

INSTRUMENTAL AND CHEMOMETRIC METHODOLOGIES TO ASSESS SENSORY QUALITY OF MEDITERRANEAN FOOD

Eva Borràs Iglesias

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Instrumental and Chemometric Methodologies to assess Sensory Quality of Mediterranean Food

Eva Borràs Iglesias

PhD. Thesis

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UNIVERSITAT ROVIRA I VIRGILI

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INSTRUMENTAL AND CHEMOMETRIC METHODOLOGIES TO ASSESS SENSORY QUALITY OF MEDITERRANEAN FOOD

Doctoral Thesis presented by

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CERTIFY

That the Doctoral Thesis entitled ***INSTRUMENTAL AND CHEMOMETRIC METHODOLOGIES TO ASSESS SENSORY QUALITY OF MEDITERRANEAN FOOD***, submitted by **Eva Borràs Iglesias** to receive the degree of Doctor with European Mention by the Rovira i Virgili University, has been carried out under our supervision, in the Department of Analytical and Organic Chemistry of this University, and all the results presented in this thesis were obtained in experiments conducted by the above mentioned student.

Tarragona, 5th April 2016

Dr. Olga Busto Busto Dr. Montserrat Mestres Solé Dr. Ricard Boqué Martí



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"*Never forget what you are, for surely the world will not.*
Make it your strength. Then it can never be your weakness.
Armour yourself in it, and it will never be used to hurt you."
George R.R. Martin, A Game of Thrones

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Presentation. Scope and Objectives

Scope

Quality assurance is a complex issue including multiple aspects. In particular, food quality is related to nutrition, safety, authenticity, genuineness or traceability, among others. In fact, quality assurance is related to the necessary actions (controls) to provide confidence that a product will fulfill the given needs [1,2]. Some of these requirements are assumed, such as safety guarantee, nutritional composition or acceptable sensory characteristics; however, other aspects depend on the context and on the consumer, such as the production frame (origin, tradition or organic agriculture) or ethical issues (environment or animal defense) [3-5].

Accordingly, food and beverage quality control often requires characterization of the products by means of chemical analysis and sensory evaluation. This is the case of olive oil, wine and tree nuts, typical products from the Mediterranean region with added quality values due to their health benefits and excellent organoleptic characteristics. Though chemical and sensory evaluations have been traditionally applied separately, their combination provides a better understanding and complete description of the product. While sensory evaluation uses human senses to perceive sensory and physical properties of the food, chemical or instrumental analyses determine the composition of the samples, complementing the information previously obtained [6].

Food and beverage sensory evaluation is aimed at analyzing and explaining the human responses to certain properties that are perceived by sight, smell, taste, hearing and touch senses. The most common methodology is based on descriptive taste panels constituted by trained panelists (experts) [7]. Despite the fact human senses are the most suitable 'techniques' to describe sensory characteristics (after exhaustive training), their adequate setup is not a simple task. This is basically because the food preferences of experts or consumers tend to be subjective, that is, they are affected by the intimate relation between sensory and psychological perceptions [3,5]. Moreover, the methods based on human senses have other important limitations, such as long-time and cost of assessors training, long analysis times, lack of standard references, number of samples restrictions (panel fatigue) and unavaliability to be applied on-line.

As a consequence, advanced instrumental methodologies, commonly applied to characterize chemical composition and physical properties of foods and beverages, have been recently developed to overcome or minimize the above limitations. These called *instrumental sensory analyses* are aimed at simulating the human perception system by finding relationships between the human responses and the instrumental signals measured. The possibility of using instrumental techniques to mimic odor, taste and vision senses (as human receptors) has generated an increasing interest during the last decades.

Many of the emerging applications are focused on non-targeted techniques emulating one specific sense and using data processing and pattern recognition techniques of the fingerprints obtained by analyzing the sample with an instrumental technique. These artificial senses are known as *electronic noses* and *electronic tongues*, and are based on different technologies to imitate olfactory and gustatory senses. The earliest attempts to detect compounds related to aroma and taste perception were based on gas and liquid sensor arrays, respectively. These sensors provide characteristic fingerprints of the samples, but their lack of specificity does not allow the identification of substances responsible of the sensory properties. Thus, other detection systems have appeared, providing spectral fingerprints that also contain specific information about the sample composition. In particular, in this doctoral thesis an electronic nose based on Mass Spectrometry (MS) and two types of electronic tongues based on Near- and Mid-Infrared spectroscopy (FT-NIR and FT-MIR) have been used.

The characteristic infrared and mass spectra are composed of multivariate data, with several instrumental responses (fingerprints) measured that can be correlated to the sensory properties using chemometric techniques. Multivariate data analysis is a powerful tool to determine those correlations and help to reduce non-relevant information. The multivariate methods more commonly applied intend to classify, discriminate or predict sample properties using the sensory attributes previously defined by a human panel, the results of a reference (often targeted) methodology or other parameters such as origin or variety. Moreover, chemometric tools also allow the identification of the variables with more relevance, which can be easily associated to certain compounds (or groups of compounds) that may explain the classification/prediction abilities obtained.

Human perceived sensory properties are usually due to the interaction of different compounds (variables) with different senses, including synergisms and other combined effects. Thus, in most cases, the artificial simulation of one specific sense using a single technique is not enough. This is the reason why the use of different instrumental techniques and the combination of the information generated, by using the so-called *data fusion*, is a good alternative to analyze sensory data trying to emulate the sensory human system. Therefore, in the Thesis, in order to provide complete information and predict sensory properties defined by a human panel, a combination of different instrumental techniques is proposed, in what is called an *electronic panel.*

Objectives

The general purpose of this Doctoral Thesis has been to further develop new instrumental methodologies to simulate human sensory responses. This implies the use of responses provided by a human taste panel, the optimization of the analytical procedures for the instrumental techniques and the development of suitable chemometric tools to build the multivariate models. The experimental developments led to define four sub-objectives:

1. To develop adequate chemometric tools to build the optimal multivariate models relating sensory and instrumental information, both for data collected from single instrumental techniques and for data collected from various techniques by applying different data fusion approaches.
2. To evaluate a preliminary instrumental sensory technique emulating an *electronic tongue* based on FT-NIR spectroscopy to discriminate samples by using one single sensory attribute related to taste perception.
3. To evaluate individual instrumental sensory techniques, such as an *electronic nose* based on Mass Spectrometry, an *electronic tongue* based on FT-Mid Infrared spectroscopy and an *electronic eye* based on UV-visible spectrophotometry, to discriminate olive oil and wine samples depending on the presence or absence of certain sensory attributes or to predict their score intensities.
4. To combine the data collected from the instrumental sensory techniques described in objective 3 using suitable data fusion strategies (*electronic panel*), in order to improve the discriminant or predictive models obtained for certain sensory descriptors.

Structure of the Thesis

This Thesis is divided into five chapters that contain the information listed below:

Chapter 1 - Introduction. This chapter introduces the actual state-of-the-art of sensory quality, and it is divided in four parts. First, the importance of food and beverage quality and authentication is briefly described, with a special focus on the Mediterranean foodstuffs studied in this Thesis: olive oil, wine and tree nuts (almonds). In the second part, an explanation about the sensory evaluation is presented including a description of the human senses and the main methodologies used in sensory analysis. In the third part, instrumental sensory analysis is introduced and the main instrumental techniques used to work as human sensory systems are explained. This part also includes the main chemometric techniques applied in this Thesis including data processing, variable selection, feature extraction, multivariate data analysis and data fusion approaches. The final part contains the published review about the state-of-the-art of data fusion methodologies to assess quality and authentication of food and beverages [**Paper 1**].

Chapter 2 - Almonds sensory analysis. This chapter describes the importance of almonds, its organoleptic characteristics and the necessity of detecting a specific sensory trait (almond bitterness) for the nuts industry. The first part describes the common methodologies applied for this purpose and the second part presents the first study of this Thesis based on FT-NIR spectroscopy to detect the presence of almonds with this characteristic tasting. The results are presented in a scientific paper [**Paper 2**].

Chapter 3 - Olive oil sensory analysis. This chapter is divided in two parts. The first part describes the main characteristics and properties of olive oil together with the main chemical and sensory analysis applied in a regulatory framework to define the different commercial olive oil categories. Also, the sensory evaluation procedure is described, as well as some instrumental methodologies applied for sensory quality assessment. The second part presents the different studies carried out using instrumental sensory analysis based on MS, FT-MIR and UV-vis, either applied individually or using data fusion strategies, to detect the presence of certain negative sensory descriptors, but also to predict most of the sensory descriptors defined by the human taste panel. The results obtained in these studies have led to four papers also presented. Two of them show the results of discriminating sensory defects using

individual data obtained by FT-MIR and HS-MS, respectively [**Paper 3** and **4**]. The other two papers show different data fusion strategies applied to discriminate sensory defects and to predict sensory descriptor intensities [**Paper 5** and **6**].

Chapter 4 - Wine sensory analysis. This chapter is similarly structured as the previous one. The first part describes the wine attributes mainly from an organoleptic point of view and includes a briefly description of both its sensory evaluation and its instrumental analyses. The second part, in this case, is only focused on a data fusion application, using the spectra coming from MS, FT-MIR and UV-vis instruments, to predict some wine sensory attributes defined by a human taste panel. The preliminary results are presented in the final part of the chapter.

Chapter 5 – General conclusions.

Appendix

A1 - List of papers presented by the author of this Thesis.

A2 - Contributions to national and international meetings attended.

A3 - Research stays and training courses attended.

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[**Paper 1**] E. Borràs, J. Ferré, R. Boqué, M. Mestres, L. Aceña, O. Busto, *Analytica Chimica Acta* 891 (2015) 1-14

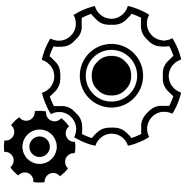
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[**Paper 6**] E. Borràs, R. Boqué, J. Ferré, M. Mestres, L. Aceña, O. Busto, *Talanta* (DOI 10.1016/j.talanta.2016.04.040)



Chapter 1. Introduction

1.1. Food and beverage quality and authentication

Quality control and authentication are fundamental in food and beverage commodities. In recent years, there has been a growing interest from consumers demanding high quality products; thus, the implementation of quality assessment systems has become the basic policy for the food industry and food control institutions.

1.1.1. Quality assessment system

The term quality has been extensively used over the years, basically to describe subjective attributes such as freshness, goodness or uniqueness. Considering that quality has different meanings depending on the context and on the person within the distribution chain, some institutions have accorded an appropriate definition [1]. Quality refers to the degree to which a set of inherent characteristics fulfills a set the requirements (ISO 9000:2015, [2]), implying a degree of excellence of a product or its suitability for a particular use. Thus, the quality of food products is defined by the characteristics expected by and/or acceptable to consumers, following a total food quality model [3]. Consumer expectations involve essentially the accomplishment of commodity, safety, nutritional and sensory requirements. However, other emerging needs should be also considered because they may play a key role in consumers' choices, such as production context, ethical issues or guarantee requirements [4].

Commodity, safety and nutrition, although being measurable and verifiable, are implicit requirements (hidden attributes) that cannot be perceived by consumers. Commodity condition is meant as the meeting of the conformity of a product to a given definition established by law. These laws also apply for safety requirements, which assure consumer health with respect to chemical and microbiological contamination, and sometimes provide the guidelines for nutritional values. The combination of these nutritional requirements with the sensory properties leads to the essence of food quality; while the nutritional value is linked to understanding and knowing the chemical constituents of the food products and their metabolism, the sensory requirements are related to perceivable properties by the human senses (e.g. flavor, taste, shape, texture, color).

Nevertheless, not only these verifiable and perceivable requirements have an impact on consumers. Requirements concerning the production context are indicators of the product origin or tradition, which are non-material properties that are related to psychological and emotional effects. Ethical requirements are related to the system of values that limits the consumers' behaviors, including environmental defense or organic agriculture, among others. Those two requirements are highly susceptible to fraud and deceit, conferring an increasing importance to the so-called guarantee requirements. Guarantee conditions are referred to certification and traceability procedures that offer consumer guarantees based on trust and credibility. In food and beverage commodities, this is represented by a product-packaging fusion (label) in a product-market system (price-to-quality ratio) [4-7].

Food authenticity and traceability

Considering all the quality requirements, determination of authenticity is an important issue in food control because it ensures the legal product description (label and law) and the detection of fraudulent statements (safety and guarantee) [8]. The authenticity control enables to get reliable information about the history of the product, its geographical region of origin, processing conditions or its species/variety [9].

Another essential element is traceability along the food chain. Traceability is defined as the ability to access to any or all the product information throughout its entire life cycle, by means of recorded identifications [10], from the producer to the final consumer (from farm to fork). It allows the identification of sources of contamination, managing crisis situations or reject from the market products that may suppose a risk for the consumers' health [11].

Food quality incidents

Food contamination, either accidental or intentional, with chemical or physical substances can be a source of serious risks with unpredictable effects in many sectors [12,13]. Accidental or unintentional incidents, also called food safety risks, are caused by hazards naturally present in foods, failures in the production system or problems during manipulation or storage [14-16]. Some famous examples are wine adulteration with methanol, bovine spongiform encephalopathy (BSE) or avian influenza [17].

However, when the act is intentional it is called a food defense incident or a food fraud. The former, also called food terrorism, generally consists on incidents perpetrated to alter the stability of a nation or its food supply. They can be motivated by extortion, discontented employees, interpersonal conflicts or coercion [18]. Food fraud, however, is related to a deliberate economical profit and is leaded by criminal actions that are specifically designed to not be detected by regulatory authorities or consumers. The absence of industrial control measures have encouraged these opportunities [13-15,19], leading to adulterated food products through the so-called Economically Motivated Adulterations (EMA).

EMA may consist on the secret addition of a foreign substance, the substitution or the removal of some ingredients or an incorrect food packaging with incomplete or misleading information [16]. Basically, these actions may either increase the apparent value of the product or reduce the cost of the production. This apparent enhancement of the value is difficult to detect by the consumer or by current analytical techniques. It can be performed by substitution or dilution of an authentic ingredient with adulterants (usually less expensive), by mislabeling or misdescripting claims about origin, by using non-consented process practices or by breaching the established legislative standards. Also, production costs are reduced by using adulterants to mask inferior quality ingredients (color or taste enhancements) or by removing authentic or demanded constituents [13,20,21]. Some of known EMA practices are the increase of milk protein content with melamine addition [8], the substitution of olive oil with other vegetable oils or the adulteration of honey with illegal antibiotics [14,22]. The main consequences of these fraudulent acts are a loss of quality of the products, a loss of consumer confidence, public health threats and important economical costs to authorities [15,19,22].

Regulatory responses

The increasing interest in products with appreciated characteristics that provide high added value, together with an ever-expanding market and more complex processing systems, are making food products more vulnerable to misleading practices [23]. As a consequence, a quality control system is crucial to safeguard consumers' safety and satisfaction, to ensure origin and product descriptions and to allow detection and even prevention of fraudulent activities or adulterations [15,22,24].

Food quality control and authentication is important not only for consumers but also for food industries, farmers and regulatory authorities. The last ones are in charge of establishing regulations to fight against deceptive practices and also of funding research projects to help in their implementation.

In the European Union, most of the food legislation is harmonized throughout a number of European Commission (EC) Directives and Regulations that protect quality and authenticity of the products. Some of them focus on preventing adulteration or substitution of high valued foodstuffs with specific characteristics with lower quality ingredients ((EEC) 2082/1992 [25]) or on protecting consumers' rights to receive truthful and complete information about the food (ECC 178/2002 [26]). Other regulations determine the specific origin and production process in the production of high valued food products, ensuring only the geographical origin of products from a specific region and allowing trade as protected designation of origin (PDO), protected geographical indication (PGI), and traditional specialties guaranteed (TSG). The indication of these designations is mandatory, simplifying traceability control ((EEC) 2081/1992 [27], (EEC) 510/2006 [28], (EEC) 1898/2006 [29]). Although deceptions regarding the geographical origin of foods have few health implications they may represent a serious commercial fraud with important economic consequences. The labeling of food is also subject to general rules, defined in the Council Directive 2000/13/EC [30] with the aim of informing and protecting the consumer. And finally, but not less remarkable, there are several regulations and norms describing instrumentation and analytical techniques to protect the public from misleading or possible fraudulent practices. These analytical tests are an essential tool in validating the different stages of foodstuffs production.

In order to improve the tools that allow guaranteeing authenticity and traceability, the European Union also have supported and supports several research programs. Some are focused on integrating cost effective analysis to verify the origin of food (TRACE [31]) and others in providing assurance about safety, authenticity and quality to consumers and other stakeholders (QSAFFE [32], FOODINTEGRITY [33]).

1.1.2. Analytical techniques for quality assessment

The alteration of food quality is a very ancient practice. First attempts were very rudimentary and involved the use of cheaper ingredients or the modification of the appearance. These fraudulent manipulations could be easily detected by using very basic tools, such as visual inspection. However, in the recent past, these procedures have been refined and have become more sophisticated and subtle, so their detection has become a more and more difficult task.

The best way to ensure the quality and authenticity of a product is defining unequivocally and before starting the production process its chemical composition, physical properties, level of chemical and microbiological contamination and sensory attributes. Moreover, it is also necessary to inspect how the products are stored, packed and labeled. To fulfill these requirements, apart from using traceability systems, it is necessary to use objective, rapid and reliable analytical methods [5,9,13,34-37]. Nowadays, there is a wide range of techniques and methods to assess authentication challenges, to detect possible adulterations and to ensure food quality. These analytical determinations can be categorized into two main approaches depending on their application purpose. The first ones, called *targeted* approaches, are classical methods that are applied to the analysis of a single compound or a group of compounds using, generally, univariate statistics. The second ones, called *non-targeted* approaches, are mainly related to the multivariate nature of food fingerprints or profiles and are evaluated using multivariate pattern recognition tools [38].

1.1.2.1. Classical targeted techniques

Targeted analyses are based on detection and identification/quantification of specific marker compounds, sometimes only present at trace levels, and a further comparison of the result with a control or regulatory limit. These assessments are mainly based on whether the results, considering also their measurement uncertainty, are in agreement or not with those regulatory limits [20,39]. Although targeted methods are still used as part of product standards or references, some limitations make these conventional testing procedures unsuitable for most of the present food assessment problems. Classical approaches, apart from being expensive and time consuming, require analytical expertise and specific knowledge of the products. Their analytical procedures are often difficult to implement and several years are

needed to become approved as official methods for regulatory institutions [23]. Besides, its suitability only for specific compounds may limit the detection of a wider range of other substances that can be related to certain parameters or attributes.

1.1.2.2. Non-targeted techniques

Unlike classical methods, non-targeted approaches do not necessarily measure the specific compounds or properties of the sample itself. Most of them measure signals that depend on the concentration of the compounds or on the magnitude of the property, and many times they need a targeted method as a reference [40]. These methods are commonly easy to carry out and cheaper than most of the targeted approaches. There are different strategies described using non-targeted methods including *food profiling, fingerprinting* and even -omic methods, in what is called *foodomics* [20].

- **Food profiling**

In the case of profiling methods, analysts need a prior knowledge of the product and, although classical determinations are typically applied, the analytes are not differentiated and sometimes neither quantified. Profiling is aimed at rapidly determining foodstuff quality based on information from multi-target screening methods, usually ending up with multivariate data analysis. These methods are based on separation techniques such as gas or liquid chromatography with different detection systems, which have more versatility for quantifying several compounds simultaneously. Despite the high reproducibility, lower costs and rapid implementation, these techniques require a tedious process of sample preparation and sometimes big databases for compound identification [20,23].

- **Foodomics**

Foodomics deals with food and nutrition domains by applying advanced -omic technologies. These powerful new methods, which are adopted to improve consumers' well-being, health and confidence [42], involve many techniques to authenticate food, such as stable isotope ratio analysis, DNA analysis, mass spectrometry, NMR and other spectroscopic techniques [20].

▸ Food fingerprinting

Nowadays, the techniques most widely used to assess food quality and authenticity are based on the so-called food fingerprinting methods. The aim of these methods is to obtain as many compounds or features as technically possible with high throughput screening of samples. To be able to carry out this type of analysis in food products, the sample preparation step should be as unspecific as possible to avoid a loss of signals that could imply possible loss of information. One of the advantages of fingerprinting is that, many times, the clean-up or enrichment of the sample is not necessary, so the sample can be directly analyzed or needs just a simple preparation [20]. The collected measurements provide information about the structure and composition of the samples, through a spectral fingerprint [36], which can be obtained using spectroscopic techniques, mass spectrometry (that can be coupled to a chromatographic separation) or sensor arrays [20,43]. Thus, a certain food product is investigated with at least one technique, making identification and mapping of patterns, to subsequently use one or more chemometric approaches for multivariate statistical evaluation. The use of chemometric models is very important to account for the simultaneous contribution of multiple effects. The determination of fingerprints provides rapid analytical information in a reproducible way, using simple procedures that are capable to handle high amount of samples or even be monitored on automatable processes. When quality or authenticity is assessed with these techniques, reliable fingerprint patterns show deviations outside the confidence thresholds of the multivariate models built [8,38,39,41].

Spectroscopic techniques

These techniques are very common in the field of food fingerprinting. They offer the possibility to analyze in a simple, rapid and non-destructive way relative small amounts of samples, often in a direct way, and detecting many compounds. These techniques are, therefore, suitable for the characterization of complex biological systems like food products. On one hand, nuclear magnetic resonance (NMR) spectroscopy is extensively used as fingerprinting technique. It allows analyzing several matrices directly in a single experiment, with simple sample preparations, high reproducibility and the possibility of performing structural analysis of key compounds. However, low sensitivity and spectral resolution are its major limitations, together with its high instrumental cost [20,22]. On the other hand,

vibrational spectroscopy has also been widely used, with successful results for authenticity and quality assessment of a broad variety of food products. These techniques include Raman, near- (NIR) and mid-infrared (MIR) spectroscopies and the most recent hyperspectral imaging (HSI) techniques. They provide rapid, non-destructive and sensitive analysis with relatively low cost, allowing qualitative and quantitative determinations. However, they have some drawbacks, such as the requirement of reference methods to calibrate the instruments, the need of expert personnel to statistically handle the data or common spectral problems with water absorption [22,43].



Mass spectrometry

Stand-alone mass spectrometry (MS) or coupled with chromatographic techniques, such as gas chromatography (GC) or high performance liquid chromatography (HPLC) is often applied as a fingerprinting technique. Good results are obtained using several ionization systems like direct analysis in real time (DART) or proton transfer reaction (PTR) [44]. Despite its high sensitivity and ability to characterize several compounds in one single sample, some limitations have to be considered, such as data complexity, the high price of the instruments and, often, the requirement of time-consuming steps, such as sample preparation and chromatographic separation [22,43].



Sensor analysis

Different electronic sensors have been developed to assess food quality. They are usually based on arrays of sensors that respond to different compounds producing a global fingerprint of food products. For example, several studies have been carried out to simulate human olfaction using a wide range of arrays with low-selective/cross-reactive sensors, as well as to simulate taste perceptions where several arrays of non-specific chemical sensors were used. Regardless the simplicity and affordability of these devices, an important drawback is that they are limited by the number of molecules that can be recognized [43,45].

Challenges when using Food Fingerprinting techniques

Notwithstanding that food fingerprinting offers many opportunities and advantages, some important challenges should be overcome before its implementation as control techniques.

Firstly, the selection of a suitable number of samples and properties that have incidence on food quality. A large number of both components should be adequate to obtain complete fingerprints, but it could be difficult to handle so much information. Therefore, it becomes necessary to select representative samples that include as much variability as possible, and to consider only those properties that allow deciding, in a simple manner, if the food fulfills the requirements of a certain quality grade [1]. Another important issue to take into account is the definition of validation systems [38]. Validation is essential to guarantee the reliability of the whole analytical procedure and the comparability within and between laboratories, including all sources of variation [1,20,43]. However, nowadays there is still a lack of validation strategies of fingerprinting results in contrast to classical targeted approaches. In order to find a solution to all these requirements, the standardization of data exchange formats and the creation of databases might be a first step. The use of reliable and accessible data and formats should help to promote transferability, which should increase the development of joint comprehensive databases. For this reason, some organizations have started to develop different official controlled databases, such as joint IAEA/FAO project [46], Food-Screener for fruit juices and wine with 1H NMR data [38], or European research projects such as the above-mentioned Trace [31], QSaffe [32] or Food Integrity [33].

Finally, in terms of instrumentation, improvements should be necessary to develop more rapid, non-destructive, robust, cheap and automated technologies able to monitor quality, safety and origin, reducing sample pre-treatment and with simpler analytical protocols [43,47]. These considerations should be sufficient to face new advanced malpractices, as to detect the ever-increasing range of analytes used in food fraud (detection of novel adulterants) or detect lower levels of certain substances to determine quality and authenticity [14].

1.1.3. Typical Mediterranean food

Among the different products susceptible to fraud, food and beverage commodities are some of the most manipulated [22]. This thesis deals with some of these products, specifically Spanish almonds (nuts), olive oils and wines. These commodities are part of the Mediterranean diet, possess healthy attributes and unique sensory characteristics and, therefore, have a high added value. These qualities have made these products very interesting for the international markets, being even target of initiatives from the European Parliament

(Joint Research Center, JRC [48]). These initiatives are focued on the development of technologies and methods to detect food fraud and to ensure differential value of these products to increase their competitiveness in world markets.

Almonds (nuts)

Tree nuts are food products with excellent sensory attributes and inherent health benefits. Nowadays, nuts have reached an important place in human diets what has implied an increasing commercial production. In particular, almonds have one of the world's largest productions [49]. Almonds can be consumed raw, but also previously processed by shelling, dry roasting, blanching, slicing, chopping or transformed into flour, paste (marzipan) or flavorings.

The desirable properties of almonds can be severely affected when they show the sensory 'bitter' defect. The genetic basis for bitterness involves a single gene so, sweet and bitter almonds come from *Prunus dulcis* but belong to two different varieties: *dulcis* and *amara*, respectively. Bitter almonds contain a toxic substance with potential health hazard (cyanide) and also have an unpleasant taste due to the presence of benzaldehyde. Although this may cause safety risks and negative commercial consequences, there are no official regulations or defined methodologies to detect bitter almonds. There are only some indirect methods that are applied to detect cyanide (biosensors or colorimetry), but these require complex and time-consuming procedures. Thus, the implementation of simpler and faster techniques would be very useful to control this problem.

Olive Oil

Virgin olive oils are highly valued commodities because of their nutritional benefits and outstanding sensory quality. These properties are related to the soft manipulation of the olives (only mechanical press) and also to the geographic origin, which confers a characteristic distinctness; however, the added value of this product has made it very susceptible of deceptive practices, such as adulterations with lower-grade olive oils (refined or lampante) or other (cheaper) vegetable oils. Therefore, there is a need to establish legal guidelines to control possible adulterations or authentication deceives. In fact, olive oil is the most regulated edible oil and institutions like the International Olive Council (IOC) [50], Codex Alimentarius [51]

or European Union Regulations [27,52] have established definitions, listed the chemical composition and organoleptic characteristics and described methods for olive oil analysis.

The methods proposed include both classical (targeted) methods to determine specific information about the product content and non-targeted methods, which rely on the whole contribution of many known or unknown compounds on the parameter studied. Classical methods define major and minor chemical components (markers), which can be responsible of the main olive oil characteristics or peculiarities. Some examples could be the analysis of fatty acids, triacylglycerol, waxes, sterols or hydrocarbons using multiple techniques [23]. In case of non-targeted analyses, these determine the olive oil profile or fingerprint, usually, through data obtained by spectroscopic techniques (NMR or vibrational spectroscopy), mass spectrometry or chromatographic techniques using mathematical algorithms (chemometrics) and databases containing chemical information [23].



Wine

As it happens with olive oil, wine is a highly valued product exported worldwide. Its production has always been associated to high quality, but in some cases, to reduce costs along the production process and get higher profits, wine is highly vulnerable to various fraudulent practices [20]. Specific quality control programs are mandatory in many countries to improve both wine traceability and authenticity of grapes and wines (mainly in terms of grape varieties, geographical origin and processing conditions). The main wine malpractices involve addition of alcohol or substances to modify color and flavor, mislabeling or blending with/replacement by wine of a lesser quality [53]. For example, alcohol, sugar or concentrated grape are added in a non-authorized way before or during fermentation. This increases the natural ethanol content and, thus, the value of the wine that achieves a higher market price [20]. As olive oil, an important part of the commercial value of a wine relies on parameters strongly related to the history and geographical origin of the product. For all these reasons, European regulations have adopted rigorous guidelines to ensure the quality and authenticity of wines [54-56]. However, due to the complexity and variability of this product, in some cases wine quality assurance through legislation, good manufacturing practices and traceability procedures is not always sufficient. To guarantee the safety and excellence of wines, regulatory bodies are

more and more interested in developing appropriate analytical procedures, based on chemical and sensory evaluations.

The most accepted analytical control methods are based on the profiling of trace elements, phenolic and volatile compounds and isotope ratios using different spectrometric and/or spectroscopic food fingerprinting techniques [20,53]. In all these cases, the use of accurate reference materials or standards has been one of the main challenges to ensure proof-of-identity of wine [53]. However, the emergence of regulated databases has been a key factor to face this problem; one example is the JRC database within EU legislation support, which established an EU wine databank in 1991 containing the isotopic composition of wines.

1.1.4. Quality assessment through sensory analysis

As explained above, when implementing a complete quality assurance system, it is necessary to cover the consumer expectations and requirements, such as the ones related to sensory attributes. Although sensory properties are easily recognizable thanks to the human senses, sensory quality is difficult to define. The sensory assessment is important for commercial purposes and from the consumers' point of view. However, since its evaluation is linked to the interaction between the food and the consumer, this evaluation tends to be subjective [1]. This subjectivity is due to human sensory perceptions that are transformed to sensations by means of the brain, which at the same time can activate memories, search in its databases or promote actions. Therefore, the union of these sensory and psychological perceptions makes very difficult the development of procedures to assess food quality through sensory analysis [4].

There are several suitable approaches in the literature that can be used in sensory quality control of food products. Their main goal is to determine all sensory factors that are likely to be important to perceive quality [1]. Human sensory evaluation is the most suitable and applied methodology; however, it has some important drawbacks, such as time/cost of assessors training and facilities conditioning, number of analyses (panel fatigue), impossibility to work on-line or subjectivity of some responses. To overcome or minimize these limitations advanced instrumental techniques have been recently developed.

Nevertheless, caution has to be taken when replacing sensory assessment through instrumental analyses, especially nowadays that scientists have almost a naive faith in data

generated by modern instrumentation. It is almost impossible to exactly simulate the wide range of sensory responses that consumers appreciate when tasting food products by any instrument (or set of instruments); and the expected correlations between instrumental responses and key sensory characteristics are not always obtained. In fact, although in past times sensory and instrumental analyses were often employed separately, recent studies have allowed the formation of an interesting tandem between these two kinds of analyses, providing more complete information and better understanding of the product. Thus, the advantages provided by instrumental techniques will always be valuable information in sensory quality control, as long as the correct measures for the sensory characteristics are established [57,58].

1.2. Sensory evaluation

Sensory evaluation is defined as the analysis of food products or other materials through the senses. It is used to measure, analyze and explain the responses generated by certain attributes, in this case by food and beverages that are perceived by sight, smell, taste, hearing and touch senses. One of the major challenges with regard to the sensory evaluation of a food product is to describe its quality and define the parameters by which it is measured. To create the product sensory specifications, the key quality attributes should be based upon human perception, where the first attributes evaluated are perceived by vision, followed by aroma, taste/flavor, texture/mouth-feel and specific after-tastes and after-feels at the end, once it is swallowed.

1.2.1. Human senses

Human senses: sight, hearing, touch, smell and taste, are very important because their stimulation leads us to act in a specific way. In food and beverage commodities, the characteristics are mainly grouped into three categories, namely appearance, flavor and texture, being connected among them and perceived by one or more senses. Among all the perceptions, flavor takes a particular place in food science. It is defined as the combined effect of odor, taste and chematosenses [59]. Many times, chemosensory is included into the taste sensation, being the result from the stimulation of receptors (trigeminal nerves) usually

associated to pain, thermal perception, touch and mouth-feel sensations, such as astringency. These receptors are mainly located at the oral, nasal and ocular mucosae [60,61]. Appearance and texture are sensory characteristics mainly perceived through the visual and touch (movement) senses, respectively [57].

Smell – Aroma perception

The olfactory perception is the most complex sense and it is perceived directly via the nose or indirectly through a retronasal path, via the mouth. Hundreds of different classes of biological receptors (specialized cells) are located in the olfactory epithelium being stimulated by aroma compounds (odorants) of diverse molecular structures (**Figure 1.1**). These aroma compounds must be volatile but, to be perceived as odorants, they should also bind to the receptor cells and then nerve impulses are triggered to be transmitted to the brain, where are decoded and finally interpreted. Thus, of all volatile compounds, only a limited number can be perceived and, of those, only few (key odorants) contribute to the characteristic aroma of a certain food [60,62].

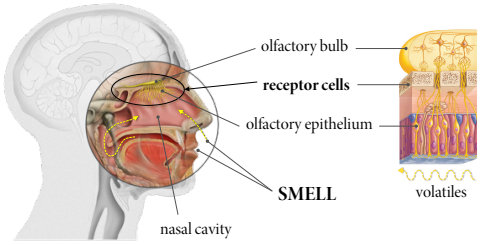


Figure 1.1. Main parts for the olfactory system.

Taste perception

The gustatory function is perceived through the tongue and produces the sensation of sweet, sour, salty, bitter and umami or savory. When the food is introduced to the mouth, the taste compounds interact with the taste receptors located in taste buds on the surface of the tongue, but also on the back and other areas of mouth and throat. These taste buds, containing the taste receptor cells, are not evenly distributed throughout the tongue, which contains different areas more receptive to certain sensations (**Figure 1.2**). As in olfactory perception, when taste cells are stimulated they trigger a nerve impulse that is transmitted to the brain hypothalamus

to interpret the final sensation. Taste compounds are generally non-volatile and include a wide array of molecules [60,61].

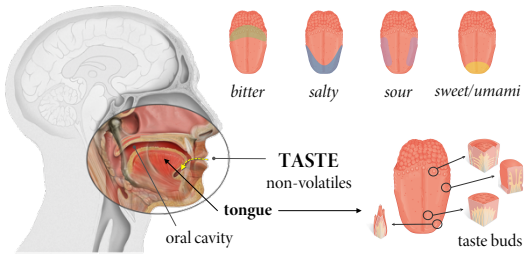


Figure 1.2. Main parts for the gustatory system.

Visual perception

The visual system determine the external sensory properties of food products, mainly perceived by color, but also by other attributes, such as appearance, shape, surface, size, brightness, uniformity and texture. To define the color it is necessary to define the spectral range of light directed to the product. Usually, the light waves are from the visible spectrum with wavelengths between 400 and 700 nm. The waves reflected by an object go through the eye and fall on the retina, where the receptor cells convert the light energy into neural impulses that travel to the brain (visual cortex) via the optic nerve. The specific photoreceptor cells are known as rods and cones and are located at the back part of the eyes retina (**Figure 1.3**). The former are responsible of different wavelength related to the color, and the latter relay information concerning to the lightness of the color (white light) [61].

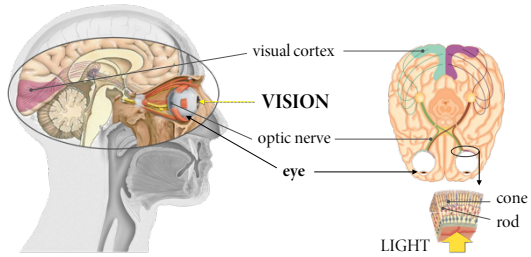


Figure 1.3. Main parts for the visual system.

1.2.2. Human sensory analysis

The best way to determine the food quality associated to the sensory characteristics is eating (or drinking) them, but this is not always possible. Accordingly, the best methods to predict human perception have to be based on human responses. Despite that human sensory analysis of food attributes considers interactions between stimuli and human senses, this can be understood from different perspectives: *affective* and *descriptive tests*. Affective tests are based on measuring the consumer responses to the sensory characteristics and explaining how flavor, texture and appearance influence the consumer acceptability [63]. These analyses are conducted by untrained individuals (representative of end-product users) and inform producers about control product maintenance and production, product shelf life or even on the development of new products. Descriptive tests are based on specific perceptions (technical aspects) and are carried out by trained assessors who measure the sensory properties. These tests provide powerful sensory tools suited for identifying attributes in a product and for discriminating sensory properties between products [64].

Descriptive taste panels

Through the different sensory methods, the descriptive taste panels based on trained panelists (experts) are the most common, reliable and powerful. This methodology provides a description of qualitative and quantitative aspects of human perception by measuring the sensory reaction of the resulting stimuli when the food is consumed. The main goal is to identify specific sensory attributes of the food or beverage evaluated, such as sensations related to aroma, taste, appearance or texture.

There are several descriptive methods in the literature [65], all of which are based on working with judges who have been trained and have a suitable sensory acuity. There are also guidelines to create a proper sensory panel by selecting and training the assessors on using an appropriate vocabulary and rating. Thus, training is carried out using a particular sample set to define a scale (intensity) for the selected attributes, allowing their final quantification for all the samples [65,66].

Using this structured procedure, good and reproducible results can be obtained. These results are commonly applied in the food and beverage industry and allow taking business decisions,

guiding product development or definition, checking the effect of ingredients or processes or tracking product changes over time. However, despite the high applicability and the advantages of sensory analysis, there are several drawbacks that are difficult to overcome. One of the main problems is the subjective information included in the results. The bias caused by the subjectivity is even more pronounced when comparing different panel tests (between laboratories or between countries). A possible solution to that could be the use of standards or quality control samples, but in most of the cases it is not possible to find stable and representative samples. Besides, there is a possibility of human fatigue or stress and panel tests cannot be implemented 'on-line' for immediate feedback. Moreover, all the training and maintenance required to work in a proper way makes the taste panel expensive and time-consuming [67]. All these reasons are increasing the interest in finding alternative methodologies to acquire more objective and unambiguous sensory information.

1.3. Instrumental sensory analysis

Nowadays, food and beverage sensory evaluation already includes diverse instrumental methods depending on the attribute of interest. What is required, basically, is to find a simple relationship between human sensory responses and the instrumental signals measured. To find those instruments with the ability to mimic the human response is not a simple task because instrumental physicochemical properties are discrete and well defined, whereas sensory perceptions are unusually discrete, mainly because of the interactions between different stimuli (physical-psychologically), the subjectivity assumed and many factors that influence the human perception [57,58,68].

Accordingly, the validation of this sensory-instrumental relationship is very important to obtain reliable predictive models. To create an analogy between biological (human) and artificial sensory systems and maximize the possible sensory-instrumental correlations, considerable research is being carried out to develop new and alternative successful methodologies. These instrumental systems try to emulate the human perception system.

Thus, as it is described in **Figure 1.4**, when a foodstuff is introduced through the sampling system (emulating human body parts) it interacts with human or artificial detectors (sensory receptors). When these receptors are stimulated they measure and transmit a signal (trigger an

impulse) to the data processors (brain or computer). These obtained responses are finally interpreted and understood using chemometric tools, which correlate instrumental signals with the sensory data (sensory descriptors).

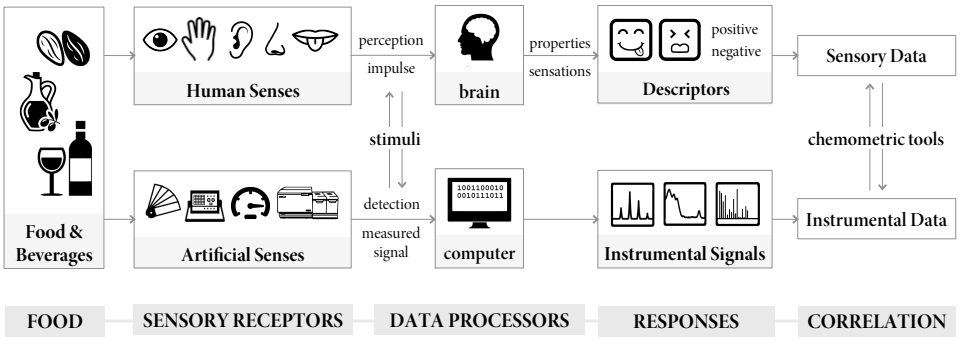


Figure 1.4. Analogy of the human and instrumental sensory analysis of food and beverages.

1.3.1. Instrumental senses

As previously described, of all senses flavor (odor and taste), followed by vision, are the most important when analyzing food and beverages. To simulate the human sensory receptors, different instrumental techniques have been applied by measuring signals related to a specific human sense, mainly in a non-targeted way (fingerprints).

Electronic nose (e-nose)

One of the most difficult tasks in sensory analysis is to instrumentally identify aroma compounds, especially in food matrices. This is because the human olfactory system is extremely sensitive to many volatile compounds, even surpassing the most sensitive instrument, being able to immediately differentiate hundreds of them. In food products these compounds are present at low concentrations so, a pre-concentration technique is needed to isolate and concentrate the volatiles to get enough intensity before instrumental detection [60]. This detection system needs a data processing system with pattern recognition methods to interpret the perceived or identified compounds.

e-nose ▸ sampling system

To obtain representative volatile fractions of the samples and to get suitable sensitivity, an appropriate sample preparation is critical. Through the many techniques aimed at isolate/concentrate volatiles from food matrices, one of the most common is the static headspace (SHS). It consists on establishing equilibrium between the sample and the gaseous phase into a sealed vial (under controlled time and temperature) to introduce, later, the gaseous phase into the detection system. The addition of an inert gas stream to purge volatile compounds is used by purge and trap (P&T) and dynamic headspace (DHS) techniques. The former injects the gas through the sample while in the latter only the headspace is purged with the gas. In both cases the equilibrium is displaced in favor of desorpting more volatiles from the matrix to the headspace zone. Solid-phase micro-extraction (SPME) is a more recent technique that is gaining more and more popularity because of its simplicity, low-cost and its relatively ease of automation. It exposes a silica microfiber coated with different adsorbents to the sample headspace to trap volatile compounds. Other recent techniques, similar to SPME, are stir bar sorptive extraction (SBSE) and inside-needle dynamic extraction (INDEX). Instead of a fiber, SBSE uses a magnetic bar coated with sorptive materials, and INDEX contains a polymer-absorbing phase inside a needle, both increasing the SPME fiber loading capacity [45,62].

e-nose ▸ detection system

The systems most commonly used to detect specific odorous molecules consist on arrays of gas sensors. These detection systems constitute the classic concept of electronic nose and they are very popular because of their high sensitivity, low-cost, fastness and simplicity (once the system has been calibrated). However, in recent years, the classical sensor types have been enhanced and complemented by other techniques, such as mass spectrometry or gas chromatography-olfactometry (GC-O).

- ***Sensor arrays***. The sensor array consists of non-specific sensors treated with different odor-sensitive materials that generate a characteristic fingerprint from odor stimuli (smellprint). This lack of specificity does not allow the identification of individual compounds, but applying pattern recognition techniques fingerprints can be used to identify or classify odors. Gas sensors are developed using various sensing materials and transduction platforms. Most of them are based on conductimetric sensors: conductive

polymer (CP) sensors, metal oxide semiconductors (MOS), and metal oxide semiconductor field-effect transistors (MOSFET). But other sensor types have been applied, such as optical transducers based on colorimetric sensor arrays, acoustic sensors (surface acoustic wave (SAW) or quartz crystal microbalance (QCM)), and new materials based on nano-structured sensors (quasi-1D metal oxides or carbon nanotubes) or biosensors [62].

- ***Mass spectrometry.*** Electronic noses based on mass spectrometry (MS) can be applied in two different configurations. The first one allows the injection of the sample headspace directly into the ionization chamber of the MS, which generates a spectrum of the entire mixture (aroma fingerprint). The second configuration consists on coupling a separation technique, usually gas chromatography, to the MS detector (GC-MS), which allows a precise separation of particular volatile components and their respective characterization through the mass spectra. This system can also be enhanced by joining an olfactometric or sniffing port (GC-O). GC-O uses human detection to determine the compounds that actually have an odor and therefore may contribute to the sample sensory flavor. However, adding these device increases both costs/analysis-times, and more skilled personnel is required [5,67,69].

Electronic tongue (e-tongue)

Frequently, molecules contributing to taste are larger and more complex than those that contribute to the aroma, which complicates their study. Taste compounds in food matrices are non-volatiles, mainly water-soluble, but also some volatiles or low water-soluble molecules can contribute to this sensation [60]. Electronic tongues are used to identify, analyze or classify tastes simulating human gustatory perception. They work in a qualitative or quantitative way by collecting a fingerprint of the multicomponent mixture. As the electronic noses, three elements can be distinguished in their configuration: sampling system, detection system and data processing system [5].

e-tongue ▸ sampling system

Food matrices may not need a prior treatment, particularly when they are in liquid phase. However, in some cases they require a prior clean-up using gel permeation chromatography

(GPC), membranes, solid phase extraction (SPE) or high-performance liquid chromatography (HPLC), or even a prior dissolution when the phase is solid [60]. Sampling systems may include other devices like a dispensing chamber when the samples are detected with sensor arrays or a mechanized flow analysis techniques, such as flow injection analysis (FIA) or sequential injection analysis (SIA), when it is necessary to shorten the time of analysis and to improve repeatability [5].

e-tongue ▸ detection system

Traditionally, taste attributes have been typically measured through specific techniques like titrimetry (acidity), refractrometry (sweetness), different HPLC detectors (bitter and umami components) and atomic absorption spectroscopy (salts). However, these techniques do not offer information on the nature of the compounds and some of them are destructive [69-71]. To overcome these limitations, increasing interest is directed onto non-destructive techniques able to obtain characteristic taste fingerprints (tasteprint) in a simple, fast and economic way. As in electronic noses, the classical concept of electronic tongue is based on sensor arrays; however, other techniques, like vibrational spectroscopy, are gaining acceptance and applicability in the last years to be used as tasting system.

- ***Sensor arrays.*** Non-specific and low-selectivity chemical sensors constitute a taste sensor array, with partial specificity (cross-sensitivity) to several components in a food solution. Different sensing modes are used depending on the application, such as optical, impedimetric, electrochemical, biosensor-based or mass change detection based on some principle like quartz-crystals. Electrochemical detection has been widely applied in food taste studies, and it can be divided into amperometry/ voltammetry (based on electric currents between electrodes) or potentiometry (based on voltage measurements) [5,72].

- ***Vibrational spectroscopy.*** The increasing widespread of vibrational spectroscopy in food sensory studies is mainly due to its advantages such as low cost, simplicity, fast analysis and versatility to be applied to solid or liquid matrices with minimal or no sample pre-treatment. The most widely applied instruments are near-infrared (NIR), Fourier transform infrared (FTIR) and Raman spectroscopy, together with multi- and hyperspectral imaging (HSI) systems [53]. All of them are based on infrared spectroscopy, and are able to acquire information about functional groups present in a molecule. NIR

ranges from 13000 to 3300 cm^{-1} and its signals are associated to overtones and combinations of fundamental vibrations. It has been widely used for both qualitative and quantitative analysis of food commodities. FTIR spectroscopy ranges from 4000 to 400 cm^{-1} with signals associated to fundamental vibrational and rotational stretching modes of molecules. In both cases, different spectral modes can be used to estimate both external and internal properties of a sample, including reflectance, transmission, interactance, and transflectance. Attenuated total reflectance (ATR) mode is the most widely adopted for food analysis. HSI has recently emerged as a powerful technique, using both spectral (usually NIR) and spatial information from an object. This enables characterization of complex heterogeneous samples [22,69].

Electronic eye (e-eye)

The visual or external perception is the first sense used by consumers and also by trained assessors. From all the possible aspects like blemishes, gloss, shape, size and color, this last is usually considered the most important visual characteristic. Chemically, color is associated to spectral vibrations resulting from differences on molecular composition and electronic configurations of chemical compounds. The color of food products is the resulting combination of their structure, which affects scattering and reflectance properties, and pigmentation, which affects absorption properties [69].

Many instrumental systems have been developed to mimic the human visual perception, going from subjective qualitative evaluation to objective quantitative techniques. Objective color measurements involve standardized color description, with clear definitions to understand and reproduce responses. These systems can be as simple as color charts but also as complex as a highly sensitive electronic instrument [57] coupled to suitable data processing methods (pattern recognition). Different color chart methods have been applied, but the CIE L^*a^*b color space is the most common system, offering an agreement between geometric and perceived color spacing [73].

