Area ***	
			'Vranja'	*"Franco"*
2.25	63.3	***Hexane***	0.14 ± 0.04	0.26 ± 0.18
2.76	73.2	***Acetaldehyde***	1.07 ± 0.44	2.04 ± 1.44
2.78	60.0	***Propane***	-	4.46 ± 1.15
3.23	60.6	***Dimethyl sulfide***	0.56 ± 0.05	1.54 ± 0.06
4.03	75.2	***Propanal. 2 methyl***	0.43 ± 0.09	1.74 ± 1.14
4.24	80.3	***Acetone***	0.22 ± 0.10	-
4.66	92.0	***2-Propenal***	0.18 ± 0.07	0.52 ± 0.19
4.90	72.0	***Furan tetrahydro***	0.26 ± 0.04	0.38 ± 0.09
5.23	31.4	***Butanal***	tr.	-
5.32	52.6	***Ethyl acetate***	tr.	0.63 ± 0.23
5.72	74.0	***2-Propen 1-ol***	-	1.21 ± 0.85
5.78	86.0	***2-Butanone***	0.33 ± 0.07	-
6.01	73.2	***Butanal 2 methyl***	0.46 ± 0.19	2.37 ± 1.67
6.17	32.9	***Isopropyl alcohol***	tr.	0.20 ± 0.14
6.43	83.0	***Ethanol***	0.35 ± 0.24	2.92 ± 0.37
7.19	59.7	***n-Propil acetate***	-	0.19 ± 0.13
7.56	79.5	***Pentanal***	0.17 ± 0.03	0.35 ± 0.01
8.29	50.5	***Acetic acid. 2 methylpropyl ester***	-	0.17 ± 0.01
8.61	39.0	***Trichloro Acetaldehyde***	tr.	-
8.96	74.0	***1-Propanol***	-	0.23 ± 0.16
9.16	33.0	***Cyclobutene. 2 propenylidene***	-	0.20 ± 0.14
9.49	44.8	***2-Butenal Z***	0.12 ± 0.03	0.16 ± 0.11
9.91	46.0	***Acetic acid. butyl ester***	-	0.23 ± 0.16
10.09	57.0	***Tetraacetyl-d-xylonic nitrile***	-	0.15 ± 0.10
10.46	53.9	***Hexanal***	56.80 ± 22.2	36.41 ± 13.32
11.02	40.3	***1.2 Cyclopentanediol. trans-***	19.56 ± 9.48	2.08 ± 0.26
11.40	19.5	***3-Methylheptyl acetate***	-	2.05 ± 0.80
12.31	19.9	***3-Hexenal Z***	12.45 ± 4.91	2.26 ± 1.59
12.36	51.4	***4-pentenal. 2 methyl***	-	2.51 ± 0.53
14.16	67.6	***1-Butanol.2 methyl***	-	9.28 ± 5.09
14.88	35.7	***2-Hexenal***	0.76 ± 0.7	1.02 ± 0.09
15.23	70.7	***2-Butenoic acid. 2 methyl- ethyl ester***	-	0.49 ± 0.20
15.48	43.0	***2-Penten 1 ol. acetate.Z***	-	0.69 ± 0.21
15.59	35.8	***1-Pentanol***	tr.	-
16.97	61.0	***Octanal***	-	0.41 ± 0.28
17.25	17.4	***1-bromo-2-fluoro Ethane***	tr.	-
17.73	84.5	***2-Buten 1 ol. 2 methyl***	-	3.12 ± 2.34
17.93	48.1	***1-Hydroxy-2-Propanone***	tr.	-
18.42	44.0	***6-Methyl-5-Hepten-2-one***	tr.	-
18.53	46.5	***1-Hexanol***	9.38 ± 4.24	17.35 ± 1.99
19.43	36.1	***3-Hexen 1 ol***	1.95 ± 0.39	4.72 ± 3.52
19.82	54.8	***Nonanal***	tr.	-

*** Mean Retention Time; ** According to Nist and Wiley library; *** Mean value of relativity percentage of Area ± SD**

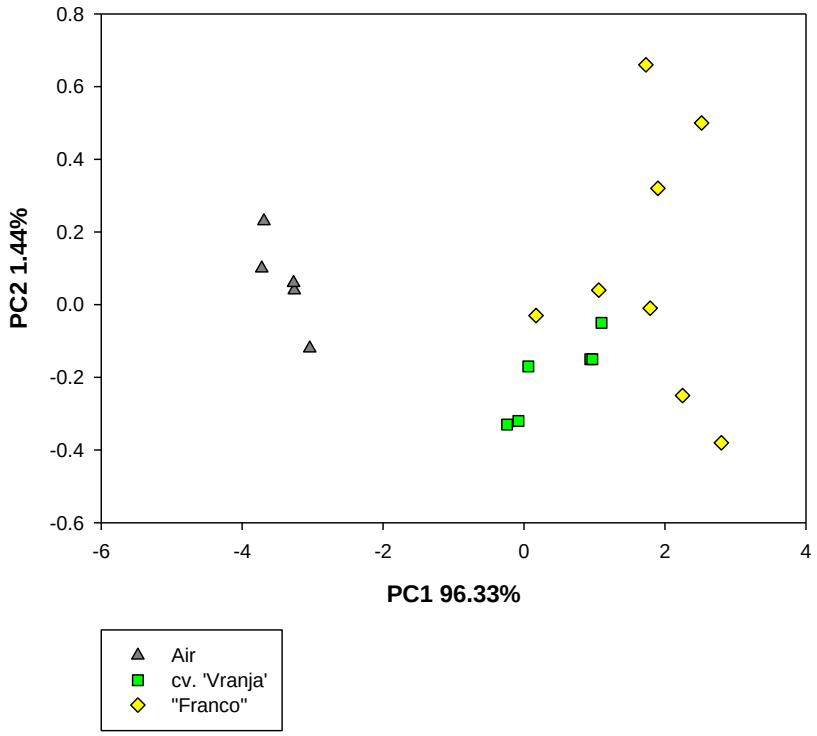


Fig. 6.2.c. PCA score plot (PC1 vs PC2) of *E-nose IQMA* data for two quince fruit: 'Vranja' cultivar and "Franco" and atmospheric air.

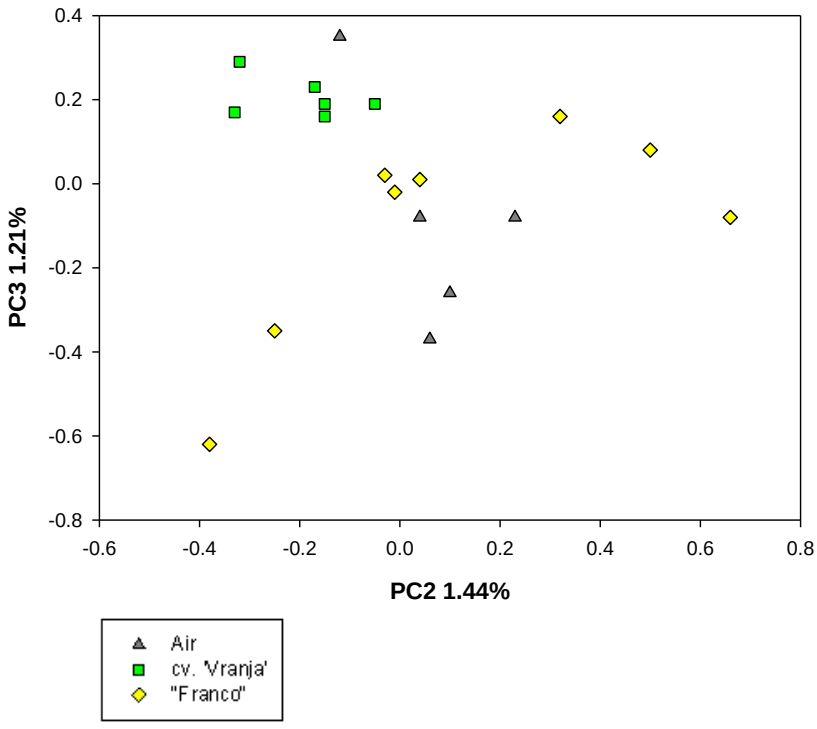


Fig. 6.2.d. PCA score plot (PC2 vs PC3) of *E-nose IQMA* data for two quince fruit: 'Vranja' cultivar and "Franco" and atmospheric air.

7. Conclusions

People use all their senses to evaluate fruit quality: sight, smell, taste, touch, and even hearing. The consumer integrates all these sensory inputs into a final judgment of the fruit acceptability.