It has to be pointed out that colorimetric measurements are the most widely used in food color assessment and they are usually constituted by lenses and integrated light sources. Mainly they can be divided into trichromatic colorimeters (filter wheel or tristimulus functions) and spectrophotometers, both devices commercially available [70].

Spectrophotometers are instruments that use a monochromator to analyze the reflected or transmitted visible light on the wavelength range from 400 to 700 nm [69].

The more recent studies are related to the use of computer image analysis, called 'computer vision'. In general, this computer system includes acquisition elements (lighting and camera) and also processing and analysis (high-resolution monitor and software) of images. This makes the visual assessment of the color, form and visual structure or texture properties of foods objective and reproducible under standardized conditions [5].

Data processing - Chemometrics

As previously mentioned, most sensory instrumental techniques that simulate human responses have in common one important element: the final data processing systems. Data processing simulates the human brain functions by understanding and interpreting measured signals. However, to build relationships between sensory attributes and instrumental variables chemometric techniques have to be used.

Sensory/instrumental relationships can be divided into direct or indirect, depending on the instrumental technique and results provided. Direct relations (linear or non-linear) are usually associated to targeted analysis, where specific compounds are related to certain sensory attributes. This can be faced, for example, when a specific volatile compound is the responsible of a specific aroma descriptor (e.g. n-hexanal with grass-green smell). However, these type of relations are not always known or proved enough because it is difficult to identify the compounds responsible of specific sensory characteristics. Moreover, sensory attributes are quite often linked to or explained by several compounds. So, considering that, finding direct relationships between specific compounds and sensory attributes is a cumbersome task for the analysts, requiring additional literature research [67]. For this reason, in food and beverage sensory analysis is more convenient to use indirect relationships, where the instrumental signal may not have a direct relation with the studied property. In these cases, a 'latent' correlation is required and at the same time the dimensional space is reduced. Non-targeted approaches are associated to these types of relationships, and usually multivariate data analysis (MVA) is needed. MVA is a powerful tool useful to determine all the variations and relations of the data matrix of study. It explores correlations or co-variations in such datasets using only minimal a priori assumptions to amplify the relevant information

(reducing the not useful one). As a consequence, to have a better understanding of the complex interactions and combined effects among components, the samples analyzed should be treated entirely (not one by one), as well as all the measured variables (if it is necessary) [67].

1.3.2. Electronic sensory panel - Data Fusion

Although there has been a great advance in the use of fingerprinting approaches for food sensory authentication, apart from the subjectivity, there are still some instrumental limitations when human responses are emulated. This is because human perceptions are often due to interactions between different stimuli or compounds that are related to one sensory property, or to a single compound that can contribute to different sensory perceptions.

Therefore, there is an increasing need to examine samples in their entireness in order to untangle the complex interactions among the components and understand the combined effects of the whole information collected. From the data analysis point of view, this requirement can be interpreted as the need to use multiple signals or measurements simultaneously [67]. The connection of different sources of information from complementary instrumental techniques is called *data fusion*. Data fusion can be applied using different strategies, which are basically classified in three levels: low-, mid- and high-level. Thus, as well as electronic noses, tongues and eyes intend to simulate their corresponding human analogous, data fusion of those instruments intends to work as an *electronic panel*, comparable to a taste panel performed by human assessors.

1.3.2.1. Levels of data fusion

Low-level data fusion is a straightforward data fusion strategy. All individual instrumental responses collected for a sample are directly concatenated into a single vector before model building. For the whole sample dataset, the resulting fused-data matrix has as many rows as the number of samples analyzed (sample-wise) and as many columns as the number of signals (variables) from all the sources. As described in **Figure 1.5**, in this level of fusion all the original single responses from each instrument are used. Coupling all data into a unique matrix can be complex and the required calculations are extremely time-consuming. For this

reason, before applying MVA it is recommended to use different data pre-treatment or variable selection methods.

The two main problems of low-level data fusion are the high dimensionality of the final fused matrix and the dominance of one instrument over the others, due to a different data scale and/or size. Dimensionality is referred to the number of variables from different nature of each independent technique. Despite describing more information and providing complementarity, the final fused array will normally contain too many variables, some of them potentially redundant. In this case, a variable selection method is usually required to reduce the dimensionality. The dominance of one block over the others can be solved by a proper data scaling, both within or between blocks [74], and also by variable selection procedures. Both alternatives lead to more balanced blocks, which ease data analysis and improve final results.

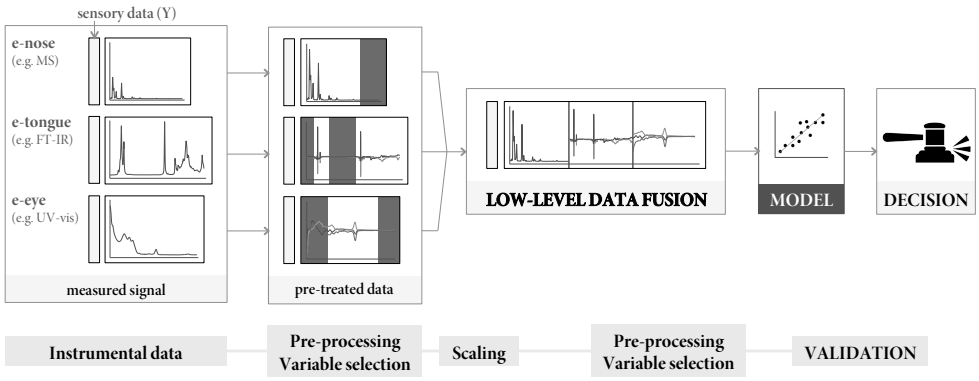


Figure 1.5. General scheme of the low-level data fusion strategy.

Mid-level (or feature level) **data fusion** consists on extracting some relevant features from the single instrumental data matrices separately and then concatenating them into a single array before the final MVA (**Figure 1.6**). This new matrix (feature-matrix) is, as in low-level, sample-wise and contains as many columns as the number of all the new features generated.

Different feature extraction methods can be applied, but the most common procedure is fusing a number of latent variables obtained from each instrument independently. Those latent variables are usually the scores extracted from principal component analysis (PCA) or partial least-squares (PLS) analysis. Mid-level fusion reduces significantly the dimensionality

problem and is a good alternative to low-level data fusion. However, the main challenge in this case is to find the optimal combination of extracted features, together with pre-treatment and variable selection methods, to describe the significant variation contained in the data.

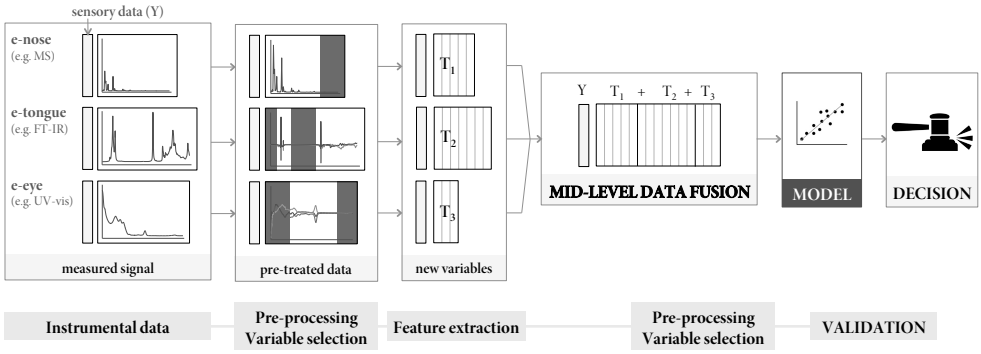


Figure 1.6. General scheme of the mid-level data fusion strategy.

High-level (or decision level) **data fusion** consists on applying a MVA to each data source individually. In this case there is not a fused matrix containing either the original variables or the possible features extracted. High-level fusion combines the individual classification/ prediction results ('identity declarations') obtained for each instrumental technique to obtain the final results (decisions) (**Figure 1.7**).

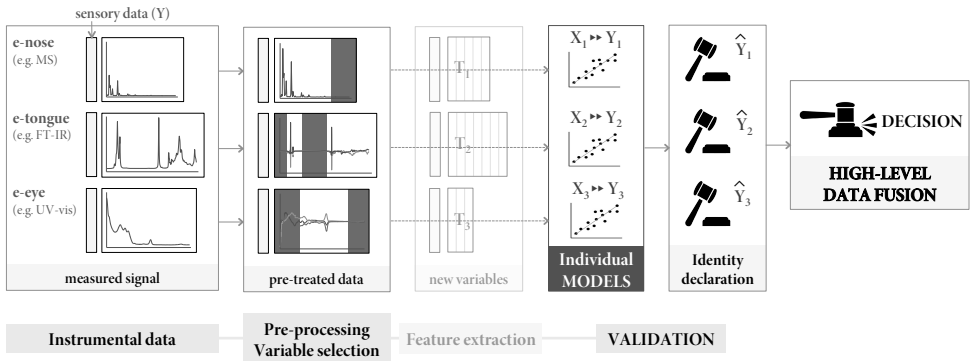


Figure 1.7. General scheme of the high-level data fusion strategy.

The most common methods to combine the individual identity declarations are Bayesian inferences or heuristic methods, and the main difficulty is to determine the optimal MVA models that work best for each data matrix to obtain a final fusion with better results than the

individual ones. However, even though with high-level the results from an inefficient MVA method do not affect the final result as in the other two fusion levels, if the correlation between datasets is not properly handled some information may be lost. In this thesis only low-level and mid-level data fusion has been applied.

1.3.2.2. Data processing

(1) Pre-processing

The main characteristic of non-targeted sensory techniques is that the responses (usually defined as 'analytes' in targeted analysis) are represented by all the instrumental variables of the 'spectral fingerprint'. Before data fusion and MVA individual datasets have to be pre-processed, in order to improve interpretability, to transform raw data into a better understandable format or to achieve a better accuracy and robustness for subsequent data mining tasks [75].

The first pre-processing steps that are usually applied, depending on the instrumental platform, are the conversion of the raw data into an appropriate format and the organization of the single sample measurements into proper data matrices. Raw data are commonly very noisy and may be affected by undesirable systematic variations, so, a proper procedure to reduce or remove these effects is extremely important to perform a good statistical analysis. These unwanted effects are mainly due to instrumental issues, experimental conditions and/or physical characteristics of the samples and, as a consequence, each analytical method requires its own typical pre-processing steps to prepare data for statistical analysis. The main data pre-processings applied to spectroscopic and spectrometric techniques used in this thesis are described below.

Spectroscopic signals collected from NIR, FT-IR, Raman or UV-Vis usually contain sources of variation not directly related to sample constituents. These include baseline offsets, instrumental drifts or light scattering, which need to be reduced without affecting the useful information [76]. The most common pre-processing techniques applied to spectroscopic data are normalization and filtering.

- **Normalization.** It is often used to minimize the spectral variability caused by scattering effects or source intensity variations. The resulting normalized spectra is scaled within a similar range and offset corrected at the same time. The most popular methods are *standard normal variate* (SNV) and *multiplicative scatter correction* (MSC) [38,75,77].
  - SNV removes slope variations and corrects baseline shifts by subtracting the average and scaling each signal (row wise) dividing by its standard deviation.
  - MSC removes wavelength-dependent effects by using linear regression on the mean of the signal (row wise).

- **Filtering.** Filters are used to minimize unwanted spectral offsets, broad baseline distortions, positive or negative slopes, and other spectral baseline effects [38,75,77].
  - *Baseline correction* corrects spectral baselines that can be distorted because of scattering, substrate absorption, environmental variability or instrumental factors. Several baseline correction methods are available, including the simplest one, *offset correction*, where a straight horizontal baseline is subtracted from the spectrum (without scaling) or *piecewise* and *polynomial baseline corrections*, where the subtracted baseline is obtained from a number of user-defined points or by an *n*th-order polynomial function, respectively.
  - *Smoothing* removes part of the random noise of instrumental signals by adjusting a polynomial to a small range of signal points (moving window averaging). Savitzky-Golay is the most applied smoothing technique [22].
  - *Derivative filters* are mainly used for smoothing spectra through the removal of baseline variations, for emphasizing spectral differences (by improving peak resolution), for accentuating small shape differences between nearly identical signals, and for correcting baseline shifts and drifts (depending on the derivative order). Savitzky-Golay (SG) polynomial derivative filters is the most popular method [22], which applies derivatives and smoothing in one single step [22].

When dealing with spectroscopic measurements the systematic differences between variables have to be corrected. For that, **mean centering** is widely applied, which consists on the subtraction of the mean value of each variable from each raw value (column wise), resulting in data with zero mean. While the average spectral intensity is removed from each spectrum the magnitude of the spectral variations is retained [78].



Mass spectrometric signal pre-processing is a critical step before performing MVA. The main objective is to arrange the instrumental signals into organized matrices containing all mass spectral signals detected across all samples. These matrices can contain different types of data. On one side, when there is no chromatographic separation, a global spectrum is obtained for each sample that contains all the abundances for each mass-to-charge ratio (m/z). On the other side, when there is a chromatographic separation, a spectrum is obtained for every retention time, which requires additional pre-processing steps like background reduction, chromatographic alignment, deconvolution of co-eluting peaks and/or peak picking. In any case, a final scaling, normalization or other mathematical transformations are necessary in both cases to improve the posterior data analysis.

- **Row profile**. It divides each signal value (abundance) of the sample by the sum of all the signal values of that sample. Row profile is useful to avoid signal drift due to differences between the first and the last analysis using a certain instrument.

- **Transformations**. *Logarithmic transformations* are applied when the instrumental signals are not linearly related to the concentrations or sensory scores (common in sensory analysis) [59].

- **Scaling**. Scaling the variables (column wise) is often recommended when working with heterogeneous variables [77,79].
  - *Autoscaling* divides each centered variable by its standard deviation. The resulting data has zero mean and unit variance. This operation is useful when variables with low signal have relevant information, giving similar importance to these variables and making scale differences comparable. However, this decreases the signal-to-noise ratio and may give the same weight to both, informative and noisy signals.
  - *Pareto-scaling* is somehow intermediate between raw data and autoscaling, where large variance variables are less down-weighted by dividing each centered variable by the inverse of the square root of the standard deviation of that variable.

(2) Variable selection

Variable selection is necessary in multivariate analysis to remove irrelevant and redundant information. In this case, the aim is to reduce the dimensionality of the data to simplify

statistical analysis and obtain better predictions. But variable selection is also necessary in sensory analysis to make the model easier to understand. For example, to explain what variables or specific regions that can be related to specific compounds are responsible for the sensory attributes described. This is very valuable to prove this 'non-direct' relationship of the non-targeted approaches, where the predicted response (e.g. sensory property) is obtained simply from the sample data (fingerprints) without any knowledge of chemical identity [59]. Moreover, variable selection can be relevant for industries too (in- or at-line applications), where using high-resolution instruments may be too expensive or measuring the whole spectrum may take too much time.

When measuring many variables it is often assumed that a large proportion of them will be irrelevant and should be rejected before data analysis. So, to select informative variables for data analysis different steps have to be followed. First, before the instrumental analysis, a preliminary variable selection based on previous knowledge of the sample or literature research has to be done to decide what variables would have expected relevance for the problem (e.g. certain spectral range). The variable selection methods should remove excessive noise, redundant information or variables non-correlated to the property of interest with the minimum loss of information. The optimal way to select the optimal variables is by trying all possible combinations to select the best ones. But this is a cumbersome and very time-consuming task, with a risk of overfitting if the number of samples is not sufficient. For these reasons, many variable selection methods have been developed to use before and while building statistical models [24,80].

In spectroscopic and spectrometric techniques the most common variable selection methods are based on model predictions. Some methods use model outputs to identify the subset of relevant variables, such as *loading weights*, *regression coefficients*, *selectivity ratios* (SR) or *variable importance in projection* (VIP) values. Other methods use these identified relevant variables to re-build reduced models with improved predictions. Examples are *genetic algorithms* (GA), *stepwise selection* (including different strategies, e.g. SELECT stepwise decorrelation) or *interval partial least-squares* (iPLS). And finally, some methods integrate the variable selection into the classification/prediction algorithm, such as *interactive variable selection* (IVS) or *soft-threshold and sparce-PLS* [81,82].

(3) Feature extraction

Another way to develop more effective MVA methods is by compressing the information contained in all measured predictors, what is called feature extraction. The use of feature extraction methods is useful to reduce the dimensionality of the individual data matrices without losing relevant information. In fact, it is a good option when the data size is still large after variable selection. The most common feature extraction techniques use PCA and PLS, this latter mainly in its discriminant mode (PLS-DA). The usual procedure is to apply the corresponding method (PCA or PLS-DA) to build an individual model for each dataset. To select the optimal number of features, different criteria can be used, most of them based on an internal validation process. Despite PCA and PLS-DA are the most common feature extraction methods, there are other alternatives depending on the data structure and the problem at hand, such as multivariate curve resolution (MCR), the Mahalanobis distance [35,83], kernel based methods or wavelet transform (WT), which provides sets of coefficients that are able to rebuild the original signal [77].

1.3.2.3. Multivariate Data Analysis (MVA)

After data pre-treatment, variable selection and/or feature extraction the information contained in the data matrix of study has to be extracted by efficient MVA techniques. In general MVA is divided into two basic categories: *unsupervised* and *supervised pattern recognition* techniques. In unsupervised techniques no prior knowledge of the sample (groups or properties) is used to build the multivariate models, while in supervised techniques known information about the samples is used in order to classify them in predefined classes or predict properties of unknown samples [84-86].

Exploratory data analysis

Exploratory data analysis is a group of unsupervised methods that does not require prior knowledge about sample properties (i.e. class or amount of compound). It provides an initial graphical tool to visualize the entire dataset and is aimed at identifying trends, patterns and clusters among samples, extracting important variables, finding relationships among them, and detecting outliers and anomalies in the data. Therefore, exploration should be the first

step in data analysis to improve data knowledge, being principal component analysis (PCA) the most common method applied [6,20].

- **Principal component analysis – PCA**

  PCA is often used as a diagnostic tool before applying other multivariate techniques. The purpose of PCA is to decompose the matrix of measured instrumental responses $\mathbf{X}_{[m,n]}$ (often mean-centered or autoscaled) into the product of smaller matrices, as described by the equation (1).

  $$\mathbf{X}_{[m,n]} = \mathbf{T}_{[m,f]}\,\mathbf{P}^{T}_{[f,n]} + \mathbf{E}_{[m,n]} \qquad \text{Eq. (1)}$$

  The new product is composed by the matrices of loadings ($\mathbf{P}_{[f,n]}$), scores ($\mathbf{T}_{[m,f]}$) and a residual matrix ($\mathbf{E}_{[m,n]}$) that contains the part of the original data not modeled by the f factors ($f \ll n$). While the loading matrix ($\mathbf{P}$) define a new coordinate system using the original set of n variables (columns), the scores matrix ($\mathbf{T}$) describe the new coordinates of the m calibration samples (rows) in this new coordinate system. The modeling factors (f), called principal components (PCs), are linear combinations of the original variables and are calculated by iteration to maximize the data variance. PCs are then hierarchical, that is, the first PC explains the maximum variance in the data, the second PC explains the maximum variance left and so on. PCs are also orthogonal to each other, that is, each consecutive PC explains the variance not explained by the previous ones, thus describing complementary information [20, 87].

Classification and Prediction methods

Supervised methods are aimed at classifying or predicting and make use of preliminary information about the membership of the samples to given predefined classes or about certain parameters of the samples (e.g. concentration or amount of a given constituent). Thus, supervised methods can be applied either for classification or prediction purposes.

Classification is the action to assign one object (sample) to one category based on a set of experimental measures. It can be done by identifying regions in the variable hyperspace corresponding to the different categories where objects share similar characteristics. However, these regions can be defined in different ways and this gives rise to discrimination or class-modeling techniques. When discrimination is the objective the hyperspace is divided in as

many regions as the number of available categories and a sample is always assigned to one of the available categories. In particular, one of the most applied discriminating techniques is Partial Least Squares - Discriminant Analysis (PLS-DA). But other methods have also been used, such as linear and quadratic discriminant analysis (LDA and QDA), canonical variate analysis (CVA), discriminant function analysis (DFA), *k*-nearest neighbors (*k*NN), discriminating Artificial Neural Networks (ANNs) and support vector machines (SVMs) [5]. Unlike discrimination techniques, in class-modeling techniques every category is modeled separately without considering the others. As a result, one sample can be accepted or rejected by one or more specific categories [6,88]. Soft independent modeling of class analogies (SIMCA) is probably the most popular class-modeling technique [5,6,89].

- **Partial-least squares - Discriminant Analysis (PLS-DA)**

  PLS-DA is a discrimination technique based on the PLS regression method (see section below). Although the PLS algorithm was firstly developed to build prediction models, it was further adapted for classification problems. In PLS-DA, the **X** matrix contains the spectra (fingerprints) of the samples and the **Y** matrix contains a dummy variable that codifies the class of the samples using a binary representation (usually zeros and ones). In case of a binary classification, the PLS-DA predicted values for unknown samples are around zero or one and are then converted into the membership class using an optimized threshold (i.e. 0.5) [88,89].

Predictive models are focused on quantification and are required when the response variables are numeric or continuous (e.g. descriptors' intensity). Different types of regression algorithms are used, but the most widely extended is partial least-squares (PLS) regression. Other methods are multiple linear regression (MLR), principal components regression (PCR) or SVM regression [87,90].

- **Partial-least squares (PLS) regression**

  Partial least squares (PLS) regression describes the relationship (regression) between the original independent matrix (**X**) and the dependent variable (**Y**). PLS performs a simultaneous decomposition of **X** and **Y,** searching a set of components (latent variables) that maximize the covariance between **X** and **Y**. An optimal regression is obtained

successively substituting the scores of the **X** matrix (**T**) by the scores of the **Y** matrix (**U**), and vice versa, up to convergence. The PLS decomposition can be expressed as described in equations (2) and (3).

$$\mathbf{X} = \sum_{f=1}^{F} \mathbf{T}\,\mathbf{W}^{T} + \mathbf{E} \qquad \text{Eq. (2)}$$

$$\mathbf{Y} = \sum_{f=1}^{F} \mathbf{U}\,\mathbf{Q}^{T} + \mathbf{F} \qquad \text{Eq. (3)}$$

The new products are composed by the score (**T**), loading (**W**) and residual (**E**) matrices of **X**, and by the score (**U**), loading (**Q**) and residual (**F**) matrices of **Y**. The loading columns (**W**), called loading weights, indicate how the **T** scores are to be computed from **X** to obtain an orthogonal decomposition. Through the several methods used to calculate the PLS parameters, NIPALS is the most common and intuitive. From the NIPALS decomposition the coefficients of the PLS models (**b**) are obtained, and for these, the predicted property ($\hat{y}$) for the new sample is calculated as $\hat{y} = \mathbf{x}^{T}\mathbf{b}$, where **x** contains the spectrum of the new sample [91].

1.3.2.4. Model validation

As it has been mentioned in previous sections (**Figures 1.5**, **1.6** and **1.7**), when models are built using MVA techniques a validation strategy is required to assess their reliability when classifying or predicting new unknown samples. It is very important to avoid overoptimistic results from overfitted models (i.e. models that fit well calibration samples but fail in predicting validation samples) and, for this reason, both an internal and an external validation should be considered. For that, datasets have to be divided into training and test sets, either randomly or using specific algorithms (Kennard-Stone [92]), and the split process should ideally be repeated several times (**Figure 1.8**). The training (calibration) set is used to build the optimal model, following an internal validation to select the number of factors (latent variables) and to avoid overfitting. Cross-validation is probably the most common internal validation procedure, dividing the objects (samples) available from the training set into different cancellation groups following a predetermined scheme (e.g. leave-one-out, venetian blind or contiguous blocks). The test (external) set is aimed at obtaining the final best model. It is composed by samples not used for building the models and provides information about the prediction ability of the models (test-set validation) [38,43,77].

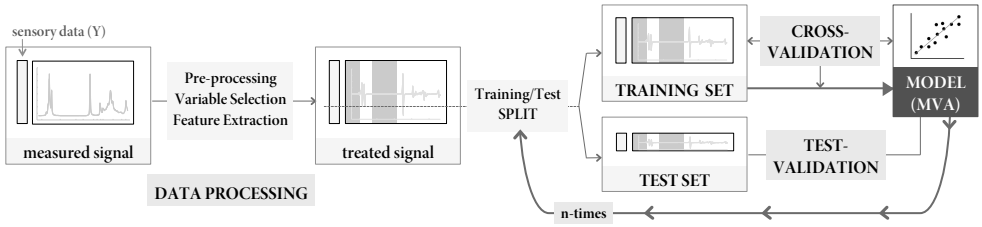


Figure 1.8. General example of model validation with internal (cross-validation) and external (test-validation) validations.

Once the validation strategies are decided, different performance indicators have to be calculated to assess the ability of the classification/prediction results. Those *parameters* depend on the MVA applied to the data, so classification and regression approaches will require different criteria to validate the final results.

Also, the methods to determine relevant variables are used to rank the importance of the individual variables according to one or several metrics. These methods evaluate the variables that carry the information related to the predicted property (**y**), by using a cutoff criterion to segment relevant/irrelevant variables, which can be determined arbitrarily (based on past experience) or through statistical assessment.

Classification methods

When classification (discrimination) methods are applied, such as PLS-DA, it is necessary to have an estimation of their classification performance. General parameters are available for that purpose (**Table 1.1**).

Table 1.1. Common ability parameters for classification models (confusion matrix).

		Measured values (real)		
		Class 1 - Positive	Class 2 - Negative	
Predicted values (model outcome)	Class 1 - Positive	TP True Positive	FP False Positive	PPV Positive Predictive Value
	Class 2 - Negative	FN False Negative	TN True Negative	NPV Negative Predictive Value
		Sensitivity	Specificity	

The classification ability is often determined by the *accuracy* (correct classification) and the *misclassification* (inaccuracy or error) rates. Both parameters express the probability of having samples well or badly classified, respectively, only considering the correctly classified samples, TP and TN (equations (4-5)).

$$\text{accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \quad \text{Eq. (4)}$$

$$\text{misclassification} = 1 - \text{accuracy} \quad \text{Eq. (5)}$$

Sensitivity and *specificity* are also parameters that express the classification success. Sensitivity is defined as the fraction of samples belonging to a given modeled class that are correctly assigned to that class. Specificity is defined as the fraction of samples rejected from the modeled class that in fact do not belong to that class. Both parameters are related to correctly classified (TP and TN) and incorrectly classified (FP and FN) samples, following the equations (6-7).

$$\text{sensitivity} = \frac{\text{true positive}}{\sum \text{condition positive}} = \frac{TP}{TP + FN} \quad \text{Eq. (6)}$$

$$\text{specificity} = \frac{\text{true negative}}{\sum \text{condition negative}} = \frac{TN}{TN + FP} \quad \text{Eq. (7)}$$

In order to find a parameter to simplify sensitivity and specificity interpretation *efficiency* and *classification error* are often described. These parameters compute the average of sensitivity and specificity values (equations (8-9)). But when the classes are unbalanced, that is, one class having a lower number of samples, this parameter gives higher values than expected, being more adequate the use of accuracy or misclassification parameters.

$$\text{efficiency} = \frac{\text{sensitivity} + \text{specificity}}{2} \quad \text{Eq. (8)}$$

$$\text{classification error} = 1 - \text{efficiency} \quad \text{Eq. (9)}$$

All these parameters used to describe the classification ability are derived from the confusion matrix (**Table 1.1**), but other diagnostic tools are also commonly studied for a better interpretation of the results. Receiver operating characteristic (ROC) curves are very popular, providing a visual and simple representation to compare classification results (sensitivity versus 1-specificity). Other common diagnostic plots for classification models are the Y-

predict plot, the distance-to-leverage-plot, the class projection into a principal component score and Pareto's decision method [35,38,77]. The last method is aimed at finding a visual tool to select the best sensitivity and specificity results, but it can also be used with regression parameters. Each model is represented in a bidimensional scatter plot reporting sensitivity and specificity on each axis, respectively, called Pareto diagram [93].

Prediction/Regression methods

An estimation of the goodness of fit and predictive performance is necessary when developing prediction/regression methods (i.e. PLS). *Determination* (R^2) and *correlation* (R) *coefficients* are indicators of the goodness of fit. R^2 derives from the y-prediction plot of measured against predicted values and is calculated as the ratio between residual sum of squares (SS_{res}) and the total sum of squares (SS_{tot}), proportional to the variance of the data. These values are determined with the sum of the differences of each measured property (y_i) and the mean of observed data ($\bar{y}$) or each predicted property ($\hat{y}_i$) computed by the model built with n samples (equations 10-12).

$$SS_{tot} = \sum_i (y_i - \bar{y})^2 \qquad \text{Eq. (10)}$$

$$SS_{res} = \sum_i (y_i - \hat{y}_i)^2 \qquad \text{Eq. (11)}$$

$$R^2 = \frac{1}{SS_{res}/SS_{tot}} \qquad \text{Eq. (12)}$$

Other important parameters are residual and error values, such as the p*redicted residual error sum of squares* (PRESS), the *root mean squared error* (RMSE) or the *standard error* (SE). Usually, the quality of the models is checked by means of the root mean square error of cross-validation (RMSECV) or prediction (RMSEP). RMSE is an absolute value having the same units as the predicted values, in a similar way to the standard deviation in univariate statistics and is calculated using the sum of the differences of each measured property (y_i) and each predicted property ($\hat{y}_i$) computed by the model built with n samples, either by internal (cross-validation, $\hat{y}_i^{CV}$) or external (test-set/prediction, $\hat{y}_i^{P}$) validations (equations (13-14)) [43].

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i^{CV})^2}{n}} \qquad \text{Eq. (13)}$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i^{P})^2}{n}} \qquad \text{Eq. (14)}$$

Variable Importance

Once the specific parameters determine the suitable models to classify or predict the samples, it is necessary to identify the significant variables (or loadings) used to build these models. There are several methods to find the relevant variables that rank them according to one or several metrics. These methods evaluate the variables that carry the information related to the predicted property (*y*), by using a cutoff criterion to segment relevant/irrelevant variables, which can be determined arbitrarily (based on past experience) or through statistical assessment. The metrics more commonly used include the magnitude of the *PLS regression coefficients*, the *PLS weight vectors* (*w*), the *selectivity ratio* (S_R) and the *variable importance in projection* (VIP) [80].

In this thesis, the VIP index has been used for judging the importance of the X-variables on Y, and has been defined for a variable *j*:

$$\mathrm{VIP}_j = \sqrt{\mathrm{N}_{\mathrm{vars}} \frac{\sum_{k=1}^{F} \left(c_k^2\, \mathbf{t}_k^{\mathrm{T}}\, \mathbf{t}_k\right) \left(w_{jk} / \|\mathbf{w}_k\|\right)}{\sum_{k=1}^{F} \left(c_k^2\, \mathbf{t}_k^{\mathrm{T}}\, \mathbf{t}_k\right)}} \qquad \text{Eq. (15)}$$

where $\mathbf{t}_k$ is the vector of sample scores along the *k* latent variable, c_k is the coefficient of the *k* PLS inner relationship, $\mathrm{N}_{\mathrm{vars}}$ is the number of experimental variables and w_{jk} and $\mathbf{w}_k$ are the weight of the *j* variable for the *k*LV and the weight vector for the *k*LV, respectively. The advantage of using VIP scores to estimate the contribution of the original variables to the PLS model is that it can be demonstrated that the average of squared VIP scores equals 1. Therefore, a criterion to identify the most significant variables generally use the *greater than one rule* [94-96].

1.3.3. Paper 1 - review

The application of data fusion strategies at different levels of abstraction in the field of food and beverages has raised the interest of researchers and food industry. The following review enumerates and critically discusses the main applications of instrumental data fusion reported in the last years. These applications are focused on authenticity and quality assessment of food and beverages and are published in *Analytica Chimica Acta* [**Paper 1**].



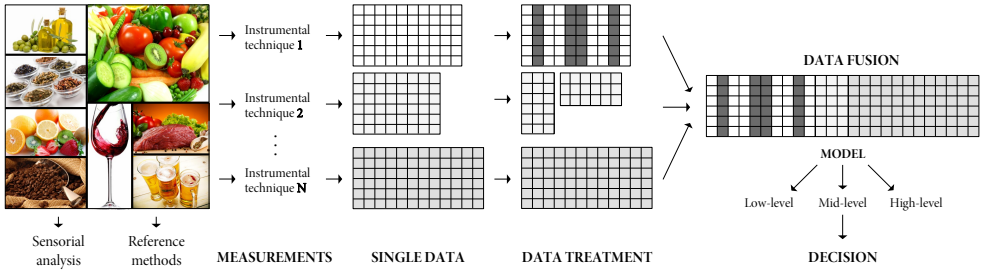
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Data fusion methodologies for food and beverage authentication and quality assessment - a review

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Graphical abstract



Abstract

The ever-increasing interest of consumers for safety, authenticity and quality of food commodities has driven the attention towards the analytical techniques used for analyzing these commodities. In recent years, rapid and reliable sensor, spectroscopic and chromatographic techniques have emerged that, together with multivariate and multiway chemometrics, have improved the whole control process by reducing the time of analysis and providing more informative results. In this progression of more and better information, the combination (fusion) of outputs of different instrumental techniques has emerged as a means for increasing the reliability of classification or prediction of foodstuff specifications as compared to using a single analytical technique. Although promising results have been obtained in food and beverage authentication and quality assessment, the combination of data from several techniques is not straightforward and represents an important challenge for chemometricians. This review provides a general overview of data fusion strategies that have been used in the field of food and beverage authentication and quality assessment.

1. Introduction

The consumer's demand for high quality food products is steadily increasing. The quality, especially of agricultural products, is specified in terms of a traceable origin, known chemical composition, adequate physical properties, satisfactory sensory evaluation, safety and health safeguards with respect to microbiological and toxic contamination and is influenced by the processing and storage of the products.

Fraudulent acts such as the adulteration with cheaper ingredients decrease the quality of the products, mislead the consumer and may imply a health risk. This is even more economically relevant for products that must comply with special laws of Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) as stated in the European Union (EU) product quality policy of agricultural and rural development. Because of their much-appreciated characteristics, such products have a high added value and are more prone to deceptive practices. In this sense, authorities are required to be able to assess the authenticity of a suspect product regarding the legal product description, detect fraudulent processing practices, prevent adulteration and control any other practices which may mislead the consumer such as mislabeling of geographical origin or composition of the product [1]. In order to protect producers and consumers from these fraudulent activities, the EU has established regulations with quality schemes determining specific origin and production processes of high valued food products [2], protecting consumers rights to receive truthful information about the food [3], assuring quality policy measures of specific products [4-6] and protecting geographical indications and designations of origin [7-9]. In addition, European research projects such as the TRACE project (Tracing Food Commodities in Europe, No.FP6-2003-FOOD-2-A 006942 (2005–2009)) were funded to advance in the practice of verifying the origin of food products. One of the outputs of the TRACE project was that the combination of classical analytical methodologies and chemometric methods could determine characteristic patterns of compounds or parameters related to a geographical origin, the adulteration of samples or some specific conditions (e.g. processes, storage, harvest or variety).

Once authentication has been granted the main basic technique for food quality assessment from the consumer point of view is sensory analysis, which has a degree of subjectivity inherent to human perception. In recent years, much research has been performed to substitute the perception of human senses with 'artificial sensors', instruments providing signals related to the sensory attributes together with suitable multivariate pattern recognition techniques. Given the complexity of the foodstuff matrices, food quality derives from a complex combination of characteristics so analytical measurements for a single analyte or technique can rarely be correlated with the quality fulfillment. It is necessary to switch to multivariate data analysis to obtain the required quality information and monitor key production parameters. Combined with rapid and reliable sensor, spectroscopic and chromatographic techniques that are available today, multivariate analysis provides more defined information about the stated quality of food, eases distinguishing between food samples and facilitates authenticity determination [10-12].

In this progression of improving the quality assessment and authentication of food, a further step is to combine - fuse - the outputs of multiple instrumental sources. Fusion of data from complementary techniques can provide more accurate knowledge about a sample, and yield better inferences (classifications with less error rate and predictions with less uncertainty) than a single technique. The concept of data fusion in food authentication is not new, as humans combine multiple senses in order to achieve more accurate inferences about food suitability and improve the chance of survival. And it is neither new for chemometricians, who have combined for a long time single chemical parameters determined by classical or instrumental analysis into a single matrix with the aim of improving food authentication results [13,14]. The challenge today is how to meaningfully combine not just single variables as it was done in the past, but blocks of them (e.g. NIR spectra and HS-MS spectra). Nowadays, analytical laboratories commonly have first- (NIR, Raman, HS-MS) and second-order (GC-MS, LC-DAD, EEM fluorescence) instruments that can successively be used to analyze the same sample. The multivariate statistical analysis of fused data from these techniques can be a powerful tool for obtaining more reliable results [15]. This requires developing new ideas for preprocessing data blocks, selecting variables and validating models. Last, but not less important, data to be fused must provide complementary information to be useful. This means that the chemical knowledge about the samples and the problem at hand is fundamental in order to select the suitable analytical techniques.

To asses quality and authenticate food and beverage products, most of the authors follow a common methodology proposed by Steinmetz in 1997 [16]. First, instrumental responses are selected so that they are related to suitable food properties such as sensory descriptors, organoleptic attributes, geographical or ripening differences or storage conditions. Reference methods to determine these properties are required. The potential instrumental techniques have to be evaluated in order to find possible redundancy and complementarity. After sample measurement and data generation the level of data fusion is decided. The choice depends on the techniques selected and the amount of data generated. Finally, results from fused data have to be evaluated by comparison with single source results and the results obtained by reference methods, and the proposed data fusion approach is accepted or rejected. We have to keep in mind that the main goal of data fusion is to enhance the synergy between the fused techniques by using complementary inputs, so to finally obtain better classification or prediction results.

In the literature there is a wide variety of means for carrying out the mentioned steps as well as very diverse applications. This work reviews the fusion of multivariate instrumental techniques that have been applied in the last years in the authentication and quality assessment of beverages and food commodities.

2. Analytical techniques used in data fusion

2.1. Sensor technology

The objective of sensor technology is to emulate human senses and to predict sensory scores of food. The most common devices on instrumental sensory analyses are electronic noses, electronic tongues

and colorimetric techniques that transform some form of input signal originated from the sample into electric, magnetic, chemical, thermal or radiation energy [17]. The responses are then correlated to aroma, taste and visual attributes or parameters respectively.

Electronic noses using this technology consist of headspace sampling, sensor arrays and pattern recognition modules that generate a characteristic odor profile. The most commonly used are conductimetric sensors such as conductive polymer sensors [18-20], metal oxide semiconductors (MOS) [21-24] and metal oxide semiconductor field-effect transistors (MOSFET) [25,26]. Piezoelectric sensors are occasionally used and combinations of different types have been studied for fruit juices [27]. Electronic tongues are amperometric/ voltammetric [22,28-30] and potentiometric [18,28,29,31,32] sensors mainly used to emulate taste and detect chemical substances in liquid samples. Finally colorimetric sensors can detect substances that cannot be detected by electrochemical sensors. They are based on phenomena such as fluorescence, reflection and absorbance. They are employed together with electronic noses and tongues to determine quality parameters [24,33,34].

2.2. Spectroscopy

Spectroscopic techniques based on infrared (IR), Raman, UV-vis, fluorescence or NMR spectroscopy are widely used for food fingerprinting. Most of these techniques offer the possibility of analyzing relatively small amounts of sample or sample extract in a non-destructive, easy and direct (minor sample preparation) way, and allow the simultaneous determination of several properties in the sample [1].

IR spectroscopy measures molecule vibrations, resulting in a spectrum that is a unique 'fingerprint' suitable for classification studies for a wide variety of food products [21,35-37]. Recently, Fourier-Transform infrared spectroscopy (FTIR) has achieved good results as an alternative system of the most conventional systems of electronic tongues [38-40]. Data fusion of NIR and MIR is a quite used strategy that improves authentication results [41-46]. UV-visible spectroscopy is widely implemented in most food quality control laboratories [47,48] and it is useful as an electronic eye for correlating visual parameters to specific geographical regions or food varieties [35,36,49]. Although this technique has some limitations for characterization and authentication purposes, its combination with other techniques such as NIR and/or MIR can enhance the results [41,42,44]. High-resolution NMR has also been applied in many food authenticity studies [50,51], despite the high cost of this technique being a major drawback for its routine application.

2.3. Mass spectrometry

Mass spectrometry (MS) is an identification and quantification technique that, after sample ionization, measures abundances of the ion fragments and differences among them. For complex samples such as food and beverages, MS is much more powerful with a previous separation by gas or liquid chromatography. However, MS has been used also without previous separation as aroma sensor

coupled to different sampling techniques. Its combination with headspace sampling systems (direct, dynamic or using sorbents like SPME or SBSE) constitutes a widely employed alternative to electronic nose sensors and has become one of the most extended methodologies to sensorilly characterize food products and assess their quality [48,52,53].

Other spectrometric techniques used in food analysis are isotope ratio mass spectrometry (IRMS) and inductively coupled plasma mass spectrometry (ICP-MS). IRMS was applied to determine geographical origin and to evaluate production factors [50,54]. ICP-MS is a fast, multi-element technique able to determine inorganic elements at low concentrations. This technique was used to screen authenticity of food products obtaining fingerprints of the element pattern for liquid and solid samples [55].

2.4. Separation techniques

Chromatography is the most used separation technique in food analysis because it allows not only the separation of the constituents of a sample but also, when coupled to a powerful detection technique such as MS, the identification of almost any molecule. Gas chromatography (GC) is suitable for the analysis of volatile or semi-volatile molecules, like aroma compounds [40,53]. Liquid chromatography, in particular HPLC, is a more versatile technique, appropriate to analyze a wide variety of compounds. HPLC is used in food authentication and quality assessments to determine compounds such as proteins, amino acids, phenolic compounds and carbohydrates [22,51]. There are numerous types of detectors, both for GC and HPLC, being MS one of the most extended, mainly because of its excellent compound identification capabilities [46,56].

2.5. Sensory analysis

Sensory analysis is the use of human responses to evaluate consumable products like food. Apart from the preference tests that use specific consumer panels, a panel of trained experts evaluates appearance, odor, flavor, color and texture among other properties. Sensory analysis implies some inherent degree of subjectivity, the responses vary over time and the number of samples that can be evaluated per day is limited. Because of these inherent difficulties, there are early attempts to replace the sensory evaluation by objective instrumental techniques [23,24,33,37,57].

2.6. Other techniques

Apart from the techniques previously described, other physical and chemical methods have been used, to analyze different food products, to authenticate them and to assess their quality. Physical properties such as conductivity and density have been measured by conductivity-meters and density-meters, respectively [58]. Texture has been measured by acoustic impulse resonance frequency (AIF) [58,60] or compression tests [60]. Firmness has been determined by acoustic firmness sensing (AFS), bioyield tests (BY), impact responses, micro-deformation and/or Magness–Taylor penetration force (MT). Composition or purity have been determined by refractometry among other techniques [61-63].

Concerning chemical analysis, they usually imply very specific methodologies, such as the determination of free acidity or peroxide index [64,65]. Other systems are the ones related to color and image, like computer vision systems based on RGB components [21,66] or CIELab methodology [64,67]. In addition, emerging techniques for product quality evaluation are being applied. Among these techniques, hyperspectral scattering image (HSI) should be named. It allows measurements of both spatial and spectral information of the samples combining conventional digital imaging with spectroscopy. HSI is increasingly used for quality and safety evaluation and control in the food industry [62,68,69].

3. Chemometric techniques used in data fusion

3.1. Descriptive models

Whatever the data fusion approach used, preliminary exploratory analysis is carried out in order to assess the repeatability of the measurements and detect clear outliers. PCA and clustering are the most used techniques for that purpose.

PCA is a dimension reduction technique that creates a few new variables called principal components (PCs) from linear combinations of the original variables. For highly collinear data, a few principal components retain the same information as many original variables, and allow the distribution of samples and variables to be easily plotted and visually analyzed. Because it is easy to use, this unsupervised exploratory technique is usually applied prior to any other more complex classification [29,51,70] or prediction [52,71] method. In some cases the single use of PCA scores can separate samples in groups. This has been used to discriminate wines [34], milks [72] and different beverages [25], and to assess texture quality of tortilla corn chips [73]. Cluster analysis (CA) separates samples into specific groups based on similarity measures. CA has been applied to improve olive oil classification [30], achieve wine quality assessment [32] and find relationships between fruit juice attributes [31].

3.2. Classification models

The classification methods most commonly used for fusion approaches for food characterization can be divided in two groups: a) class discriminating techniques that define delimiters between established classes so that problem samples are always assigned to one of the classes, and b) class modeling techniques that calculate a separate model for each established class, so that an unknown sample can be either assigned to that class or rejected.

3.2.1. Class discrimination techniques

Class discriminating techniques include linear discriminant analysis (LDA), k nearest neighbors (kNN), Partial Least Squares – Discriminant Analysis (PLS-DA), support vector machine (SVM) and some kind of discriminating Artificial Neural Networks (ANNs).

LDA is one of the most frequently used discriminant techniques in food analysis. It is based on maximizing the ratio between-class vs within-class variance using linear combinations of the original variables to achieve class discrimination. LDA has been used to classify beer, wine, tea or olive oil using fused data, for example. Beer samples were discriminated using two types of electronic tongues [29,74] or combining MIR, HS-MS and UV-Vis [39]. Wine cultivars or varieties were differentiated with artificial noses and tongues [35,40], tea was characterized with electronic sensor systems [18,75] and extra-virgin olive oil was acceptably classified using NIR, MIR and other electronic devices [47,48]. Also honey and syrups were discriminated using sensor technology [19]. Other discriminant methods include quadratic discriminant analysis (QDA) and Discriminant Function Analysis (DFA). QDA, which establishes parabolic boundaries, was used to authenticate rainbow trout fillets using NIR outperforming linear classifiers like LDA or PLS-DA [60]. DFA was applied to origin discrimination to very different food products like coffee [43], potato [53] and cheese [64] and to assess fish quality [67]. Canonical variate analysis (CVA) is another discriminant technique similar to LDA, but CVA can use a matrix containing the membership information. This technique was successfully applied to detect adulteration levels in tomato fruit juices [71,76].

A common situation in data fusion is that the number of variables is much larger than the number of objects. This is a problem for classical LDA that requires a lower or equal number of variables and samples. This restriction is usually overcome either by using PCA scores for LDA [77] or by using discriminant techniques such as PLS-DA in which feature extraction and discrimination is carried out by the same model. In PLS-DA a regression model is calculated relating an X block of instrumental signals to a Y block of sample classes in coded units (zeros and ones). For each sample, the model predicts a value around zero or one that is then converted into a class label using an optimized threshold. PLS-DA has been widely applied to classify alcoholic beverages, olive oils, fruits and other food products. Combining electronic systems, spectroscopic/ spectrometric techniques or chemical analysis, wines [51,78-80], musts [81] and beers [41] were discriminated according to its composition or origin by PLS-DA. This technique also allowed classifying olive oils according to their varieties [46,47] and predicting some properties on apples [38,61], peaches [82] and orange juices [56]. Other foodstuffs studied using PLS-DA have been coffees [43], fish products [66], cheeses [64], culinary spices dyes [83] and yellow peas [84]. In the last example, another classification technique called orthogonal projection analysis (OPA) provided better results than PLS-DA.

For data showing non-linear behavior, artificial neural networks (ANNs) have shown superior performance. ANNs mimic a biological system that collects and transfers signals to the central nervous system, processes the data, and makes specific decisions depending on the identified objects. The most frequently used ANNs in data fusion include back propagation (BPNN) and modified versions, like radial basis function (RBF) and probabilistic neural networks (PNN). The most classical and common feed-forward multilayer network is BPNN. It consists of neurons arranged in layers, being the unidirectional connection from input to output. It has been used to recognize patterns in fruit juices

[80], potato chips cream [85], rice wine [24] and pork meat [21] using electronic sensor systems. RBF is embedded in a two layer neural network, where each hidden unit implements a radial activation function. This has also been used with fruit juices [80]. PNN is basically the implementation of a statistical algorithm called the kernel discriminant analysis in which the operations are organized in a multilayered feed forward network with four layers: an input layer, a pattern layer, a summation layer and an output layer [75].