Instruments that measure quality attributes like human senses, are very important for several applicative purposes. Indeed, instrumental measurements are to be preferred to sensory evaluations in many research and commercial applications because instruments reduce variations among individuals, are more precise, and can provide a common language among researchers, industry and consumers.

The electronic nose can be considered a supplementary tool to human sensory panel assessment especially in food quality assurance and food quality control.

An 'electronic nose' is a system originally created to mimic the function of the animal nose. However, this analytical instrument is more a 'multi-sensor array technology' than a real 'nose'. Whatever the sensor technology, it is still far from the sensitivity and selectivity of a mammalian nose. Therefore, its aim is not that of totally replace the human nose or other analytical methods. A sensory panel is always necessary to define the desired product quality that can be used to train the system. Traditional analytical methods such as GC-analysis to determine on a qualitative or/and quantitative base in what one food sample differs from others. The 'electronic nose' can only perform quick 'yes or no' tests of comparison with other products. Therefore, an 'electronic nose' can be regarded as an interesting tool for a quick quality test in various food applications.

Today many electronic noses are commercially available for quality investigation and control. They have a wide range of applications in various markets and industries ranging from food processing, industrial manufacturing, quality control, environmental protection, security, safety and military applications to various pharmaceutical, medical, microbiological and diagnostic applications.

An universal electronic nose capable of identifying or discriminating any type of gas sample with high efficiency in all possible applications has not yet been built. This is largely due to the limitations in selectivity and sensitivity of e-nose sensor arrays for specific analytic gases. Electronic noses are not designed to be universally appropriate sensor systems for every conceivable gas-sensing application nor they are capable of serving every possible analytical need. Thus, the suitability of an electronic nose for a specific application is highly dependent on the required operating conditions of the sensors in the array and the composition of the analyte gases being detected.

Consequently, the process of electronic-nose sensing of analyte gases is a part of an art form involving not only proper instrument and sensor-array selection, but also some experience

and training in proper e-nose operational protocols, although training requirements for electronic noses are much less rigorous than those for complex analytical instruments.

Electronic nose sensors do not require chemical reagents, have good sensitivity and specificity and provide rapid results. Furthermore, e-noses generally are far less expensive than analytical systems, easier and cheaper to be operated, have greater potential for portability and larger field of use compared with complex analytical laboratory instruments.

The electronic noses will never completely replace complex analytical equipment or odor panels in all applications, because they presents real problems with reproducibility, recovery, and negative effects of humidity and temperature on the sensor responses. Anyway, new emerging technologies are continuously providing means for e-noses improvement.

The current trend is toward the development of electronic noses for specific purposes in a fairly narrow range of applications. This strategy points to increase the e-nose efficiency by minimizing the number of sensors, thus reducing the instrument cost and allowing for greater portability through miniaturization. New potential discoveries are expected in this relatively new sector of sensor technology as new products, machines, and industrial processes are continuously developing. This could provide future advantages for the use of electronic noses in the field of fruit quality control.

Appendix A :

Mathematical descriptions of covariance and correlation.

In probability theory and statistics, the mathematical descriptions of **covariance and correlation** are very similar. Both describe the degree of similarity between two random variables or sets of random variables.

Correlation: $\phi_{XY} = E[(X - E[X])(Y - E[Y])]/(\sigma_X \sigma_Y)$

Covariance: $\gamma_{XY} = E[(X - E[X])\,(Y - E[Y])]$

where σ_X and σ_Y are the standard deviations of X and Y respectively. Notably, correlation is dimensionless while covariance is in units obtained by multiplying the units of the two variables. The correlation of a variable with itself is always 1 (except in the degenerate case where the two variances are zero, in which case the correlation does not exist). The covariance of a variable with itself (i.e. $X = Y$) is called the variance.