Other less used technique is k nearest neighbors (kNN) where distances (usually Euclidean ones) between an unknown sample and the modeling samples used to decide the class. This technique is simple to use but it cannot work properly when the number of samples for each class is very different and it does not give information about the structure of the classes or the importance of the variables. Although kNN is rarely used, it has been applied to different instrumental data to authenticate and assess quality of potato chips and cream [85], rainbow trout [60], yellow peas [84]. Another scarcely used technique is classification and regression trees (CART) analysis. This is a form of binary recursive partitioning where data are split repeatedly into groups in a way that each group of samples is represented by a 'node' in a decision tree. It has to be noted that CART applications such as wine origin characterization do not provide enough discrimination as other techniques like LDA [86].

Finally, support vector machine (SVM) is a technique applicable to classification and regression problems. In the case of classification, SVM is focused on obtaining the 'optimal' boundary of two classes in a vector space independently on the probabilistic distributions of training vectors in the dataset. This technique is increasingly popular and it has been used to classify olive oils origin with sensors [30,87] and to authenticate fresh cherry tomato juice with sensors [71,77].

3.1.2. Class modeling techniques

Class modeling techniques include soft independent modeling of class analogy (SIMCA) and unequal class models (UNEQ).

SIMCA calculates a PCA model for each specific class. SIMCA models provides better results than discriminant models (PLS-DA) in beer and wine geographical classification [41,70]; but there were less effectiveness to assess wine origin than when using LDA [48]. UNEQ is based on the assumption of multivariate normally distributed groups, using Mahalanobis distance (or generalized distances) from the centroid of the modeled class. It can be only applied when the number of variables is relatively low and it is very sensitive to unbalanced datasets. Some studies used both SMICA and UNEQ class modeling techniques to characterize samples, such as olive oils origin authentication with MIR, NIR and UV-vis obtaining similar results [36,42].

3.3. Prediction models

Partial least squares regression (PLSR) is probably the most popular latent variable regression method and is especially suited for datasets with more variables than samples. PLSR seeks to maximize the

covariance between the X and Y blocks, in a way that the new latent variables not only explain the variability of X but are also maximally correlated to Y. PLSR has been used in food analysis to predict properties or composition parameters. Wine [22,33,52,57] and olive oil [46,88] samples have been successfully predicted from combinations of different instrumental techniques. Also, fruit and vegetables like apples [38,61,62], peaches [82], bell peppers [69], meat [44,68], cocoa [45] and soybean flour [89] have been studied. Other regression methods, although less popular, are principal component regression (PCR) [69,71,76], multiple linear regression (MLR) [76] and SVM regression [69].

4. Data fusion strategies in food authentication and quality assessment

4.1. Fusion approaches

The data from the techniques mentioned in the previous section 2 have been combined in different ways to enhance the classification of food products and the prediction of their properties. The combination of data can be carried out basically at three levels. These are represented in **Figure 1**.

In the low-level fusion data from all sources are simply concatenated sample-wise into a single matrix that has as many rows as samples analyzed and as many columns as signals (variables) measured by the different instruments. This is then used for calculating a single model that provides the final classification or prediction. Although concatenation can be done without additional mathematical preprocessing, specific operations may be necessary on the data from each source. Pre-processing methods, variable selection methods and feature extraction techniques are discussed in the sections below. Low-level fusion also includes the "outer product" of signal vectors [38,82]. All outputs of one instrument are multiplied by all outputs of another instrument, resulting in a three dimensional merged data matrix that consists of all possible combinations of the signal values from both instruments. The first dimension is equal to the number of samples, the second dimension represents the signals of the first instrument (variables) and the third dimension represents the signals of the other instrument (variables). This matrix can be analyzed with multiway methods or can be unfolded to two dimensions and analyzed with suitable multivariate methods.

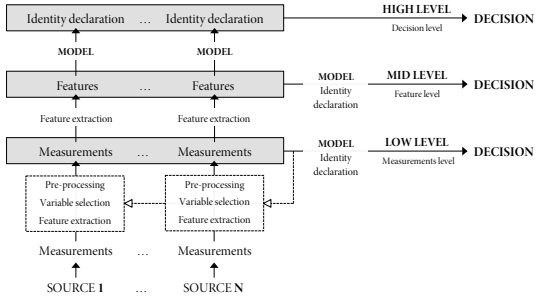


Figure 1. Data fusion shceme at low-, mid- and high-levels.

Intermediate or mid-level fusion (also called feature level fusion) first extracts some relevant features from each data source separately and then concatenates them into a single array that is used for multivariate classification and regression (**Figure 1**). The most common approach is to fuse a number of latent variables obtained independently from the signals of each instrument. Usually scores from Principal Component Analysis (PCA) or Partial-Least squares discriminant analysis (PLS-DA) are used [41]. The challenge is to find the optimal combination of extracted features and pre-processing that describe the significant variation of the instrumental responses and provides the best final model.

In the high-level fusion, also called decision level fusion, separate classification or regression models are calculated from each data source, and the results from each individual model are combined to obtain the final identity declaration (**Figure 1**). The challenge in this case is to determine the classification or regression models that work best for each block so that their combination performs better than individual models. High-level data fusion in food analysis has mostly focused on classification problems. Bayesian inference based on probability estimation is the most used decision fusion technique [90]. For each source, an estimation of the probability that samples belong to a specific class is provided a priori and these preliminary identity declarations are combined to provide an updated joint probability for each possible entity. Heuristic methods, based on voting or scoring schemes, are also used. In voting schemes a democratic (weighted) process is addressed to fuse the identities; however, in scoring schemes a ranking of scores for each data source is specified for each candidate hypothesis [90,91]. Less used are the classical inferences, such as the Dempster–Shafer's method [92] and the generalized evidence processing (GEP) theory [93]. Classical inferences draw conclusions about an underlying distribution based on an observed sample of data, typically assuming an empirical probability model. Dempster–Shafer's method and GEP theory, which are generalizations of the Bayesian techniques, have been applied to data with high level of uncertainty.

In summary, data fusion is mainly a data-driven approach. Low-level fusion is conceptually simple, uses a single model and can pick up correlations between variables of different blocks. Some limitations are a high data volume and the possible predominance of one data source over the others. This is partially overcome by mid-level fusion. Feature extraction significantly reduces the data dimensionality and allows each block to be treated individually. Also, mid-level is useful to filter block noise and enables interpretation of the results, since the contribution of each individual block can be visualized more easily than in low-level fusion. However, since many combinations of feature extraction methods and preprocessing are possible, testing all the combinations makes the whole process cumbersome, computationally intensive and difficult to validate. High-level fusion, on the other hand, allows focusing on the particularities of each individual technique, but the final identity declaration is obtained from only a few values that must accurately embed the main information from each technique. One advantage of this type of fusion is that every individual matrix is treated independently and the results from inefficient techniques do not worsen the overall performance as much as in the other fusion levels. However, this level requires accurate data preprocessing and if the correlation of the responses between

sources is not taken into account information can be lost [21,81]. It must be noted that the nomenclature "low-level", "mid-level" and "high-level" is sometimes used interchangeably in the literature and different denomi-nations have been used. An example is coupled matrix and tensor factorization approach applied to the simultaneous analysis of two- and three-way matrices [94]. Even 'hybrid' data fusion methodologies, for example combining low- and mid-level approaches [69,73]. In this paper we adopted what seems to be the most accepted nomenclature.

4.2. Pre-processing

Since multivariate analysis is scale dependent, data from single techniques are usually preprocessed in order to properly scale the data, and also remove uninformative systematic variations and reduce noise. Data from each source are treated specifically depending on their specific characteristics. For example, standard normal variate (SNV) was used for mid-infrared (MIR) data [41], multiplicative scatter correction (MSC) was used for near-(NIR) and mid-infrared (MIR) spectra [41,89] and derivatives were used to eliminate baseline shifts in infrared spectra [39,42,47], baseline corrections and derivatives were used with UV-visible (UV-vis) spectra [39] scaling/normalization with mass spectra (MS) [35,39,42], and misalignment peak correction with nuclear magnetic resonance (NMR) spectra [50]. In addition to the above, low-level data fusion may require additional preprocessing aimed to compensating for the different measuring scales and variability of each technique to prevent one block from being dominant [95]. In this sense each data block is weighted separately (block-scaling) usually with auto-scaling, root square scaling and log scaling. Finally, after data are merged, they are usually mean-centered before building the model. As in the case of low-level methodologies, extracted features in mid-level data fusion can also have different typology so scaling of the fused matrix can be required to build the model [39,41,51].

4.3. Variable selection

Variable selection is a necessary step in multivariate analysis. A preliminary variable selection is performed before any measurements are made when, based on previous expertise or literature, the analyst decides the variables that are expected to be relevant for the problem at hand and that should be measured (e.g., a certain NIR spectral range). In spectroscopic techniques, it is also common to measure wide spectral ranges and the most informative spectral regions are selected after the measurements, based on model predictions, so that the uninformative variables with excessive noise or the ones non-correlated to the property of interest are discarded with the minimum loss of information. Since the various techniques may generate blocks of data with a very different number of variables, predominance of one large matrix over the others may decrease the performance of low-level data fusion. In that aspect, data reduction is advised. The combined data matrix can still have a very large dimension and contain redundant information from the different techniques. In this case, variable selection may avoid useless and time-consuming calculations.

The most common variable selection method is stepwise selection, where variables are chosen to enter or leave the model following a selected criterion. Stepwise strategies include forward stepwise, backward stepwise or forward entry and backward removal. In the forward methods, variables successively enter the model, whereas in the backward methods the variables are successively removed from the model [77]. As an example, the SELECT is a stepwise decorrelation algorithm that generates decorrelated variables depending on the Fisher weights, and searches at each step for the variable with the largest classification weight [36,48,96]. Other methodologies for selecting variables are based on parameters and diagnostics of the model. Variable Importance in Projection (VIP) scores and selectivity ratios for PLS models discriminate variables depending on the importance of their information [41]. When data are highly correlated, regions of variables are selected instead of single variables. Interval PLS (iPLS) was also used, performing forward/reverse variable selection of variable intervals based on the cross-validation error obtained for each individual interval of variables [97]. Clustering around Latent Variables (CLV) uses hierarchical cluster analysis and consists on simultaneously determining K clusters of variables and K latent components so that the variables in each cluster are as correlated as possible to the corresponding latent component [50]. Genetic Algorithms (GAs) are a group of search methods that, inspired by the theory of evolution, create populations of solutions that evolve to optimize an objective function. Using the populations code subsets of variables GAs become a powerful tool to select the variables that maximize the predictive performance of the models [33]. Other methodologies used for variable selection include analysis of variance (ANOVA) variable selection, which evaluates the inter-intra category variance ratio (F-ratio) for each variable to rate its prediction ability, or a method based on kernel functions using predefined response 'bell-shaped-windowing' curves [22]. Although variable reduction is the most common way to regularize the datasets size, variable generation using combinations of original variables has also been used, for example, to compare different sensor responses [27].

4.4. Feature extraction

In mid-level data fusion, the features extracted from the different sources are concatenated to build a single array which is then processed by chemometric techniques. The feature extraction process is useful to reduce dimensionality and keep the relevant information when data volume is still large after variable selection. Commonly feature extraction uses principal components or latent variables from PLS-DA. Nevertheless, other methods have been applied to extract features depending on data structure and the particular problem. Some applications have used PARAFAC [51], multivariate curve resolution (MCR) [54], kernel based methods [98], independent component analysis (ICA) [57] and wavelet transform, where the signal was decomposed into several components with different frequency [96]. For computer vision (CV) systems, specific feature extraction is performed from images based on RGB color mode [21].

Feature extraction is also common when linear discriminant analysis (LDA) is the chosen technique and the number of features is higher than the number of observations because of a dimensionality problem [74,75]. Another way to deal with many variables is by using multiblock methods, such as hierarchical PCA/PLS, multiblock PCA/PLS or serial-PLS. Multiblock methods provide distinctive information from each data set and the relative importance of each block can be evaluated. Hierarchical PCA and PLS (H-PCA/ H-PLS) try to discriminate between blocks using the scores of PCA to build a new variable matrix and then build a PCA or PLS model [99,100]. In multiblock PCA and PLS (MB-PCA/PLS) the predictors are separated into sub-blocks, followed by PLS model building of each sub-block. Those sub-block models are used to build other models [101]. The main difference between hierarchical methods and multiblock methods is a normalization step. In serial-PLS (S-PLS) the predictors are separated into subsets or blocks, according to a meaningful criterion or process knowledge [100]. In the last years new multiblok PLS approaches have been proposed which, by including an orthogonalization step, handle the problems of redundancy or block scale differences. One of these methods is parallel orthogonalized-PLS (PO-PLS), which uses a combination of PLS regression and GCA (Generalized Correlation Analysis) to identify common and unique components across several data blocks [102]. A second method is sequentially orthogonalized-PLS (SO-PLS) [103], and its classification analogue SO-PLS-LDA [104]. The SO-PLS method is based on fitting the property matrix (Y) to the first block, and then fitting the estimated residuals to a second block after orthogonalization with respect to the first block.

5. Applications

5.1. Low-level fusion

Low-level data fusion has been applied to a wide range of food and beverages to authenticate origin and assess quality. **Table 1** summarizes the main applications.

Most of the analyses combine gas sensor devices (emulating an electronic nose) and liquid sensor devices (emulating an electronic tongue) and, sometimes, also UV-Vis spectroscopy or vision systems, considered an electronic eye. These techniques used all together are called "electronic panel" since they emulate the human panel responses when sensory analyzing the products. Data from these techniques were fused at low level by simply concatenating the data [25,26,32,65,67,79,86] although other approaches based on pre-processing and statistics [18,31,76,85,88], variable selection using kernel functions [22,78], stepwise decorrelation [19,29,71], ANOVA [30,87] and Gas [27,33,80] were also used. Low-level data fusion allowed improving the prediction of polyphenol and oxygen content in wines [22], a better discrimination of the origin and enhanced the authenticity determination of virgin olive oils [30,87,88], a better recognition of different types of fruit juices [25-27,31], commercial teas [18] and mold species [28], improving the identification of fresh tomato cherry juices adulterations [71,76] and enhancing the correlation of Tilapia pellets sensory scores [67]. Data fusion did not always improve

Table 1. Application of low-level data fusion

Food	Objective	Input data	Data fusion treatment	Multivariate analysis	Ref.
Alcoholic beverages					
Red wine	Oxygen & polyphenol content	Gas sensors & liquid sensors	(VS) kernel methods	PLS-DA & PLS	[22]
Beer	Brand & variety discrimination	Two liquid sensors	(VS) statistics & stepwise	PCA & LDA	[29]
Wine	Quality assessment (spoilage)	Gas sensors & liquid sensors	Simple concatenation	PCA & CA	[32]
Red wine	PDOs sensory correlation	Gas sensors, liquid sensors & UV–Vis	(VS) statistics & GA	PLS	[33]
Wine	Origin, variety & year assessment	^{1}H NMR & stable isotope	Pre-processed data, (VS) ANOVA & CLV	ICA, LDA, DFA & PLS-DA	[50]
Wine	Sensory properties assessment	HS-MS & Vis-NIR	Pre-processed data	PCA & PLS	[52]
White wine	Origin authentication	NIR, MIR & UV-Vis	Simple concatenation	PCA, SIMCA & PLS-DA	[70]
Red wine	Oxygen, polyphenol content & closure OTR sensory evolution	Gas sensors, liquid sensors & UV–Vis	Pre-processed data	PCA & PLS-DA	[78]
Red wine	Quality aging & physico-chemical correlation	Gas sensors, liquid sensors & CIELab	Simple concatenation	PCA & PLS-DA	[79]
Wine	PDO authentication & characterization	Gas sensors, liquid sensors & UV–Vis	Simple concatenation	PCA, LDA & CART	[86]
Extra Virgin (EVOO) and Virgin (VOO) Olive Oil					
VOO	Origin & variety characterization	Gas sensors & liquid sensors	(VS) statistics & ANOVA	PCA, CA & SVMs	[30]
EVOO	PDO authentication & component correlation	NIR, MIR & UV-Vis	Pre-processed data, (VS) stepwise	PCA,UNEQ, SIMCA & PLS	[42]
EVOO	Quality assessment (oxidation)	Gas sensors, liquid sensors & chem. param.	Simple concatenation	PCA & LDA	[65]
EVOO	Origin authentication	Gas sensors & liquid sensors	(VS) ANOVA	PCA & SVMs	[87]
VOO	Sensory & polyphenols correlation	Gas sensors, liquid sensors & UV–Vis	Pre-processed data	PCA, PLSDA & PLS1/2	[88]
EVOO	Cultivar authentication	NIR & MIR	Pre-processed data, (VS) stepwise, (FE) wavelet transform	LDA	[96]
Non-alcoholic beverages					
Tea	Type discrimination (brands)	Gas sensors & liquid sensors	Pre-processed data	PCA & LDA	[18]
Black tea	Sensory quality assessment	Gas & liquid sensors & sensory panel	(FE) wavelet transform	Bayesian classifier	[23]
Liquids	Quality assessment & discriminate liquid	Gas sensors & liquid sensors	Simple concatenation	PCA	[25]
Fruit juices	Type discrimination & prediction	Gas sensors & liquid sensors	Simple concatenation	PCA & PLS	[26]
Fruit juices	Characterization	Four gas sensors	(VS) GA, statistics & PCA loadings	RBF & PNN	[27]
Fruit juices	Type discrimination	Gas sensors & liquid sensors	VS – statistics (average)	PCA & CA, Fuzzy ANN	[31]
Orange juice	Flavor quality & chemical differences	HS-GC-TOF & UHPLC-TOF	(VS) ANOVA	PCA & PLS-DA	[56]
Liquids	Characterization & %milk & fat correlation	Conductivity-meter, density-meter & optical/ turbidity	Simple concatenation	Weighted non-linear least squares	[58]

Table 1 *(continued)*

Food	Objective	Input data	Data fusion treatment	Multivariate analysis	Ref.
Tomato juice	Adulteration & param. correlation (pH, SSC)	Gas sensors & liquid sensors	(VS) stepwise, factor F, (FE) PCA scores	PCA/CVA, LVQ, LibSVM & PCR	[71]
Tomato juice	Adulteration & param. correlation (pH, SSC)	Gas sensors & liquid sensors	(VS) ANOVA & stepwise	PCA & CVA, PCR & MLR	[76]
Must	Sensory discrimination	Gas sensors, FTIR & UV	(VS) GA	PLS-DA	[80]
Fruit & vegetables					
Potatoes	Origin authentication	HS-GC-MS & IRMS	(VS) forward stepwise	DFA	[53]
Apple	Characterization & parameters correlation (firmness & SSC)	Acoustic sensor, Vis & NIR	Simple concatenation	PLS-DA & PLS	[61]
Apple	Characterization & parameters correlation (firmness & SSC)	Acoustic sensor, HSI, Vis-SWNIR & bioyield tester	(VS) specific for each technique	PLS	[62]
Peaches	Quality maturity & harvest assessment	Impact response, titration, taste panel, Vis, physical parameters & refractometer	Simple concatenation	PCA, multilinear regression	[63]
Peaches	Characterization & parameters correlation	Vis & TSMR	Outer Product analysis, (FE) PCA scores	PLS & PLS-DA	[82]
Apple	Sensory correlation (sugar content)	RGB vision system & NIR	Pre-processed data	Multilayer NNs	[105]
Meat & fish					
Minced beef	Adulteration	UV-Vis, NIR & MIR	(VS) stepwise	PCA, LDA & PLS	[44]
Rainbow trout	Authentication & quality assessment	NIR, compression test & CIELab	Simple concatenation	PLS-DA, LDA, QDA & kNN	[60]
Goatfish	Fresh/frozen-thawed authentication	Vis/NIR, texture analyzer & RGB system	Simple concatenation	PCA & PLS-DA	[66]
Tilapia	Sensory quality assessment	Gas sensors & vision system	Simple concatenation	DFA	[67]
Others					
Honey & syrups	Quality assessment	Gas sensors & liquid sensors	VS -backward & forward stepwise	LDA	[19]
Mold & yeast	Species discrimination	Two liquid sensors	(VS) statistics	PCA, LDA & PLS	[28]
Coffee	Varietal authentication	NIR & MIR	Pre-processed data	DFA & PLS-DA	[43]
Cocoa powder	Quality correlation (fat, nitrogen & moisture)	NIR & MIR	Outer Product analysis	PLS	[45]
Yellow split peas	PDO authentication	ICP-MS: REEs & trace elements	Simple concatenation	OPA, Mahalanobis, PLS-DA & kNN	[84]
Soybean flour	Quality correlation (crude protein & moisture)	NIR & MIR	Pre-processed data	PLS	[89]

(VS): Variable selection; (FE): Feature extraction; PDO: Protected Designation of Origin; OTR: Oxygen transfer rate; SSC: Soluble solid content;

individual results [19,79,65] and in some cases affected negatively to the classification if both data blocks variables were correlated or noisy [80].

Other techniques used as electronic panels are NIR, MIR, UV-Vis and NMR spectroscopies. Reported applications fused NIR, MIR and UV-Vis [42,44,70], NIR and MIR [43,45,89,96] and H1-NMR with stable isotope data [50]. Although these techniques can share some repeated pieces of information, they are mostly complementary, a requirement for improving discrimination and prediction with fused data. Common fusion techniques are simple concatenation [70] with pre-processing [43,89] or variable selection such as stepwise [42,44,96], CLV and ANOVA [50]. Sometimes, combinations of instrumental responses of different nature can lead to better results using data fusion synergies, like using HS-MS (emulating an electronic nose) combined with Vis-NIR [52]. It must be again stressed the importance of appropriate pre-processing when, like in the mentioned cases, the raw data have very different magnitude. The most common samples studied by these techniques have been olive oils and wines, improving origin characterization [42,70,96] and sensory scores prediction [52]. Low-level data fusion of only NIR and MIR spectroscopic techniques could enhance the robustness when discriminating coffee varieties [43], predict better quality properties of soybean flour [89] and predict fat, nitrogen and moisture content in cocoa powder [45]. Combination of mass spectrometry techniques could also achieve good discrimination of potato origin with HS-GC-MS and IRMS [53], correlate chemical compounds for orange juice samples with HS-GC and UHPLC both coupled with TOF MS spectrometer [56] and discriminate yellow split peas origin with fusion of ICP-MS rare earth element (REEs) and trace elements [84]. In spite of the improved results, the enhancement in some applications was too small to justify the use of more than a single technique in terms of cost-benefit ratio [26,42,44].

Other techniques, mostly based on physical measurements, also improved results when low-level fusion was used. The use of vision systems, Vis, NIR and acoustic sensors could improve apple characterization [61,105]. NIR, vision systems, color, texture and shear parameters improved fish species authentication by rearing farm and genetic strain [60,66]. The use of multiple instruments (conductivity-meters, density-meters, acoustic sensors, HSI, etc.) could lead to problems of redundancy of information. In these cases data preprocessing was critical before data fusion, and sometimes the use of fewer instruments was sufficient to improve the results [58].

5.2. Mid-level fusion

Mid-level data fusion has also been applied to characterize, authenticate and assess quality of a wide range of food and beverage samples. **Table 2** summarizes its main applications.

As in low-level examples, most of the cited references use sensors, spectroscopic and spectrometric instruments, and the fusion of their data lead, in general, to enhance classification and prediction results. In mid-level fusion, the feature extraction is a fundamental step, and in most cases latent variables (mainly principal components) are used. This simplifies the fusion when data sources are very

different and there is a significant data variation. The performance of mid-level applications has commonly been compared to low-level fusion as well as to single instrument models.

Sensory quality evaluation and origin authentication are the main objectives when mid-level data fusion is applied to wines and olive oils. Some applications are based on sensor technology, emulating human sensory responses, such as gas and liquid sensors fusion [57,74] combined with color evaluation systems [34]. Different combinations of spectroscopic and mass spectrometric techniques were also reported: MIR and NIR [46], MIR, UV-Vis and HS-MS [39], NIR, UV-Vis and HS-MS [36], UV-Vis and HS-MS [48] among other combinations [24,35,40,41]. Other used techniques for wines and olive oils are NMR spectroscopy coupled to HPLC and fluorescence [51] and X-ray power diffraction fused with isotopic abundance ratio [54]. In most of them PCA was used for feature extraction (scores of the most relevant principal components), although other studies used ICA [57], MCR [51,54] and PARAFAC [51] scores. Mid-level data fusion improved the discrimination capability of different styles of beers combining two different liquid sensors [74] and combining NIR, MIR, UV-Vis and thermogravimetry [41]. Improved geographical authentication of virgin olive oils was also achieved by fusing HS-MS, NIR, MIR and UV-Vis [36,46-48]. However, the improvement in some studies was not relevant enough to recommend combining the techniques as, for example, to discriminate varieties of red wine [35]. Sometimes, fusion of different techniques was even worse than using single techniques, such as for cultivar authentication and characterization of white wines [40].

Other products such as black tea [75] and milk [72] were studied with gas and liquid sensor devices, and bell peppers [69], pork [21,68] and tortilla corn chips [73] were studied with NIR, hyperspectral imaging, physical sensors and other techniques. In all of them the prediction of sensory properties improved and some sample specific parameters like pH, texture or total volatile basic nitrogen could better be correlated using mid-level data fusion. It has to be noted that, sometimes, some important features can be lost during variable reduction with low-level fusion, thus worsening the prediction and that mid-level can better preserve the information content. However, in some cases data fusion did not improve individual results such as in the quality assessment of potato chips using sensor analysis [85]. Chinese rice wine achieved better correlation of sensory parameters using low-level fusion than mid-level [24].Also apple analysis achieved an enhancement in variety differentiation using outer product (low-level), but quantification of apples organic content showed no differences using fused data, due to the similar chemical information provided by both the electronic tongues used in the study (sensors and ATR-FTIR) [38]. Other studies showed no differences when working with low or mid level of data fusion, as it happens for some studies related to beer sensory and origin characterization [39] and class modeling of different origins of olive oils [36]. This can be attributed to the fact that low- and mid-level, though using different strategies (variable selection or feature reduction), if done properly both end up with data matrices carrying similar amount of information.

Table 2. Application of mid-level data fusion

Food	Objective	Input data	Data fusion treatment	Multivariate analysis	Ref.
Alcoholic beverages					
Chinese rice wine	Sensory evaluation & sensory parameters correlation	Gas sensors, FTIR & colorimeter	**LL** – Pre-processed data **ML** – (FE) PCA scores	MLR, BP-ANN & SVM	[24]
Wine	Origin & ages assessment	Gas sensors, liquid sensors & CIELab	(FE) PCA scores	PCA	[34]
Red wine	Varietal authentication	liquid sensors, HS-MS, NIR & UV-Vis	Pre-processed data, (FE) PCA scores	LDA	[35]
Beers	Sensory & origin characterization	HS-MS, MIR & UV-Vis	**LL** – Pre-processed data **ML** – (FE) PCA scores	PCA & Fisher-LDA	[39]
Wine	Characterization & cultivar authentication	GC & MIR	(FE) PCA scores	PCA & LDA	[40]
Beer	Quality adulteration & authentication	MIR, NIR, UV, Vis & thermogravimetry	**LL** – Pre-processed data **ML** – (FE) PCA scores	SIMCA & PLS-DA	[41]
Lambrusco wine	Type discrimination & PDO authentication	^{1}H-NMR, fluorescence & HPLC	(FE) PCA scores, MCR & PARAFAC	PCA, PLS-DA & NPLS-DA	[51]
Lambrusco wine soil	Characterization & correlations	X-ray powder diffraction & isotopic abundance ratio	(FE) MCR C matrices	MCR	[54]
Red wine	Sensory classification & correlation	Gas sensors & liquid sensors	(FE) ICA scores	PCA, PLS-DA & PLS	[57]
Beer	Type discrimination (styles)	Two liquid sensors	(VS) statistics, (FE) wavelet & PCAscores	PCA & LDA	[74]
Extra Virgin (EVOO) and Virgin (VOO) Olive Oil					
EVOO	Class modeling of geographical origin	HS-MS, UV-Vis & NIR	**LL** – (VS) stepwise **ML** – (FE) PCA scores	UNEQ-QDA & SIMCA	[36]
VOO	PDO characterization & param. correlation	NIR & MIR	**LL** – Simple concatenation **ML** – (FE) PCA scores	PLS-DA & PLS	[46]
EVOO	Origin authentication	UV-Vis & physico-chemical parameters	**LL** – Pre-processed data **ML** – (FE) PCA scores	PCA, LDA & PLS-DA	[47]
EVOO	PDO^{c} authentication	HS-MS (e-nose) & UV-Vis	**LL** – Pre-processed data, (VS) stepwise **ML** – (FE) PCA scores	LDA & SIMCA	[48]
Non-alcoholic beverages					
Milk	Quality assessment	Gas sensors & liquid sensors	**LL** – Simple concatenation **ML** – (FE) PCA scores	PCA	[72]
Black tea	Sensory quality assessment	Gas sensors & liquid sensors	(FE) Wavelet & PCA scores	PCA, LDA & NNs (BP-MLP, RBF & PNN)	[75]

Table 2 *(continued)*

Food	Objective	Input data	Data fusion treatment	Multivariate analysis	Ref.
Fruit & vegetables					
Apple	Varietal discrimination & param. correlation (organic acids content)	Liquid sensors & ATR-FTIR	LL – (VS) statistics, Outer Product ML – (FE) PCA scores	PCA & PLS-DA, PLS	[38]
Bell peppers	Quality assessment & param. correlation	Colorimeter, Vis-NIR, SWIR, HSI, relaxation test & US pulse generator	(FE) spectral indices, PLS & PCA scores	PLS, PCR, kernel methods & SVMs	[69]
Meat & fish					
Pork	Quality & TVB-N correlation	NIR, Vision system & gas sensors	(FE) iPLS + GA-PLS, PCA scores & pearson correlation	PCA, BP-ANN	[21]
Pork	Quality assessment & pH correlation	NIR & HSI	(FE) PCA scores	PLS	[68]
Others					
Umami flavor	Sensory quality & parameter correlation	Liquid sensors & NIR	(FE) PCA scores	PCA & PLS	[37]
Honey	Origin & brand discrimination	Liquid sensors, Vis-NIR & UV-Vis	(FE) PCA scores	PCA &CA, Multi-way-PCA	[49]
Cheese	Origin discrimination	NIR, CIELab & chemical parameters	(VS) ANOVA, (FE) PCA scores	DFA & PLS-DA	[64]
Tortilla corn chips	Sensory quality assessment (texture)	Visual blister level appraisal, break force & sensory panel	(FE) wavelet & PCA scores	PCA	[73]
Potato chips & cream	Quality assessment	Gas sensors & liquid sensors	Pre-processed data, (FE) PCA scores	Fuzzy C-means & ANFIS BPNN, RBF & KNN	[85]

LL: Low-level data fusion; ML: Mid-level data fusion; (VS): Variable selection; (FE): Feature extraction;
PDO: Protected Designation of Origin; TVB-N: Total Volatile Basic Nitrogen

5.3. High-level fusion

High-level data fusion is performed when predictions are calculated from each individual technique and the results are then combined to obtain the final decision. High-level fusion has been less explored than low-level and mid-level fusion, and it has been mainly applied in classification problems. Some of these applications are summarized in **Table 3**.

Most applications refer to quality assessment of fruits such as tomatoes, apples and peaches, and also grape products like wine or must. Tomatoes were analyzed for color, firmness and weight lost and Bayesian classifiers were combined to determine the ripening stage of the fruits [59,106]. The quality of apples was studied combining: a) gas sensors with liquid sensor [20] and b) gas sensors with vision system and NIR [107]. In a) three levels of data fusion were compared using neural networks (PNN and BPNN) of simple concatenated data (low-level), PCA scores and covariance matrix adaptation evolutionary strategy (CMAES) (mid-level) and Bayesian network of concatenated BP-NN of the PCA scores (high-level). Mid-level was the best approach for detecting defective apples, and high-level was better than individual results [20]. In b) two high-level approaches were studied to assess apple quality and correlate measurements with sugar content. First, stepwise decorrelation followed by NNs was applied individually to each data source to enhance quality results. Then, a decision tree model was applied using MCR, PLS and PCR without better sugar estimation using high-level data fusion [107]. Sensory properties and firmness were evaluated in peaches with two data fusion strategies: multilayer NN (mid-level) gave better results than the combination of Bayesian classifiers using majority voting (high-level) [16]. To asses sensory quality and variety authentication of grape products electronic panel (gas and liquid sensors, FTIR and UV) data and fuzzy logic were used to merge the results followed by pseudo outer-product- fuzzy NN (POFNN) and gave better classification of wine sensory parameters than other common methods [108]. Also the Bayesian inferences of PLS-DA results slightly improved must classification, obtaining the best approximation with the responses of FTIR and UV using the minimum risk rule approach [81]. UV-Vis and 1H-NMR spectra were also fused to differentiate and characterize commercial culinary species adulterated with Sudan I-IV dyes using two levels of data fusion. PLS-DA of the fused source responses (low-level) was compared to fuzzy set theory of the PLS-DA individual results (high-level). Both approaches achieved better discrimination between pure and spiked samples than individual techniques [83]. Finally, quality assessment of visual parameters was studied for granular wheat. The classification of semolina types (from grinding wheat grains) was not improved. HCA was applied to individual data sources, obtained by an image system and a camera with stroboscope, to be then fused with NNs [109].

It has to be noted that, in the cases where the three fusion levels have been compared, high-level fusion often gave worse results than low- and mid- levels of fusion. An advantage of high-level, however, is that new features (techniques) can be added to the classification decision when a new type of data becomes available thus increasing the versatility of the decision process.

Table 3. Application of high-level data fusion

Food	Objective	Input data	Data fusion treatment	Multivariate analysis		Ref.
				Identity declaration	Final decision	
Alcoholic & non-alcoholic beverages						
Must	Sensory assessment & variety authent.	Gas sensors, FTIR & UV	ID concatenation	PLS-DA	Bayesian inference	[81]
Wine	Sensory quality assessment	Gas sensors & liquid sensors	ID concatenation	Fuzzy C-means	POFNN	[108]
Fruits & vegetables						
Peaches	Sensory quality assessment	Vision system, NIR, sniffer & impact sensor	ML – NNs HL – ID concatenation	Multilayer NNs Bayesian classifier	 Majority voting	[16]
Apple	Quality assessment for defect detection	Gas sensors & liquid sensors	LL – Simple concatenation ML – (FE) PCAscores & CMAES HL – ID concatenation	PNN PNN & BP-NN BP-NN	 Bayesian network	[20]
Tomato	Quality assessment (ripening stage)	Weight lost, CIELab & impact acoustic test	ID concatenation	Bayesian classifier		[59]
Tomato	Quality assessment (ripening stage)	CIELab & impact acoustic test	ID concatenation	Bayesian classifier (Bhattacharyya distance)		[106]
Apples	Quality assessment & param. correlation	NIR, vision system & gas sensors	(a) (VS) stepwise (b) Decision tree	(a) NNs (b) MCR, PLS & PCR	 (b) Decision tree	[107]
Others						
Culinary spices	Type discrimination & characterization	UV-Vis & ^{1}H NMR	LL – (VS) iPLS-DA HL – ID concatenation	PLS-DA	Fuzzy set theory	[83]
Granular wheat	Vision quality assessment	Image system & camera with stroboscope	ID concatenation	HCA	NNs	[109]

LL: Low-level data fusion; ML: Mid-level data fusion; HL: High-level data fusion; ID: Identity Declaration;
(VS): Variable selection; (FE): Feature extraction; POFNN: Pseudo outer-product- fuzzy neural network

6. Conclusions

This paper reviewed data fusion strategies for authentication and quality assessment of food and beverages. Applications developed for a variety of products were listed and explained briefly.

Of the three levels of data fusion, low-, mid- and high level, low- and mid-level fusion approaches are the most used. More than 50% of the applications reviewed used low-level fusion and improved classification and/or prediction results with respect to using individual techniques. This approach is a common, conceptually simple, first attempt when data from different sources are available. When data are very different in size or scale, the more tunable mid-level fusion, used in 30% of the applications considered in this review, can yield better results. In the literature, high level fusion was the least used, only in 10% of the applications.

The ever increasing availability of rapid and non-destructive analytical techniques in industrial processes and laboratories will keep promoting advances in data fusion in a variety of fields. Advances in authentication and quality assessment of foodstuff products, especially with the prediction of intrinsically complex properties which cannot be detected by a single technique, such as sensory properties, fraud, adulteration, origin, harvest and raw material control will benefit from advances in data fusion. A clear example is food sensory analysis, where the use of different techniques is needed in order to model the different attributes described by different senses (smell, taste, texture, visual, etc,), as a human panel does. There is still room for improvement in the different levels of fusion and data processing techniques. As far as the performance of the models is concerned, it is expected that data fusion will not only increase the global classification/prediction ability but also decrease the uncertainty of each individual result and enable better outlier detection in prediction. This still needs to be studied more thoroughly. Finally, new approaches are emerging such as hybrid applications combining low and mid-level data fusion, fusion of second and higher-order data, i.e. hyperspectral images or data from hyphenated techniques, and the combination of first order-data from evolving systems, i.e. on-line monitored systems, to predict the quality of finished products.

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Chapter 2. Almond Sensory Analysis

2.1. Introduction

Almonds (*Prunus amygdalus*) belong to the Rosaceae family that also includes apples, pears, prunes and raspberries. The fruit is catalogued as a drupe and has three distinct parts: an outer green cover or hull (exocarp), a middle hard shell portion (endocarp) and the inner kernel (seed) (**Figure 2.1**). The kernel (seed) is the commercial product and consists of a seed coat and an embryo, which contain two cotyledons and a small radicle [1,2].

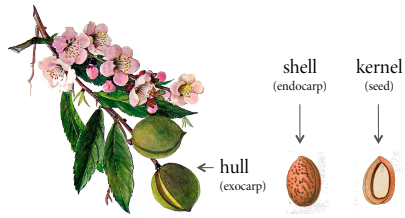


Figure 2.1. Parts of the almond.

In terms of commercial production, almond is one of the most important nut crops worldwide [3]. The almond production is limited to areas characterized by a Mediterranean climate with mild to cool wet winters and warm to hot dry summers [4]. This typical climate of lands around the Mediterranean Sea is also found in other areas such as the region of Central Valley of California. In fact, this region is the principal producer of almond with the 80% of global production, thanks to its highly developed marketing system and the favorable soil and climate combination of the area [5]. Despite the domain of the American production, the growing concern on maintaining the biodiversity has reawakened the interest mainly in traditional varieties of the smaller producers from the Mediterranean but also from other areas [3,6]. Thus, other important producers are Australia and Spain, with 7% and 4% of the world production respectively, followed by Turkey, Greece, Chile, Italy, and China [7].

Although the American production has remained stable, the rest of the regions vary its production from year to year, depending on pollination success and climatic conditions as well as insect damage and other diseases [8]. These oscillations may influence the final almond properties such as composition, fruit weight or yield.

Almond can be sold either shelled or unshelled, but also processed with dry roasting, blanching, slicing or chopping. It can be consumed as it is (as snack food) or being transformed to butter, paste, milk, flour or even oil, the latter used in pharmaceutical and cosmetic industries [8]. Moreover, it is incorporated as an ingredient in a wide variety of foods including bakery, flavor extracts and confectionery products, such as marzipan, nougat, sweets, cereals, ice creams or chocolates. Basically, the different almond uses depend on the population habits and/or the tree types availability of the region [9-11].

In any case, almond and its products have attained an important place in human diets, mainly due to their pleasant taste, high nutritional value and potential health benefits [10]. From the nutritional point of view almonds are an important source of macronutrients. The kernels have high content on lipids, proteins, fiber and minerals. Despite its high fat content, almonds have substantial quantities of triacylglycerol and unsaturated fatty acids. In addition they are also an important source of unique phytonutrients, such as vitamin E (α-tocopherol), polyphenols and folic, oleic and linoleic acids [4,8,12]. Thanks to this composition, the regular consumption of almonds has been associated to a wide range of health benefits including prevention of heart related diseases, blood lipid profile (cholesterol-lowering effects), cancer protection, obesity, diabetes and potential prebiotic properties [1,10,12-15].

2.1.1. Almonds quality assessment

As mentioned in Chapter 1 (section 1.1) quality assessment is always difficult, especially when dealing with food commodities, due to the complexity of their physical and chemical composition and the constant evolution of the components during ripening and postharvest processes. In the case of almonds (and related nuts) it is even more complicated because of the scarcity of quality regulations or well-defined evaluation guidelines.

Despite the valuable nutritional properties of the almonds presuming significant health benefits there is little information to define their biochemical characteristics such as fatty acid profiles or oil stability. In fact, almond quality has been defined almost exclusively by physical parameters: basically size, shape, double kernel or possible kernel damage. Thus, almond physical quality demands large kernels fully formed (not shriveled), unbroken skin with uniform dark brown color, and with a combined fravor of sweet and oily notes (without musty or rancid flavors) [16].

However, this expected nutritional and physical quality shows an important variability among cultivars (varieties) and is highly dependent on the final commodity (end use), which comprises a wide variety of products developed in different regions and cultures and determines the market acceptance. For this reason, agronomic and production controls are also required, aimed at selecting self-compatible and late-blooming cultivars (breeding) to assure the final product quality. Moreover, it has to be considered that some compounds are an important source of food allergens and, in some cases of toxicity, which can affect consumer health negatively. For this reason, there is a demand for maximizing sensory and phytonutrient characterization and minimizing food safety concerns [13,16,17].

Sensory quality - Kernel bitterness

From an organoleptic point of view almonds are mainly differentiated between sweet and bitter types. This particularity is basically perceptible in the fruit kernel, which has a characteristic non-bitter (sweet) or bitter fravor. The almond bitterness is an unpleasant fravor that is caused by a single recessive gene and, as a consequence, this trait can be easily domesticated through breeding selection [18]. Therefore, sweet almond is the predominant cultivated type by means of a progressive reduction of the alleles responsible for the bitter fravors [8].

This preference on the prevalence of sweet almond varieties is not only to prevent the characteristic off-fravor, but also because they do not contain a toxic substance present on the bitter types. This toxic substance comes from cyanogenic glycosides, naturally-occurring plant toxins within plant cells that are widely distributed, being present in more than 2500 species [19]. Although these compounds are not toxic when intact, when the tissues are disrupted, for example by crushing, bruising or chewing; they become toxic because of the action of the enzymes liberated, being an important defense against insects and herbivores [20]. Among all the natural cyanogenic glycosides, amygdalyn and prunasin are the ones present in almonds. Prunasin is a monoglucoside found in the roots and leaves or when the kernel is unripe. During the ripening process prunasin is converted to its related diglucoside, amygdalin, which is found only in the kernels where is more susceptible to be disrupted or eaten. When this happens, amygdalin comes into contact with endogenous enzymes (β-glucosidases and α-

hydroxynitrile lyases) that hydrolize amygdalin into benzaldehyde and hydrogen cyanide (**Figure 2.2**) [21].

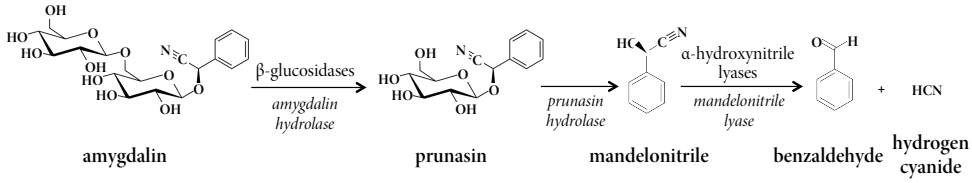


Figure 2.2. Metabolic pathway involved in the catabolism of amygdalin in almonds.

Benzaldehyde is an aromatic aldehyde that confers the disgusting taste of bitter almonds ('amaretto' fravor), and hydrogen cyanide is a well-known poisonous substance [22]. Cyanide toxicity can occur in humans by the ingestion of a high amount of almonds (one bitter almond produces from 4 to 9 mg of hydrogen cyanide), causing both acute and subacute health problems (depending on the dose) and, in extreme cases, death [23]. Despite the danger, some people still grow bitter almonds to obtain the extract oil used as fravoring in sweets and cooking. In these cases reduction of toxicity is required through processing techniques such as chopping, grinding, soaking, fermentation, drying, roasting, boiling, and steaming [24].

As a consequence of the potential hazard risk the major producers (America and Australia) have decided to cultivate exclusively sweet varieties. However, countries from the Mediterranean area like Spain still produce bitter almonds (approximately 1%). The main reason is because some traditional varieties of these regions, such as 'Desmayo Largueta' or 'Marcona', despite being of the sweet type still carry the recessive bitter gen. This is an important problem not only to prevent health risks, but also to prevent economical losses for sensory contamination through possible intentional or unintentional sweet and bitter almond mixtures. This sensory spoilage is significant due to the low fravor threshold perception of benzaldehyde [25] that may damage huge sweet almond batches with a mere presence of a small amount of bitter almonds. Thus, nowadays there is an increasing interest to find quality control measures to differentiate the bitter almonds from the sweet ones, particularly when some strategic markets, like United States and emergent Asian countries, have adopted extreme controls to completely avoid the presence of bitter almonds from their importations.

2.1.2. Instrumental sensory analysis

Despite the above-mentioned safety risk and possible economic losses, there are still insufficient official regulations with defined methodologies to detect bitter almonds. Most methodologies are based on costly and/or time consuming conventional methods without accurate and/or immediate response. This may encourage farmers and producers to practice economic adulterations by trading with bitter species hidden beneath sweet almonds.

The most reasonable and simple methodology to detect the presence of bitter almonds in batches of sweet almonds would be by a human sensory panel, using trained assessors able to detect and distinguish the bitter taste caused by the benzaldehyde. However, apart from the cost or time limitations, the use of a human panel would suppose a health risk; so, the application of instrumental techniques is required.

The first attempts to detect bitter almonds instrumentally were conducted using conventional targeted analysis. This was not complicated, due to the proved relationship between cyanogenic glucosides and the bitter taste (considered the indicator or target) [26]. The relationship between bitter taste and targeted analysis is described in **Figure 2.3.**

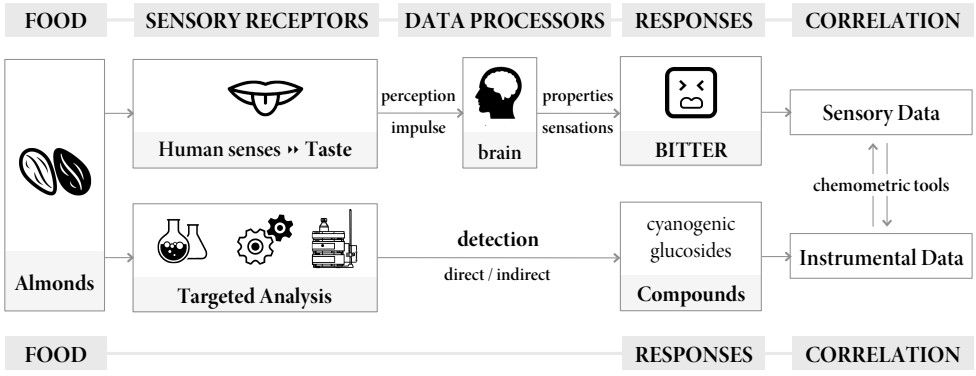


Figure 2.3. Correlation between human sensory analysis and instrumental classical approaches.

These targeted analyses are classified depending on their suitability to detect the genuine cyanogenic compounds directly or only by estimation of their content with an indirect method, both including destructive procedures. Indirect methods determine the cyanide obtained by enzymatic or chemical hydrolysis, which is subsequently measured using

gravimetry, colorimetry, spectrophotometry or biosensors. But to identify and quantify the specific cyanogenic compounds, direct methods are needed. Usually, these consist on the use of a separation technique (chromatography or electrophoresis) coupled to a detection system, mainly ultraviolet–visible (UV–Vis) spectrophotometry, refractive index or mass spectrometry (MS). However, all these methods are time-consuming, making impossible their on-line application [20,27]. Immunoassay methods, like enzyme-linked immunosorbent assay (ELISA), are a good alternative because they are simple and fast on detecting amygdalin. However, their results are not reliable enough because of their low specificity that does not consider other possible cyanogenic compounds (e.g. prunasin) when the immunogenic conjugate is prepared [24,28].

As already explained, to avoid the main drawbacks of these analyses, non-targeted approaches are required, which provide a characteristic sample profile that can be correlated to the studied attribute. In this case, the appropriate instruments are those that emulate the tongue sensory responses and, among these, a good choice, because of their simplicity and rapid response, are techniques like Raman and infrared.

Raman spectroscopy provides strongly intense bands containing selective chemical information. It shows a characteristic band assignable to the nitrile group (CN), typical of the cyanogenic compounds, which can be detected with a direct measure (on or near the sample surface) without any sample pretreatment [27,29-31]. However, this technique has some important drawbacks, such as interferences from fluorescent compounds that affect the final spectral response, alteration (peeled sample) or destruction of the sample with the heat generated by the laser and the high cost of the instrument. To overcome these limitations, another good option is Near-infrared (NIR) spectroscopy. NIR is a cost-effective, non-destructive and high-throughput technique that provides information about molecular structures in situ without any sample pre-treatment, similarly as Raman does. NIR absorption bands are typically broad but are related to organic and some inorganic compounds that show good reflectance or transmission properties. Up to now, there are only few studies related to NIR almond bitterness detection, mainly because the lower intensity of the nitrile bands when compared to Raman spectroscopy and also to the low specificity of the technique. Despite these limitations, NIR spectroscopy is simple and suitable for on-line processes, and coupled to multivariate analysis becomes a very powerful technique. This was the motivation to carry

out the first study presented in this PhD. Thesis, which consisted on finding relationships between NIR spectral fingerprints and almond bitterness perception.

Thus, this chapter describes a new methodology based on NIR spectroscopy that is applied as a 'preliminary electronic tongue' to discriminate bitter almonds from non-bitter ones. We call it 'preliminary' because it attempts to simulate the human tongue by evaluating only one single attribute (bitterness) whereas a real tongue evaluates all the different taste perceptions at the same time.

2.2. Preliminary electronic tongue based on NIR

The aim of this study was the discrimination of bitter almonds from sweet ones using near-infrared (NIR) spectroscopy. Although the reference method in the case of taste perceptions should be the sensory analysis, in this case and due to the toxic character of the almond samples (cyanide content), an instrumental method was required as a reference. According to the literature, Raman spectroscopy had provided good results on detecting amygdalin in bitter almonds [27,29-31], so obtained spectra with this technique were selected to be correlated with the obtained spectra by NIR spectroscopy applying partial least squares discriminant analysis (PLS-DA) as classification technique. A brief description of the strategy followed is shown in **Figure 2.4.**

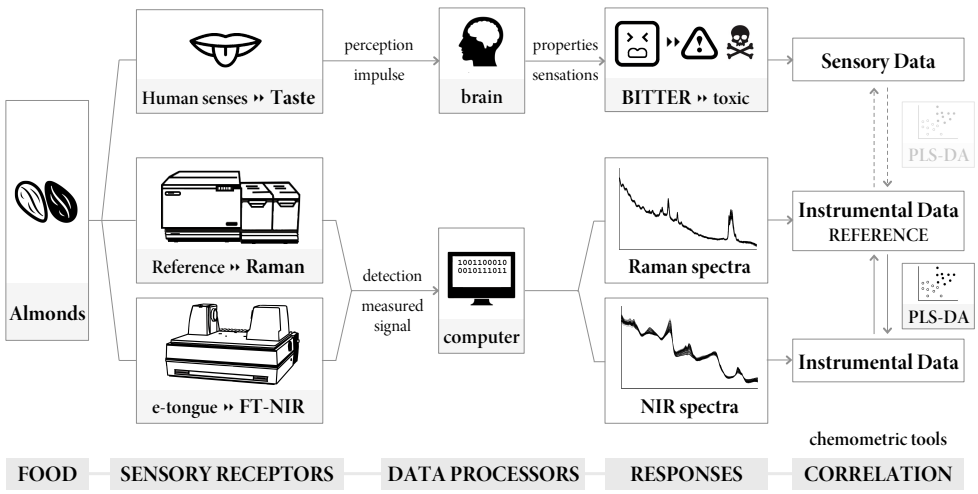


Figure 2.4. Preliminary electronic tongue scheme. Correlation between human sensory analysis, reference Raman spectroscopy and NIR spectroscopy.

2.2.1. NIR and Raman spectroscopy

NIR and Raman are considered rapid, non-destructive and molecular specific vibrational spectroscopic techniques. In both cases, Fourier Transform (FT) configuration was used to collect data using an interferometer. The interferometer receives the radiation through a beam-splitter and mirrors, as detailed in **Figure 2.5**, and then radiation is divided into two equal beams that are transmitted to a fixed mirror and reflected to a moving mirror, respectively. These beams are recombined into a modulated beam with a unique frequency by introducing a continuously-varying optical path difference for various values. The mirror position is controlled by a helium neon laser (HeNe at 633 nm, "red" excitation) that works as an internal reference (moduled monochromatic beam).

This FT structure provides good data repeatability, fluorescence-free measurements and wavelength accuracy, compared to filter or dispersive techniques [32,33]. The main instrumental components of both techniques, FT-NIR and FT-Raman, are the radiation source, an interferometer, a sampling system and, finally, a signal detector. This final detected signal converts the modulated light (intensity) to an electrical signal that is represented by an interferogram. The interferogram frequencies (intensity versus time) are demodulated via a mathematical function, called Fourier Transform, which transforms it into the corresponding optical spectrum (intensity versus frequency) using the data processor (software).

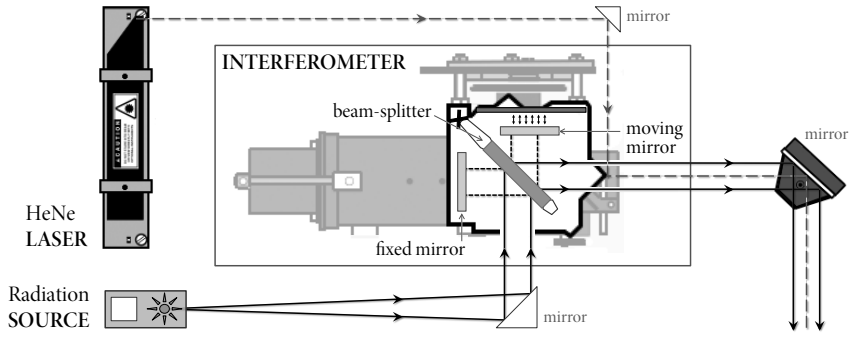


Figure 2.5. Typical Michelson interferometer with radiation source and laser. Interferometer includes the beam-splitter, the fixed mirror and the moving mirror.

Raman spectroscopy. In FT-Raman spectroscopy an excitation source generates a monochromatic light (usually from a laser) that is scattered by molecular interactions with vibrational and rotational transitions in such way that the laser photons energy shifts down or up. The shift in energy gives information about the vibrational and rotational modes in the system, so the signals obtained provide information about the chemical composition of the samples.

Since FT-Raman was the reference method for the FT-NIR analysis, a NdYAG laser emitting a monochromatic light that produces photons in the near-IR region (1064 nm) was chosen. A line filter was used to filter plasma lines that focus the light to the sample compartment. Then, the scattered light was collected using back-scattering geometry, passing through a dielectric filter (indium-gallium-arsenide, InGaAs) before entering the detector (**Figure 2.6**) [32,34].

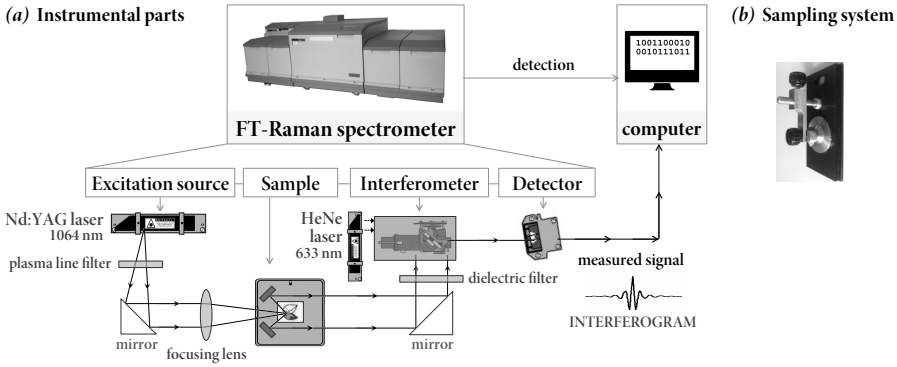


Figure 2.6. Description of the main parts of FT-Raman spectrometer with excitation source, interferometer, sample compartment, detector and data processor (a) and Raman sample system (b).

NIR spectroscopy. When using FT-NIR the radiation is generated by a polychromatic IR light source that provides an electromagnetic energy in a wavenumber range of 4000 – 12.000 cm^{-1}. This radiation is directed to the interferometer, where it is divided into two beams (as previously described in **Fig 2.5**) and then directed to interact with the sample by varying the optical path difference (from zero to a maximum length that depends on the resolution required) (**Figure 2.7**).

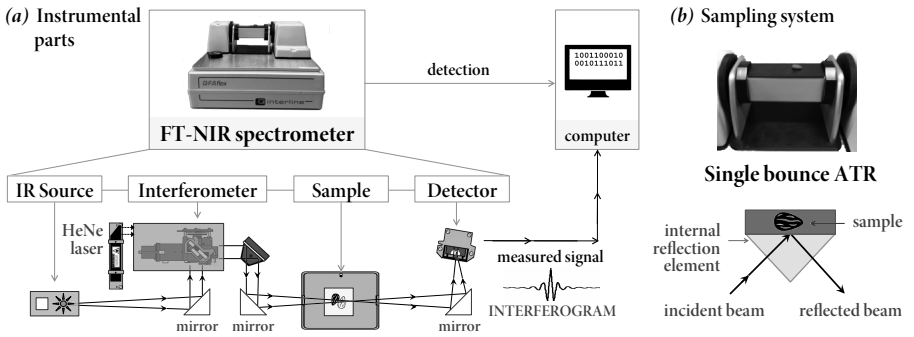


Figure 2.7. Description of the main FT-NIR spectrometer parts with IR source, interferometer, sample compartment, detector and data processor (a); and attenuated total reflection system (ATR) (b).

The most common sampling system when working with solid samples is attenuated total reflection (ATR) with a single or multiple bounce. In this case, single-bounce ATR was used, using an ATR crystal made with diamond, a material with a high refractive index. The infrared radiation goes through the crystal in such a way that it reflects along its length (with a single bounce) the internal surface in contact with the sample, to finally measure this reflection with the detector (**Figure 2.7**) [33-35].

Although both are vibrational spectroscopic techniques, NIR and Raman are based on different principles and the results obtained with each technique do not provide the same information. In fact, infrared and Raman information is considered partially complementary (high signals on IR spectra are often weak in Raman spectra, and vice versa).

They are based on vibrational movements of the molecules, combined discrete energy transitions and changes of electromagnetic radiation frequencies during absorption (infrared) or scattering (Raman). While in NIR spectroscopy the sample absorbs the polychromatic light and changes the dipole moment of the molecules, in Raman spectroscopy scattering occurs when monochromatic light irradiates the sample and changes the polarizability of the molecules (by deforming the electron cloud). One important difference between these techniques is the O-H stretching vibration due to OH bonds that are weakly polaritzable. Their bands are very weak in Raman, whereas they are very strong in IR spectra. As a consequence, samples with high amounts of water may not be analyzed by NIR [31].

In addition, FT-Raman can also provide information about functional/chemical groups, similarly as mid-infrared (MIR) spectroscopy. In the case of almond components, for example, the characteristic substances related to bitterness are the nitrile groups (2300-2100 cm^{-1}). Nitrile bands are strongly active in Raman. Moreover, these nitrile-compounds present in almonds (cyanogenic glucosides) are rarely found in most of natural compounds, including sweet almonds. Since now, this clear and direct measure of characteristic signals (cyanide band intensities) of bitter almonds has made FT-Raman a suitable technique for this kind of studies [31,36-38]. The main absorbance bands related to Raman spectroscopy and the characteristic compounds are shown in **Table 2.1**.

Table 2.1. Raman characteristic group frequencies.

Frequency (cm^{-1})		**Molecular vibrations**	**Compounds**
3500 - 3100	Vw	stretching ν -OH	Hydroxyl ↠ *liquid phase*
3100 - 3000	M	stretching ν =C-H	Unsaturated ↠ *lipids*
3000 - 2700	M	stretching ν -C-H	Saturated ↠ *lipids*
2300 - 2100	**S**	**stretching ν -C≡N**	**Nitrile**
1750 - 1700	m-w	stretching ν C=O	Ester ↠ *lipids, aminoacids*
~1700	w-m	stretching ν C=O	Carboxylic acid ↠ *lipids, aminoacids*
1700 - 1600	S	stretching ν C=C	Not conjugated ↠ *lipids*
1650 - 1600	S	stretching ν C=C	*Trans* ↠ *lipids*
~1600	s	stretching ν C=C	*Cis* ↠ *lipids*
~1400	m-w	scissoring δ C-H	Aliphatic $-CH_2$ ↠ *lipids*
1400 - 1350	-	stretching ν C-O	Carboxylates ↠ *lipids, aminoacids*
1250 - 400	-	-	Fingerprint region
~1200	m-w	stretching ν C-O	Ether ↠ *carbohydrates*
~700	vw	rocking ρ C-H	Aliphatic $-CH_2$ ↠ *lipids*

vw: very weak, *w:* weak, *m:* medium band, *s:* strong, *vs:* very strong intensities

Unlike MIR and Raman spectra, usually NIR spectra provide little interpretable structural information, mainly because their peaks are broad and weak due to combinations and overtones of functional groups of chemical constituents. These signals may help to predict the presence of functional groups but it is more complicated and NIR is basically used for quantitative applications (**Table 2.2** shown characteristic NIR regions [32,35]). However, despite the apparent lack of information in NIR spectra (compared to MIR and Raman), NIR spectroscopy has been shown to be a powerful tool, especially when it is used together with chemometric techniques. Moreover, NIR has been extensively used due to its rapid measurement capability, on-line application potential for direct process control and relatively

low cost (compared to Raman spectroscopy). So, NIR could be a good alternative to Raman or conventional methods for industries to discriminate bitter from sweet almonds.

Table 2.2. NIR absorption bands of characteristic functional groups.

Frequency		Bond vibration
Wavelength (nm)	**Wavenumber (cm^{-1})**	
2500 – 2200	4000 – 4545	C-H combinations
2200 – 1800	4545 – 5555	O-H, N-H combinations
1800 – 1600	5555 – 6250	C-H 1st overtone
1600 – 1420	6250 – 7042	O-H, N-H 1st overtone
1420 – 1300	7042 - 7692	C-H combinations
1300 – 1100	7692 – 9090	C-H 2nd overtone / O-H combination
1100 – 800	9090 – 12500	N-H 2nd overtone / O-H 2nd overtone / C-H 3rd overtone

2.2.2. Almonds and preliminary e-tongue

The instrumental response (NIR and Raman spectra) obtained by almond samples should be treated with multivariate techniques to find proper correlations between collected spectra and bitterness. In both cases a preliminary data exploration was performed (PCA) before building the final discriminant PLS-DA models. Additionally, since these spectral data also contain chemical information, these were studied using the Variable Importance in Projection (VIP) scores. VIP scores select the relevant variables (wavenumbers) that help to understand the final model that best discriminates the samples.

Raman spectra (reference method). Direct spectral data obtained by Raman spectroscopy showed clear differences between almond classes. Accordingly, these visual differences were confirmed by the PLS-DA model, which achieved high values of sensitivity/specificity, with almost all the samples correctly classified (**Figure 2.8a**). These results allowed the identification of specific variables using the VIP scores plot, and provided information about the characteristic bands corresponding to cyanogenic substances (e.g. amygdalin) that are present in bitter samples (**Figure 2.8b**).

Thus, variables (wavenumbers) with VIP>1 were considered the most important ones to discriminate bitter almonds, comprising regions at 600-1000, 1604, 2245 and 3060 cm-1 from the Raman spectra (sections a-d in **Figure 2.9**). In particular, a band at 1003 cm^{-1} (**Fig. 2.9a**) was the most significant variable, showing higher differences between samples, together with

other spectral regions (**Fig. 2.9 a, b, d**). The peak at 1003 cm^{-1} was associated to the aromatic ring present in amygdaline and other cyanogenic molecules. Also, a characteristic band at 2245 cm^{-1} (**Fig. 2.9c**) was related to the nitrile group.

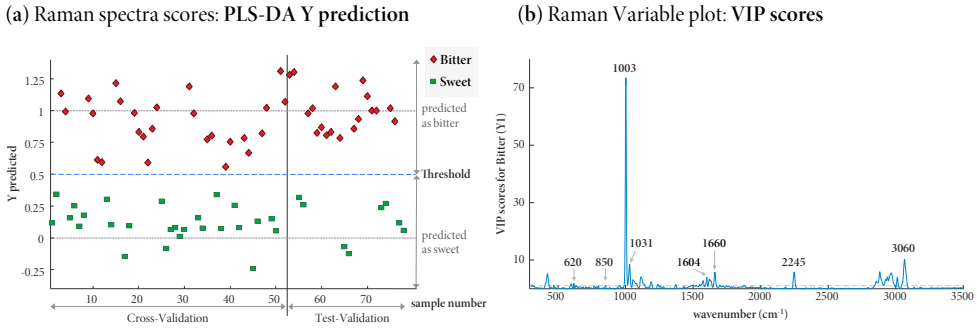


Figure 2.8. PLS-DA results obtained by Raman spectroscopy. Y prediction values for cross- and test-validation sets of sweet (y=0) and bitter (y=1) samples (a) and VIP scores plot (b).

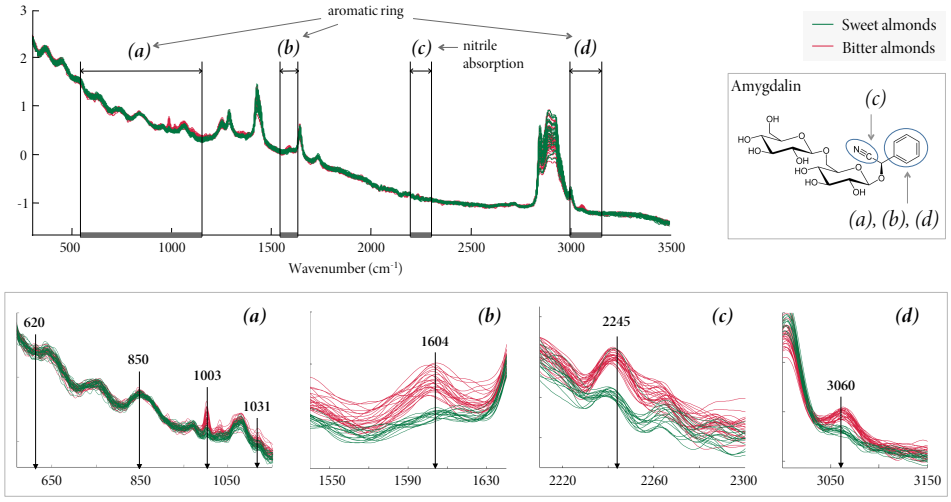


Figure 2.9. Raman spectra of all samples analyzed with the characteristic bands (wavenumbers) of amygdalin presence, with specific bands associated to nitrile (a) and aromatic rings (b-d).

NIR spectra. Despite NIR spectroscopy responses are often more complex and include weaker nitrile absorptions, wider and more overlapping bands than Raman, NIR spectra of almond samples showed clearer and more differentiated signals for the two studied classes (**Figure 2.10**).

Thus, a simple PCA model revealed the sample differences (**Figure 2.11a**) and the characteristic variables (loadings) responsible to discriminate sweet (**Fig. 2.10** and **2.11 (b)**: green lines) from bitter (**Fig. 2.10** and **2.11 (b)**: red lines) almonds.

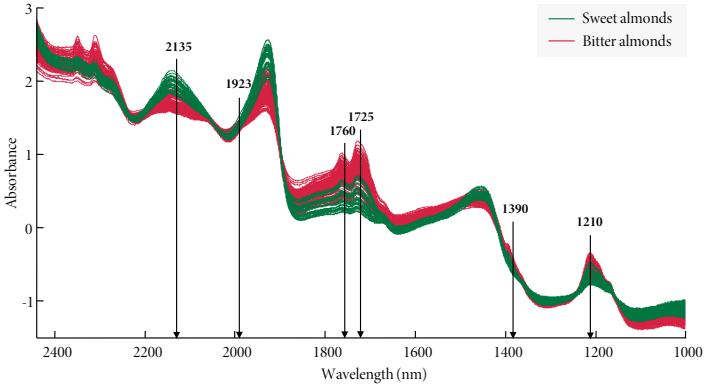


Figure 2.10. NIR spectra of all the almond samples analyzed and main bitter/sweet characteristic wavelengths.

(a) NIR spectra: **PCA scores plot (PC1 vs. PC2)** **(b)** NIR Variable plot: **PCA loading plot**

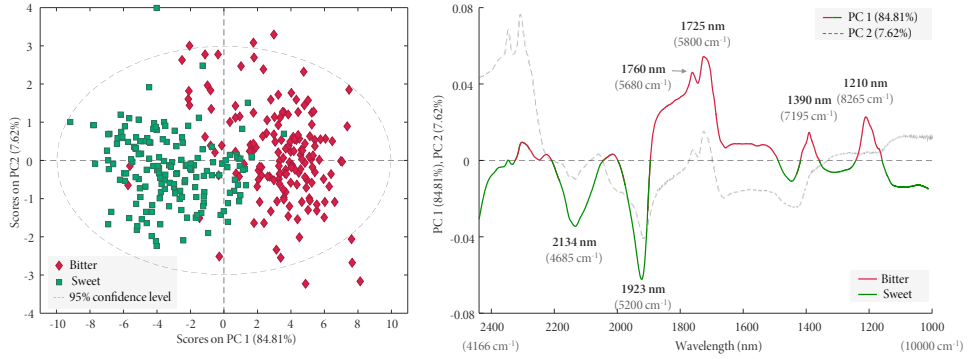


Figure 2.11. PCA results obtained by NIR spectroscopy. Scores plot for duplicates (a) and Loading plot with important wavelengths (dark) and wavenumbers (light)(b).

These encouraging preliminary results were the starting point of a final study, where PLS-DA models were built to discriminate bitter from sweet almonds, both from NIR and Raman spectra. The results of this work were published in *Food Chemistry* [**Paper 2**].

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Fast and robust discrimination of almonds (*Prunus amygdalus*) with respect to their bitterness by using near infrared and partial least squares-discriminant analysis

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Fast and robust discrimination of almonds (*Prunus amygdalus*) with respect to their bitterness by using Near Infrared and Partial Least Squares-Discriminant Analysis

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Abstract

In this study, near-infrared spectroscopy (NIR) coupled to chemometrics is used to develop a fast, simple, non-destructive and robust method for discriminating sweet and bitter almonds (*Prunus amygdalus*) by the in situ measurement of the kernel surface without any sample pre-treatment.

Principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) models were built to discriminate both types of almonds, obtaining high levels of sensitivity and specificity for both classes, with more than 95% of the samples correctly classified and discriminated. Moreover, the almonds were also analysed by Raman spectroscopy, the reference technique for this type of analysis, to validate and confirm the results obtained by NIR.

Keywords

Sweet almonds; Bitter almonds; *Prunus amygdalus*; NIR; Raman ; PLS-DA; Classification

1. Introduction

Almonds (*Prunus amygdalus*) are members of the family Rosaceae and the fruit is classified as a drupe in which the edible seed or kernel is the commercial product (Gradziel, 2009). Two different species can be distinguished depending on the kernel bitterness: the bitter and the non-bitter/sweet almonds. The bitter fravor of the almond is a consequence of the presence of cyanogenic glucosides, such as amygdalin and prunasin, concentrated in the kernel. When the seed tissue is damaged an enzymatic hydrolysis (beta-glucosidases) occurs that produces benzaldehyde (that confers the bitter fravor), sugars and hydrogen cyanide (HCN), which is highly toxic, though providing an effective chemical defence against herbivores, insects and pathogens (Arrázola et al., 2012; Franks et al., 2008; Gradziel, 2009; Krafft, 2012; Micklander et al., 2002; Sánchez-Pérez et al., 2008, 2009, 2010; Zagrobelny et al., 2008)

The sweet or bitter fravor characteristic of almond kernels is an inherited monogenic trait, bitter being recessive. The sweet almond is the predominant type cultivated globally, and a constant selection in the breeding is carried out to obtain sweet kernelled almond trees, with a progressive reduction of the alleles responsible for the bitter fravors. Nevertheless, some varieties, which have been grown for years and are commercially viable, carry these alleles and can produce seedlings with bitter kernels when combined with each other (Sánchez-Pérez et al., 2012).

Since cyanogenic glucosides are not found in sweet almonds its detection might be a specific indicator of bitterness in the almonds (Mirrahimi et al., 2011). Prunasin (**Figure 1a**), a monoglucoside, is found in unripe almonds, and is converted to amygdalin (**Figure 1b**), a diglucoside, during the ripening process.

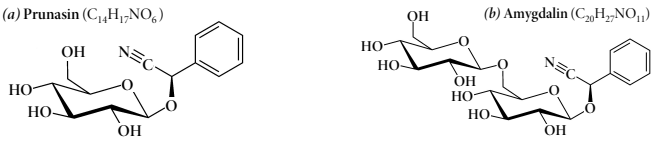


Figure 1. Structures of the molecules of prunasin (a) and amygdalin (b)

The most commonly consumed is the sweet almond, which has been recognized as a source of nutrients (Yada et al., 2011). Nevertheless, Bitter almonds are also used, primarily in the production of fravor extracts, being processed before consumption to remove the poisonous substances. Sometimes, though, bitter almonds are mixed up with sweet almonds, causing unpleasant taste of the final processed products and, what is more important, poisoning. Some cases of poisoning through the ingestion have been described in the literature (Sánchez-Pérez et al., 2012; Shragg et al., 1982; Toomey et al., 2012). One bitter almond produces from 4 to 9 mg of hydrogen cyanide and a high consumption can lead to death. Because of the potential health hazard associated with the ingestion of cyanide or for possible economic adulterations it is important to differentiate between sweet and bitter almonds.

There are indirect ways to estimate the presence or content of cyanogenic compounds, based on enzymatic or chemical hydrolysis of the cyanogenic compounds to cyanide and measured with methods

such as gravimetry, spectrophotometry or biosensors. These methods do not allow the identification and quantification of the specific compounds responsible for cyanide release. Therefore, direct ways should be used to detect the genuine cyanogenic substances. In consequence, separation techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and micellar capillary electrophoresis (MCE), coupled with detection systems like ultraviolet-visible (UV-Vis) spectrophotometry and mass spectrometry (MS) have been applied for the analysis of the cyanogenic glucosides (Dicenta et al., 2002; Gradziel, 2009; Yıldırım et al., 2009). However, all these methodologies are complex, time and reagent consuming and sample destructive. Thus simpler, faster and non-destructive techniques are required.

Vibrational spectroscopy techniques like Near Infrared (NIR) (Gálvez-Sola et al., 2010; Guidetti et al., 2012; Roggo et al., 2007) and Raman (Micklander et al., 2002; Thygesen et al., 2003) are complementary techniques that provide information on the molecular structure and are able to measure the sample in situ without any sample pre-treatment. Most of the studies related to spectroscopy are based on Raman analysis because it provides strongly intense bands containing selective chemical information. Nevertheless, Raman is usually affected by the interference from fluorescent compounds, it is more expensive than NIR and the heat generated by the laser may alter or destroy the sample during the measurement, becoming a potentially sample destructive technique (Thygesen et al., 2003). On the contrary, NIR has the advantage of being extremely fast in the measurement, robust and no alteration of the sample whatsoever is needed. The main drawback of NIR is the wide non selective bands of the spectral profile. This has promoted very few studies of almond with NIR (Galvez-Sola et al., 2010; Pearson, 1999). With the evolution of chemometrics (multivariate data analysis and modelling) NIR is gaining strong acceptance in food science and technology. Multivariate exploratory, classification and prediction methods are required to extract information from the spectral data that is related to the desired predictor (bitterness in our case).

The aim of this study is to develop a simple, fast, non-destructive and robust methodology to discriminate bitter and sweet almonds by the in situ measurement of the kernel surface without any sample pre-treatment and employing NIR spectroscopy. Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) were applied to develop a method to classify almonds with respect to their bitterness. Raman spectroscopy was used as a reference technique to validate and confirm the results obtained by NIR.

2. Materials and methods

2.1. Almond samples

One kilogram of both bitter and sweet almonds was provided by La Morella Nuts (Group Barry Callebaut, A/S, Zurich). The almonds were stored in vacuum sealed in bags and in dark and cold place (fridge at 4 ºC). Therefore, no significant oxidation was observed. All analyses were completed within 2 months after opening the bags in order to protect them against oxidation reactions.

2.2. Spectroscopic analysis

2.2.1. Near Infrared (NIR) analysis

Spectral reflectance NIR measurements were collected by using a Bomen FT-NIR MB160PH Aridzone spectrometer equipped with an InGa detector, a reflection accessory, and the Grams/LT 7.00 data collection software program. A representative surface of the almond with skin was directly deposited on the reflection measurement area with a complete and reproducible contact between the sample and the window. Spectra were recorded in the reflectance mode from 4100 to 10000 cm^{-1} (1000 to 2500 nm), at 4 cm^{-1} resolution and taking 16 scans per sample. The absorbance was computed against a background spectrum of Spectralon. The reflection window plate was carefully cleaned with a soft tissue to eliminate the presence of residues between measurements. Two replicates were recorded for each sample.

2.2.2. Raman analysis

Raman spectra were collected with a Perkin Elmer System 2000 FT-Raman spectrometer. The radiation source was a Nd:YAG laser centred at 1064 nm with a power adjusted to 100 mW at the sample. The spectra were collected at a resolution of 8 cm^{-1} in the range of 100 to 3500 cm^{-1}, and represented the average of 32 scans. The reference method for Raman measurements states that the almond must be measured on the skin peeled surface (Micklander et al., 2002; Thygesen et al., 2003). Two replicates were recorded for each sample.

2.3. Data Analysis

A final matrix of 320 samples and 3060 variables was built from the NIR spectra and a matrix of 78 samples and 3401 variables was obtained in the case of Raman measurements. Both NIR and Raman spectra were pre-processed with Standard Normal Variate (SNV) scaling and mean centering previous any multivariate analysis.

2.3.1. Principal Component Analysis (PCA)

PCA is a bilinear decomposition method that decomposes the **X** matrix of spectra into a score matrix, **T**, and a loading matrix, **P**, which describe the original data in a more condensed way, being the residuals collected in matrix **E** (Eq. (1)):

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathbf{T}} + \mathbf{E} \quad (1)$$

The goal of PCA is to express the main information contained in the original variables in a lower number of variables, called principal components (PCs), which describe the main sources of variation in the data. These PCs are linear combinations of the original variables (Lavine, 2001). Some properties of PCs are that they are orthogonal (i.e. uncorrelated to each other), hierarchical (i.e. the first PC retains the main information of the data, the second PC retains the main information that is not included in the first, and successively), and they are calculated sequentially (Bro & Anderson, 1998).

2.3.2. Partial least squares-discriminant analysis (PLS-DA)

In PLS-DA, a PLS regression model is calculated that relates the independent variables (**X** matrix of spectra) to a vector **y** containing the codified classes as integer numbers, for instance ones (1) if the training sample belongs to a given class of interest, and zeros (0) if the sample belongs to a different class. Classification of an unknown sample is derived from the value predicted by the PLS model. This value is a real number, not an integer, which should ideally be close to the values used to codify the class (here either 0 or 1). A cut-off value between 0 and 1 is established so that a sample is assigned to class 1 if the prediction is larger than the cut-off value, or assigned to class 0 otherwise. The method uses the appropriate number of latent variables (or factors); which are linear combinations of the initial selected variables that maximize the discrimination among the groups (Roggo et al., 2007).

The selection of the optimal number of latent variables in PLS-DA was done using the criterion of lowest prediction error in leave-one-out cross validation (LOOCV). Additionally, the optimal PLS-DA model was further validated by predicting an external validation set. Quality assessment of the results was performed by computing the sensitivity (samples of the class of interest correctly assigned to their class), specificity (samples not belonging to the class of interest correctly not assigned to that class) and overall classification error of calibration (Cal), cross-validation (CV) and prediction (Pred).

The collected spectra were imported into MATLAB v. 7.8 (The Mathworks AS, Massachusetts, USA). Two data subsets were randomly prepared, one for calibration and another one for externally validate the calibration model. All calculations were performed using the PLS_Toolbox v. 6.2 (Eigenvector Research, Manson, WA, USA) working under MATLAB environment.

3. Results and discussion

3.1. Near Infrared (NIR) analysis

A set of 160 almond samples of sweet and bitter types were measured on both sides of the outer skin and in different days, to account for the instrumental and day-to-day variability. The corresponding raw spectra are plotted in **figure 2a**. The spectra were pre-processed with Standard Normal Variate (SNV) and mean centred previous to PCA (**Figure 2b**).

A preliminary data exploration with PCA was carried out in the whole dataset after spectral pre-processing. The PCA score plot shows a clear differentiation of the samples corresponding to the sweet and bitter types. The first component (PC1) is the main responsible of the separation, explaining the 85.70% of the original variance. The combination of PC1 and PC4 (explaining a total of 87.45% of the original variance), shows the best separation of the groups. No significant differences between days of analysis were observed, thus indicating a robust model. PC1 loadings shows the variables related to each type of almond: negative loadings mainly describe sweet almonds and positive loadings mainly describe bitter almonds.

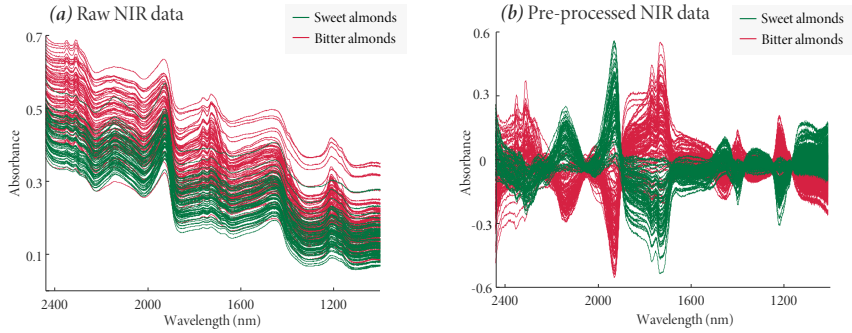


Fig. 2. (a) Raw spectra and (b) pre-processed (SNV and mean centered) spectra of all the almonds analyzed by NIR (gray: sweet almonds; black: bitter almonds)

A PLS-DA calibration model was built by using the almonds measured on the first day (80 spectra). The model was leave-one-out cross validated and the optimal number of factors was chosen on the basis of the minimum value of the Root Mean Square Error of Cross-Validation (RMSECV). The optimal model was built with two factors (explaining a total of 90.18% of the variation in the spectra (X) and 81.37% of the variation in the vector of classes (y)). Using this calibration model, the other measurement set (240 almonds measured on different days) was used as external prediction set. The results from the PLS-DA model are shown in **Figure 3a** (scores plot), **Figure 3b** (loadings plot) and **Table 1**.

The PLS-DA scores plot shows an outstanding discrimination between bitter and sweet almonds. The loadings plot (**Figure 3b**) shows the wavelengths responsible for the class separation. Mainly, the sweet almonds (green, positive values in factor 1) are explained by wavelengths 2135 nm (i) and 1923 nm (ii), due to O-H bend/ C-O stretch combinations and O-H stretch/ HOH deformation combination (water), respectively.

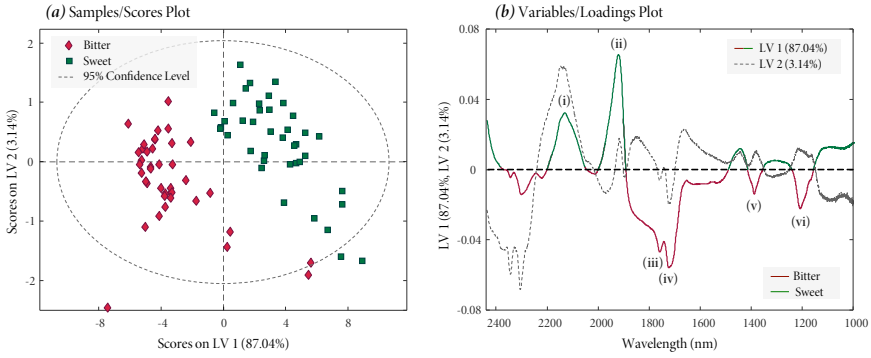


Fig. 3. (a) Scores plot and (b) loadings plot of the first two factors of the PLS-DA model built with the NIR spectra of the calibration set of almonds.

Bitter almonds (red, negative values for factor 1) are mainly described by wavelengths 1760 nm (iii) and 1726 nm (iv), corresponding to C-H stretch 1st overtone; 1390 nm (v) for C-H combination and 1210 nm (vi) for stretch 2nd overtone.

Table 1 shows the classification results obtained with the NIR data. High sensitivity and specificity levels were obtained for both classes, with more than 95% of the samples correctly classified as well as low classification errors (below 2.5%). The false positive/negative values are below 5% both in the cross-validation and the prediction confusion matrices.

3.2. Raman Spectroscopy

Raman spectroscopy was applied to a set of 78 samples, both sweet (34) and bitter (44). The measurements were performed on peeled almonds, as previous studies proved to contain the higher amount of amygdalin (Micklander, 2002; Thygesen, 2003). From the collected raw spectra some of the characteristic bands related to the chemistry of amygdalin molecule were selected (previously selected by PCA). Each region was first pre-processed individually by Standard Normal Variate (SNV) and mean centered and then joined to build the final PLS-DA model.

The whole dataset was split into a training set (52 samples) and a validation set (26 samples) using Nearest Neighbour Thinning algorithm (selecting a subset of samples by removing nearest neighbours) (Shenk, 1991). The optimal number of factors of the PLS-DA model, found by leave-one-out cross-validation, was two. The results from the PLS-DA model are shown in **Table 2.**

The model obtained explained a 44.32% of the initial variance (X) and 86.44% of the variation in the vector of classes (y). In the scores plot of the samples on these two factors sweet and bitter almonds are well separated. High sensitivity and specificity levels were obtained (**Table 2**), with a percentage of correct classification greater than 95.7% and low classification errors (below 2.2%). Low false positive/negative values (below 4.3%) were obtained both for cross-validation and prediction samples.

Table 1. PLS-DA model statistics for NIR data

Modeled Class (%)	Bitter	Sweet
Sensitivity (Cal[a])	95	100
Specificity (Cal[a])	100	95
Sensitivity (CV[b])	95	100
Specificity (CV[b])	100	95
Sensitivity (Pred[c])	99.2	96.7
Specificity (Pred[c])	96.7	99.2
Class. Err (Cal[a])	2.5	2.5
Class. Err (CV[b])	2.5	2.5
Class. Err (Pred[c])	2.1	2.1

[a] Cal: Calibration; [b] CV: Cross-Validation;
[c] Pred: Prediction.

Table 2. PLS-DA model statistics for Raman data

Modeled Class (%)	Bitter	Sweet
Sensitivity (Cal[a])	100	100
Specificity (Cal[a])	100	100
Sensitivity (CV[b])	100	95.7
Specificity (CV[b])	95.7	100
Sensitivity (Pred[c])	100	100
Specificity (Pred[c])	100	100
Class. Err (Cal[a])	0.0	0.0
Class. Err (CV[b])	2.2	2.2
Class. Err (Pred[c])	0.0	0.0

[a] Cal: Calibration; [b] CV: Cross-Validation;
[c] Pred: Prediction

The wavenumbers responsible of the Raman classification correspond to the characteristic bands of the selected amygdalin regions. These results correspond to the negative values of the first factor, associated to the bitter almonds that contain this compound. Nitrile vibrational band at 2245 cm^{-1} is a highly specific signal from the cyanide. Aromatic ring bands agree with the rest of the bitter representative loadings obtained at 3060, 1604, 1031, 1003, 850 and 620 cm^{-1}.

Figure 4 shows the predicted class of the samples of the test set for both techniques (NIR and Raman). The confidence intervals are centered at the mean value of the predictions for each class and built with two times the standard deviation of the predictions. The plot shows a good discrimination for both techniques between sweet and bitter almonds. Good class division for both techniques is achieved with clear separation of the classes.

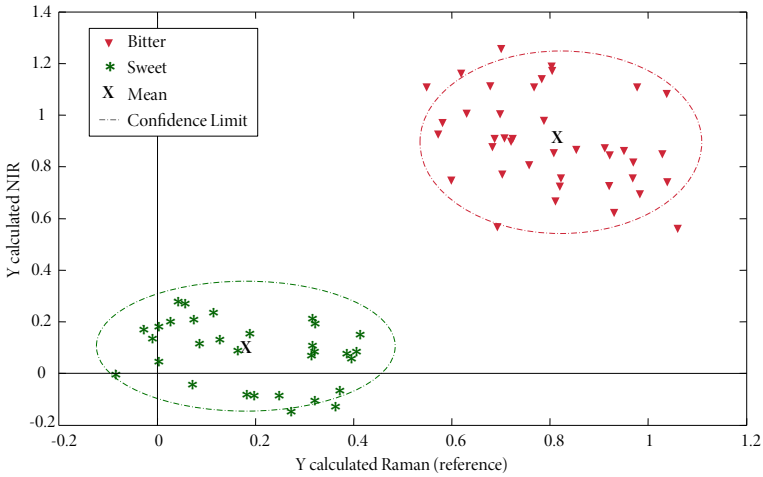


Fig. 4. Discrimination plot between sweet and bitter almonds for the two instrumental techniques used (NIR and Raman). Confidence intervals are centered on the mean of the predicted values and built with two times the standard deviation of the predictions.

4. Conclusion

This work demonstrates the feasibility of building a robust, fast and non-destructive methodology to discriminate between sweet and bitter almonds by combining NIR and PLS-DA obtaining an outstanding discrimination of the classes with high sensitivity and specificity levels. Raman methodology requires pre-treatment of the samples and dictates more control over measurement parameters and, therefore, more skilled operators than with NIR. NIR methodology is simpler, economically attractive and with a direct measurement on the sample it can be easily adapted and used as an automated method in industry, suitable to be implemented for quality assurance and control of raw material or final product (i.e. final packaged almonds or almonds intended for baking or other secondary products).

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Chapter 3. Olive Oil Sensory Analysis

3.1. Introduction

Olive oil is obtained from the fruit of the olive tree (*Olea europaea L.*), a traditional cultivation through the Mediterranean region. The press of ripe and healthy olives produces a fresh olive juice that can be consumed directly in the crude form without any chemical treatment [1]. The simplicity of the production keeps intact its organoleptic and nutritional properties, which make olive oil distinguishable from other vegetable oils, in such way that it has become a highly valued commodity essential in the Mediterranean diet. Its positive effects in health are mainly due to some major components like unsaturated fatty acids, but also to other minor constituents. Thus, the balanced fatty acid composition (oleic and linoleic acid) protects the body from cardiovascular diseases and reduces LDL cholesterol levels. Moreover, the high content of antioxidants (e.g. polyphenols, tocopherols, chlorophylls or carotenoids) in olive oil also is also related to the reduction of degradative processes, preventing heart diseases, tumors and degenerative aging diseases [2,3].

All these properties, together with the new trends on consumer preferences focused on searching the combination of a pleasant fravor with nutritional benefits in food products, involve an increasing demand and popularity of olive oils and, therefore, a rapid expansion of its market [4,5]. Most of the world production of olive oil is concentrated in Europe, with approximately 70%, followed by Turkey, Syria, Tunisia and Morrocco with near 20% of the world production. The main contributors of the European countries are Spain, Italy and Greece, with 45%, 25% and 20% of the world olive oil production, respectively [6].

Olive oil characteristics and the limited production because of the climatic requirements have made it the highest priced oil in the food market. As a result, it is highly exposed to fraudulent activities such as adulteration, mislabeling or deception about the geographical area or botanical variety of the fruit. The most common adulteration practices consist on blending different categories of olive oil or mixing it with other vegetable oils [7-9], mainly to achieve greater economical benefit. For this reason, it is very important to establish suitable systems to assess the olive oil quality and its authenticity in order to protect consumers against fraud. There are strict and specific legal guidelines controlled by international institutions, such as the International Olive Council (IOC) [10], the Codex Alimentarius [11] or the European Union [1,12]. These regulatory bodies establish product definitions, uniform labeling

regulations [13,14], define physicochemical and sensory parameters and their detection limits, and define as well rapid, easy and accurate official methodologies to determine those quality parameters. Thereby, the accepted product definitions mainly describe *quality commercial grades* or categories that determine the olive oil economic value and specific *geographical regions* (like protected designations of origin (PDO)) that depend on the olive fruit varieties, environmental factors, agronomic techniques/cultivation, and production and storage conditions [12,15].

3.1.1. Olive oil quality categories

According to IOC trade standards [16] and European Community Regulations (EU 29/2012 [17], EU 61/2011 [18], EC 865/2004 [19], EC 1234/2007 [20] and EC 1019/2002 [13]) different commercial and quality categories of olive oil are defined. As it can be observed in **Figure 3.1**, there are two main categories depending on the raw material used to obtain the final oil: olive oil and olive-pomace oil.

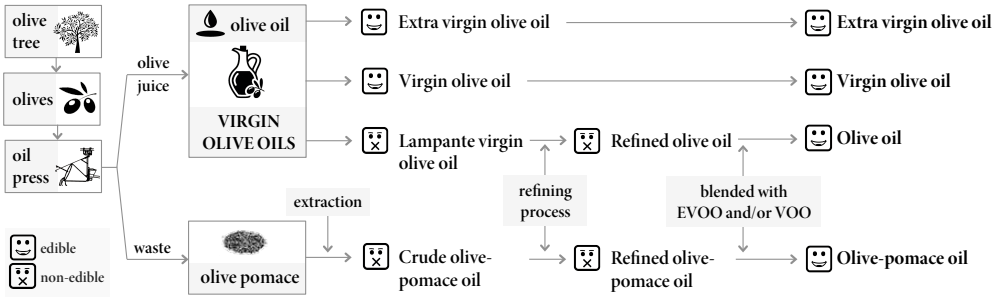


Figure 3.1. Classification of different olive oil categories and grades [16].

Olive oil is solely obtained by the juice of the olives without using solvents or re-esterification processes nor mixtures with oils of other kinds. Olive-pomace oil is obtained by treating the olive pomace with solvents or other physical treatments. From each olive oil provenances different quality grades are clearly described.

3.1.1.1. Virgin olive oil

Virgin olive oils are obtained from the olive fruit juice only by mechanical or other physical means under conditions (particularly thermal) that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration [16]. However, depending on different factors, such as agronomy, elaboration conditions or storage and transport, the quality may vary, as well as the sensory and chemical characteristics.

- **Extra virgin olive oil (EVOO).** It is the superior quality of virgin olive oil and should have a clear fravor that reflects the fruit from which it was made. It requires a total absence of any sensory defective attributes, with less than 0.8% of free acidity and conform all the standard parameters listed in its category [16]. EVOO can be very different from one another, depending on the fruit varieties, ripeness, growing region and extraction technique.
- **Virgin olive oil (VOO).** This oil has a slightly lower quality than EVOO. It has some sensory defectiveness (less than 3.5), free acidity below 2% and comply the standards of its category [16].
- **Lampante virgin olive oil (LOO).** This is the lowest quality grade from virgin olive oils, coming from inadequate fruits or improper handling or processing. This oil has severe organoleptic defects (higher than 3.5), free acidity greater than 3.3% and its category corresponding standard characteristics (also called lampante olive oil) [16].

While both EVOO and VOO can be bottled and consumed directly without any further treatment, LOO is not suitable for human consumption and is intended to be refined or used for technical applications. When the refining process is necessary, oils like LOO must be treated without altering its initial glyceride structure to obtain *refined olive oil*, which has free acidity values lower than 0.3% and must not come from the solvent extraction of pomace. The most common refining procedures consist on neutralizing free acidity (with sodium hydroxide), washing, drying, odor removal, color removal, and filtration. The resulting oils are usually odorless, tasteless and colorless [3]. As they are not fitted for consumption, refined olive oils have to be blended with VOO or EVOO to obtain the *olive oil*. This category has

higher acidity (less than 1%) and other defined standard characteristics that enables the human consumption.