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List of papers and reports:

- ✓ **"FRUIT QUALITY EVALUATION OF FOUR LOQUAT (*Eriabotrya japonica* Lindl.) CULTIVARS GROWN IN SICILY (ITALY)"**
  Autors: V. Guarrasi, V. Farina, A. Mazzaglia, P.L. San Biagio, M. A. Germanà.
  Proceedings of III. International Symposium on Loquat. 03-06 May 2010. Antakya(Turkey). *Acta Horticulturae.*(http://www.actahort.org/books/887/887_51.htm)

- ✓ **"STUDIO PRELIMINARE SULLE CARATTERISTICHE QUALITATIVE E SALUTISTICHE DI 4 CULTIVAR DI MANGO (MANGIFERA INDICA L.) COLTIVATE IN SICILIA"**
  Autors: V. Guarrasi, F. Barone, P.L. San Biagio, M. Amenta, P. Rapisarda, M.A. Germanà.
  Proceedings of VIII. Congresso Nazionale Chimica degli Alimenti, p. 362-365. 20-24 September 2010. Marsala (TP)

- ✓ "**NASO ELETTRONICO CON SENSORI MOS PER DISCRIMINARE 3 CV. DI *ERIOBOTRYA JAPONICA* Lindl."**
  Autors: V. Guarrasi, V. Farina, P.L. San Biagio, M.A. Germanà. *Italus Hortus vol.17 (3), p. 103-108*. (2010)

- ✓ **"LA QUALITÀ DEI FRUTTI DI MANGO COLTIVATI IN SICILIA"**
  Seminary: "La coltivazione del mango in Sicilia: Stato dell'arte e linee guida per l'impianto". SOAT Capo d'Orlando - PROGETTO: Rete di filiera fruttiferi tropicali. 11 December 2010. Capo d'Orlando (ME).

- ✓ "**INHIBITION OF FOOD-BORNE PATHOGENS BY ESSENTIAL OILS EXTRACTED FROM CITRUS FRUITS CULTIVATED IN SICILY"**
  Autors: L. Settanni, E. Palazzolo, V. Guarrasi, A. Aleo, C. Mammina, G. Moschetti and M. A. Germanà; accepted from *Food Control* (January 2012).

- ✓ **"MULTIVARIATE DATA ANALYSIS OF THERMALLY TREATED SICILIAN EXTRAVIRGIN OLIVE OILS. COUPLING OF ELECTRONIC NOSE. GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND RHEOLOGY TECHNIQUES"**
  Authors: Amenta M., Monge M., Lizarraga L., Giacomazza D., Guarrasi. V, San Biagio P. L., Bulone D.; submitted to *Journal of Food Lipids* (December 2011).

Meetings participation:

Title	Place	Organization	Period
"NUOVI MARKERS PER LA RINTRACCIABILITÀ DELLA FRUTTA"	Catania	C.R.A di Acireale	24 /07/2009
"ORTOFRUTTA DI IV GAMMA: LA RICERCA INCONTRA L'INDUSTRIA"	Università di Foggia	Dip.PRIME Università di Foggia	04 /09/2010
"LA MODIFICAZIONE DELL'ATMOSFERA NELLA FASE POSTRACCOLTA DEI PRODOTTI ORTOFLOROFRUTTICOLI"	Palazzo dei Congressi Firenze	IX Giornate Scientifiche SOI	10-12 /03/ 2010
III. INTERNATIONAL SYMPOSIUM ON LOQUAT	University of Antakya Hatay (Turchia)	Mustafa Kemal University Faculty of Agriculture. Dept. of Horticulture	03-06 /05/ 2010
IV Course "QUALITÀ E SICUREZZA DEI PRODOTTI IV GAMMA"	Castello Normanno Mesagne (BR)	Dip.PRIME Università di Foggia	27-29 /09/ 2010

DEMETRA's seminary participations:

Professor	University
Yildiz Aka Kacar	Faculty of Agriculture, Cukurova University (Turkey)
Turgut Yesiloglu	Department of Horticulture University of Cukurova , Adana (Turkey)
Rodrigo Rocha Latado	Centro de Citricultura Instituto Agronomico de Campinas Cordeiropolis (Brasil)
Patan Shaik Sha Valli Kan	Department of botany, Yogi vemana Univerity, Kadapa-517502, Andhra Pradesch (India)
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Pasquale Losciale	Dipartimento di Colture Arboree. Università degli studi di Bologna (Italy)
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Muhammad Usman	Institute of Horticultural Sciences, University of Agriculture, Faisalabad (Pakistan)
Rosario Muleo	Dipartimento di Produzione Vegetale Università della Tuscia , Viterbo (Italy).
Maria Carmen Reig Valor	Instituto Agroforestal Mediterràneo Universidad Politécnica De Valencia (Spain)
Manuel Agusti	Instituto Agroforestal Mediterràneo Universidad Politécnica De Valencia (Spain)