3.1.1.2. Olive-pomace oil

The remaining residue after the pressing process is the pomace and its oil is extracted using solvents to obtain the *crude olive-pomace oil*. This oil is not suitable for human consumption so, it should be refined (using the same procedures as the refined olive oil) to obtain the *refined olive-pomace oil* and blended afterwards with small amounts of virgin olive oils (5-10%) to produce the final *olive-pomace oil*. This is the worst quality among the different olive oil categories that can be consumed.

3.1.2. Olive oil quality assessment

As previously explained, due the high value of virgin olive oil it is susceptible to suffer illegal deceptions to obtain higher economic profits. As previously mentioned, to detect and prevent these practices several international and national organizations have established strict rules to continuously control the properties of olive oil products and also for commercial activities. These regulations are based on well-established methods that mainly evaluate physico-chemical parameters although the sensory analysis is also determinant on these controls.

3.1.2.1. Physico-chemical evaluation

Most of the physico-chemical parameters evaluated are related to the components of olive oil. Basically, more than the 98% of the olive oil is composed by triacylglycerols esterified mainly with oleic, linoleic, palmitic and stearic acid. In lower proportion olive oil contain free fatty acids (FFA), mono- and diacylglycerols, waxes and an many other lipids including hydrocarbons, sterols, aliphatic alcohols, tocopherols, and pigments, in addition to a wide number of phenolic and volatile compounds [21-24].

Among the different physico-chemical parameters, the most traditional and usual are free acidity, peroxide value and UV spectroscopy.

- **Free acidity.** The amount of free fatty acids determines olive oil acidity degree, expressed as oleic acid (%). The concentration of free fatty acids increases due to the degradation of tricaylglycerols that mainly occurs through poor handling during processing or deficient

harvesting or storage of the fruit. Therefore, high values are considered a sign of deterioration of oil quality, and specific values are defined for the different categories of olive oils (**Table 3.1**).

- **Peroxide value**. The peroxide value determines the primary oxidation state of oil long before fravor perception of rancidity. As in many other foodstuffs, olive oils exposed to oxygen suffer some chemical and enzymatic reactions that lead to the formation of peroxides so, high values of this parameter indicate deterioration of the oil caused by fruit damages or long inappropriate storages. It is measured in miliequivalents (meq) of active oxygen per kg, and legal limits for olive oil categories are defined in **Table 3.1**.
- **UV spectroscopy (ΔK, K_{232}, K_{270}).** This analysis is based on the absorbance of ultraviolet radiation of some specific compounds that results on different degradative processes. Thus, K_{232} is a signal of peroxides and conjugated dienes, and K_{270} detects compounds from more advanced oxidative stages, where peroxides evolve to carbonylic compounds and conjugated trienes. These values are useful to assess the oxidation during storage, identifying oils age or refinement treatments. Delta K (ΔK) measures the difference between absorbances at 270 nm and 266–274 nm, which detects oil treatments and presence of refined or pomace oil with color-removing substances. Limit values for each olive oil category are also presented in **Table 3.1**.

Table 3.1. Physico-chemical quality limits for olive oil categories by IOC [16] and EU [18] regulations.

	Edible	Free acidity (%)	Peroxide value (mEq O_2/kg)	UV spectroscopy		
				K_{270}	K_{232}	ΔK
Extra virgin olive oil	Yes	≤ 0.8	≤ 20	≤ 0.22	≤ 2.50	≤ 0.01
Virgin olive oil	Yes	≤ 2.0	≤ 20	≤ 0.25	≤ 2.60	≤ 0.01
Lampante olive oil	No	> 3.3	-	-	-	-
Refined olive oil	No	≤ 0.3	≤ 5	≤ 1.10	-	≤ 0.16
Olive oil	Yes	≤ 1.0	≤ 15	≤ 0.90	-	≤ 0.15
Olive-pomace oil	Yes	≤ 1.0	≤ 15	≤ 1.70	-	≤ 0.18

Apart from these three parameters, the regulations define other parameters (and their values depending on the oil quality) and also specify the methodologies to determine them. These include *moisture and volatile matter* to control the oil extraction method, *insoluble impurities* to detect poor manufacturing practices, *trace metals* (iron, copper) present from

contamination during processing and storage, *flash point* (related to free acidity) or *halogenated solvents* from solvent-extraction residues. But also *fatty acid, ethyl ester, waxes* and *sterol content* to determine the purity and detect possible mixtures with other vegetable oils.

3.1.2.2. Sensory evaluation

The most important parameter to ensure the final olive oil acceptance for consumption is the organoleptic quality. This is related to smell, taste and color sensations, although the last one is not entirely considered in official olive oil sensory analyses. This sensory assessment consists on evaluating both *positive* and *negative* descriptors of olive oil using standards and specific scales defined by the regulation bodies. The positive descriptors are principally fruity, bitter and pungent; and the negative ones include fusty, musty, winey, metallic and rancid. According to IOC and UE standards, extra virgin olive oil should not have any sensory defect and should have some fruitiness. Therefore, when defects are present the olive oil should be classified into a lower category: virgin olive oil (intensity of the defect lower than 3.5) or lampante olive oil (intensity of the defect higher than 3.5) (**Table 3.2**).

Table 3.2. Sensory quality limits for olive oil categories by IOC [16] and EU [18] regulations.

	Edible	Odor and taste	Median of defects (Md)	Median of fruity attribute (Mf)	Color
Extra virgin olive oil	Yes	-	$Md = 0$	$Mf > 0$	-
Virgin olive oil	Yes	-	$0 < Md \leq 3.5$	$Mf > 0$	-
Lampante olive oil	No	-	$Md > 3.5$	-	-
Refined olive oil	No	acceptable	-	-	light yellow
Olive oil	Yes	good	-	-	light yellow to green
Olive-pomace oil	Yes	good	-	-	light yellow to green

▸ Human sensory taste panel

As described in Chapter 1, there is a general criterion to develop descriptive taste panels for different foodstufs, but in the case of olive oil this procedure is specified through several documents and regulations. The olive oil taste panel is the only accepted and homologated method by IOC (COI/T.20/Doc. No 15/Rev. 7 [25]) and European Union (EEC Regulation No 640/2008 [26]) focused on evaluating the sensory properties. These institutions define the guidelines referred to the whole sensory analysis, including the general and specific vocabulary applied (COI/T.20/Doc.04), the optional terminology for labeling purposes, the

panel selection, training and monitoring, the oil tasting glass (COI/T20/Doc.05), the test room installation (COI/T20/Doc.06), the description of the profile sheet used by tasters, as well as the processing of the sensory data obtained [25,26]. The aim of the olive oil sensory assessment is to classify this product into the different categories according to detection and intensity of sensory descriptors in the most accurate and reproducible manner.

The panel consists on one person responsible for the panel and its organization (the head panel) and from eight to twelve tasters, previously selected and trained (distinguishing similar samples). Each assessor must smell and then taste the oil submitted marking the intensity perceived of each descriptor on a structured 10-cm scale provided on a profile sheet (**Figure 3.2**).

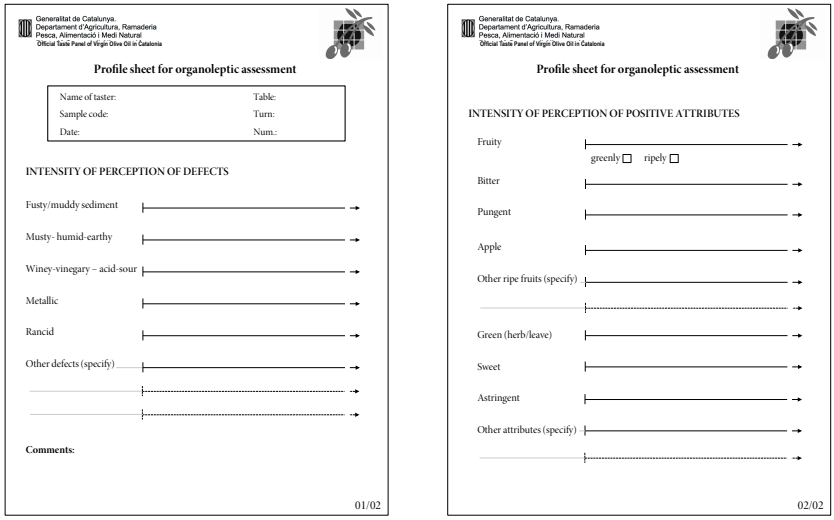


Figure 3.2. Example of a profile sheet used by the Official Taste Panel Of Virgin Olive Oils in Catalonia, following the EU Regulation 640/2008 [26].

According to the regulation guidelines, the analyzed oil samples shall be presented in standardized tasting glasses containing 14–16 ml of oil kept in the glasses at 28ºC ± 2ºC. These samples (not many to avoid fatigue) are randomly presented and with unknown information about them. The median value is calculated for each descriptor, using the highest value of the defects and the intensity of the fruity attribute to identify the olive oil category [25-27]. In fact, the most important values of sensory analysis are values that identify defect presence (to classify the commercial grades) rather than positive attributes.

However, even with all these specifications the methodology has some fundamental drawbacks. One of the main problems is the subjectivity of the experts that, despite their training, may cause variability of the responses, as well as fatigue of the human sensory organs over the time that limits the tested number of samples by day. In addition, the required training and maintenance of the panel test is time-consuming, expensive and makes impossible the process automation. Another limitation is the lack of stable standards for all the descriptors studied, which are often semantically fuzzy, even with the specific definitions provided [28].

Sensory descriptors

Descriptors or attributes are the different properties perceived through direct stimulation of human sensory receptors. These are related to pleasant and healthy sensations or undesirable off-fravors (defects). Among the different positive attributes, the most important ones are described by *fruity*, *bitter* and *pungent* sensations. From those, fruity is the only one related to smell, resulting from sound and fresh olives, either ripe or unripe. It is perceived via nasal but also retronasal and its specific description and intensity depends on the variety of the fruit. Bitter is a characteristic primary taste directly perceived, mainly on the back of our tongue, that appears from green olives and also during the first stages of olive veraison. Pungency is a chemestetic or tactile sensation, perceived both on mouth and throat, and it is characteristic of new oils at the beginning of the new crop year and also of oils obtained from still unripe olives [26,27]. There are other pleasant descriptors also considered, such as *green-grass* odor that gives a reminiscent sensation of freshly cut grass, *green* odor that comes from green fruits, *sweet* and *astringent* notes, among others [29].

Regarding the negative attributes, these are related to defective olive oils. The most critical stage on the off-fravors formation is the fruit preservation, mainly when olives are stored in piles with high temperatures during long periods. When this happens several degradative phenomena occur giving rise to *fusty* and *winey-vinegary* fravors. Moreover, when conditions are additionally too humid, great amounts of fungi and yeasts can grow up with the subsequent characteristic *musty-humid* flavor release. There are many other inappropriate procedures that could imply the generation of negative attributes, such as the harvesting process (when olives fall and remain on the ground several days, sensations of *mouldy* and

earthy may appear), the washing olives (if the fruit is not washed prior to the press, *wet earth* smell appears), crop care (careless plant conditions such as the use of metallic surfaces in prolonged contact with olive oils, reminiscent *metallic* fravors appear) or olive oil storage (unsuitable temperature conditions or prolonged storage periods cause oil oxidations leading to *rancid* odors) [24].

Compounds related to sensory descriptors

The different sensory perceptions are caused by the different components present in virgin olive oil [30]. Some of these are non-volatile compounds, which are usually related to taste perceptions because they stimulate the taste receptors (and free endings of trigeminal nerve) located in the mouth and throat. From these, phenolic compounds, besides being protecting agents against oxidative modifications (either for oil spoilage or human health) play a very important role in olive oils because they have a direct influence on bitter, pungent, astringent and metallic sensations [31-33]. Specifically, the main responsible of these perceptions are phenolic acids, lignans, flavones and sercoidoid derivatives, which are linked to compounds like hydroytyrosol, tyrosol, oleuropein and ligstroside, considered the main bioactive molecules in virgin olive oil [3,33,34].

However, it has to be taken into account that the most representative sensory perception considered in olive oil evaluation is the aroma. Aroma is constituted by different volatile compounds that stimulate the olfactory receptors via nasal and retronasal ways. In fact, a unique balance of fruity, green and other pleasant attributes contribute to the distinctive fravor of edible oils in the same way that the presence of off-fravors characterizes a low-quality olive oil. The perception of the aroma is related to the concentration and odor threshold of each volatile compound. Thus, very low concentration of a compound with low odor-threshold might be more crucial to the final aroma that substances with high concentrations but with high odor-thresholds. This fact, together with the synergism and antagonism effects of several olive oil compounds over the aroma perception, makes the determination of the aroma profiles a very difficult task [35]. Many of these volatiles are C6 and C5 compounds, such as aldehydes, alcohols, esters, hydrocarbons, ketones, furans, and probably other volatile compounds still unidentified. They derive from the degradation of polyunsaturated fatty acids through the lipoxygenase pathway by enzymatic reactions and autoxidation [30,36]. This

occurs during the oil extraction process and produce pleasant sensations like fruity, freshly cut-grass, sweetness, green fruits (apple or banana) or vegetables (tomato or artichoke) [37,38]. Different reactions and pathways generate the volatiles that define the undesirable odors. In this case, chemical oxidations and exogenous enzymes, usually from microbial activity produce volatile compounds like C7–C11 monounsaturated aldehydes, C6–C10 dienals, C5 branched aldehydes and alcohols or some C8 ketones [39,40]. Also several volatile phenols contribute to the negative fravor perception, comprising methyl, ethyl and vinyl derivatives of phenol and guaiacol [41,42]. Particularly, sugar fermentations originates winey and vinegary fravors, anaerobic microorganisms give muddy and fusty perceptions and moulds enzymatic activities and auto-oxidative processes confers mustiness and rancidity, respectively [31,32].

▸ Instrumental analysis

The increasing interest to determine olive oil sensory descriptors, together with the already mentioned limitations of human expert tasters, has motivated an increasing demand on alternative analytical tools to support taste panel evaluations. The so-called instrumental sensometry is aimed at defining sensory properties using objective, non-invasive, non-destructive, economic, fast, precise and easily automated techniques. In the last years, several instrumental techniques have been proposed for that purpose, including classical targeted methods that study specific sensory compounds, and non-targeted methods that make use of all the information obtained by the instruments.

Classical (targeted) methodologies

Many of the techniques used to determine the sensory properties are based on identifying the chemical compounds (targets or makers) responsible of specific taste, aroma and vision perceptions, such as volatile, phenolic or even colored compounds. To determine olive oil volatile compounds gas chromatography (GC) is the most common technique, offering fully objective results [41,43]. Despite the GC ability to identify several volatiles in a mixture, it does not consider the real aromatic compounds that contribute to the aroma. Up to now, only the human nose is able to detect an aromatic substance. This is the reason why in the GC–olfactometry system, this human detector (nose) is 'coupled' to a GC to determine the odor-active compounds [37,44].

To determine phenolic compounds high-performance liquid chromatography (HPLC) is widely used, as well as other analytical procedures, such as the K-225 value, Folin–Ciocalteau colorimetry, photometric-pH gradient or fluorimetry. Despite the non-specificity of the latter methodologies, these are well correlated to the bitterness taste attribute [45]. Visual sensory descriptors are not considered by the official method, but they are much related to the final acceptance of the olive oil. For this reason, different techniques are used to determine color compounds, such as chromatographic techniques that determine pigments (chloropylls and carotenoids) or non-specific methods that assess visually olive oil color by comparison with color scales (e.g. bromothymol blue method or CIE L^*a^*b color space) [46,47].

However, there are still many challenges when classical analytical procedures are used to replace the sensory panel. The main limitation is that there is a large set of currently unidentified compounds when investigating volatile and phenolic compounds responsible of the sensory descriptors [48,49]. Moreover, sometimes the problem is not in the analytical part but in the descriptors themselves, mainly because of the considerable vagueness on the description of some attributes by the assessors (e.g. fruity and green), which does not allow the proper identification of the chemical compounds, resulting in a vicious circle [28, 50]. In addition, the expected relationship between compounds and sensory attributes is not simple and casual, but several factors may affect this correlation, such as sensory interactions through synergism/antagonism of different aromatic compounds or even between taste and odor perceptions [36]. To overcome these problems, non-targeted analytical methods, combined with statistical procedures, might be a good option.

Non-targeted methodologies

As described in Chapter 1, there are many non-targeted approaches, mainly using spectroscopic, spectrometric and sensor techniques, where multivariate information is used to find mathematical relationships not between specific compounds (markers) and descriptors, but between spectral fingerprints and sensory descriptors.

The first instruments developed to evaluate olive oil sensory parameters were based on sensor arrays, using either liquid-chemical or gas sensor arrays to detect non-volatiles or volatiles, respectively. Non-volatile compounds (taste-causing chemical substances) are detected by non-specific sensor arrays, such as potentiometric [51-54] or amperometric [55] sensors,

which are applied to analyze the phenolic content of the olive oil. To study volatile substances cross-reactive gas sensors arrays are applied, such as metal oxide semiconductor (MOS) [36,56], polymeric [57,58] among others [59]. Both gas and liquid sensor systems are low cost, simple and fast techniques that collect signal responses from several compounds when they interact with the arrays. However, their responses are limited to the number of molecules that each sensor can recognize. This is an important limitation in olive oil sensory assessment, where a high number of sensory-related compounds are unknown and sometimes show complex interactions. For this reason, alternative techniques have been recently applied to obtain more general or non-specific responses. Despite the fact these sensor systems were first called 'electronic tongues' and 'electronic noses', nowadays this terminology has changed, and the 'electronic sensory simulator' requires to be complemented with a *'based on'* specifying the instrumental technique used.

In the case of *electronic tongues* several applications have been described in the literature. Some studies determined bitter and pungent related compounds using HPLC coupled to MS [45, 60], and other intended to correlate sensory descriptors using NMR spectroscopy (also called 'magnetic tongue') [61, 62] or vibrational spectroscopies like NIR or MIR [63, 64]. NIR- and MIR-based methodologies are simple (with no or minimal sample pre-treatments), low-cost, clean and fast, and are able to provide significant information about the components of complex matrices through a spectral fingerprint. Despite these advantages, there are still few applications of electronic tongues based on NIR/MIR to describe olive oil sensory descriptors, most of them focused on determining chemical composition [64-69], detecting adulterations [70-74] or authenticating olive varieties [75-78] and geographical origins [79-82].

In the case of *electronic noses* the only alternative technique to sensor arrays is based on mass spectrometry (MS). This 'e-nose based on MS' may use different sampling techniques to extract the aromatic part of the olive oil (SPME, static and/or dynamic HS) and different mass analyzer configurations (quadrupole, ion trap (IT) or time of flight (TOF)). MS-based e-noses can be coupled to a prior separation technique, such as GC [42,58,83-86], or to an olfactometric system (GC-O) to detect active-odor compounds [44,87]. GC methods have long analysis and data processing times, requiring usually an expert assessment. Therefore, a good alternative to reduce the analysis time is the removal of the chromatographic separation, thus providing all olive oil volatile information in a single mass spectral fingerprint. Few

applications are found in the literature that relate volatile fingerprints to olive oil sensory descriptors [88-91], and most of them do not include enough number of samples to consider the whole variability in the study.

In the case of color analyses the use of spectrophotometric techniques in the UV-Visible (UV-Vis) region may provide a useful tool to work as an *electronic eye*. Although color may be related to many different chemical species in olive oil, mainly pigments and carotenoids, some studies have proposed to use color-spectral fingerprints to find correlations with sensory parameters [92-94].

All these mentioned techniques can also be used together to emulate a taste panel, which performs the sensory assessment using smell, taste and, sometimes, color perceptions simultaneously. Thanks to the recent advances in chemometric tools, a more realistic and suitable emulation of the human sensory system is to join different sensory instrumental devices. This combination is called *data fusion*, and the final combined responses act as an *electronic panel*, simulating a human panel. Despite the increasing interest on data fusion applications, few studies have focused on olive oil sensory quality assessment [95], most of them based on olive oil geographical authentication [96-100].

In this chapter, new methodologies aimed at emulating olive oil sensory panel responses were applied. Our first studies were focused on identifying the presence or absence of sensory defects in olive oil samples previously analyzed by an official taste panel. To identify these negative attributes, and to define olive oil quality categories, individual instrumental techniques were initially used, such as an 'e-tongue based on FT-MIR spectroscopy' and an 'e-nose based on headspace-mass spectroscopy (HS-MS)'. Afterwards, these techniques, together with an 'e-eye based on UV-Vis spectro-photometry', were combined to enhance the results. Apart from discriminating olive oils by the presence of sensory defects, the intensities (sensory scores) of those defects and other positive descriptors were also modeled using regression models.

3.2. Electronic tongue based on FT-MIR spectroscopy

The usefulness of spectroscopic techniques working as electronic tongues is being more and more frequently proved. One example has already been described in Chapter 2, where NIR

spectroscopic responses were correlated to the bitterness of almonds in an effective way. In this section, MIR spectroscopy has been used as an electronic tongue, emulating taste perceptions on olive oil samples, and particularly to identify sensory defects. Olive oil is a complex matrix and MIR spectroscopy offers the possibility to obtain chemical information of the samples, because the mid-infrared spectral region can be used to identify functional groups and characterize molecular structures, especially from non-volatile substances that are related to gustatory perceptions. Moreover, it has to be noted that MIR spectroscopy is a fast, non-destructive and simple technique (with no or minimal sample preparation), so it could be easily automated (i.e. on-line).

3.2.1. MIR spectroscopy

An FT-MIR spectrometer is composed by an IR radiation source, an interferometer, a sampling system, a detector and a computer to process the data collected, in a similar manner as FT-NIR (Chapter 2). The mid-infrared region ranges between 400 and 4000 cm^{-1}, where most of the fundamental structural information can be found. The beam emitted by the source is guided through an interferometer, where it is divided by the beamsplitter using fixed and moving mirrors. As the mirror moves, each wavelength in the beam is periodically blocked or transmitted, and then the detector measures the recombined and interfered beam after passing through the sample using a reference laser.

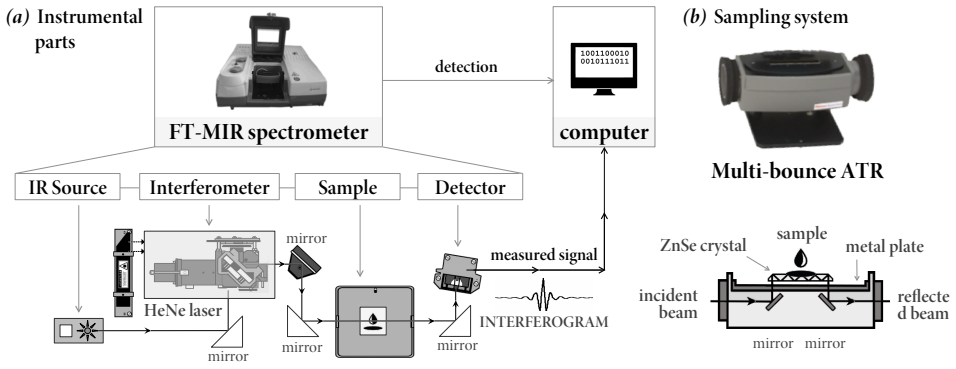


Figure 3.3. Description of the main parts of the FT-MIR spectrometer (a) and the sample compartment and multi-bounce ATR principle (b).

The recorded electrical signal is an interferogram, which contains all the acquired information. The final spectra are obtained by processing the interferogram with a Fourier Transform algorithm (**Figure 3.3a**). The sampling system consists on a multi bounce horizontal attenuated total reflection (HATR or ARK). It measures the infrared beam changes when it comes into contact with the sample. The totally internal reflected beam is directed to a ZnSe crystal (optically dense with a high refractive index) and creates an evanescent wave that is extended beyond the crystal's surface into the sample (12 reflections). If the sample (in contact with the crystal) absorbs energy, that wave is attenuated and its energy passes back to the IR beam, which then exits to the opposite side of the crystal to be measured as an interferogram when reaches the detector (**Figure 3.3b**).

MIR spectral information describes frequencies corresponding to vibrations and rotations of certain bonds that are representative of characteristic molecules. This ability to identify molecular structures is due to the high content of information of the MIR spectra and the possibility to assign most of the peaks and shoulders (absorption bands) to specific functional groups. In general, active fundamental vibrational bands in mid-IR region have stronger line strengths than overtone and combination bands used in near-IR regions. Molecular vibrations can be classified as stretching, changes on distances along bond axes, and bending, changes on the angle between two bonds (**Figure 3.4**).

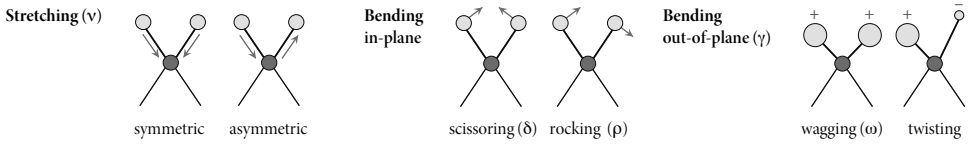


Figure 3.4. Main categories of molecular vibrations occurred with mid-infrared.

3.2.2. Olive oil and e-tongue

Taking into account all the properties above explained, the aim of the second study presented in this PhD Thesis was to assess the capacity of MIR spectroscopy as an electronic tongue to analyze olive oils. This study consisted on the application of MIR spectroscopy to a set of olive oil samples previously assessed by an official taste panel (to define its sensory profile) following the procedure described in **Figure 3.5**.

Since the quality categories: extra virgin (EVOO), virgin (VOO) and lampante (LOO) olive oil, mostly depend on the evaluation of the defects, only the negative attributes were chosen to be correlated to MIR spectral data.

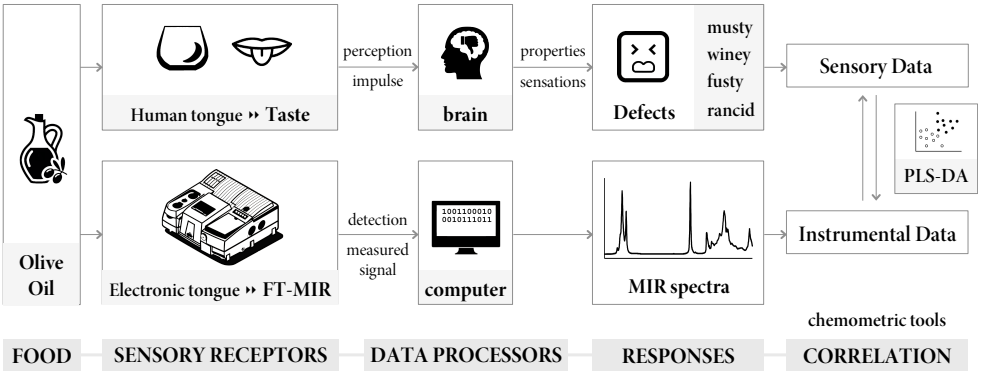


Figure 3.5. Correlation between human sensory analysis and the e-tongue based on MIR spectroscopy.

Regarding the different absorption bands obtained in the MIR region, only the most significant regions were considered according to the literature. In **Table 3.3** the main absorption bands, molecular vibrations, functional groups and substances associated to olive oil are described [71,72,77,78,81,101-114]. Given the complexity of the full olive oil MIR spectrum, it was divided into five principal regions corresponding to specific molecular vibrations. These regions and main absorption bands are also shown in **Figure 3.6**, where the non-informative signals between 2000 and 2500 cm^{-1} (carbon dioxide) and higher than 3100 cm^{-1} (from water, oil oxidation processes and instrumental noise) were removed.

By combining the information in **Table 3.3** and the bands observed in **Figure 3.6** it is possible to get very interesting information. The highest frequencies (>3000 cm^{-1}) are associated to water, primary oil oxidation products (hydroperoxides and its breakdown compounds (alcohols) [101,102]) and also olefins from unsaturated fatty acids and triacylglycerols [72,103-105]. Then, very strong absorbances at 3000 to 2800 cm^{-1} appear, due to hydrogen stretching vibrations related to symmetric and asymmetric movements of aliphatic methyl and methylene groups from fatty acid chains (**Figure 3.6a**) [72,77,81,101-103,105-108].

Although these regions are very informative of the olive oil structure and oxidative processes, they are not so correlated to sensory defects as regions from 1900 to 700 cm^{-1} (**Figure 3.6b**). These include a very strong absorption band near 1746 cm^{-1} corresponding to carbonyl

double bond stretching vibrations (-C=O) that are connected to olive oil ester bonds (triacylglicerols) and the presence of free fatty acids (1711 and 1653 cm^{-1}) [77,78,102,105,106, 109], specially from non-conjugated cis-olefins like oleic and linoleic acids [71,105,110].

Table 3.3. Molecular vibrations and compounds related to olive oil MIR spectral bands.

Frequency (cm^{-1})		Functional groups	Molecular vibrations	Compounds
3400-3700		-OH	stretching, ν	H_2O, hydroperoxides, alcohols ›› *water & oxidation products*
Oxidation process fingerprint				
3470		-C=O	overtone	ester ›› *triacylglycerols*
3025	*vw*	=C-H trans-	stretching, ν	olefins ›› *triacylglycerols & unsaturated fatty acids*
3007	*m*	=C-H cis-	stretching, ν	olefins ›› *triacylglycerols & unsaturated fatty acids*
Hydrogen stretching region				
2955	*sh*	-C-H	stretching, ν_{asym}	aliphatic methyl (CH_3) group
2925	*vs*	-C-H	stretching, ν_{asym}	aliphatic methylene (CH_2) and methyl (CH_3) group
2854	*vs*	-C-H	stretching, ν_{sym}	aliphatic methylene (CH_2) and methyl (CH_3) group
Double bond's stretching region				
1746	*vs*	-C=O	stretching, ν	ester ›› *triacylglycerols*
1728	*vw*	-C=O	stretching, ν	saturated aldehydes (secondary oxidation)
1715	*vw*	-C=O	stretching, ν	ketones (secondary oxidation)
1711	*sh*	-C=O	stretching, ν	acid ›› *free fatty acids*
1653	*vw*	-C=C- cis-	stretching, ν	disubstit. cis-olefins (unconjugated) ›› *oleic & linoleic acids*
1463	*M*	-C-H	bending, $\delta_{scissoring}$	aliphatic methylene (CH_2) and methyl (CH_3) group ››
			stretching, ν_{asym}	›› *aliphatic hydrocarbons*
1418	*w*	=C-H cis-	bending, $\rho_{rocking}$	disubstituted cis-olefins (unconjugated)
1400	*vw*	=C-H cis-		
1377	*m*	-C-H	bending, $\delta_{scissoring}$	aliphatic methyl (CH_3) group ›› *aliphatic hydrocarbons*
1363	*sh*	-C-H	bending, $\delta_{scissoring}$	ether methyl (CH_3) group
Esters fingerprint region				
1235	*m*	-CH_2-	bending, $\gamma_{out\text{-}of\text{-}plane}$	aliphatic methylene (CH_2) group ›› *saturated acyl groups*
		-C-O	stretching, ν_{asym}	esters from C-C(=O)-O and O-C-C
1220	*sh*	-C-O	stretching, ν	Epoxides
1160	*s*	-C-O	stretching, ν	aliphatic esters ›› saturated acyl groups
		-CH_2-	bending	
1138	*sh*	-C-O	stretching, ν	esters ›› saturated acyl groups
1118	*m*	-C-O	stretching, ν	esters ›› saturated acyl groups
1097	*m*	-C-O	stretching, ν	esters from C-C(=O)-O and O-C-C
1035	*sh*	-C-O	stretching, ν	esters from C-C(=O)-O and O-C-C
965	*w*	-HC=CH- trans-	bending, $\gamma_{out\text{-}of\text{-}plane}$	disubstituted tans-olefins (isolated) ›› estimation of trans
912	*vw*	-HC=CH- cis-	bending, $\gamma_{out\text{-}of\text{-}plane}$	double bonds and free fatty acids
850	*vw*	=CH_2	bending, $\omega_{wagging}$	-
723	*m*	-HC=CH- cis-	bending, $\rho_{rocking}$	aliphatic methylene (CH_2) group
			bending, $\omega_{wagging}$	disubstituted cis-olefins

vw: very weak band; *w*: weak band; *m*: medium band; *s*: strong band; *vs*: very strong band; *sh*: shoulder (band intensities)

Spectral signals of their secondary oxidation products, such as aldehydes and ketones (1728 and 1715 cm^{-1}) that may be related to degraded oils responsible of the main sensory off-fravors [110-112] can also be observed. However, not only stretching vibrations are important: other significant region is the one related to bending vibrations (1500-1250 cm^{-1}). Most of them are in-plane vibrations, like -C-H scissoring at 1463, 1377 and 1363 cm^{-1} from aliphatic hydrocarbons [72,78,81,103,105, 106,108,113] or ether groups [111], and =C-H rocking at 1418 and 1400 cm^{-1} from disubstituted cis-olefins [72,78,103,105,106,113].

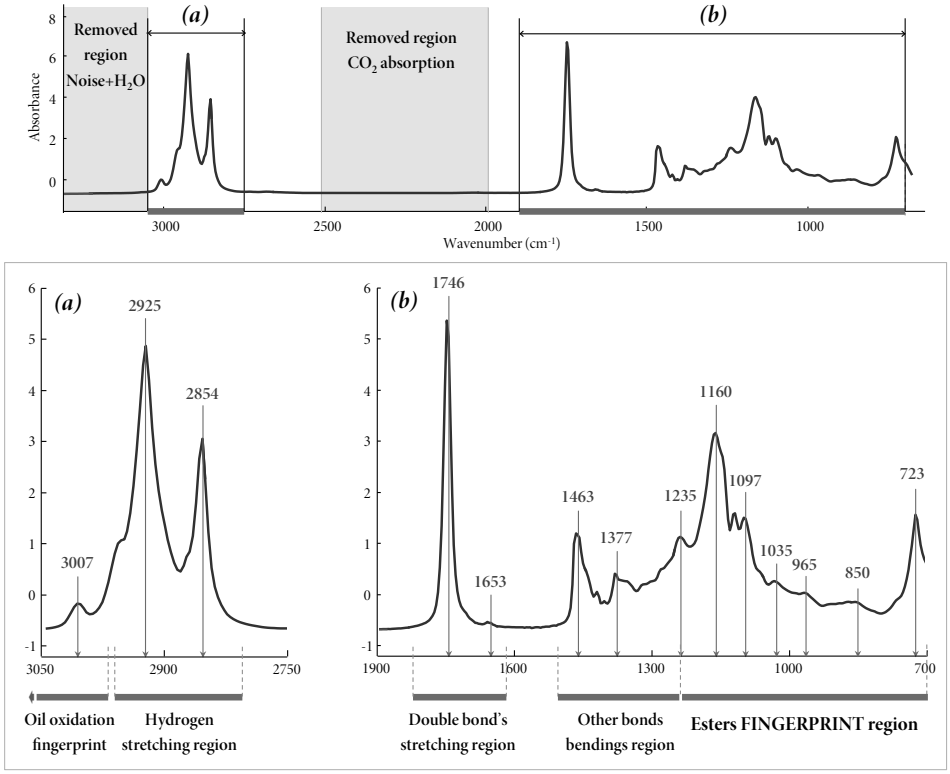


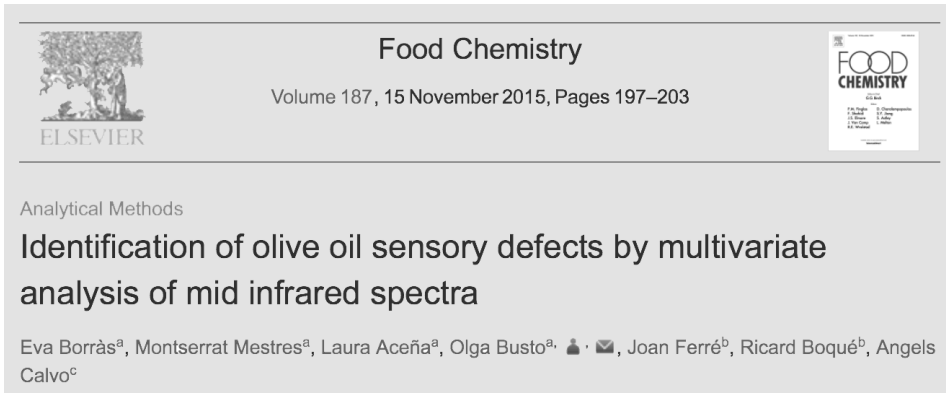
Figure 3.6. Full FT-MIR spectra of olive oil samples and removed spectral regions, with characteristic absorption frequencies from 3050 to 2750 cm^{-1} (region ***a***) and from 1900 to 700 cm^{-1} (region ***b***).

Finally, a characteristic pattern of bands of molecular composition from 1240 to 700 cm^{-1} is described as the esters fingerprint of olive oil. It mainly contains stretching -C-O vibration bands from esters forming saturated acyl groups at 1235, 1160, 1138, 1118, 1097 and 1035 cm^{-1} [72,78,81,105,106] and epoxides at 1220 cm^{-1} [111]. At lower wavenumbers, bending vibrations, mostly out-of plane, are related to -HC=CH- disubstituted trans- and cis-olefins at

965, 912 and 723 cm^{-1} that are used to estimate trans-double bonds (965 cm^{-1}) and free fatty acids [72,78, 81,101,105,106,114].

Although all this information was very important to determine the olive oil composition, it was not enough when applying FT-MIR as an electronic tongue. To find correlations between MIR spectra and sensory attributes chemometric tools are needed. Multivariate discriminant models were built to identify the four main off-fravors recognizable in olive oils: musty, winey, fusty and rancid. These sensory attributes and the MIR signals ('taste fingerprints') were correlated using PLS-DA.

In order to select the optimal results, several PLS-DA models were calculated by testing different pre-processing methods and regions (variables) of the MIR spectra. These conditions, together with the number of model factors (latent variables) were selected using internal validation (cross-validation). The final model and conditions were chosen by performing an iterative external validation. The results were published in a relevant technical journal related to analytical chemistry methods in food products [**Paper 3**].



[**Paper 3**] E. Borràs, M. Mestres, L. Aceña, O. Busto, J. Ferré, R. Boqué, A. Calvo, Food Chemistry 187 (2015) 197-203

Identification of Olive Oil Sensory Defects by Multivariate Analysis of Mid Infrared spectra

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Abstract

Mid Infrared (MIR) spectra (4000 to 600 cm^{-1}) of olive oils were analyzed using chemometric methods to identify the four main sensory defects, musty, winey, fusty and rancid, previously evaluated by an expert sensory panel. Classification models were developed using partial least squares discriminant analysis (PLS-DA) to distinguish between extra-virgin olive oils (defect absent) and lower quality olive oils (defect present). The most important spectral ranges responsible for the discrimination were identified. PLS-DA models were able to discriminate between defective and non-defective oils with predictive abilities around 87% for the musty defect and around 77% for winey, fusty and rancid defects. This methodology makes an advance towards the instrumental determination results from a human test panel.

Keywords

Virgin olive oil; Mid infrared spectroscopy; Multivariate analysis; Partial least squares discriminant analysis (PLS-DA); Classification; Sensory analysis

1. Introduction

Virgin olive oil (VOO) has well-known nutritional and sensory qualities. Because of its high economic value, VOO might be subject to blending with cheaper vegetable oils or olive oil grades, which constitute, in addition to economic fraud, a health threat for consumers. Furthermore, oils of lower quality can be unintentionally produced due to inappropriate production practices and storage conditions. In order to assure product quality and to protect consumers from frauds, it is important to be able to authenticate products both chemically and based on sensory characteristics. In this sense, there is an extensive regulatory framework by the European Community (ECC/2568/1991, ECC/796/2002, ECC/1089/2003 and ECC/640/2008), the International Olive Oil Council (IOOC, 2013) and the Codex Alimentarius (FAO-OMS) that establish different categories of olive oil, analytical parameters and sensory evaluation criteria. Moreover, the Protected Designation of Origin (PDO) councils also control the typicity of the product from a particular area, adding additional quality assurance to the VOOs. At present, Catalonia (in the northeast of Spain) has five of such PDOs for extra virgin olive: Les Garrigues, Siurana, Oli de Terra Alta, Oli del Baix Ebre-Montsià and Oli de l'Empordà (EC/510/2006, EC 1989/2006).

Olive oils can be graded in three quality categories, extra-virgin, virgin and lampante, based on values of four parameters the limits of which are defined by legislation (**Table 1**). Among these, three are chemical parameters (free acidity, peroxide value and UV absorbance) and the fourth is related to sensory properties.

Table 1. Olive oils grading according to Regulation (EC) 640/2008

	Free acidity (%)	Peroxide value (mEq O_2/kg)	UV spectroscopy		Sensory analysis	
			K_{232}	K_{270}	Md	Mf
Extra virgin olive oil	≤ 0.8	≤ 20	≤ 2.50	≤ 0.22	= 0	> 0
Virgin olive oil	≤ 2.0	≤ 20	≤ 2.60	≤ 0.25	≤ 3.5	> 0
Lampante olive oil	> 3.3	-	-	-	> 3.5	= 0

Md, median for any negative attribute; Mf, median for the "fruity" attribute

There are well established analytical methods for determining the chemical parameters and even additional methods for detecting fraud from blending or inadequate manufacturing of the oil (Aparicio et al. 2013). The present study deals with the fourth requisite: sensory analysis. The organoleptic characteristics of olive oils are determined by two procedures: the sensory panel test and study of volatile compounds. The only homologated method to evaluate the sensory quality is based on assessment by trained assessors (panel test) who recognize and evaluate sensory attributes represented by several descriptors (Monteleone & Langstaff, 2014). The body responsible for the organoleptic evaluation of the VOOs produced and commercialized in Catalonia is the "Panell de Tast d'Olis Verges d'Oliva de Catalunya" (Official Taste Panel of Virgin Olive Oil in Catalonia), from the Ministry of Agriculture, Food and Rural Action following the official Decree 473/2004 (DOGC 4291-30/12/2004 and DOGC 2396-22/05/1997).

Sensory attributes of olive oil can be classified as positive or negative. The positive ones determine the particular olive oil fravor, which produces a balance of green, fruity, bitter and pungent sensory notes (Peres et al., 2013). Among them, bitterness and pungency are phenolic compounds in VOO (Recchia et al., 2012). On the opposite side, the most frequent off-flavors are grouped into five main defects: musty/humid, winey/vinegary/acid, fusty/muddy, rancid and metallic, which may be caused by microorganisms and the oxidation of fatty acids, mainly due to inadequate storage of the olive fruit and oil. Whereas the positive sensory attributes are desirable, the negative ones determine labeling. If sensory defects are present, olive oil cannot be labeled as EVOO and, if the median of any defect is higher than 3.5 (**Table 1**), the oil has to be subjected to further refining and be labeled as lampante, which has lower economic value (Delgado & Guinard, 2011).

In the recent years, the rise in olive production and improvement in cultivation, harvesting, and processing techniques, together with consumers' demands for higher quality products, has led producers to increase the production of higher-value EVOO (Inarejos-García et al., 2013). In order to fulfill legal requirements, this oil must be sensory analyzed before commercialization. The panel responsible for such evaluation has some inherent operative problems such as experts' subjectivity, variability of the responses over time and the limited number of samples that can be evaluated per day. Moreover the lack of standards makes quite difficult to standardize the sensory results (Busch et al., 2006; Sinelli et al., 2010). Finally, to guarantee the quality of the results, the creation, training and maintenance of expert tasting panels involves complex logistics and a significant investment of both time and money. Therefore, the development of alternative instrumental techniques able to assess the sensory properties of oils in an objective, fast, automated and precise way, would be advantageous for the olive oil sector (Escuderos et al., 2007; Inarejos-Garcia et al., 2009).

Some previous attempts for describing the sensory properties have been based on the determination of the volatile compounds of the oils, which are responsible for the aroma. Gas chromatography (GC), headspace-mass spectrometry (HS-MS), HS-GC-olfactometry, HS-GC-MS, and e-noses based on metal-oxide sensors have been used for this purpose (Aparicio et al., 2012). Applications based on HS-MS (Morales, Luna, & Aparicio, 2005; Procida et al., 2005; López-Feria et al., 2007a; López-Feria et al., 2007b; López-Feria et al., 2008) and direct-MS (ESI-MS (Alves et al., 2013) and DART-TOFMS (Vaclavik et al., 2009)) were focused on achieving rapid determination of volatile compounds as well as correlating this information with negative and positive attributes to classify olive oils in different categories. The relationship between volatile and phenolic composition and bitterness intensity perception was studied using high performance liquid chromatography coupled with diode array or mass detectors (HPLC-DAD/MS) (Inarejos-García et al., 2013) as well as physicochemical determinations like K-values (Favati et al., 2013). Nuclear magnetic resonance (NMR) spectroscopy using the phenol and aldehyde signals was also used to predict sensory descriptors (Lauri et al., 2013). Most of these methods are time consuming, expensive or not widely available and, despite the extensive knowledge on the volatile and phenolic composition of olive oil, they were not always successful in

reproducing some results of the panelists (Busch et al., 2006). An alternative is the use of arrays of sensors for detecting volatile compounds (electronic noses) (Escuderos at al., 2013; García-González & Aparicio, 2004) and multisensory systems for liquid samples (electronic tongues) (Apetrei et al., 2010) to discriminate virgin olive oils with different organoleptic characteristics. However, the use of arrays of electrochemical sensors is highly complex due to the low conductivity of the oil samples, their viscosity and their reduced solubility in the typical solvents employed for electrochemical analysis. Near and mid infrared (NIR and MIR) spectroscopies are fast and affordable and have been proposed as an alternative to sensor systems, offering significant information about individual components in complex samples and showing their usefulness for describing quality attributes (Inarejos-García et al., 2013; Gertz, 2013; Sinelli et al., 2010).

The present study focuses on the identification of sensory defects in olive oil by MIR spectroscopy. For this purpose, infrared spectra of raw olive oil samples were collected, treated and correlated by Partial Least Squares Discriminant Analysis (PLS-DA) to the sensory results provided by an official taste panel.

2. Materials and methods

2.1. Olive Oil Samples

A total of 146 samples were supplied by the 'Official Taste Panel of Virgin Olive Oil in Catalonia' in Reus (Government of Catalonia, Spain) during the seasons 2012 and 2013. Samples were stored in dark bottles at –20ºC under nitrogen atmosphere until instrumental analysis. All the samples were analyzed within three months after the sensory analysis. Based on the sensory results provided by the panel, 84 were graded as extra virgin olive oils (EVOO), 48 as virgin olive oils (VOO) and 14 as lampante olive oils (LOO). The number of olive oil samples with each defect was: 49 fusty, 49 winey, 46 musty and 16 rancid.

2.2. Sensory analysis

Sensory analysis was carried out by the official tasting panel following the official method of the Olive Oil Council (COI/T20/Doc15) and within the framework of ECC regulation 640/2008. The panel is also accredited according to the ISO17025 norm.

15 ml of each sample were tasted in a normalized colored cup to mask the color differences. The temperature of the oils was kept at 28 ± 2ºC. Samples were labeled with a digit code and served following a balanced rotation plan.

The positive scored attributes were fruitiness, bitterness, pungency, grassy green, astringent, sweet and apple. The scored sensory attributes indicating defectiveness or unpleasantness were musty, winey, fusty, rancid and metallic. The samples tested did not have the metallic attribute, and for this reason this defect was not studied. Descriptors were evaluated on a continuous, unlabelled, intensity scale (10cm), and then transformed into numeric variables between 0 and 10. Each sample was tasted by 8 to 10 panelists and the median value was provided for the predominant attributes.

2.3. MIR spectroscopy

Fourier-transform mid infrared spectra were measured on an FT-MIR Nexus (Thermo Nicolet, USA) spectrometer equipped with a deuterated triglycine sulfate (dTGS) detector. The spectra were collected at room temperature in the 4000–600 cm^{-1} range, at 4 cm^{-1} resolution and with 36 scans both for background and samples. Three replicates were measured per sample. A thick film of each oil sample was homogenously placed over the ZnSe crystal ARK multi-bounce (horizontal attenuated total reflectance, HATR) with 12 reflections. The OMNIC software version 6.2 from Thermo Nicolet was used for spectral acquisition, instrument control and preliminary file manipulation. The spectra were compensated to eliminate disturbing H_2O and CO_2 bands by running a blank with air. After that only the regions with informative signals were selected for modeling, namely the ranges 3257.3–2604.3 cm^{-1} and 1951.4–682.8 cm^{-1}.

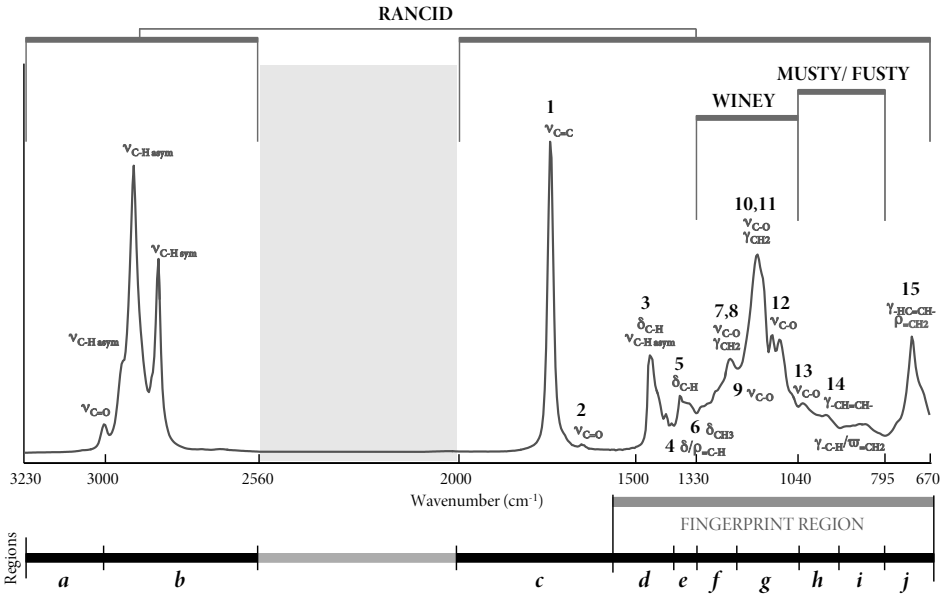


Figure 1. MIR spectra of olive oils used for PLS-DA models after SNV pre-treatment. Vibrations: (ν) stretching, (δ) bending (scissoring)/ in-plane deformation, (γ) out-of-plane deformation, (ρ) rocking, (ϖ) wagging. Characteristic compounds by region of the spectra: (1) *esters* of triglicerides and free fatty acids (FFA), (2) *acids* of FFA, saturated *aldehydes* and *ketones*, (3) *aliphatic* methyl and methylene groups, (4) *cis-olefines*, (5) *aliphatic* hydrocarbons, (6) *ethers*, (7, 10, 12, 13) *ester* fingerprint, (8, 11) methylene groups, (9) *epoxides*, (14) *trans-olefines*, (15) *cis*-disubstituted *olefines*. (a) to (j) indicate the spectral regions tested.

2.4. Data processing and models

Calculations were carried out using in-house routines written in Matlab v.7.8 (Mathworks, MA, USA) and the PLS Toolbox software v.6.2 (Eigenvector Research, Manson, WA, USA).

After a preliminary exploratory analysis with principal component analysis (PCA) of the spectra, PLS-DA classification models were developed to predict the presence or absence of each of the four defects (musty, winey, fusty and rancid). To find the optimal classification model for each defect, ten different spectral regions and combinations of them were considered (**Figure 1**) together with different preprocessing options (Lasch, 2012).

The optimization process of the spectral preprocessing is described in **Figure 2**. Several PLS-DA models were calculated for all possible combinations of preprocessing methods (offset correction, baseline correction, normalization, first- and second-derivative, smoothing, and combinations of them, **Fig 2 – step a**), spectral regions (**Fig 2 – step b**) and number of factors.

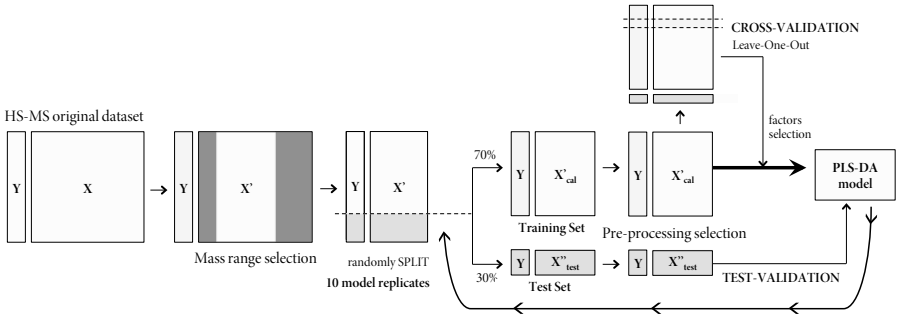


Figure 2. Steps followed to optimize the classification models.

The optimal wavelength ranges, preprocessing and number of factors were decided based on the maximum number of samples correctly classified (sensitivity) and misclassified (specificity) obtained by leave-one-out cross-validation, considering the triplicates as a single sample. The final performance of the models was confirmed by means of a test set. For that purpose, the dataset was randomly split into a training and test set, containing the 70% and 30% of the samples, respectively. The split between training and test was done by keeping the ratio defective/non-defective samples as in the original set. In order to avoid test results depending on the particular split, the training-test set split procedure was repeated ten times. Mean values and standard deviations of sensitivity (true positive rate), specificity (true negative rate) and classification error were calculated and expressed as percentages. In all cases, data were mean-centered before the classification model was calculated.

3. Results and Discussion

3.1. Principal Component Analysis

Preliminary exploratory analysis with PCA was performed on the whole set of triplicate spectra (438 spectra) in order to check the repeatability of the measurements, to detect outliers, and to recognize patterns in the samples' distribution due to the olive oil grade, harvest year, measurement day or presence of a certain attribute or defect. Preprocessing with standard normal variate (SNV) transform

followed by first derivative yielded the highest resemblance between triplicate spectra. The scores plot (not shown) of the two first principal components (94.68% of total variance explained) neither showed clusters or patterns in the data, nor a clear distinction between defective and non-defective samples. Six samples were removed before building the classification models, showing Hotelling's T^2 values and Q residuals well above 95% confidence limits.

3.2. Partial Least Squares-Discriminant Analysis

The main goal of the study was to discriminate olive oils having the key off-flavors detected by the official panel: fusty, musty, winey and rancid, from those without defects. If one or more of these defects is detected, then the oil can not be labeled as EVOO, which has important economic consequences for the producer. The interest was focused on the spectral ranges that could be assigned to each defect. For this reason, individual PLS-DA models were developed for each defect. At this stage, no intends were made to combine the results of the models for each defect.

Table 2. Optimal conditions and classification parameters obtained with PLS-DA (a)

	Musty	**Winey**	**Fusty**	**Rancid**
Range (cm^{-1})	1040 - 795	1330 - 1045	1040 – 795	3230-2560 + 2110-670
Preprocessing	SNV + 1DRV(b)	SNV + 1DRV (b)	Offset + 1DRV (c)	Offset
Number of Factors	3	5	4	5
Cross validation (%)				
Sensitivity	88.2 (± 2.6)	77.2 (± 3.8)	75.5 (± 6.4)	67.4 (± 5.7)
Specificity	83.0 (± 4.5)	75.2 (± 5.3)	72.3 (± 6.3)	87.5 (± 5.7)
Class. Error	14.4 (± 2.7)	23.8 (± 3.4)	26.1 (± 4.6)	22.6 (± 4.5)
Test set validation (%)				
Sensitivity	86.6 (± 5.9)	76.4 (± 6.3)	76.5 (± 6.7)	76.8 (± 6.4)
Specificity	81.5 (± 10.0)	77.1 (± 11.0)	71.0 (± 13.8)	80.7 (± 18.3)
Class. Error	15.9 (± 3.9)	23.2 (± 5.5)	26.3 (± 7.9)	21.2 (± 9.0)

(a) The results are indicated as a percentage and presented as the mean (± standard deviation) of the 10 models.
(b) SNV + 1 DRV: Standard Normal Variate and 1st derivative.
(c) Offset + 1DRV: Offset and 1st derivative.

For developing the PLS-DA models, the **X** matrix contained the spectra in rows and **y** was a vector with zeroes (for non-defective oils) and ones (for oils with the defect). The optimal combination of spectral region, preprocessing method and number of PLS-DA factors was chosen as described in section 2.3. For the best PLS-DA model, the classification parameters sensitivity, specificity and classification error are shown in **Table 2**. The classification results were obtained by cross-validation (leave-one-out) and test set prediction.

Figure 1 shows the spectra of the oil samples in the infrared region from 3230 to 670cm^{-1}. The meaningful spectral regions corresponding to the model with optimal classification performance are indicated for each defect studied.

3.2.1. Musty defect

The optimal classification model was calculated using a spectral range from 1040 to 795 cm^{-1}, preprocessed with SNV followed by first derivative and with three factors. The classification results for this model, for a certain split of training and test set, are shown in **Fig. 3**. The threshold value for the prediction of the PLS model (0.42) was set as the value that minimized the prediction error rate in the training step. Samples whose prediction is below or above the threshold are classified as non-defective and defective, respectively. Correct classifications (filled symbols in **Fig. 3**) are obtained when the samples measured as defective by the panel are predicted as defective by the model, likewise for the non-defective samples. Misclassifications (empty symbols in **Fig. 3**) occur when defective and non-defective oils described by the panel are wrongly predicted by the model. Among the four modeled defects, the best discriminatory results were achieved for the musty defect, both with both cross-validation and test set validation.

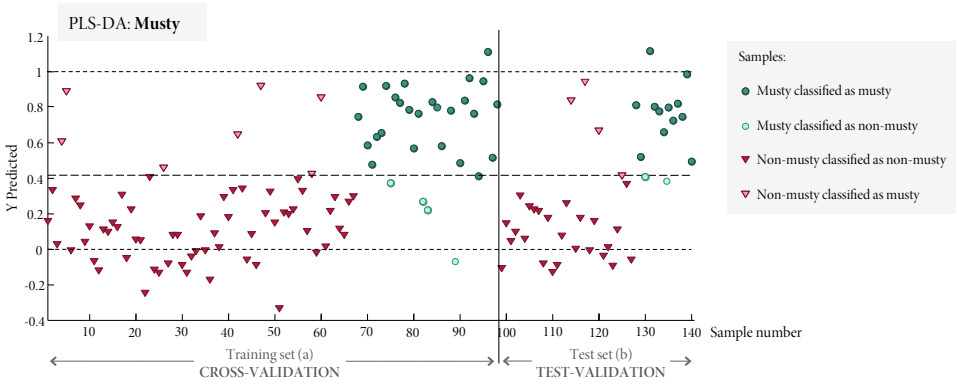


Figure 3. Predictions for the training (a) and test (b) set of musty defect. Mean of triplicates of one PLS-DA model of the 10 calculated models. Oils with musty defect (●) and without musty defect (▼). Samples correctly classified are filled symbols (●/▼) and samples badly classified are light symbols (○/▽).

With a similar classification error of about 15%, both for cross-validation and test set validation, the sensitivity (musty oils correctly identified as defective) is around 88% and the specificity (non-musty oils correctly identified as non-defective) is around 83%. The similarity between the cross-validation and the test set validation results ensures the reliability of the PLS-DA model. By considering the remaining information from the misclassified samples (other descriptors, defects) no obvious reason was found that could explain the wrong assignment of the misclassified samples. Interestingly, the non-musty samples (according to the panel classification) misclassified by the model as having the musty defect had similar spectra as the samples described as defect presence. Likewise for the musty samples misclassified as not having the musty defect by the model. This particular fact warns about the limits of the MIR technique with respect to its ability to differentiate samples with and without this defect, which should be further studied.

The loadings of the PLS-DA model (not shown) reveal the spectral regions that best explain the differences between classes for the musty defect. Highly influential wavenumbers in this model are around 1035, 965 and 850 cm^{-1}, corresponding to C-O (stretching) and -HC=CH- (cis/trans bending). Signals around 1035 cm^{-1} explain mainly the musty samples and the shoulder observed in the spectra could be assigned to the stretching vibrations of the C-O group in esters ($\nu_{C\text{-}O}$), which agrees with the fact that some compounds of this family can be related to the musty defect (Procida et al., 2005). Wavenumbers close to 965 and 850 cm^{-1} show higher influence when explaining the non-musty samples. The absorbance at 965 cm^{-1} is related to the trans double bonds due to bending out-of-plane deformation of trans-olefines ($\gamma_{\text{-HC=CH-}}$), and the absorbance at 850 cm^{-1} is related to bending vibrations of $=CH_2$ wagging ($\omega_{=CH2}$) and out-of-plane deformations of -C-H ($\gamma_{\text{-C-H}}$) (Guillén & Cabo, 1997). All these are general rotations, vibrations and other deformations that could be assigned to a wide number of organic molecules so it is difficult to correlate the mustiness fravor with a specific compound from the MIR spectrum. This agrees with the fact that mustiness appears in olives with a large number of fungi and yeasts growing when olives are stored for several days in humidity conditions that activate complex microorganism metabolisms liberating several metabolites on the growing media.

3.2.2. Winey defect

The best classification results were achieved using the fingerprint region of the spectra, from 1330 to 1045 cm^{-1}, with SNV and first derivative preprocessing and five PLS-DA factors. The prediction plot of the winey defect is shown in **Figure 4**. Sensitivities and specificities near 77% were obtained for both cross-validation and test set validation using a threshold of 0.4.

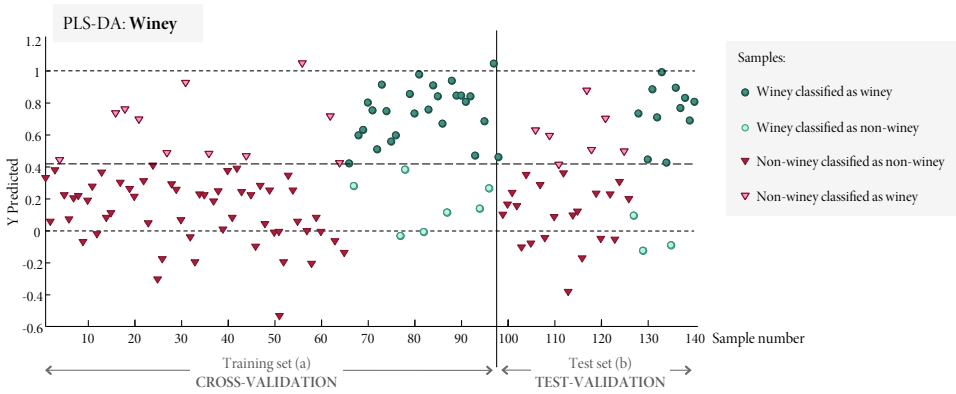


Figure 4. Predictions for the training (a) and test (b) set of winey defect. Mean of triplicates of one PLS-DA model of the 10 calculated models. Oils with winey defect (●) and without winey defect (▼). Samples correctly classified are filled symbols (●/▼) and samples badly classified are light symbols (○/▽).

The loadings (plot not shown) of the first two factors indicate that wavenumbers from 1100 to 1160 cm^{-1} are the ones with the highest discriminative power for the winey defective samples. This region is

associated with stretching vibrations of C-O aliphatic ester (esters fingerprint), with a strong band at 1160 cm^{-1} due to bending vibrations of CH_2 groups (γ_{-CH2-}). A band at 1100 cm^{-1} is related to oleic acid (Guillén & Cabo, 1997).

Although it is known that the winey fravor is mainly due to acetic acid, ethyl acetate and ethanol formed as a consequence of fermentation processes in olives, the complexity of the sample matrix complicates the assignation of each of these compounds to the discriminative spectral bands found.

3.2.3. Fusty defect

The best PLS-DA model to discriminate fusty samples was obtained using the same region as for the musty defect, that is, from 1040 to 795 cm^{-1}. In this case, preprocessing with offset correction followed by first derivative and four factors was used. Classification errors of 26% both for cross-validation and test set validation were obtained, with around 76% of the fusty samples and 72% of the non-fusty samples correctly classified.

Based on the PLS-DA loadings (not shown) the region around 1030–1040 cm^{-1} is the most important in discriminating the samples with the fusty defect. Samples with such a defect show a weak shoulder in that region that could be assigned to the C-O stretching vibration of esters (ν_{C-O}) (Procida et al., 2005). Indeed, the fusty defect has been described as being caused by olives stored in piles that have undergone an advanced stage of anaerobic fermentation, resulting in the presence of esters and acids, being butyl acetate and ethyl propanoate the main responsible of this defect (García-González & Aparicio, 2010). In addition, wavenumbers 965–980 cm^{-1} and 1015 cm^{-1} are remarkable, showing larger absorbances for the samples without the fusty defect. The first range is related to trans double bonds due to bending out-of-plane deformation of trans-olefins ($\gamma_{-HC=CH-}$), and the second is specifically related to the C-O stretching region of esters (Guillén & Cabo, 1997).

3.2.4. Rancid defect

In this case the best model was obtained using the whole spectrum, from 3230 to 2562 and 2110 to 670 cm^{-1}, using offset correction as preprocessing technique and five PLS-DA factors.

The classification errors were around 22% both for cross-validation and test set validation; however, sensitivities and specificities are quite different. Correctly classified rancid samples (sensitivity) were between 67–77%, lower than correctly classified non-rancid samples (specificity), with 80–88%. This difference could be due that most of the samples collected did not have the rancid defect (less than 10% of the samples).

Wavenumbers around 1740 cm^{-1} are the characteristic ones to interpret the rancid defect. In this region a strong band arises from the stretching vibration of the ester carbonyl ($\nu_{C=O}$) functional groups of the triglycerides, which are related to C=O stretching vibrations from ester linkages of carbonyl triglycerides

and the C=O absorption of the free fatty acids present in olive oils. Rancidity is a common sensory characteristic of oils that have undergone a process of auto-oxidation caused by a prolonged contact with air, and major contributors are aldehydes and acids (Morales et al., 2005) that may be correlated with some of the bands obtained from the samples presenting rancidity.

4. Conclusions

In this study a methodology using mid infrared (MIR) spectroscopy in combination with multivariate analysis has been developed for the identification of musty, winey, fusty and rancid defects in olive oil samples previously analyzed by an official sensory panel. Promising results were obtained for all the defects studied, mainly for musty and winey. PLS-DA models were able to discriminate the defective vs non-defective oil categories with predictive abilities between 70–90%, as evaluated by cross-validation and test set validation.

This study makes an advance towards the development of an objective instrumental analysis methodology able to distinguish EVOOs (defects absent) from lower quality oils. This instrumental approach, once fully validated, could be an alternative to the complex and time-consuming official olive oil sensory evaluation, because of its advantages in terms of cost, time and simplicity.

Although the predictive models may allow a good discrimination of the oils based on their defect presence, sensory determination implies a complex mixture of fravors and tastes. A single technique to study the broad sensory characteristics defined by a human panel may be difficult to find; however, the implementation of instrumental data fusion with different sensitive methodologies is expected to improve the results.

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3.3. Electronic nose based on HS-MS spectrometry

As explained before, in olive oil sensory evaluation the aroma is a determinant factor, so the next step was to apply an electronic nose to the analysis of olive oils. In this Thesis, mass spectrometry (MS) was used as the electronic nose after a preliminary extraction and pre-concentration of the volatile fraction of the olive oil samples. The extracted volatile compounds are sent to the MS detection system, which provides the final and characteristic digital mass-odor fingerprints (mass spectra) of the samples. It has to be remembered that apart from being a 'fingerprint', the spectrum brings chemical information about the components of the olive oil aroma, what is an advantage over other detection systems.

3.3.1. MS spectrometry

In electronic noses based on MS systems two essential parts are required to obtain instrumental data suited for pattern recognition purposes: the sampling system and the detector system.

Concerning the sampling system, and because of its simplicity, one of the most common techniques to extract and concentrate volatiles from samples is solid-phase micro-extraction (SPME). SPME uses a small fiber coated with an extracting phase that is exposed to the headspace sample (under optimal temperature and time conditions). Then, the volatile compounds are retained on the adsorbent coating of the fiber until it is inserted directly into the chromatograph system (GC) for desorption and analysis. There are many extracting phases commercially available but, from the expertise of our research group, the proper absorbent to extract the volatile compounds is the one composed by divinilbenzene, carboxen and polydimethylsiloxane (DVB/CAR/PDMS). The sample extraction procedure is described in **Figure 3.7a**, using an automatic sampling system (CTC-PAL Autosampler, **Figure 3.7b**) to achieve reproducible responses.

Once the volatiles are desorbed they are directly transferred to the mass spectrometer through the GC system, but avoiding chromatographic separation (**Figure 3.8**). For this, an HP5-MS column (30m x 0.25mm x 0,25 μm) was used with steep temperature ramp, helium mobile phase and splitless injection mode, reaching the MS system in 5 min.

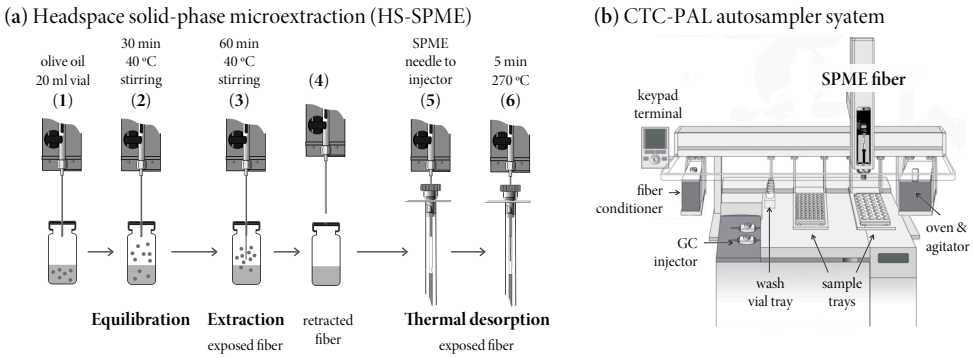


Figure 3.7. Sampling system used to extract volatiles. (a) Conditions and steps required applying SPME, and (b) main components of CTC-PAL autosampler working in SPME mode.

When the compounds arrive to the MS ionization source, they are ionized with electronic impact (EI) mode at 70 eV. Fragmented ions are then accelerated from the ionization chamber to a quadrupole mass analyzer, composed by four parallel cylindrical rods, which filter the ions based on their mass-to-charge ratio (m/z) using oscillating magnetic fields. The filtering can be applied either by selecting specific ions (SIM, selective ion monitoring) and/or scanning a previously selected range of masses (Scan). For fingerprinting analyses it is recommended to use Scan filtering, because it allows detecting the maximum representative (unknown) masses. Finally, the selected ions go through focus lenses to reach the detector, an electron multiplier, which generates an electronic signal proportional to the number of ions striking it.

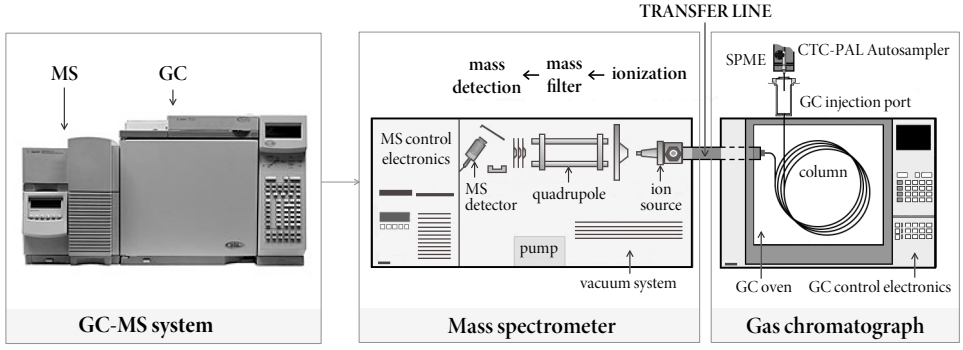


Figure 3.8. Structure of the GC-MS system used to detect olive oil volatiles. Description gas chromatography and mass spectrometry main components.

3.3.2. Olive oil and e-nose

Since the aim of this study was to reproduce the response of a taste panel, the experimental procedure, described in **Figure 3.9**, is similar to the one explained in the case of FT-MIR electronic tongue (section 3.3). So, the first step was the sensory analysis of the olive oil samples, which was carried on by an official taste panel. In this case, only the negative descriptors (musty, winey, fusty and rancid) were considered to build the discriminant PLS-DA models.

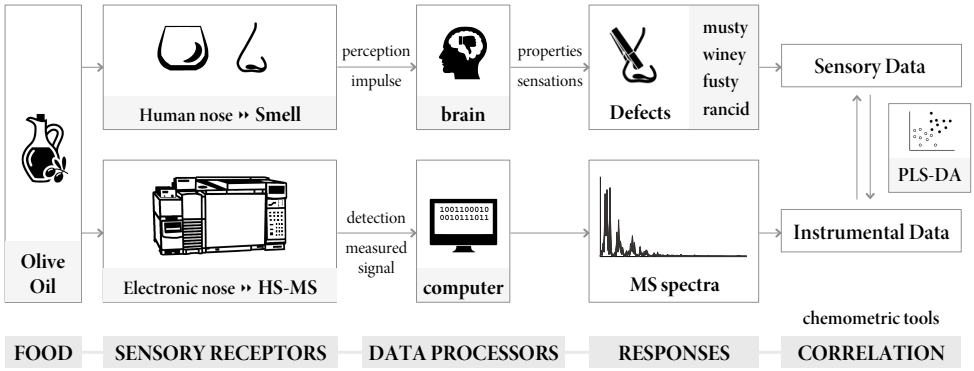


Figure 3.9. Electronic nose based on HS-MS system. Correlation between human sensory analysis and HS-MS spectrometry.

Instrumental data were obtained by extracting the headspace (HS) sample with SPME, and measuring them with a GC-MS system (HS-MS) without chromatographic separation. The resulting signal from the mass spectrometer detector is represented by a pseudo-chromatogram (MS 'chromatogram') with abundances of mass-to-charge ratios (m/z) detected through time (**Figure 3.10a**). As it can be seen, because of the short analysis time there is no clear separation of the compounds so, the final spectral fingerprint is only composed by the sum of all the abundances of each mass (m/z) detected during all the analysis time (**Figure 3.10b**). This provides non-targeted but useful information to be correlated with the specific off-fravors identified by the taste panel. Despite MS is an instrument with high sensitivity, this can be lost during consecutive analyses. To correct the differences between initial and final spectra (along time), row profile pre-processing of the spectra was required (**Figure 3.10c**).

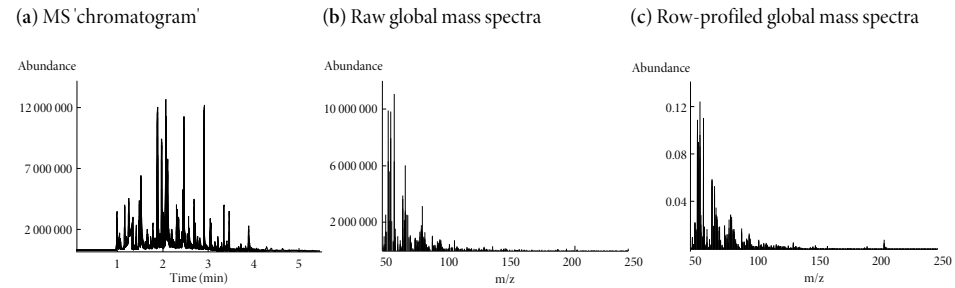


Figure 3.10. GC-MS signals obtained for all olive oil samples with e-nose system. (a) Initial 'chromatogram' obtained with ions abundance vs. time. (b) Global mass spectra (fingerprint) and (c) row-profiled global mass spectra, both with ions abundance vs. ions (m/z).

The collected mass-fingerprints are representative of each olive oil sample; however, it is very difficult to identify the possible compounds present in the samples. Nowadays, there is a huge interest on interpreting the impact of volatile compounds on olive oil aroma, and several intensive research studies have identified a great number of chemical compounds. From this literature review, some of these compounds have been identified as mainly responsible of olive oil sensory defects, as well as positive sensory descriptors, but several compounds are still unknown. **Table 3.4** summarizes the main volatile compounds identified and present in olive oil and the principal descriptors related (positive and negative), together with other sensory notes (gray colored) commonly associated to each compound in the literature. The most characteristic descriptors for each compound are highlighted (bold) and the main MS peaks obtained in EI mode are also provided.

Despite the complex identification of the compounds responsible of the sensory descriptors, it is important to note that the use of pattern recognition techniques on the global mass spectra fingerprints obtained by MS-electronic nose is capable to recognize important variables (m/z) for each model using the VIP scores. These variables provide information about the characteristic ions (m/z) generated (**Table 3.4** MS peak values) and allow identifying the possible volatile compounds responsible for the defects modeled. In particular **Table 3.5** shows the compounds most commonly correlated to the studied descriptors (musty, winey/vinegary, fusty and rancid).

Table 3.4. Summary of main volatile compounds identified in literature

Family	Compound	Sensory positive attributes	Negative descriptors (defects)	MS peaks[1]	CAS num.	References
Alcohols	ethanol	**apple**, sweet, pungent (floral)	**winey (alcoholic)**	< 50	64-17-5	[31,38,49,50,83,87,115-122]
	1-propanol	fruity, pungent	musty (**alcoholic**)	59	71-23-8	[122,123]
	isopropanol	**green,** apple, bitter (solvent)	fusty, winey (muddy-sediment)	55, 67	123-51-3	[31,115,116,122,124-127]
	1-butanol	sweet (fruit)	musty, winey, vinegary (**medicine, fat**)	55	71-36-3	[49,50,83,122,123]
	2-butanol	**fruity**	**fusty, winey** (medicine, muddy)	59, 57	78-92-2	[49,83,87,119,122,128]
	1,3-butanediol	-	**winey-vinegary**	72,57	-	[38]
	2-methyl-1-butanol	sweet (spicy, malt)	**winey-vinegary**, musty, fusty (**fish oil**)	55	137-32-6	[31,38,119,122,124-128]
	3-methyl-1-butanol	**sweet** (spicy, malt)	musty, **fusty, winey-vinegary (whiskey)**	55, 70	123-51-3	[50,115,119-124,128,129]
	2-methyl-3-buten-2-ol	cut-grass , fruity (herbal)	fusty, rancid, muddy (earth, oil)	71, 59, 53	115-18-4	[116,119,125]
	1-pentanol	**fruity, pungent**, sweet (**balsamic, ripe**)	fusty, musty, winey, rancid (**sticky, strong**)	55, 70	71-41-0	[115,116,120-122,124-130]
	3-pentanol	green	vinegary (defective)	59, 58, 57	584-02-1	[32,50]
	2Z-penten-1-ol	**green**, fruity, grass, sweet (banana)	(plastic, alcoholic)	57, 68	1576-95-0	[115-117,124-128,131]
	2E-penten-1-ol	-	(mushroom)	57, 67	1576-96-1	[125]
	3-penten-2-ol	green, grass (perfume)	musty (**woody**)	71, 53, 67	1569-50-2	[38,44,87,122]
	1-penten-3-ol	**green**, fruity, pungent (hay, lawn)	fusty, rancid (**wet earth, butter, undesirable**)	57	616-25-1	[115,116,120-127,131,132]
	1-hexanol	**fruity**, cut-grass, astringent (**soft, banana**)	rancid, fusty (**undesirable**, woody)	56, 55	111-27-3	[115,116,120-122,124-134]
	2E-hexen-1-ol	**green, ripe-fruit**, sweet (**leaves**)	**winey-vinegary** (fat, undesirable)	57, 82	928-95-0	[115,116,118-121,126-134]
	2Z-hexen-1-ol	**green, cut-grass**, astringent (almond)	winey	57, 82, 67	928-94-9	[31,115,116,124,135]
	3E-hexen-1-ol	**grass, green, bitter (bitter almond)**	vinegary, rancid (fat)	67, 69, 82	928-97-2	[115,116,124,125,128,133]
	3Z-hexen-1-ol	**green, grass**, pungent, apple (**banana**)	vinegary	67, 55, 82	928-96-1	[115,116,119-127,132,134]
	heptanol	sweet, green	musty, fusty, rancid (**earth**, mushroom)	55, 83	543-49-7	[36,38,49,83,89,122]
	6-methyl-5-hepten-2-ol	(perfume, nutty)	musty, mould	95, 69, 67, 110	1569-60-4	[38,50,87,122]
	1-octanol	green, sweet (herbal)	musty, fusty (wax, burnt, fat)	55, 70, 69, 83	111–87-5	[115,122]
	2-octanol	-	musty-humid, mould (earth, fat)	55, 56, 57, 70	123-96-6	[38,50,87,122,128]
	3-octanol	-	musty, mould (earth)	59, 55, 83, 101	589-98-0	[38]
	1-octen-3-ol	-	**musty, mould,** fusty (**earth, mushroom**)	57, 72, 55, 85	3391-86-4	[36,83,87,120,122,128,129]
	nonanol	green (citrus)	**rancid (fat)**	56, 55, 70	143-08-8	[38,44,58,87,122]
	2E-nonen-1-ol	fruity	(waxy)	57, 55, 68, 82	31502-14-4	[44,122]
Aldehydes	acetaldehyde	**pungent**, sweet	-	< 50	75-07-0	[31,38,122]
	propanal	pungent, sweet	-	58, 57	123-38-6	[31]
	butanal	green, pungent, sweet	musty, fusty	72, 57	123-72-8	[49,50,119,122]
	isobutanal	green	-	72	78-84-2	[119]
	2-methylbutanal	apple, pungent, sweet (malt)	musty, vinegary	57, 58, 86, 55	96-17-3	[31,38,115,116,122,125]
	3-methylbutanal	**sweet, fruity**, apple (malt)	fusty, vinegary	58, 57, 71	590-86-3	[115,116,120,122,124-127]
	pentanal	**bitter**, pungent, green (almond)	rancid, fusty (woody, oil, burnt)	58, 57	110-62-3	[36,38,87,120,122,127,131]
	2E-pentenal	**green**, fruity-pungent, grass (bitter almond)	winey-vinegary , fusty, rancid	55, 84, 83	1576-87-0	[115,116,122,124-128,130]
	hexanal	**cut-grass, green-sweet** (green apple)	**rancid**, winey-vinegary, mould	56, 57, 67, 72	66-25-1	[117,119-128,131-134,136]
	2E-hexenal	**green, fruity,** green astringent, bitter	fusty, winey-vinegary, rancid, musty, mould	83, 69, 55, 57	6728-26-3	[35,124]
	2Z-hexenal	fruity, bitter, green, sweet	-	55, 69	16635-54-4	[35,124]
	3E-hexenal	green	-	55, 69	69112-21-6	[115,116,124,127,133,135]
	3Z-hexenal	**green, cut-grass**, fruity, apple	-	55, 69	6789-80-6	[115,116,122,125,132,135]
	2E,4E-hexadienal	**green**, grass	-	81, 53, 67	142-83-6	[132]
	2-ethyl-E2-butenal	grass, floral	-	98, 55, 69, 83	19780-25-7	[31,36,44,49,122,127,132]
	heptanal	green (citrus, nutty)	**rancid (fat, oil, woody)**	70, 55, 57	111-71-7	[36,38,49,87,122,125,132]

Table 3.4. *Continued*

Family	Compound	Sensory positive attributes	Negative descriptors (defects)	MS peaks[1]	CAS num.	References
Aldehydes *(cont.)*	2E-heptenal	**pungent** (soap, almond)	**rancid, musty, mould (oxidized)**	55, 83, 57, 56	18829-55-5	[36,38,49,87,122,125,132]
	2Z-heptenal	-	rancid	55, 57, 83	57266-86-1	[50]
	2,4-heptadienal	cinnamon	**rancid**, musty, mould (**fat**)	81, 110, 53, 67	5910-85-0	[31,50,58,87,122,125,127]
	octanal	green (**citrus**, soap, lemon)	**rancid**, mould, fusty, musty (**fat**)	56, 84, 57, 55	124-13-0	[31,36,38,122,127,128,132]
	2E-octenal	**green,** fruity (**herb, spicy**)	**rancid (fat)**	55, 70, 83	2548-87-0	[115,116,120,122,127,128]
	nonanal	**pungent**, green, grass (citrus)	**rancid,** fusty (**fat, wax**)	57, 56, 55, 70	124-19-6	[117,120,122,123,127,132]
	2E-nonenal	cut-grass, fruity	rancid (**fat, paper-like,** wax)	55, 70, 83, 57	18829-56-6	[31,38,39,50,122,127,132]
	2,4-nonadienal	-	(deep-fried, penetrating)	81, 67	5910-87-2	[31,38,122]
	decanal	**sweet** (citrus, orange, soap)	**rancid** (**wax, penetrating**, fat)	57, 55, 70, 56	112-31-2	[31,37,87,122,127,128,132]
	2E-decenal	-	**rancid (fat, fish, paint)**	55, 70, 83, 57	3913-81-3	[31,50,120,122,127,128,132]
	2,4-decadienal	-	rancid (**fat, solvent, deep-fried**)	81, 67, 55, 53	25152-84-5	[38,44,50,87,122,127,132]
	E4,5-epoxy-E2-decenal	-	**metallic**	-	134454-31-2	[38,122]
	2E-undecenal	olive, fruity (**fresh, orange peel**)	**(fat)**	70, 55, 57, 83	53448-07-0	[127,132]
	benzaldehyde	(almond)	-	77, 105, 106	100-52-7	[31]
	benzenacetaldeyde	pungent (phenolic)	-	91, 92, 120	122-78-1	[31]
Ketones	acetone	sweet	winey	58	67-64-1	[119]
	2-butanone	**fruity**, sweet (**ethereal, pleasant**)	fusty	72, 57	78-93-3	[115,116,119,122,124-128]
	2-pentanone	fruity, green, sweet (ethereal)	vinegary	86, 71, 58	107-87-9	[20,50,116]
	3-pentanone	**sweet**, fruity, green (ethereal)	fusty (woody, oil)	86, 57	96-22-0	[115,116,121,124,126,127]
	1-penten-3-one	**sweet, pungent, green-spicy, fruity**, bitter	metallic, musty, rancid, muddy	55, 84, 57, 56	1629-58-9	[35-38,124-128,134,136]
	4-methyl-2-pentanone	**sweet, fruity**, green (**strawberry**)	fusty, rancid	58, 57, 85	108-10-1	[115,116,120,122,125-127]
	2-heptanone	**fruity, sweet (cinnamon)**	musty, winey-vinegary	58, 71, 59	110-43-0	[115,116,119,122,124-127]
	6-methyl-5-hepten-2-one	**pungent, green, fruity**, grass, bitter (herb)	musty, fusty, rancid, muddy (mushroom)	69, 108, 111	110-93-0	[115,116,120,122,124-127]
	3E-6-methylhepta-3,5-dien-2-one		vinegary, musty, mould	109, 81, 79	1604-28-0	[50]
	2-octanone	**green**, fruity (soap)	winey-vinegary, fusty, **mould**	58, 71, 59	111-13-7	[115,116,122,124-127]
	3-octanone	grass, green (nut, herb)	(mushroom, butter)	57, 71, 71, 99	106-68-3	[49,83,122,123]
	1-octen-3-one	pungent	musty, winey, metallic, **mould (mushroom)**	55, 70, 71	4312-99-6	[31,36,38,50,83,87,132]
	(Z)-1,5-octadien-3-one	(geranium-like)	metallic		65767-22-8	[36,38,122]
	3E-octa-3,5-dien-2-one	-	vinegary, musty, mould	95, 81, 109	30086-02-3	[50]
	2-nonanone	**fruity**, sweet (floral, hot milk)	musty-humid	58, 57, 71, 59	821-55-6	[35,115,116,122,124-128]
Acids	acetic acid	pungent	**winey-vinegary,** rancid, musty, fusty (**sour**)	60	64-19-7	[83,87,115,120-122,129,131]
	propionic acid	**pungent** (aromatic)	musty, fusty, winey, rancid, mould (**sour,** soy)	74, 73, 57	79-09-4	[36,38,83,87,120-122,129]
	methyl propanoic acid	fruity	fusty	73, 88	79-31-2	[83,87]
	butyric acid	pungent	**rancid, fusty**, musty, winey (**cheese**, sweat)	60, 73, 55	107-92-6	[49,58,83,87,120,122,129]
	2-methylbutyric acid	-	fusty (sweat)	74, 57, 87	116-53-0	[31,38]
	3-methylbutyric acid	-	winey, rancid	60, 87	503-74-2	[31,38]
	valeric acid	**pungent**	musty, fusty, winey, rancid (**unpleasant,** sweat)	60, 73	109-52-4	[31,38,49,83,87,120,122]
	caproic acid	**pungent**	**rancid**, musty, winey (sweat)	60, 73, 87	142-62-1	[31,38,49,58,120,122,132]
	heptanoic acid	-	**rancid**, fusty, winey (fat)	60, 73, 87	111-14-8	[38,49,58,87,122]
Esters	methyl acetate	**green**, sweet (ethereal, fruit)	winey (solvent)	74, 59	79-20-9	[49,115,116,119-122,124]
	ethyl acetate	**sweet**, bitter/pungent, astringent	**winey-vinegary**, musty, fusty (sticky)	61, 70, 88	141-78-6	[115,116,119-124,126,129]
	butyl acetate	**green, pungent**, fruity, sweet	fusty, rancid	56, 73, 61	123-86-4	[115,116,120,122,124-127]
	isobutyl acetate	fruity, green (apple, banana)	-	56, 61	110-19-0	[116,125]
	hexyl acetate	**fruity, green, sweet** (apple)	fusty, rancid	56, 55, 84	142-92-7	[115,116,124-127,133,136]

Table 3.4. *Continued*

Family	Compound	Sensory positive attributes	Negative descriptors (defects)	MS peaks[1]	CAS num.	References
Esters *(cont.)*	3Z-hexenyl acetate	**fruity (green-leaves, banana)**	-	67, 82	3681-78-1	[115,124-127,132,133,136]
	2-methylbutyl acetate	**fruity, green (banana)**	-	70, 73, 55	624-41-9	[125]
	3-methylbutyl acetate	fruity, bitter (banana)	fusty, rancid	70, 55	123-92-2	[31,35,37,115,124,126]
	3-methyl-2-butenyl acetate	pungent	(unpleasant, putty)	68, 67, 69	1191-16-8	[35,116,124-127]
	ethyl propionate	**sweet-green, fruity (strawberry)**	fusty, rancid (strong)	57, 102	105-37-3	[116,120,122,124,126,127]
	methyl butyrate	sweet	fusty	74, 71, 59, 87	623-42-7	[50,128]
	ethyl butyrate	**fruity**, sweet (apple)	**fusty** (cheese)	71, 88, 60	105-54-4	[31,35,50,83,87,122,129]
	propyl butyrate	(pineapple)	fusty (sharp)	71, 89	105-66-8	[38,87,122,128]
	ethyl isobutyrate	fruity, sweet	fusty (rubber)	71, 116, 88	97-62-1	[31,37,38,122]
	methyl 2-methylbutyrate	fruity (apple)	fusty	57, 88, 85	868-57-5	[31,37]
	ethyl 2-methylbutyrate	fruity (citrus)	fusty	57, 102, 74	7452-79-1	[31,37,38,122]
	ethyl valerate	-	musty	85, 88, 57	539-82-2	[50]
	isoamyl isovalerate	fruity	-	70, 85, 57, 71	659-70-1	[132]
	ethyl caprylate	(fruit)	vinegary	55, 88, 101	106–32-1	[50]
	ethyl caprate	green, bitter	vinegary	88, 101, 155	110–38-3	[50,115,124]
	ethyl cyclohexanoate	fruity (aromatic)	-	83, 55, 101	3289-28-9	[50,122]
	methyl palmitate	fruity	-	74, 87, 55	112-39-0	[44,132]
	ethyl palmitate	fruity	-	88, 101, 55	628-97-7	[44,132]
Phenols	ethylguaiacol	-	musty	137, 109	2785-89-9	[38,50,122,132]
	guaiacol	-	musty, mould (woody, smoky)	81, 109, 110	90-05-1	[38,44,50,87,122,132]
Hydrocarbons	octane	**sweet** (alcane, solvent)	fusty, winey-vinegary, rancid	57, 85, 71	111-65-9	[115,116,120,122,125,131]
	1-octene	green	musty (solvent)	55, 56, 70	111-66-0	[35,50,115,125-127]
	3-ethyl-1,5-octadiene	green	-	69, 68, 67, 109	-	[135]
	tetradecane	pungent	-	57, 71, 85	629-59-4	[135]
	styrene	(balsamic)	(gasoline, plastic)	104, 103, 78	100-42-5	[123,125]
	methylbenzene	(overripe fruit)	**(glue, solvent)**	91, 92	108-88-3	[31,35,115,124,126,127]
	ethylbenzene	fruity, bitter, sweet (ether, floral)	fusty (**strong**)	91, 106	100-41-4	[31,35,115,116,122,124-127]
	1,2,4-trimethylbenzene	-	fusty (fish oil, unpleasant)	105, 120, 77	95-63-6	[35]
Sulphur compounds	dimethylsulfone	sweet	-	79, 94	67-71-0	[119]
	dimethyl sulfide	green	(organic, wet earth)	62, 61	75-18-3	[31,119]
Others	3-ethyl-2(5H)-furanone	fruity (artichoke+tomato)	-	83, 55	2407-43-4	[135]
	2-ethyl furan	**sweet**, pungent (artichoke+tomato, ether)	burnt, rancid	81, 96, 53	3208-16-0	[115,116,124,126,127,134,135]
	2(5H)-furanone	fruity	-	55, 84	497-23-4	[132]
	δ-decalactone	fruity	-	57, 85, 99	706-14-9	[44,132]
	γ-dodecalactone	fruity	-	85	2305-05-7	[44,132]
	pentanal + 3-pentanone	fruity, green, sweet, bitter	(woody, oily)	-	-	[125]
	ratio 3Z-hexenal/2E-hexenal	fruity, pungent	-	-	-	[135]
	C5 alcohols	pungent, bitter (high quality olive oils)	-	-	-	[38,130]
	C6-LOX[2] alcohols	**positive attributes**, green	-	-	-	[32,38,135]
	C5 aldehydes	pungent, bitter	-	-	-	[38,130]
	C6-LOX[2] aldehydes	green, cut-grass (almond)	-	-	-	[38,130]
	C6-LOX[2] esters	**positive attributes**	-	-	-	[38,130]
	C6 alcohols + C6 aldehydes	fruity	-	-	-	[38,130]

[1] Only m/z higher than 50 (acquired range by MS based e-nose: 50-250), [2] LOX - lipoxygenase pathway

Basically, lower quality olive oils (defective) use to have many saturated and unsaturated aldehydes from chemical oxidations and degenerative processes, and have absence of C5 and C6 aldehydes, alcohols and esters from the lipoxygenase pathway. The most important volatiles are hexanal, C5 branched aldehydes and alcohols, C7-C11 monounsaturated aldehydes, C6-C9 dienals and some C8 ketones [38,39,121], but also some volatile phenols, such as methyl, ethyl and vinyl derivatives of phenol and guaiacol [3,42,137,138].

Table 3.5. Main volatile compounds related to the most common sensory defects detected in olive oil.

Musty	Winey[1]/ vinegary[2]	Fusty	Rancid
Alcohols: 1-propanol 1-butanol 1-pentanol 3-penten-2-ol 2-/3*-methyl-1-butanol heptanol 1-/2-/3-octanol 1-octen-3-ol* 6-methyl-5-hepten-2-ol	**Alcohols:** ethanol[1*], isopropanol[1] 1[12]-/[21*]-butanol[12] 1,3-butanediol[12*] 2-/3-methyl-1-butanol[12*] 1-pentanol[12], 3-pentanol[2] 2E/Z-hexen-1-ol[12*] 3E/Z-hexen-1-ol[2]	**Alcohols:** isopropanol, 2-butanol 1-pentanol, 1-penten-3-ol 2-/3*-methyl-1-butanol 2-methyl-3-buten-2-ol hexanol, 2-heptanol 1-octanol, 1-octen-3-ol	**Alcohols:** 2-methyl-3-buten-2-ol 1-pentanol 1-penten-3-ol hexanol 3E-hexen-1-ol heptanol, nonanol*
Aldehydes: butanal, hexanal 2-methylbutanal hexanal, 2E-hexenal 2E-heptenal* 2,4-heptadienal octanal	**Aldehydes:** 2E-pentenal[2] hexanal[12*], 2E-hexenal[12*] 2-/3-methylbutanal[2]	**Aldehydes:** butanal, pentanal 2/3-methylbutanal hexanal, octanal, nonanal 2E-pentenal	**Aldehydes:** pentanal, hexanal* heptanal, octanal nonanal*, decanal 2E-pentenal 2E-hexenal 2E/Z-heptenal* 2,4-heptadienal 2E-octenal 2E-nonenal 2,4-nonadienal 2E-decenal, 2,4-decadienal
Ketones: 1-penten-3-one 2-heptanone, 2-nonanone 6-methyl-5-hepten-2-one 3E-6-methylhepta-3,5-dien-2-one 1-octen-3-one* 3E-octa-3,5-dien-2-one	**Ketones:** acetone[1], 2-pentanone[2] 2-heptanone[12] 2-octanone[12] 1-octen-3-one[1] 3E-6-methylhepta-3,5-dien-2-one[2]	**Ketones:** 2-butanone, 3-pentanone 4-methyl-2-pentanone 6-methyl-5-hepten-2-one* 2-octanone	**Ketones:** 1-penten-3-one 4-methylpentanone 6-methyl-5-hepten-2-one
Acids: acetic acid, propanoic acid butyric acid, valeric acid caproic acid	**Acids:** acetic acid[12*] propionic acid[1] butyric acid[1] pentanoic acid[1] 3-methylbutyric acid[1] valeric acid[1], caproic acid[1] heptanoic acid[1]	**Acids:** acetic acid, propionic acid* methyl propanoic acid butyric acid, valeric acid heptanoic acid	**Acids:** acetic acid, propionic acid butyric acid* 3-methylbutyric acid valeric acid, caproic acid* heptanoic acid*
Esters: ethyl acetate	**Esters:** methyl[1]/ethyl[12*] acetate ethyl caprylate[2] ethyl caprate[2]	**Esters:** ethyl/butyl*/hexyl acetate 3-methylbutyl acetate ethyl* propionate methyl/ethyl*/propyl butyrate ethyl isobutanoate metyl ethyl2metylbutyrate	**Esters:** butyl/hexyl acetate 3-methylbutyl acetate ethyl propanoate
Others: guaiacol*, ethylguaiacol* 1-octene	**Others:** octane[12]	**Others:** octane*, ethylbenzene 1,2,4-trimethylbenzene	**Others:** octane, 2-ethyl furan

* Compounds highly correlated with the specified defect
[1] Compounds correlated with winey off-fravor, [2] Compounds related with vinegary off-fravor

The MS spectral fingerprints obtained for each olive oil were correlated to the detected defects using PLS-DA models. The same iterative process as in section 3.2.2 (MIR data) was applied, by selecting the optimal mass ranges (variables) and testing different pre-processing methods with internal and external validations. The results were submitted to a technical journal [**Paper 4**].

[**Paper 4**] E. Borràs, R. Boqué, J. Ferré, M. Mestres, L. Aceña, O. Busto, *Food Research International*, Submitted

Modeling olive oil sensory defects using an electronic nose based on headspace-mass spectrometry

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Abstract

Olive oils are graded as extra-virgin, virgin or lampante olive oil according to the International Olive Oil Council (IOC) quality standards which are based on physical, chemical and organoleptic characteristics. An accredited taste panel is mandatory to carry out the sensory analysis but even the best well-trained taste panel suffers from subjectivity and variability and presents time-consuming limitations. However, these problems may be overcome by the use of alternative instrumental techniques simulating the human sensory responses. In this study, an electronic nose based on headspace solid-phase micro-extraction coupled to mass spectrometry (HS-SPME-MS) has been applied to detect defective edible olive oils and their four main sensory off-flavors: *musty, winey, fusty* and *rancid*. 146 different olive oil samples, all of them previously evaluated by an expert and accredited sensory panel, have been used to create the predictive models. Classification models were developed using partial least squares discriminant analysis (PLS-DA) being able to discriminate between defective and non-defective oils with predictive abilities between 80-90% for the *musty* descriptor, near 80% for the *winey* descriptor and between 70-85% for both the *fusty* and the *rancid* descriptors. The use of this kind of electronic nose allowed determining the most important variables (ions) responsible for the discrimination and they can be related to specific volatile compounds. The proposed method has proved to be a fast technique able to distinguish between extra-virgin olive oils and lower quality olive oils.

Keywords

Olive oil; Electronic nose; Solid-Phase Micro-Extraction (SPME); Mass spectrometry; Multivariate analysis; Partial least squares discriminant analysis (PLS-DA); Sensory evaluation

1. Introduction

Olive oil is a typical product from the Mediterranean region with well-known and highly appreciated nutritional and sensory properties. Thanks to its health benefits and quality, its popularity has increased considerably during the last decades. In fact, nowadays it is the most demanded edible oil worldwide (IOC, 2016) in spite of being the most expensive vegetal oil as well. This high demand together with the high market price have encouraged some producers to carry out different fraudulent practices, mainly the blending with lower quality olive oils or even with other vegetable oils (Esslinger, Riedl, & Fauhl-Hassek, 2014). Some international organizations such as the International Olive Oil Council (IOC) and the European Union (EU) have adopted a series of regulations to control and detect these activities. The aim of these regulations based on physical, chemical and organoleptic analyses is the evaluation of specific olive oil quality parameters to allow classifying them as extra-virgin (highest quality), virgin and lampante (lowest quality). Among the different analyses, the importance of the sensory evaluation when dealing with olive oil has to be noted as the detection of positive and negative descriptors is the main criteria to determine olive oil quality categories. According to International Olive Oil Council (IOC, 2015) and European Union (EC 640/2008) standards, *extra virgin* olive oil must have some fruity notes and must not present any sensory defect. Olive oils are classified as *virgin* olive oil if they present one or more defects with an intensity value ≤ 3.5 (on a scale from 0 to 10) and they are categorized as *lampante* olive oil if the intensity of the defects is higher than this value. Therefore, the sensory analysis that only human beings can carry out is compulsory. However, the human taste panels have some important limitations such as subjectivity, inter-day and inter-panelists response variability, assessors' fatigue (what limit the number of analysis per day), and impossibility to automate the analysis (García-González & Aparicio, 2010). To overcome these problems, alternative and objective instrumental techniques to emulate the responses given by the human senses are being developed. Thus, we obtained promising results on detecting the presence of the most commonly studied olive oil defects (fusty, musty, winery/vinegary, rancid, metallic and frostbitten) in a previous study by using an electronic tongue based on infrared spectroscopy (Borràs et al., 2015). However, most of the olive oil off-flavors are produced by defective volatile compounds only perceptible through the olfactory system (nasally or rethronasally) (Wiesman, 2009). It stands to reason that instrumental techniques aimed to detect the sample volatile fraction should be more adequate. One of the earliest techniques designed to emulate the human nose was based on gas sensor arrays together with pattern recognition systems. These so-called electronic noses are commonly made of metal oxide semiconductors and polymeric sensors (Guadarrama et al., 2000; Aparicio et al., 2012) which are able to detect the compounds from the volatile fraction of the olive oil. But there is a problem because of the limited number of substances that the sensor arrays can detect while olive oil is a really complex matrix with many volatile compounds with different chemical properties and present at different concentration levels, all of them responsible for the aroma. Thus, sometimes the aroma perceived is due to unidentified compounds or even to several compounds interacting at the same time through positive and negative synergisms (Tena et al., 2015).

For this reason 'electronic noses based on mass spectrometry' (MS) are becoming a good alternative to the traditional sensor arrays devices as they do not suffer from the detection limitation (López-Feria et al., 2008). Though there are applications using the MS technique to determine the relationship between volatile composition and sensory properties (Procida, et al., 2005; Cimato et al., 2006; Servili et al., 2008), most of them require long times of analysis to separate the compounds. However, the direct analysis of the whole volatile fraction by MS (avoiding the chromatographic separation) allows the simultaneous fragmentation of all the volatile compounds, providing a smell-fingerprint of the sample (ion abundance) in a fast way. This fingerprint contains a lot of information that can be used to predict and detect sensory descriptors linked to olive oil aroma perception if combined with multivariate analysis techniques (Vera et al., 2010, 2011).

From these premises, the aim of this study was to apply an electronic nose based on the headspace solid-phase micro-extraction coupled to mass spectrometry (HS-SPME–MS) as a fast technique to discriminate defective olive oil samples and to detect the presence or absence of the main sensory defects.

2. Experimental part

A total of 146 samples from the 2012 and 2013 seasons were supplied by the 'Official Taste Panel of Virgin Olive Oil' located in Reus (Government of Catalonia, Spain). All samples were stored in dark glass bottles at -20 ºC under nitrogen atmosphere until their instrumental analysis that took place within three months after the sensory analysis. The official taste panel worked with confidential-codified samples and we were provided only with information about the sensory results. According to the sensory results given by the panel, 84 samples were graded as extra virgin olive oils (EVOO), 48 as virgin olive oils (VOO) and 14 as lampante virgin olive oils (LOO).

2.1. Sensory evaluation

The 'Official Taste Panel of Virgin Olive Oil' is accredited according to the ISO17025 regulation and performs the sensory analyses following the International Olive Oil Council official method (IOC, 2015) within the framework of the European Commission Regulation (EC 640/2008).

Each sample (15 ml) was smelled and then tasted from a normalized colored cup (to mask color interferences) at a controlled temperature (28 ± 2 ºC). Three main positive attributes (fruity, bitter and pungency) together with five defective descriptors (musty, winey, fusty, rancid and metallic) were scored. Because of none of the tested samples showed metallic notes this defect was not studied. All the descriptors were evaluated on a continuous, unlabeled 10 cm intensity scale and this evaluation was then transformed into numeric scores between 0 and 10. Each sample was tasted by eight to ten panelists whose evaluations for each predominant attribute were finally provided as median values.

2.2. Electronic nose based on MS

Samples analyses were performed with a headspace solid-phase micro-extraction coupled to mass spectrometry (HS-SPME-MS) system composed of a CTC CombiPAL autosampler (CTC Analytics, Switzerland) and a HP 6890N gas chromatograph equipped with an HP 5973 mass selective detector (MSD) (Hewlett-Packard, USA). For sample preparation (i.e. to extract the volatile compounds) a 2 cm length StableFlex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm SPME fiber (Supelco, USA) was used. The extraction was performed into the headspace area of 20 ml vials containing 5 g of olive oil tightly capped with a PTFE/silicone septum under nitrogen atmosphere. All samples were prepared and analyzed in duplicate. Previously to the extraction, sample headspace was stabilized during 30 min at 40 ºC to reach the equilibrium. Then, the fiber was exposed to the vial headspace for 1 h at 40 ºC under continuous mechanical stirring. Finally volatile compounds were automatically desorbed into the GC injection port for 5 min in the splitless mode at 270 ºC. Since no chromatographic separation was required, the HP-5MS column (30 m × 0.25 mm × 0.25 μm) employed only worked as a transfer line between the injector and the detector. Thus, the oven temperature was sharply increased from 40 to 270 ºC with a helium flow rate of 1.8 ml min-1 to guarantee the complete sample transference to the MS system in less than 5 min. The mass spectrometer scanned a range from 50 to 250 m/z operating in the electron impact ionization (70 eV) mode while ion source and mass quadrupole temperatures were 250 ºC and 150 ºC, respectively.

The analytical signal obtained from the fragmentation of the volatiles of each sample was expressed as the sum of abundances of all ions (as m/z) during all the acquisition time. These data were organized in a data matrix with samples/oils (rows) and m/z values (columns) using the Chemstation E.2.0 (Agilent, USA) and the Pirouette v.4.0 (Infometrix, USA) software.

2.3. Statistical and multivariate analysis

All multivariate models and calculations were performed using in-house routines programmed in Matlab v.7.8 (Mathworks, USA) and the PLS Toolbox software v.6.2 (Eigenvector, USA).

Principal component analysis (PCA) was firstly applied as an exploratory method to check the repeatability of the replicates, to find patterns and to recognize outliers in the MS spectra of the 146 olive oils analyzed. PCA is a dimension reduction technique that compresses the information into a few variables, called principal components (PCs), which are linear combinations of the original variables. While the first PC covers the maximum information, the consecutive ones are orthogonal and they explain the remaining information in a hierarchical way (PC1 explains more information than PC2 and so on). PCA provides a graphical tool useful to detect sample groups, sample trends or outlier samples (Li Vigni, Durante, & Cocchi, 2013). Partial least squares discriminant analysis (PLS-DA) was used as a supervised discrimination technique to discriminate between samples belonging to different classes. Thus, this analysis was applied to discriminate between samples without any defect (non-defective) and with defects or high scored defects (non-edible). Also to discriminate between samples presenting

specific musty, fusty, winey and rancid notes. PLS-DA is based on the partial least squares regression method and it regresses the spectra contained in the **X** matrix against a **y** vector containing a dummy variable. In this case zeros for defect absence and ones for defect presence were considered (Bevilacqua et al., 2013).

All mass spectra were initially row-profiled to avoid differences between samples analyzed throughout the study due to MS instrumental variability. Prior to model building, different spectral pre-processing methods such as autoscaling, mean centering, logarithm transformation and Pareto scaling were tested. Additionally, different spectral ranges to find optimal classification conditions for each descriptor were also tested. Then, an iterative procedure described in **Figure 1** was followed to build PLS-DA models and achieve optimal results.

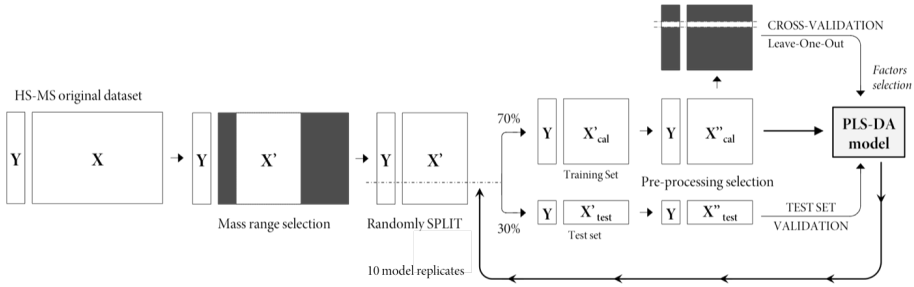


Figure 1. Iterative procedure to optimize the class-modeling method.

Replicate PLS-DA models were performed by randomly dividing the whole data matrix into calibration and test sets (70/30% of the samples, respectively). Each iterative procedure was applied to different combinations of spectral pre-processing techniques and mass ranges. As all the pre-processing techniques are column-wise, they were applied after splitting the data matrix. The number of factors (latent variables) was selected by leave-one-out cross-validation using the calibration set. An external test-validation was applied using the random test sets to obtain the final PLS-DA classification results. This iterative procedure was repeated ten times in order to avoid results depending on a particular split. Results were obtained as the mean values and the standard deviations (S.D.) of classification parameters such as sensitivity, specificity and inaccuracy (misclassification) expressed as percentages. In all cases, the variability of the 10 PLS-DA models was controlled by obtaining a variation coefficient (%VC) lower than 20% (%VC = S.D./mean x 100) (EC 640/2008). Sensitivity is defined as the number of samples without defects modeled as non-defective while specificity is the number of samples with defects modeled as defective. Inaccuracy is defined as the probability of samples from both classes being incorrectly classified. The criteria to choose the final predictive models were based on the maximum values of sensitivity and specificity, together with the minimum values of inaccuracy.

As the specific spectral regions selected contained discriminant information related to the presence of defects, it was possible to identify those mass ranges more correlated to each off-flavor, thus providing

helpful information to identify some of the compounds associated to the presence of musty, winey, fusty and rancid notes (**Figure 2**). Moreover, a PLS-DA parameter called variable importance in projection (VIP) within the characteristic mass ranges was studied. VIP method evaluates the variables that carry the information related to the predicted class by using the greater-than-one criterion, where variables (m/z ratios) with VIP > 1 are considered to have a high influence on the selected model

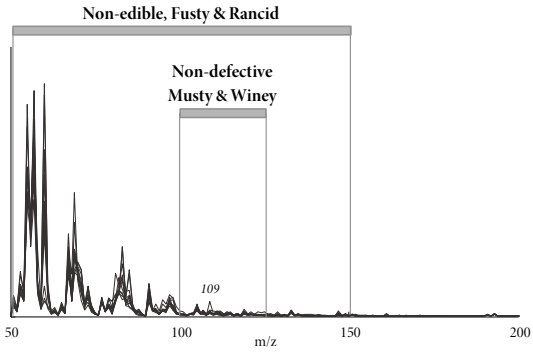


Figure 2. Selected mass ranges for each descriptor studied.

3. Results and discussion

Preliminary PCA models did not show clear patterns for the analyzed samples (data not shown). To check the presence of outliers the Hotelling T^2 and Q residuals were considered at a confidence level of 95%. According to these criteria, six samples were removed before building the classification models. Several PLS-DA models were built for each studied class by applying different pre-processing treatments and selecting different spectral regions. The optimal models were selected using a test set of samples (test-validation/prediction) according to the procedure described in section 2.4 and the results are shown in **Table 1**.

As can be seen, the resulting predictive parameters (inaccuracy, sensitivity and specificity) obtained by cross-validation (CV) and test-validation (TV) were similar, assuring the reliability of the applied discriminant models. With regard to the specificity/selectivity values, specificity results were lower and with higher standard deviations than the sensitivity ones. This is because most of the models were not balanced against the number of samples of each subclass: for example, when dealing with the defective olive oils class, more than the 60% of the samples used to build the models were not defective so this fact reduces the ability to recognize defective samples (class with less samples). These differences are more noteworthy for non-edible and rancid classifications, also showing unusual differences between CV and TV results, due to the lack of sufficient samples with these two negative properties (less than 10%). Related to the other descriptors studied, the musty attribute achieved the lowest number of misclassifications (13.7% inaccuracy), followed by the fusty descriptor (18% inaccuracy), and the non-defective and the winey ones, both around 20%. Sensitivities and specificities were higher for musty (between 80 and 90%) and lower than 80% for all the other classifications.

Table 1. Optimal conditions selected and classification results obtained by the test-validation PLS-DA models of the sensory defects studied.

		Musty	Winey	Fusty	Rancid	Defective	Non-edible
Mass range selected (m/z)		100 - 125	100 - 125	50 – 150	50 - 150	100 - 125	50 - 150
Pre-processing selected[1]		log10 + autoscale	log10 + mean center	log10 + autoscale	log10 + mean center	autoscale	log10 + mean center
Number LV		2	2	2	2	2	2
% inaccuracy[2]	CV	15,2 (±1,8)	21,2 (±2,2)	18,7 (±2,6)	34,5 (±6,7)	20,9 (±2,8)	33,0 (±4,2)
	TV	13,7 (±2,3)	20,2 (±4,2)	18,0 (±3,7)	24,4 (±4,8)	19,8 (±4,6)	30,2 (±5,7)
% sensitivity[2]	CV	85,9 (±2,9)	83,9 (±2,7)	88,7 (±2,5)	63,5 (±7,9)	84,9 (±3,7)	66,8 (±3,4)
	TV	87,9 (±6,8)	80,7 (±5,7)	87,4 (±6,6)	75,9 (±9,1)	82,5 (±6,6)	70,5 (±6,8)
% specificity[2]	CV	82,3 (±3,2)	69,1 (±2,3)	66,1 (±4,1)	84,4 (±7,8)	71,3 (±3,4)	70,0 (±10,5)
	TV	83,1 (±9,5)	77,9 (±6,3)	71,4 (±7,5)	72,5 (±14,9)	77,0 (±7.1)	62,5 (±13,4)

[1] All pre-processings applied after row profile normalization

[2] Percentage as mean (± standard deviation) of 10 random validations.

LV: latent variables, CV: cross-validation; TV: Test-validation

With regard to the different ions selected (m/z ranges) for the different classes, musty, winey and defective olive oils were better discriminated using the range between 100 and 125 m/z. Fusty, rancid and non-edible classes required a wider range, between 50-150 m/z, to explain the differences among those classes. However, a mass of 109 was the common one with the highest VIP values for most of the studied classes. It is difficult to find a single compound associated to this mass because, according to the literature, there are different volatile compounds able to provide this m/z ion. Among them, guaiacol, phenol and its methyl, ethyl and vinyl derivatives or ketones (like 3-(E)-6-methylhepta-3,5-dien-2-one) are the most characteristic ones that have been reported as key-odorants that may contribute to off-flavored defective olive oils (Vichi et al., 2009; Monteleone & Langstaff, 2014).

3.1. Musty descriptor

Musty is a characteristic flavor that appears when olives are stored several days under humid conditions before milling, thus developing fungi and yeasts responsible for the production of this unpleasant volatile compounds (De Santis & Frangipane, 2015). The musty presence was basically defined by a mass range between 100 and 125 m/z and the use of logarithmic and autoscaled pre-processed data. To visualize the classification results obtained when applying the best predictive developed model, the actual values for the training and the test sets were plotted versus their PLS-DA predicted values (**Figure 3a**). A threshold limit (0.43) was then defined and samples above and below this limit were classified as musty or non-musty samples, respectively. Misclassifications are shown as empty symbols in **Figure 3a**, corresponding to wrongly predicted samples by the model.

Within the selected region, the mass with the highest influence in the model (VIP > 1) was 109, together with 102, 104 and 110 masses (**Figure 3b**). As it was said before, and although it is difficult to correlate specific compounds to these masses, some of the most cited volatile compounds in the literature that are

related to the musty defect in olive oils do have some of these masses. This is the case of those volatiles mainly generated by enzymatic activities that oxidize fatty acids to alcohols, aldehydes and ketones (hexanal, 1-octen-3-ol, 1-octen-3-one, 2-heptenal, 2-heptanone, 2-nonanone, 3-(E)-6-methylhepta-3,5-dien-2-one and 6-methyl-5-hepten-2-one) (Angerosa, 2002; Morales et al., 2005; Bendini et al., 2012), and also due to the presence of phenolic volatiles such as ethylguaiacol, guaiacol and methyl, ethyl and vinyl derivatives (Monteleone & Langstaff, 2014).

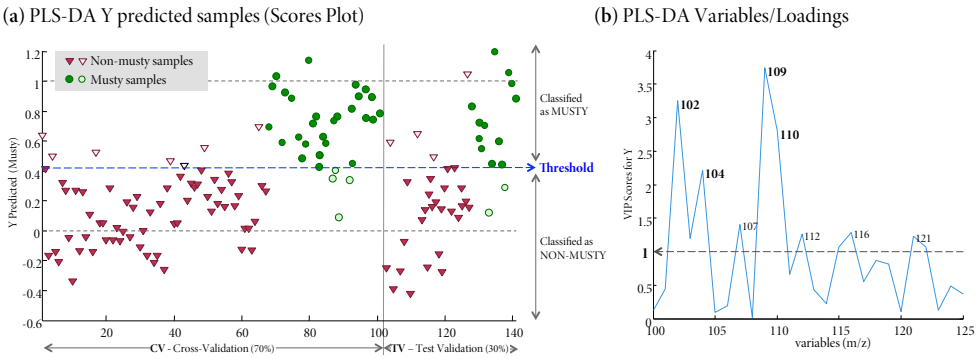


Figure 3. PLS-DA model results for musty defective samples using the optimal pre-processing and region (variables). (a) Y predicted values for cross-validation (CV) and test-validation (TV) sets of samples where filled symbols correspond to correctly classified samples and unfilled to incorrectly classified samples. (b) VIP score values obtained by all the variables (region).

3.2. Winey descriptor

When an olive oil presents a certain reminiscence of wine or vinegar this is mainly due to the anaerobic fermentation produced in olives or the olive paste when left on improperly cleaned processing mats (De Santis & Frangipane, 2015). Olive oils with winey presence were predicted using the same conditions as mustiness: with a mass range between 100 and 125 m/z and with logarithmically transformed and mean-centered data. Compared to the musty defect, higher inaccuracies were obtained (20%) with sensitivity/specificity values near 80% (**Table 1**). The representative volatile compounds generally correlated to this sensory attribute in the available literature are ethanol, ethyl acetate, acetic acid, 1-pentanol and 2-butanol (Morales et al., 2005; Procida et al., 2005; Dierkes et al., 2012) whose main m/z values range from 50 to 70. However, the masses with higher VIP scores for our winey/vinegary PLS-DA model (m/z ratios 109, 102 and 104) are mostly associated to the ketone 3-(E)-6-methylhepta-3,5-dien-2-one, considered as a characteristic compound of vinegary defective oils (Purcaro et al., 2014).

3.3. Fusty descriptor

Fustiness is the characteristic flavor of olive oil obtained from olives that have been piled or stored in contact with sediments settled in underground tanks and vats. These unadequate production conditions allow advanced stages of anaerobic fermentations (Monteleone & Langstaff, 2014; De Santis &

Frangipane, 2015). Fusty oils were defined by a wider mass range than the two previous descriptors, from 50 to 150 m/z, and using logarithm and autoscaled data. In this case, similar classifications to the winey case were obtained, with slightly lower inaccuracies (18%) and sensitivity and specificity around 85% and 70%, respectively (**Table 1**). This unpleasant off-flavor is linked to some esters and branched aldehydes, alcohols and short chain acids (Angerosa, 2002; García-González & Aparicio, 2002; Lerma-García et al., 2010). Among these different chemical families, the results showed that the acids seems to have the greatest influence in this sensory defect because the highest VIP scores were obtained for 60 and 104 m/z ratios, which are mainly related to short acids such as butanoic, pentanoic and hexanoic (Morales, Aparicio-Ruíz, & Aparicio, 2013). Moreover, the results also showed an interesting contribution of some esters because significant VIP scores for 54 to 109 m/z values were also found, which could be linked to ethyl and butyl acetate and ethyl butyrate presence (Angerosa, 2002; Morales et al., 2005).

3.4. Rancid descriptor

The rancid flavor appears when olive oils have undergone intense processes of oxidation caused by a prolonged contact with air (Monteleone & Langstaff, 2014; De Santis & Frangipane, 2015). The mass range from 50 to 150 m/z, together with the use of logarithmic transform and autoscaled data, were indicative of its presence. Although this specific descriptor presented a higher inaccuracy than the other descriptors as well as lower sensitivity and specificity values (**Table 1**), the VIP scores can be easily related to specific compounds. Thus, the m/z values of 50 and 72 and the range 60-88 with VIP values > 1 can be associated to some suggested markers of rancidity, such as (E)-2-heptenal and C5 - C11 aldehydes (mainly nonanal). Although the promising results obtained to the rancid models, more samples with the presence of this defect should be necessary to build more reliable and accurate discriminations.

4. Conclusions

The electronic nose based on headspace mass spectrometry (HS-MS) combined with chemometric tools have demonstrated to be useful to discriminate olive oils according to specific defective sensory descriptors. Best classifications were achieved for the musty descriptor (80-90% correct classifications), followed by the winey (80%) and the fusty and rancid (70-85%) ones. Moreover, this technique enables getting information about the chemical compounds responsible for each unpleasant attribute and this constitutes an advantage over other types of electronic noses. The results obtained on the discrimination of defective samples showed that this technique is a very fast and powerful tool that would help or complement human taste panels offering more objective and reliable results.

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3.4. Electronic Panel

Discrimination of olive oil defects using FT-IR spectroscopy and MS spectrometry (described in Sections 3.3 and 3.4, respectively) are just two of the many examples found in the literature [63,85,86,88,89,132] that try to correlate individual instrumental responses with sensory properties. However, these sensory properties are the consequence of not a single perception but a combination of gustatory, tactile, olfactive, visual and, sometimes, even auditory perceptions. Accordingly, to mimic a human sensory panel it would be reasonable to combine instrumental responses coming from different sources in order to consider the wide variability of the compounds involved (e.g. volatiles and non-volatiles) and even the possible interactions between them (positive and negative synergisms). Thus, the use of different instrumental techniques may provide complementary information that allows improving the models obtained by the individual techniques.

As explained in Chapter 1, there are several available data fusion approaches to combine those instrumental responses and, in this Thesis, some of them have been applied to the spectra obtained from the analyses of olive oil samples.

3.4.1. Instrumental sensory analysis

The instrumental techniques used to emulate the taste panel were the electronic tongue described in Section 3.2.1 and the electronic nose described in Section 3.3.1, together with an electronic eye based on UV-visible spectroscopy. It has to be noted that, although the visible spectral range is limited to 400-700 nm approximately, the experimental measurements were performed in the range 300-1000 nm (including information from the ultraviolet and near-infrared regions). This wider range was considered because the colored substances may also absorb at other wavelengths, thus providing more instrumental information. UV-Visible spectrophotometers are composed of light sources, a monochromator, a sample container, a detector and a signal processor (**Figure 3.11**).

To allow the absorption at the whole range considered, two separate light sources are required: a deuterium lamp that emits radiation in the UV range (160–375 nm), and a tungsten filament lamp to provide the visible light (350–2500 nm). These sources generate

polychromatic radiation that comes into the monochromator through the entrance slit. This beam, previously collimated through a lens, strikes onto a dispersing device (grating) and it is split into the different wavelengths. This multi-component beam is directed to an exit slit that only allows a particular (selected) wavelength to leave the monochromator. Then, the radiation goes through the sampling compartment where it is partially absorbed by the sample placed into quartz cuvettes. Finally, the radiation not absorbed (transmitted) arrives to the detector, which consists on a photodiode array that measures the signal and generates the UV-Vis spectrum [139,140].

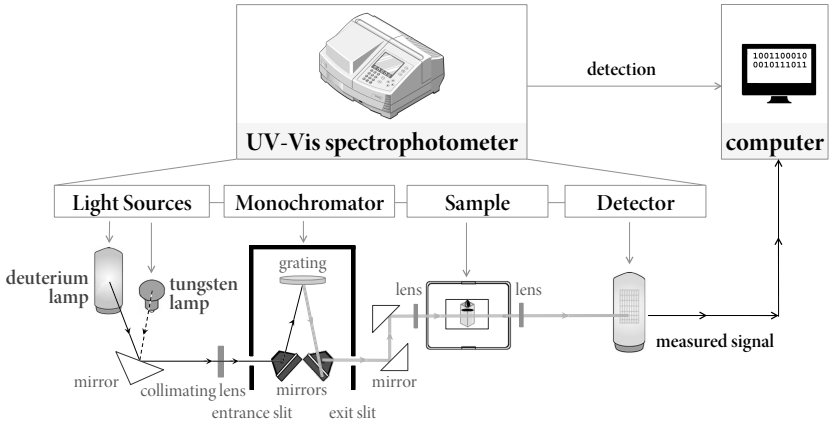


Figure 3.11. Structure of the UV-Visible system used to detect olive oil colored compounds. Description of the main components UV-Vis spectrophotometer.

3.4.2. Olive oil and e-panel

Once the different instruments were chosen and the olive oil samples were analyzed, it was necessary to combine the collected spectra in order to get a global response as similar as possible to that provided by the official olive oil panel. The procedure used in this study is shown in **Figure 3.12**.

All the spectra collected by the different instrumental techniques were first treated individually to then apply the selected data fusion strategy. The information described in sections 3.3.2 and 3.4.2 was used to select the optimal data pre-processings and spectral regions for the individual spectra obtained from FT-MIR and HS-MS. Similar considerations were taken into account for the UV-Vis data.

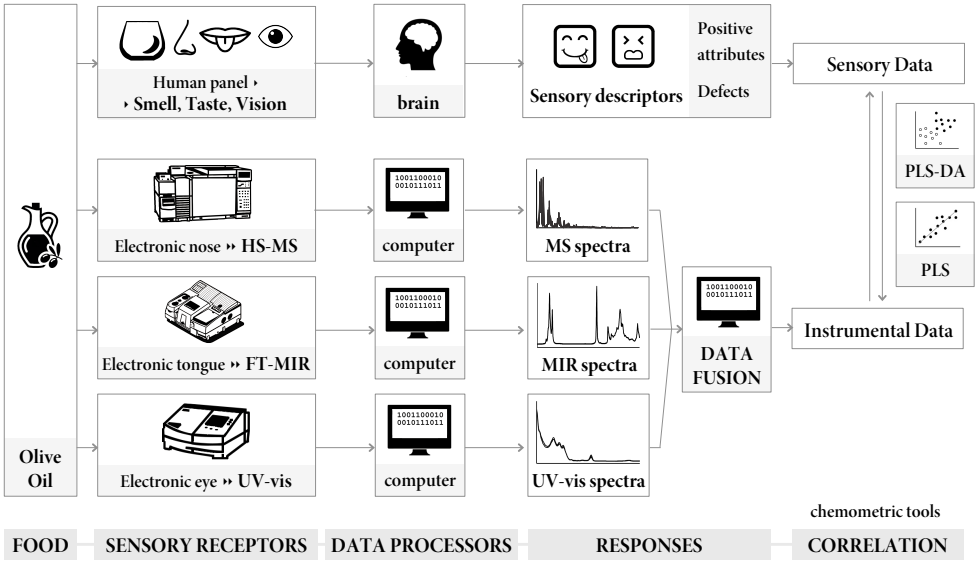


Figure 3.12. Electronic panel system for modelling olive oil sensory descriptors. Correlation between human sensory analysis and data fusion techniques.

The spectra obtained with each one of the instruments contain much chemical information about the samples, including information about both positive and negative sensory attributes. Thus, although defects are the attributes that better classify the olive oils into the different categories, the taste panel does not only evaluate these so, it seemed more appropriate to carry out this study by considering all the descriptors. Therefore, for **HS-MS spectra** volatile compounds present in olive oil (listed in **Table 3.4**) were examined. Moreover, the compounds associated to the studied positive sensory descriptors were also considered. As it has be seen summarized in **Table 3.6**, some of the most representative compounds for olive fruity notes are C6 alcohols and aldehydes, 3Z-hexenal/2E-hexenal ratio, ketones (e.g. 2-butanone, 1-penten-3-one, 6-methyl-5-hepten-2-one and 2-nonanone), different aromatic esters (e.g. hexyl and 3Z-hexenyl acetate) [3,27,141].

Bitterness, pungency and astringency are not directly olfactory-related attributes, but some compounds may be correlated or may influence their perception. Although not contributing to taste perceptions, 1-penten-3-one is positively correlated and 3Z-hexen-1-ol and hexanal are negatively correlated to bitter and pungent tastes [30]. Finally, sweet and cut-grass (or grassy) notes were mainly associated to C5 and C6 compounds, basically hexanal, esters and ketones for sweetness and alcohols and aldehydes for grassy notes [27,38].

Table 3.6. Main volatile compounds related to the six olive oil sensory positive attributes studied.

Fruity		Bitter	Pungent	Sweet	Cut-grass	Astringent
Alcohols: 1-propanol 2-butanol* 1-pentanol* 1-hexanol* 2Z-penten-1-ol 1-penten-3-ol 2E-hexen-1-ol* 2E-nonen-1-ol	**Esters:** isobutyl acetate hexyl acetate* 3Z-hexenyl acetate* 2-methylbutyl acetate 3-methylbutyl acetate methyl acetate butyl acetate ethyl propionte * ethyl butyrate * ethyl isobutyrate methyl 2-metylbutyrate ethyl 2-methylbutyrate isoamyl isovalerate ethyl cyclohexanoate methyl palmitate ethyl palmitate	**Alcohols:** 3E-hexen-1-ol*	**Alcohols:** ethanol, 1-propanol 1-pentanol*, 1-penten-3-ol 3Z-hexen-1-ol	**Alcohols:** ethanol, 1-butanol 2-methyl-1-butanol 3-methyl-1-butanol* 1-pentanol 2Z-penten-1-ol 2E-hexen-1-ol heptanol, 1-octanol	**Alcohols:** 2-methyl-3-buten-2-ol 2Z-penten-1-ol 3-penten-2-ol 1-hexanol 2Z-hexen-1-ol* 3E-/3Z-hexen-1-ol*	**Alcohols:** 1-hexanol 2Z-hexen-1-ol
Aldehydes: 3-methylbutanal* 2E-pentenal 3Z-hexenal 2E-/2Z-hexenal* 2E-octenal 2E-nonenal 2E-undecenal	**Others:** ethylbenzene 3-ethyl-2(5H)-furanone 2(5H)-furanone δ-decalactone γ-dodecalactone pentanal + 3-pentanone 3Z-hexenal/2E-hexenal C6alcohols + C6aldehydes	**Aldehydes:** pentanal* 2E-pentenal 2E-/2Z-hexenal	**Aldehydes:** acetaldehyde* propanal, butanal 2-methylbutanal pentanal, nonanal* 2E-pentenal, 2E-heptenal* benzenacetaldeyde	**Aldehydes:** acetaldehyde propanal, butanal 2-methylbutanal 3-methylbutanal* hexanal*, 2Z-hexenal decanal*	**Aldehydes:** 2E-pentenal hexanal*, 3Z-hexenal* 2E,4E-hexadienal 2-ethyl-E2-butenal nonanal, 2E-nonenal	**Aldehydes:** 2E-hexenal
Ketones: 2-butanone* 2-pentanone 3-pentanone 1-penten-3-one* 4-methyl-2-pentanone* 2-heptanone* 6-methyl-5-hepten-2-one* 2-octanone, 2-nonanone*		**Ketones:** 1-penten-3-one 6-methyl-5-hepten-2one	**Ketones:** 1-penten-3-one* 6-methyl-5-hepten-2-one* 1-octen-3-one	**Ketones:** acetone, 2-butanone 2-pentanone, 3-pentanone* 1-penten-3-one* 4-methyl-2-pentanone* 2-heptanone*, 2-nonanone	**Ketones:** 6-methyl-5hepten-2one 3-octanone	**Esters:** ethyl acetate
Acids: methyl propanoic acid		**Esters:** ethyl acetate 3-methylbutyl acetate ethyl caprate	**Acids:** acetic acid, propionic acid* butyric acid, valeric acid* caproic acid*	**Esters:** methyl acetate ethyl acetate*, butyl acetate hexyl acetate* ethyl propionate* methyl butyrate ethyl butyrate ethyl isobutyrate	**Others:** C6-LOX aldehydes	
		Others: ethylbenzene pentanal + 3-pentanone C5alcohols C5aldehydes	**Esters:** ethyl acetate, butyl acetate* 3-methyl-2-butenyl acetate	**Others:** octane*, ethylbenzene dimethylsulfone 2-ethyl furan* pentanal + 3-pentanone		
			Others: tetradecane, 2-ethyl furan C5alcohols, C5aldehydes			

* compounds highly correlated to the specified attribute

Regarding **FT-MIR spectra**, these showed relevant peaks located at 2925, 2854, 1746, 1463, 1377, 1235, 1160, 1114, 1097 and 723 cm^{-1} [63], as detailed in **Figure 3.6** and **Table 3.3** in Section 3.3. However, when comparing olive oil samples with different sensory attributes, there are no obvious differences from visual inspection. Since the use of an electronic tongue based on MIR spectroscopy is quite recent and few applications have been published on olive oil descriptors we set the challenge of improving the results obtained in the previous study of this Thesis (section 3.3), where negative descriptors were modeled. However, considering that defects are basically correlated to volatile compounds, in this new investigation it seemed more adequate to also model some positive attributes, like bitterness, pungency and astringency, which are mainly connected to taste (mouthfeel) perceptions generated by non-volatile compounds.

These compounds are mainly polyphenols, like secoiridoid derivates of hydroxytyrosol, oleuropein or deacetoxy ligstroside aglycones [27, 55, 142], which present a broad band at 3700 - 3000 cm^{-1} due to OH stretching vibrations (ν_{OH}) (e.g. oleuropein, cellulose, organic acids) and two bands at 3000 - 2800 cm^{-1} from symmetric and asymmetric CH stretching vibrations (ν_{CH}, alkyl). Also, regions at 1800 - 1500 and 1500 - 1200 cm^{-1} may correspond to phenolic alcohols. The former is due to -C=O and -C=C- stretching vibrations from esters, acid, carboxylate and aromatic ring, and the latter (more complex) with especially -C-H, -C-O stretching and OH deformation vibrations should be related to phenols [143].

It has to be noted that polyphenols in olive oils are more frequently measured by spectrophotometry, which allows the determination of the total phenol content as a measure of oil quality related to oxidation [27]. Therefore, **UV-vis spectral data** should provide valuable information to study sensory properties like bitterness or pungency, and maybe also other descriptors apparently not related to color composition. In fact, MIR and UV-vis spectra were pre-processed in a similar manner, by selecting also different spectral ranges to find the optimal models for each purpose. The compounds with more influence in the UV-Vis spectra are the ones responsible of olive oil color, ranging from yellow-green to greenish-gold. The green components are associated to chlorophyll pigments, whereas the yellow color is due to the presence of carotenoids. Chlorophylls (and pheophytins) show important absorption peaks in two regions of the visible spectra: one around 400 - 500 nm (blue region)

and the other at 600 - 700 nm (red region). Typical absorbances of the carotenoids ranged between 400 and 500 nm (blue region) (**Figure 3.13**) [3,96].

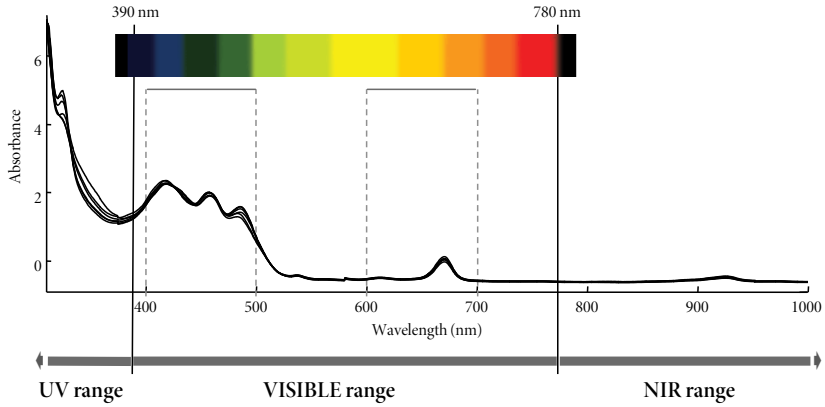


Figure 3.13. Full UV-Visible spectra of olive oil samples and characteristic spectral ranges of the visible region.

All these considerations greatly helped to select the spectral regions of the different techniques, but the fusion of all this information was still necessary. Related to the experimental part, two studies were performed. The aim of the first study was to discriminate main defects (musty, winey, fusty and rancid) of olive oils analyzed during two seasons (2012-2013) and using PLS-DA models. The second study was focused on building predictive models using PLS regression for all the sensory descriptors defined by the taste panel, including positive attributes (fruity, cut-grass or grassy, bitter, pungent, sweet and astringent) and defects (musty, winey, fusty and rancid). In this case, more variability was added, with samples collected during four seasons (2010-2014).

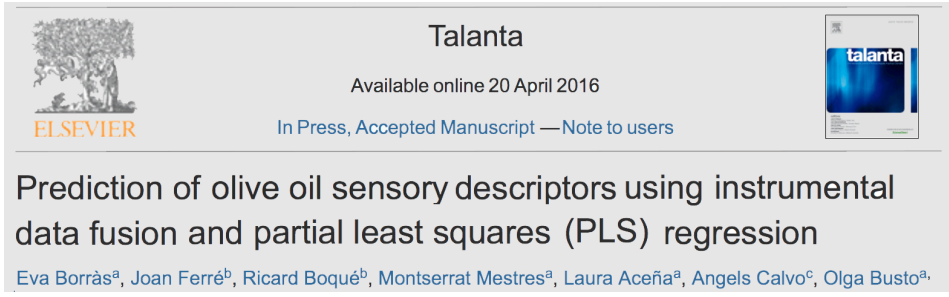
Both discriminant (PLS-DA) and predictive (PLS) models were performed for the individual techniques as well as for combinations of them, using different data fusion strategies (i.e. joining two-blocks of data (MS and MIR) and three-blocks of data (MS, MIR and UV-Vis)). These block combinations were tested separately, considering that the visual perception of olive oils is omitted in the human taste panel. Although this color information is not used in the human taste panel assessment, the instrumental results may provide additional information about the samples. For this reason, UV-visible spectra were also collected. Two different data fusion strategies were studied: low level and mid-level data fusion. In low-level

data fusion, the data matrices were simply concatenated prior to building the multivariate model. In mid-level data fusion, extraction of relevant features was performed for each data matrix before PLS-DA or PLS models. In both cases, the number of latent variables was chosen with leave-one-out cross-validation using a random subset of the data (65% of the samples in the calibration set). The criterion of selection was the lowest inaccuracy and the highest sensitivity/specificity values for PLS-DA models and the lowest root mean squared errors (RMSECV) for PLS regression models. To select the final model conditions, a random subset of samples was chosen and used as an external test set (35% of the samples). To evaluate the high number of results obtained, Pareto diagrams were developed to plot two validation parameters: sensitivity versus specificity for PLS-DA models, and RMSE versus determination coefficient (R^2) for PLS regression models.

The results of the first data fusion study to discriminate olive oil sensory defects by PLS-DA, were published in a relevant technical journal [**Paper 5**].



The results of the second data fusion study, focused on predicting the estimated intensities for both, positive and negative descriptors, by PLS were accepted in Talanta [**Paper 6**].



[**Paper 5**] E. Borràs, J. Ferré, R. Boqué, M. Mestres, L. Aceña, A. Calvo, O. Busto, *Food Chemistry* 203 (2016) 314-322

[**Paper 6**] E. Borràs, J. Ferré, R. Boqué, M. Mestres, L. Aceña, A. Calvo, O. Busto, *Talanta* (DOI 10.1016/j.talanta.2016.04.040)

Olive oil sensory defects classification with data fusion of instrumental techniques and multivariate analysis (PLS-DA)

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Abstract

Three instrumental techniques, headspace-mass spectrometry (HS-MS), mid-infrared spectroscopy (MIR) and UV–visible spectrophotometry (UV–vis), have been combined to classify virgin olive oil samples based on the presence or absence of sensory defects. The reference sensory values were provided by an official taste panel. Different data fusion strategies were studied to improve the discrimination capability compared to using each instrumental technique individually. A general model was applied to discriminate high-quality non-defective olive oils (extra-virgin) and the lowest-quality olive oils considered non-edible (lampante). A specific identification of key off-fravors, such as musty, winey, fusty and rancid, was also studied. The data fusion of the three techniques improved the classification results in most of the cases. Low-level data fusion was the best strategy to discriminate musty, winey and fusty defects, using HS-MS, MIR and UV–vis, and the rancid defect using only HS-MS and MIR. The mid-level data fusion approach using partial least squares-discriminant analysis (PLS-DA) scores was found to be the best strategy for defective vs non-defective and edible vs non-edible oil discrimination. However, the data fusion did not sufficiently improve the results obtained by a single technique (HS-MS) to classify non-defective classes. These results indicate that instrumental data fusion can be useful for the identification of sensory defects in virgin olive oils.

Keywords

Virgin olive oil; Headspace-mass spectrometry (HS-MS); Mid infrared spectroscopy (MIR); UV–vis spectrophotometry; Data fusion; Multivariate analysis; Partial least squares-discriminant analysis (PLS-DA); Classification; Sensory analysis

1. Introduction

Virgin olive oil (VOO) is a valuable and highly appreciated vegetable oil. It is extracted from fresh and healthy olive fruits (Olea europeae L.) by mechanical and other physical methods without additional refining (Cecchi & Alfei, 2013). Due to its unique nutritional and organoleptic properties there is an increasing interest of consumers for olive oil quality. Olive oil sensory and chemical quality characteristics depend on olive variety, environmental factors, agronomic techniques and cultivation, production and storage conditions (Bendini, Valli, Barbieri, & Gallina Toschi, 2012; García-González & Aparicio, 2010b; Karabagias et al., 2013). Furthermore, oils of higher quality can be intentionally blended with cheaper vegetable oils or olive oil grades, which constitute, in addition to economic fraud, a health threat to the consumer. To guarantee VOO quality different international institutions (ECC/2568/1991; ECC/796/2002; ECC/1089/2003 and ECC/640/2008; International Olive Council, 2013 and the Codex Alimentarius (FAO-OMS, 1981)) have accorded maximum values of specific parameters that cannot be exceeded by each olive oil category.

Three physico-chemical parameters (free acidity, peroxide value and specific ultraviolet (UV) absorptions) and a sensory evaluation (based on taste and aroma) determine the three quality categories of VOOs: (1) extra-virgin olive oil (EVOO), which has the maximum quality and no sensory defects; (2) virgin olive oil (VOO), which may have some negative sensory attributes if they are of low intensity (63.5); and (3) and lampante olive oil (LOO), with intense defects (>3.5). LOO cannot be directly consumed and must undergo a prior refining process, while EVOOs and VOOs can be bottled and directly consumed. From an economic point of view, it is important to know how an olive oil is qualified through the presence of off-fravors (Monteleone & Langstaff, 2014), discriminating the lower-quality category (LOO), called non-edible from the highest quality olive oils (EVOO), called non-defective (Escuderos, García, Jiménez, & Horrillo, 2013). The only homologated method to assess the sensory attributes of olive oils is the evaluation by an official taste panel, following well-standardized protocols with highly and permanently trained panelists, which evaluate the sight, aroma, taste, texture and aftertaste of the oil (Monteleone, 2014). The sensory descriptors of olive oil are classified as 'positive attributes', such as fruity, bitter and pungent, and 'negative' attributes. The latter describes the defects of olive oil, and include fusty (along with muddy sediment (Purcaro, Cordero, Liberto, Bicchi, & Conte, 2014), musty-humidity, winey-vinegary, rancid and metallic. An intense process of auto oxidation caused by unsuitable storage conditions is the main cause of rancidity in olive oils, whereas the presence of exogenous enzymes, mostly due to microbial activity is mainly responsible for musty (by fungi and yeast), fusty and winey-vinegary defects (Morales, Luna, & Aparicio, 2005).

Sensory descriptors mainly depend on the content of volatile and non-volatile minor components. Non-volatile compounds, such as phenolic compounds, stimulate the taste receptors, evoking the perception of bitterness, pungency, astringency and metallic attributes. Volatile compounds, mainly arising from the oxidation of the fatty acids, stimulate the olfactory receptors, responsible for the whole

VOO aroma (Angerosa, 2002). This combined effect of taste, odor and chemical interactions, perceived as 'fravor', is well-established by the sensory human panel. However, this methodology has some inherent problems, such as experts' subjectivity, variability of responses over time, the lack of reference standards and low number of analyses per day (Sinelli et al., 2010).

The development of an objective, rapid, automated, low-cost and precise methodology to assess sensory properties using instrumental techniques might be an alternative solution to taste panels. Several approaches have been proposed as alternatives to human sensory panels to evaluate the quality of olive oil. The first attempts focused on the characterization of the chemical species responsible for the fravor, by correlating negative and positive attributes to specific responses of chemical compounds, such as volatile and phenolic compounds. Volatile compounds, which are responsible for the aroma, were initially studied with traditional electronic-noses based on gas sensor arrays. Metal-oxide sensors (MOS) were applied to find relationships between organoleptic descriptors and sensor responses (Aparicio, Rocha, Delgadillo, & Morales, 2000; Escuderos et al., 2013). However, due to the lack of sensitivity and selectivity of the sensor devices, electronic-noses based on mass spectrometry gained popularity. Different methods have been used to pre-concentrate the volatile fraction: static headspace (HS), dynamic headspace (DHS) (Aparicio, Luna, & Morales, 2000; Morales et al., 2005) and solid-phase micro-extraction (HS-SPME) (Purcaro et al., 2014; Vichi, Romero, Tous, Tamames, & Buxaderas, 2008) providing a characteristic fingerprint of the volatile compounds. The most common strategy is the combination of gas chromatography (GC) and a pre-concentration method. Another option to analyze volatile compounds is by directly coupling a headspace system to a mass spectrometer (HS-MS) without performing a chromatographic separation. Although the characterization of the volatile compounds is not achieved, the global volatile 'spectral fingerprint' is considered to be correlated with the main sensory defects (López-Feria, Cárdenas, García-Mesa, & Valcárcel, 2008).

The non-volatile compounds, like phenolic compounds, have also been studied using electronic-tongues based on liquid chemical sensor arrays (Apetrei et al., 2010). Nevertheless, problems, such as low conductivity, viscosity and limited solubility of the oil samples, make the liquid sensors a complex system to work with. Vibrational spectroscopy, such as near- and mid-infrared (NIR and MIR), is an alternative to the liquid sensor systems due to its simplicity, rapidness and affordability. NIR and MIR spectra offer significant 'fingerprint' information about individual components in complex samples that can be connected to the sensory attributes described by the human panel (Sinelli et al., 2010). Related to the color measurements, another alternative fingerprint technique is ultraviolet–Visible spectrophotometry (UV–vis). Although the color is not a descriptor considered by the official taste panel, it may influence the quality of the olive oil. Few works have been conducted to study the color sensory descriptors (Kružlicová, Mocák, Katsoyannos, & Lankmayr, 2008), and have mainly focused on region authentication (Pizarro, Rodríguez-Tecedor, Pérez-del-Notario, Esteban-Díez, & González-Sáiz, 2013) and time evolution (Tarakowski, Malanowski, Kościesza, & Siegoczyński, 2014).

Panelists perceive sensory attributes and defects of olive oils as a mixture of gustatory, olfactory and tactile perceptions. These particular sensory properties are due to the presence of several compounds that may interact through synergisms and antagonisms. So, for example, one single compound may contribute to different sensory properties and at the same time one single property may depend on many compounds for explanation. For this reason, to model the sensory parameters, a combination of different instrumental techniques is required, which provide complementary information from different sources to make sensory description easier. To process the data suitable multivariate pattern recognition techniques are required, especially from non-selective spectral data. The combination of the information from different instruments is called data fusion and essentially it is carried out at three levels. The low-level data from different instruments are simply concatenated ('fused') into a single matrix prior to multivariate modeling. Mid-level data fusion first extracts features from the individual matrices and then fuses the new variables into a 'scores-matrix' which is used to build the final model. The new features are usually extracted by Principal Component Analysis (PCA) or partial least squares-discriminant analysis (PLS-DA). Finally, high-level fusion multivariate models are built on each individual technique and the individual predictions are combined to produce the final result (Borràs et al., 2015). Some data fusion approaches have been proposed to assess olive oil quality by fusing responses from electronic sensor devices, such as gas sensors (MOX or MOS) and liquid sensors (Apetrei et al., 2010), from spectroscopic measurements, such as MIR and NIR (Dupuy, Galtier, Ollivier, Vanloot, & Artaud, 2010), mass spectrometry (MS) with optical instruments, such as UV–vis spectrophotometry, (Casale, Armanino, Casolino, & Forina, 2007) or CIELab color space, chemical parameters and combinations of them (Pizarro et al., 2013). Most of the studies cited were applied to the authentication of olive oil samples according to their origin and only a few studies applied data fusion to correlate human sensory responses (Apetrei et al., 2010).

This study evaluates the feasibility of combining an electronic nose based on headspace mass spectrometry (HS-MS), an electronic tongue based on MIR spectroscopy and an electronic eye based on UV–vis spectrophotometry to discriminate the main olive oil categories (extra-virgin, virgin and lampante) and to detect the main off-fravors (musty, winey-vinegary, fusty and rancid) of olive oils. The instrumental data were correlated to the sensory results using PLS-DA to finally find the best data fusion strategy for improving the discriminating capability of the techniques. For this purpose olive oil samples from the Catalonia region with the presence of a variety of defects were evaluated by an official taste panel and also analyzed by the three instrumental techniques.

2. Materials and methods

2.1. Olive oil samples

A total of 146 samples were supplied by the 'Official Taste Panel of Virgin Olive Oil in Catalonia' in Reus (Government of Catalonia, Spain) during the seasons 2012 and 2013. Samples were stored in dark bottles at 20 C under nitrogen atmosphere until instrumental analysis. All of the samples were analyzed

within three months after the sensory analysis. Based on the sensory results provided by the panel, 84 were graded as EVOO or non-defective, 48 as VOO and 14 as LOO or non-edible. 30–40% of the olive oils presented the musty, winery or fusty defects, and only around 10% had the rancid defect.

2.2. Sensory analysis

Sensory analysis was carried out by the official panel following the official method of the Olive Oil Council (COI/T20/Doc15) and within the framework of ECC regulation 640/2008. The panel is also accredited according to the ISO17025 norm. 15 ml of each sample was tasted in a normalized colored cup to mask the color differences. The temperature of the oils was kept at 28 ± 2ºC. Samples were labeled with a digital code and served following a balanced rotation plan.

The positive scored attributes were fruitiness, bitterness, pungency, grassy green, astringent, sweet and apple. The scored sensory attributes indicating defectiveness or unpleasantness were musty, winey-vinegary, fusty, rancid and metallic. The samples tested did not show the metallic attribute, and for this reason this defect was not studied. Descriptors were evaluated on a continuous, unlabeled, intensity scale (10 cm), and then transformed into numeric variables between 0 and 10. Each sample was tested by 8–10 panelists and the median value was provided for the predominant attributes.

2.3. Instrumental analysis

The volatile composition was determined by a headspace-mass spectrometry based electronic nose (HS-MS). The volatiles were extracted and concentrated using a solid phase micro-extraction (SPME) fibre in the samples' headspace and detected with a HP5973N Mass Selective Detector. All of the extractions were made by an Autosampler CTC-PAL (Agilent) with divinylbenzene/car boxen/poly-dimethylsiloxane (DVB/CAR/PDMS) SPME fibres of 50/30 lm film thickness and 2 cm length. Olive oil samples (5 g) were weighed and placed into a 20 ml vial closed with a PTFE/silicone septum. Before extraction, the stabilization of the headspace in the vial was reached by equilibration for 30 min at 40 C. Then the fibre was exposed at 40 C for 1 h with stirring. After extraction, 5 min of thermal desorption of the fibre was sent into the GC system. To transfer the volatiles from the fibre to the MS detector, a HP-5MS column (30m x 0.25mm x 0.25μm) on a 6890 N gas chromatograph was kept at the suitable temperature to transfer the volatiles to the MS and avoid chromatographic separation. Mass spectra were obtained in the electron impact mode (70 eV) in a mass range from 50 to 350 amu. Two replicates were measured per sample.

To analyze the liquid composition, Fourier-transform mid infrared spectra (MIR) were measured on an FT-MIR Nexus (Thermo Nicolet, USA) spectrometer equipped with a deuterated triglycine sulphate (dTGS) detector. The spectra were collected at room temperature over the range 4000–600 cm^{-1}, at 4 cm^{-1} resolution and with 36 scans both for background and samples. A thick film of each oil sample was homogenously placed over the ZnSe crystal

ARK multi-bounce (horizontal attenuated total reflectance, HATR) with 12 reflections. The OMNIC software version 6.2 from Thermo Nicolet was used for spectral acquisition, instrument control and preliminary file manipulation. The spectra were compensated to eliminate interfering H_2O and CO_2 bands by running a blank with air. After that only the intervals with informative signals were selected for modeling, namely the ranges 3257.3–2604.3 cm^{-1} and 1951.4–682.8 cm^{-1}. Three replicates were measured per sample.

The color of the samples was determined by a single beam UV-Visible Hekios Gamma spectrophotometer (Thermo) equipped with diode array technology. The radiation source was a combination of a deuterium-discharge and a tungsten lamp for the ultraviolet and visible wavelength range respectively. The instrument was controlled by a compatible PC equipped with Vision Thermo software. Spectral data were collected at room temperature within the wavelength range 300–1000 nm at 2 nm resolution. It is referred to as 'UV–Vis spectra', despite acquired data also contained a small region of the near-infrared (NIR) (800–1000 nm) region. All samples were analyzed in duplicate in a quartz cuvette with a path length of 10 mm.

2.4. Statistical data analysis

2.4.1. General

Chemometric models were applied to the experimental data collected by the different instrumental techniques. The main purpose was to classify the olive oils with respect to their sensory defects presence using individual and fused data model strategies.

Preliminary PCA models of each instrumental technique data and for each data fusion approach were studied (Kozak & Scaman, 2008). Afterwards, different modeling strategies using PLS-DA were considered. PLS-DA is a discrimination technique useful when data matrices have more objects than variables. A regression model is calculated relating the independent variables X to an integer Y that designates the class of the sample with a binary response (called 'dummy' matrix). The X matrix (instrumental signals) contained the spectra in rows and Y was a vector with zeroes (for negative, i.e. non-defective) and ones (positive, i.e. defective oils). The class of each sample is determined from the predictions of the PLS model (ranging from zero to one) according to a threshold value that delimits the classes (Bevilacqua, Bucci, Magrì, Magrì, & Nescatelli, 2013).

2.4.2. Optimal model selection

To find the optimal classification model for each class studied, different spectral regions (variables) and combinations of them were considered along with different pre-processing options. The classification models were built with a training set using leave-one-out cross validation (CV) and the lowest classification error was the criterion used to select the optimal number of PLS-DA latent variables. The final models' performance was confirmed by a test set validation (TV). For that purpose, the dataset was randomly split into a training and test set, with 65% and 35% of the samples, respectively. The split

between training and test was done by keeping the ratio of samples of each class like in the original set. The random split procedure between training and test sets was repeated ten times and the mean of the classification parameters (sensitivity, specificity and misclassification rate) of the ten models was used as the final result, thus avoiding results depending on a particular split. The mean values and standard deviations were reported as percentages.

The quality of the models was assessed from the classification and prediction abilities. The optimal conditions were decided by means of sensitivity, specificity and inaccuracy. Sensitivity and specificity are defined as the number of samples correctly classified as negatives (non-defectives) or positives (defectives), respectively. To select the best sensitivity and specificity results a multicriteria Pareto's decision method was applied, where each model was represented in a bidimensional scatter plot reporting sensitivity and specificity on each axis, respectively. A compromise between high sensitivity and specificity was used to find the optimal solutions in the Pareto diagram (Oliveri et al., 2014). Minimum inaccuracy values were also considered. Inaccuracy is defined as the probability of samples from both classes being wrongly classified and it is useful to avoid artificially high errors when classes are unbalanced. The final selection criterion was defined considering inaccuracy and sensitivity/specificity Paretos' multicriteria values for the test-validation results.

2.4.3. Individual and data fusion strategies

In order to find the best combinations to classify the olive oil samples three modeling strategies were tested: individual techniques, and low- and mid-level data fusion approaches (**Fig. 1**).

Before the data fusion, individual data from HS-MS (MS), MIR and UV–vis analyses were processed, giving three different fingerprints per sample. The strategy described in **Fig. 1a** was followed in order to find the most representative variables (regions) and preprocesses for each model. Each spectral matrix was split into a training/test set (65/35) before building PLS-DA models.

In the low-level (LL) fusion approach (**Fig. 1b**), raw data from MS, MIR and UV–vis were concatenated (joined together in a single matrix) before model calculation. Scaling methods were tested considering the different scale of each data type (especially for the MS). Scaling may be done separately for each data block, but also between blocks. After that, the data fused matrix was randomly split into a training/test set (65/35) to build several PLS-DA models.

In the mid-level (ML) fusion approach (**Fig. 1b**), relevant features were extracted from the different data blocks and were concatenated into a single matrix. This reduced the dimensionality and allowed each block to be treated individually. Before that, individual matrices were randomly split into a training/test set (65/35). Scores from PCA or PLS-DA models obtained independently from each data matrix were then fused and named MLpca and MLpls respectively (Louw et al., 2009). These new variables selected, which kept enough original information, were subsequently combined to build a 'score-matrix', which was preprocessed by different scaling methodologies before building the final PLS-DA model. To

extract the possible features, different strategies were chosen. For PCA features the number of principal components (PCs) was chosen in order to give more than 90% of cumulative variance. To extract PLS-DA features, the number of latent variables (LVs) was chosen when the classification error obtained by cross-validation was minimized. The challenge was to find the optimal combination of extracted features and pre-processing methods that provided the best model. However, examining all of the combinations can make the process cumbersome and computationally intense.

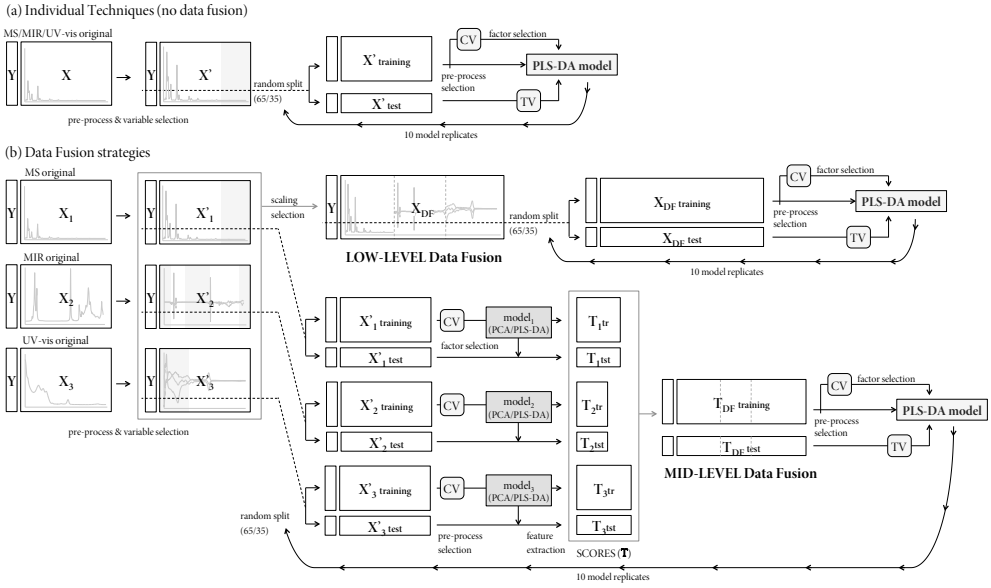


Fig. 1. Individual (a) and data fusion (b) procedures followed to obtain the final optimal PLS models.

In both cases, CV was applied to each training set to decide the optimal number of latent variables or scores used for the new 'score-matrices'. Several final PLS-DA models were applied to the fused score-matrices starting from the training-test set split procedure.

The different strategies were performed using two techniques (MS + MIR), called data fusion 2 (2LL, 2MLpca and 2MLpls); and three techniques (MS + MIR + UV–vis), called data fusion 3 (3LL, 3MLpca and 3MLpls). This is because the official taste panel does not evaluate visual characteristics of the olive oil, so the UV–vis technique might not provide enough information. At this stage, no attempts were made to combine the results of the models (high-level data fusion).

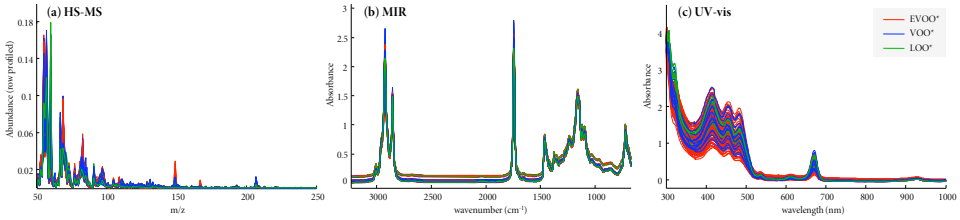
All of the data processing was carried out with in-house Matlab v.7.8 routines (Mathworks, MA, USA) and PLS Toolbox software v.6.2 (Eigenvector Research, Manson, WA, USA).

3. Results and discussion

3.1. Individual data results

3.1.1. General

In the first stage, individual headspace/mass spectrometry (MS), mid-infrared (MIR) and UV–visible (UV–vis) spectroscopy data were treated to build the discrimination models. The measurements (**Fig. 2**) were organized in matrices of dimensions 146 301 (MS), 146 594 (MIR) and 146 701 (UV–vis).



* EVOO: extra virgin olive oil (non-defective/edible); VOO: virgin olive oil (defective/edible); LOO: lampante virgin olive oil (defective/edible)

Fig. 2. Instrumental signals obtained by the different techniques to study olive oil samples: (a) HS-MS mass spectra (row profiled); (b) MIR mid-infrared spectra; (c) UV–vis ultra-violet/visible spectra.

To check the repeatability of the measurements, detect outliers and recognize patterns in the samples' distribution (harvest year, analysis day or other attribute's presence) PCA was performed for each technique. No clear patterns were observed. Considering the measured spectra, six samples for MIR and five samples of both, MS and UV–vis, were removed based on Hotelling's T^2 values and Q residuals well above 95% of the confidence limits.

Then separate PLS-DA models were studied for each instrument. The best pre-process and region (variables), together with the optimal configuration of the PLS-DA models, such as the number of latent variables retained, were selected as those leading to the lowest inaccuracy and highest sensitivity and specificity obtained with the validation set (TV). The optimal conditions for each discrimination model are summarized in **Table 1**.

Each defect and technique were defined by different optimal regions (variables) carrying discriminant information. It is possible to identify which regions of the specific fingerprints (variables) are the most influential in discriminating between defective and non-defective samples by studying the values of the variable importance in projection (VIP) index. Important variables are indicated by a VIP index greater than one (Wold, Johansson, & Cocchi, 1993).

3.1.2. Mass spectra

In order to avoid differences caused by instrumental analysis over time all mass spectra were row profiled. Logarithmic pre-processing was applied to all of the classes, except fusty and non-edible. For

these two classes data were autoscaled while the others were only mean-centred before building the final PLS-DA model.

Table 1. Optimal PLS-DA models on each instrumental technique for each class studied.

Class	Technique	Region (variables)	Preprocess	factors (LV)
non-defective (EVOO)	MS	100 – 125 m/z	Row profile + autoscaling	2
	MIR	1040 – 795 cm^{-1}	SNV + 1st derivative + mean-centering	3
	UV-vis	300 – 1000 nm	Baseline correction (order 3) + mean-centering	4
non-edible (LOO)	MS	50 – 150 m/z	Row profile + logarithm + mean-centering	2
	MIR	1040 – 795 cm^{-1}	SNV + mean-centering	2
	UV-vis	300 – 1000 nm	Offset correction + mean-centering	2
Musty	MS	100 – 125 m/z	Row profile + logarithm + mean-centering	2
	MIR	1040 – 795 cm^{-1}	SNV + 1st derivative + mean-centering	3
	UV-vis	580 – 1000 nm	SNV + 1st derivative + mean-centering	3
Winey	MS	100 – 125 m/z	Row profile + logarithm + mean-centering	2
	MIR	1330 – 1045 cm^{-1}	SNV + 1st derivative + mean-centering	5
	UV-vis	580 – 1000 nm	SNV + 1st derivative + mean-centering	4
Fusty	MS	50 – 150 m/z	Row profile + autoscaling	2
	MIR	1040 – 795 cm^{-1}	Offset correction + 1st derivative + mean-centering	4
	UV-vis	580 – 1000 nm	Offset correction + 1st derivative + mean-centering	3
Rancid	MS	50 – 150 m/z	Row profile + logarithm + mean-centering	2
	MIR	3230 – 670 cm^{-1}	Offset correction + mean-centering	5
	UV-vis	580 – 1000 nm	SNV + 1st derivative + mean-centering	3

MS: headspace/mass spectrometry, MIR: mid-infrared spectroscopy, UV-vis: UV-vis spectroscopy
SNV: standard normal variate

Although mass spectrometry is commonly used to provide information about the volatile composition, the use of direct-coupling HS-MS, where the information of the individual compounds is avoided, makes the interpretation difficult. The highest discriminant potential was obtained from 100 to 125 m/z for musty, winey and defective, and from 50 to 150 m/z for fusty, rancid and non-edible oils. For all of the classes, in particular for musty, winey, fusty and defective, a mass of 109 was the one with the highest VIP. Although it is difficult to find the compounds associated with certain masses, the literature indicates that the 109 base peak is indicative of compounds like guaiacol or 3E-6-methylhepta-3,5-dien-2-one. A few studies have indicated that some key-odorants that contribute to these off-fravored defective oils are volatile phenols, such as guaiacol, phenol and its methyl, ethyl and vinyl derivatives (Monteleone, 2014; Vichi et al., 2009a, 2009b, 2008). Other studies assign the ketone as a characteristic compound of vinegary and mould defective oils (Purcaro et al., 2014). Other significant musty and winey variables with VIP > 1 were 102, 104, 110, 107, 112 and 119 m/z. Most of these are characteristic of guaiacol and phenol derivatives, however, some masses can be fragments of other compounds like 1-octen-3-ol, 1-octen-3-one, (E)-2-heptanal and hexanal, related to the musty defect (Monteleone, 2014); and acetic acid and ethyl acetate related to the winey defect (Angerosa, 2002). High VIP scores at 60, 104 and between 54 and 109 m/z were found in the fusty model. These masses could be related to alcohols, aldehydes, ketones, esters and short chain fatty acids, such as 3-hexenol, 2-hexenal, 2,4-heptadienal, ethyl and butyl acetate, ethyl butyrate, acetic and butanoic acid (Angerosa, 2002; Monteleone, 2014). Finally, rancidity was mostly explained by 50, 72 (VIP > 5) and between 60 and 88 m/z, which could be

attributed to aldehydes, alcohols and acids. In particular aldehydes, such as trans-2-heptenal or the hexanal/nonanal ratio, were suggested as good markers of rancidity and indicators of oxidative status of olive oils (Angerosa, 2002; Kotti, Cerretani, Gargouri, Chiavaro, & Bendini, 2011).

3.1.3. Infrared spectra

Most of the optimal MIR models were pretreated with SNV, except for fusty and rancid where an offset correction was applied. Then, except for rancidity and non-edible, first-derivatives were applied, followed by mean-centering of all the resulting spectra before building the PLS-DA model. The olive oil fingerprint region between 1500 and 700 cm^{-1} was included in all of the classification models. Optimal variables for musty, fusty, non-defective and non-edible belonged to 1040–795 cm^{-1} region, with high VIP values at 1040–1025 cm^{-1}. This zone is assigned to the stretching vibrations of the C-O ester group ($\nu_{C\text{-}O}$) which can be attributed to ethyl acetate, butyl acetate, ethyl propanoate or ethyl butyrate that mainly contribute to the fusty perception (García-González & Aparicio, 2010a). Bands around 925–920 and 910–900 cm^{-1} had an important discriminant capacity, however they are difficult to assign. Other common representative wavenumbers were from a region close to 980 and 950 cm^{-1}, due to bending out-of-plane deformation of trans-olefins ($\gamma_{\text{-HC=CH-}}$), and a region near 850 cm^{-1}, related to bending vibrations of $=CH_2$ wagging ($\omega_{=CH2}$) and out-of-plane deformations ($\gamma_{\text{-CH}}$). These are general rotations, vibrations and deformations that could be assigned to many different organic molecules, and interpreting their correlation with the defects is very difficult. In addition, fusty oils had marked absorbances at 1015 cm^{-1}, often associated with the C–O stretching zone of esters. Another zone from the fingerprint was characteristic of winey-vinegary oils (1330–1045 cm^{-1}). A band at 1115–1100 cm^{-1} related to oleic acid had a higher discriminant power. Other important bands were at 1160, 1135 and 1040 cm^{-1}, belonging to the esters fingerprint zone and associated with stretching vibrations of C–O aliphatic esters, with a strong band at 1160 cm^{-1} due to bending vibrations of CH_2 groups ($\gamma_{\text{-CH2-}}$). The complexity of the matrices makes it difficult to assign the main compounds responsible for the winey defect (acetic acid, ethyl acetate and ethanol) for the experimental spectral bands. Finally, all spectral ranges registered, from 3230 to 2562 and from 2110 to 670 cm^{-1}, were selected to classify rancid oils. Wavenumbers with high VIP values are 2850 and 2920 cm^{-1}, from the CH stretching of cis-double bond vibrations ($\nu_{\text{asymm/symm C-O}}$) (Guillén & Cabo, 1997), called the fingerprint of oxidation process resulting

in a presence of secondary oxidation products, such as aldehydes and ketones. Auto-oxidation is the main cause of rancidity, nonanal and trans-2-heptenal are two examples of the compounds that cause this (Angerosa, 2002; Morales et al., 2005). Other important regions are 1745 and 1155 cm^{-1}, both bands related to ester compounds. The first one is related to C=O stretching vibrations of the ester carbonyl ($\nu_{C=O}$) of triglycerides and free fatty acids and the latter is related to C–O stretching vibrations of aliphatic esters (Guillén & Cabo, 1997).

3.1.4. UV–vis spectra

All of the specific defect models were pretreated with first-derivatives using second-order smoothing polynomials through seven points. Standard normal variate (SNV), offset and baseline (third-order) corrections were applied before the derivatisation, followed by mean-centering of the resulting spectra. From all of the studied variables, the range from 580 to 1000 nm provided the best results to classify the specific defects and the whole spectra (300–1000 nm) was suitable for the general non-defective and non-edible oils. The first region corresponds to the visible range, which shows the presence of dyes and pigments (Grazia Mignani et al., 2012). A range from 380 to 450 nm, belongs to carotenoid pigments that have high stability. However, chlorophylls and pheophytins, with an exclusive absorption band at 650–700 nm, had a high influence in the model for all of the defects. This may be attributed to the defective samples which have undergone a degradation process, for different reasons, which may have affected the composition of these substances (Sikorska et al., 2007). Another band near 935 nm had significant influence on the classification model for winey and rancid samples. In general, peaks at 610 and 670 nm are reduced for all of the degenerated or defective olive oils.

3.2. Classification results

In order to select the best classification models, all of the data fusion strategies were tested using combinations of some of the optimal regions and pre-processing obtained from the classification achieved by the individual techniques (Section 3.1). All of the data fusion strategies were studied with 135 samples. Twelve samples were initially removed based on individual PCA of fused data where the Hotelling's T^2 and Q residual values were above 95% of the confidence limits.

Each optimal model, for all the data fusion strategies, was selected based on the criteria described in Section 2.4. The optimal results for the conditions selected by all of the data fusion strategies are plotted in **Figs. 3** and **4**. **Figs. 3a** and **4a** show bar charts of the inaccuracies (%) obtained by test-validation (TV) of the optimum data fusion strategies compared to the individual models. Standard deviations of the 10 replicated models are also plotted. A striped line indicates the lowest inaccuracy obtained by the best individual model for each class studied. The best fusion strategies are shown outside the grey region. Figs. **3b** and **4b** show the sensitivity vs. specificity Pareto diagram of all the data fusion strategies together with the individual results. Best prediction abilities for each defect or class (balance between selectivity and specificity) are connected by a black line, called Pareto front. Final selection of the best classification models for each class and their results, considering inaccuracy, sensitivity and specificity, are summarized in **Table 2**.

The results obtained by CV (not shown) and TV were similar, ensuring the reliability of the models built. Most of the models are unbalanced (60–70% of samples of one class) and this might cause the specificity, which is the ability to correctly recognize positive samples with defect presence (class with fewer samples), to show higher standard deviations. This is even more noticeable for non-edible and rancid classifications, due to lack of enough positive samples with these two properties (less than 10%).

Table 2. Test-validation PLS-DA results of the best individual and data fusion strategies for each class studied (*). Bold and highlighted techniques are the best strategies selected for each class.

Defect	Strategy	Technique	% inaccuracy		% sensitivity		% specificity	
non-defective (EVOO)	**Individual**	**MS**	19.8	(4.6)	77.0	(7.1)	82.5	(6.6)
	Mid-level PLS-DA (2MLpls)	MS + MIR	21.7	(7.7)	78.0	(5.9)	78.6	(12.3)
	Mid-level PLS-DA (3MLpls)	MS + MIR + UVv	18.5	(4.4)	75.3	(7.5)	85.7	(5.6)
non-edible (LOO)	Individual	UV-vis	24.2	(6.7)	77.4	(4.9)	65.0	(24.1)
	Mid-level PLS-DA (2MLpls)	MS + MIR	22.6	(9.3)	79.1	(13.2)	60.0	(37.6)
	Mid-level PLS-DA (3MLpls)	**MS + MIR + UVv**	20.2	(6.5)	81.0	(7.0)	67.5	(26.5)
Musty	Individual	MS	13.7	(4.3)	87.9	(6.8)	83.1	(9.5)
	Mid-level PLS-DA (2MLpls)	MS + MIR	10.7	(3.7)	91.8	(4.5)	83.8	(8.5)
	Low-level (3LL)	**MS + MIR + UVv**	10.5	(4.8)	90.0	(6.8)	88.3	(7.0)
Winey	Individual	UV-vis	15.0	(4.5)	90.4	(4.8)	74.3	(10.2)
	Mid-level PLS-DA (2MLpls)	MS + MIR	15.9	(5.9)	85.9	(8.5)	80.7	(9.6)
	Low-level (3LL)	**MS + MIR + UVv**	15.4	(4.8)	83.8	(6.2)	86.2	(10.1)
Fusty	Individual	MS	18.0	(3.7)	87.4	(6.6)	71.4	(7.5)
	Mid-level PLS-DA (2MLpls)	MS + MIR	16.6	(2.5)	86.3	(4.6)	77.9	(8.6)
	Low-level (3LL)	**MS + MIR + UVv**	14.9	(3.4)	88.1	(5.3)	79.2	(7.3)
Rancid	Individual	UV-vis	17.4	(8.0)	82.9	(8.5)	80.0	(15.8)
	Low-level (2LL)	**MS + MIR**	14.9	(6.0)	85.9	(6.8)	77.5	(18.4)
	Mid-level PLS-DA (3MLpls)	MS + MIR + UVv	19.7	(8.3)	79.7	(8.7)	86.7	(17.2)

(*) Results indicated as a percentage and presented as mean (standard deviation) of the 10 models.
MS: headspace/mass spectrometry, MIR: mid-infrared spectroscopy, UV-vis: UV-vis spectroscopy

To select the best technique or data fusion strategy to classify olive oils based on the presence of defects, the compromise between lower inaccuracy and Pareto results were examined. In most of the models the best classification results were obtained by fusing MS, MIR and UV–vis using low- or mid-level approaches, but in some cases the improvement was not sufficient to justify the use of the three instruments, in such cases MS and MIR or even a single technique being an acceptable option. For all of the defects, mid-level data fusion approaches using PCA scores with two or three techniques did not sufficiently improve (or even worsened) the results obtained by the best individual model.

Non-defective and non-edible oil classifications are detailed in **Fig. 3**, obtaining significantly better results from the non-defectiveness classifications.

The best individual technique for non-defective oils was MS with inaccuracies of around 20% and sensitivity and specificity above 75%. Compared to data fusion strategies slightly lower inaccuracy results were obtained, using both low- and mid-level fusion of MS, MIR and UV–vis (**Fig. 3a** – non-defective). In addition, similar sensitivity and specificity to MS were achieved (Fig. 3b). In this case, data fusion was not necessary to improve the results and the best classification was obtained with MS. For the non-edible oils the best classifications were achieved by UV–vis spectra (near 25% inaccuracy) and 77/65% (sensitivity/specificity). However, in this case data fusion did improve the best individual results. Inaccuracies were lower in all data fusion strategies using the three techniques (Fig. 3a – non-edible) and the best Pareto results were observed using mid-level data fusion of the three techniques (3MLpls) using PLS-DA scores (**Fig. 3b**).

This approach was the best option, improving sensitivity/specificity from 77/65 to 80/67.5%, respectively, and reducing inaccuracy from 24% (UV–vis) to 20%. To check relevant contribution of all the mid level blocks, the loadings and highest VIP scores were observed. For all the optimized strategies selected, loadings of every data block were considered to be contributing to the final model.

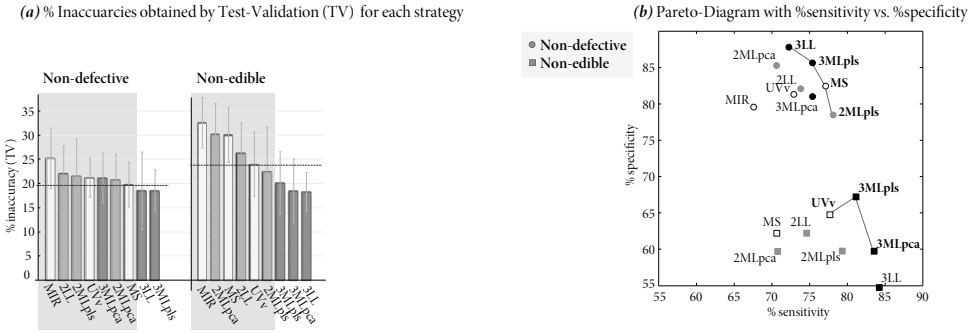


Fig. 3. Final PLS-DA classification parameters (TV) for non-defective and non-edible classes. (a) Inaccuracy values (%) and (b) sensitivity vs. specificity Pareto diagram.

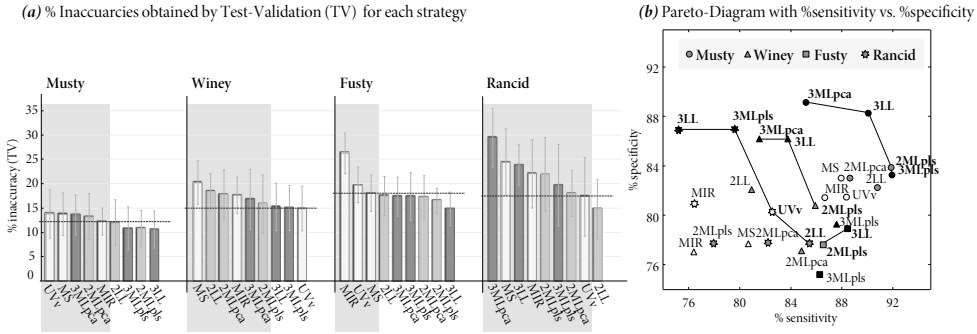


Fig. 4. Final PLS-DA classification parameters (TV) for musty, winey, fusty and rancid defects. (a) Inaccuracy values (%) and (b) sensitivity vs. specificity Pareto diagram.

Specific defects, such as mustiness, fustiness, winery and rancidity, are detailed in **Fig. 4**. The best classification results using individual techniques were obtained with MIR data for musty defective oils with sensitivities and specificities over 80% and around 13% of the samples incorrectly classified. Mustiness showed no significant differences among the three single techniques applied. Winey-vinegary oils had inaccuracies ranging from 15% to 20%, the UV–vis spectra being the one with the lowest value. The highest sensitivity and specificity were 75 and 90%, respectively. Fustiness and rancidity achieved lower inaccuracies (less than 20%) with MS and MIR, respectively.

The classification results of musty, winey and fusty defective oils improved by fusing the three instruments and using low-level data fusion. Mustiness reached lower inaccuracies (**Fig. 4a** – musty) by fusing two or three techniques with low- and mid-level data fusion, the mid-level strategy always using

PLS-DA scores. However, Pareto results (**Fig. 4b**) indicated that low-level data fusion of all the techniques (3LL) was the best strategy, enhancing sensitivity/specificity from 88/83 to 90/88%, respectively. The inaccuracy was reduced from near 14%, using MS, to 10.5%, using 3LL (**Table 2**). Winey-vinegary defect achieved similar inaccuracies compared to UV-vis using data fusion (**Fig. 4a** – winey), but prediction abilities improved when 3LL and 2ML were applied (**Fig. 4b**). Low-level data fusion of the three instruments was considered the best strategy, improving 75% of specificity obtained by the best individual technique for more than 85%, even though inaccuracy was maintained at around 15% (**Table 2**). Fustiness reduced its inaccuracies when all of the data fusion strategies tested were used, the 3LL being the best combination (**Fig. 4a** - fusty). These results were supported by the sensitivity/ specificity results (**Fig 4b**). The inaccuracy was finally reduced from 18% (by MS) to 15% using low-level data fusion of three techniques, and specificity was improved from near 70% to almost 80% (**Table 2**). In the case of rancidity, an improvement in the classification of the oils was achieved using only MS and MIR low-level data fusion (2LL). Lowering of the inaccuracy of the best individual technique (UV–vis) was achieved (**Fig. 4a** – rancid), decreasing from 17% to 15% (**Table 2**); however, sensitivity and specificity showed greatest similarity when PLS-DA (3MLpls) was used rather than the single UV–vis technique or other data fusion strategies, such as mid-level data fusion (**Fig 4b**). Of all the low-level data fusion approaches, it was found that all the merged blocks had relevance to the final fused model, assuring the complementarity of the data measured by the three instruments. Of all the particular defects, best classifications were obtained for the musty defect. Winey, fusty and rancid defects achieved similar classification abilities using low-level data fusion (at around 15% inaccuracy).

4. Conclusions

The combination of three different instrumental techniques, headspace-mass spectrometry (HS-MS), mid-infrared spectroscopy (MIR) and UV-visible spectrophotometry (UV–vis), can be a useful tool to classify olive oil samples based on their category and the presence of certain sensory defects. The application of different data fusion approaches together with a PLS-DA strategy to select specific regions and pre-processings for each case improved the discrimination capability of the olive oils.

Only in the case of the discrimination of high-quality non-defective olive oils (extra-virgin), did data fusion not improve the inaccuracy and sensitivity/specificity values obtained with single MS. For the lowest-quality of olive oils considered non-edible (lampante) data fusion of the three techniques (3MLpls) using PLS-DA scores improved individual results. Low-level data fusion was the most suitable strategy to discriminate musty, winey and fusty defects, using HS-MS, MIR and UV–vis, and the rancid defect using HS-MS and MIR. Of all the specific defects, the best classifications were obtained for the musty defect, with inaccuracies of approximately 10% and sensitivity/ specificity values near 90%. Winey, fusty and rancid achieved similar classification abilities (around 15% inaccuracy) using low-level data fusion.

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Prediction of olive oil sensory descriptors using instrumental data fusion and Partial Least Squares (PLS) regression

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Abstract

Headspace-Mass Spectrometry (HS-MS), Fourier Transform Mid-Infrared spectroscopy (FT-MIR) and UV-Visible spectrophotometry (UV-Vis) instrumental responses have been combined to predict virgin olive oil sensory descriptors. 343 olive oil samples analyzed during four consecutive harvests (2010-2014) were used to build multivariate calibration models using partial least squares (PLS) regression. The reference values of the sensory attributes were provided by expert assessors from an official taste panel. The instrumental data were modeled individually and also using data fusion approaches. The use of fused data with both low- and mid-level of abstraction improved PLS predictions for all the olive oil descriptors. The best PLS models were obtained for two positive attributes (fruity and bitter) and two defective descriptors (fusty and musty), all of them using data fusion of MS and MIR spectral fingerprints. Although good predictions were not obtained for some sensory descriptors, the results are encouraging, specially considering that the legal categorization of virgin olive oils only requires the determination of fruity and defective descriptors.

Keywords

Olive oil; Electronic panel; Mass spectrometry; MIR spectroscopy; UV-Vis spectrophotometry; Data fusion; Sensory evaluation; Partial least squares (PLS) regression; Multivariate analysis

1. Introduction

Olive oil is obtained from the fresh fruit of the olive tree (*Olea europaea L.*) exclusively by mechanical or other physical means [1]. It is a fundamental ingredient of the Mediterranean diet that is usually consumed in its crude form. The olive oil unique aroma and delicate fravor, mainly conferred by minor compounds, together with its claimed health benefits [2] has contributed to an increase in the global demand for this food commodity. As a consequence, olive oil prices have increased, encouraging some farmers and producers to practice fraudulent activities, such as mixing high quality olive oils with cheaper vegetable oils or with lower quality olive oils. Some international institutions such as the European Union, the International Olive Council and the Codex Alimentarius have adopted a series of regulations to detect possible adulterations and to guarantee olive oil quality and safety [1,3]. These control measures have made olive oil one of the most strictly regulated food products [4], with defined physico-chemical parameters (free acidity, peroxide value, fatty acids and specific ultraviolet (UV) absorptions) as well as present organoleptic characteristics determined by official methods. According to maximum values that cannot be exceeded, these parameters differentiate three main quality categories that determine the olive oil economic value (**Table 1**). The two first categories, extra virgin (EVOO) and virgin (VOO) olive oils can be bottled and directly consumed, while lampante olive oil (LOO) must be previously refined [5].

Table 1. Quality limits for olive oil categories established by IOC [6] and EU [7] regulations.

	Free acidity (%)	Peroxide value (mEq O_2/kg)	UV spectroscopy			Median of defects (Md)	Median of fruity attribute (Mf)
			K_{270}	K_{232}	ΔK		
Extra virgin olive oil	≤ 0.8	≤ 20	≤ 0.22	≤ 2.50	≤ 0.01	Md = 0	Mf > 0
Virgin olive oil	≤ 2.0	≤ 20	≤ 0.25	≤ 2.60	≤ 0.01	0 < Md ≤ 3.5	Mf > 0
Lampante olive oil	> 3.3	-	-	-	-	Md > 3.5	-

The sensory assessment plays a crucial role in the determination of olive oil categories. The only homologated method is performed by the taste panel, which employs well-standardized protocols as well as continuously and well-trained panelists. The main task of the taste panel is to evaluate the so-called positive and negative sensory attributes. The positive notes, perceived by consumers as "healthy" indicators [8], are mainly attributable to fruity (green or ripe), bitter and pungent sensations. Reminiscent sensation of freshly cut grass (grassy), green fruits (green odor), sweet and astringent notes can be also considered as positive characteristics of good quality olive oils. On the contrary, winery-vinegary, fusty, mustiness-humidity, rancid and metallic are the most frequent off-fravors [9]. The classification of the olive oils in different commercial categories depends on the presence of fruity notes, defects and their intensities [8]. The absence of sensory defects is mandatory to classify olive oil as EVOO, the maximum quality olive oil. When sensory defects are detected their intensity has to be specified, being low intensities (≤3.5) allowed for VOO and high intensities (>3.5) or absence of the fruity attribute for LOO (**Table 1**) [1,3,10].

The sensory attributes that define the unique olive oil fravor are a combination of aroma, taste, sight and texture sensations directly related to the content of minor volatile and non-volatile compounds. The tasting receptors and the free endings of the trigeminal nerve are stimulated by non-volatiles such as phenolic compounds evoking bitter, pungent, astringent and metallic perception [11]. In particular, bitterness and pungency perceptions have been linked to the content of specific secoiridoid derivatives [12]. However, the most important factor that defines the sensory attributes of the olive oil is the aroma, composed by volatile chemical compounds that stimulate the olfactive receptors. These volatile compounds, mainly produced by the oxidation of fatty acids through the lipoxygenase (LOX) pathway, include the main compounds responsible for the positive fruity attribute; and secondary pleasant olfactory notes in VOO, such as green notes, grassy or sweetness [13]. Otherwise, the unpleasant sensory notes are mainly originated by volatiles that can alter the initial olive oil sensory profile through several processes. Additional compounds are produced by sugar fermentation (winey), anaerobic microorganisms (muddy), branched amino acid production (fusty), enzymatic activities of moulds (musty) and to auto-oxidative processes (rancid), thus providing more complex sensory profiles for defective or lower quality olive oils (VOO and LOO) [14].

Considering that sensory assessment has some inherent complications, there is an increasing need for developing analytical methodologies to support the human panels and overcome their limitations [EU, HORIZON 2020 Work Programme]. Basically a taste panel lacks standardized reference oils, suffers from assessors' subjectivity and fatigue, carry-over effects, high costs of training and maintenance and cannot be automated. Several analytical approaches have been proposed as an alternative to a human panel [15], most of them focused on determining the chemical compounds that are responsible for the sensory descriptors. However, the correlation of these compounds (volatiles and non-volatiles) to the human sensory responses is highly complex mainly due to the difficulty to identify many of the compounds [4,16,17], as well as the semantics fuzziness of some of the descriptors [16,18,19]. Moreover, sensory perceptions are also affected by positive and negative synergisms caused by complex interactions in the olfactory and gustative receptors or by kinetic components of the fravor when olive oil is in the mouth (saliva and movements) [15,17].

To overcome these limitations, alternative analytical instrumental techniques have been proposed that, together with multivariate analysis techniques, can find relationships between instrumental signals (fingerprints) and sensory quality attributes [4]. These instrumental techniques simulate the human perceptions and are basically classified according to the detection of volatile or non-volatile compounds. Electronic-noses simulate the human olfaction, detecting volatile compounds by means of gas sensor arrays [10,20] or mass spectrometers [21,22]. In both cases a pre-concentration step (i.e. headspace or solid-phase micro extraction) is required to enhance response of the volatiles. Electronic-tongues simulate the tasting human receptors, detecting the presence of non-volatile compounds. First applications were based on liquid sensor arrays [23], but vibrational spectroscopy (near- and mid-infrared) is becoming a simpler alternative to determine taste attributes [24,25]. Although color is not

among the sensory quality parameters required to categorize olive oils, the visual perception is an important aspect with a decisive influence on the consumers' acceptance and it is associated to chemical and physical properties. Electronic-eyes are based on color measurements from visual methods using color scales (e.g. bromothymol blue method or CIE L·a·b colour space) or spectroscopic methods, such as Ultra-Violet Visible spectrophotometry (UV-Vis) [26].

However, when sensory attributes are detected through a human taste panel, the final responses are the result of the combined effects of human senses (i.e. odor, taste and vision). Analogously, instrumental techniques working as the so-called electronic panel, should combine the characteristic responses (fingerprints) obtained by the individual techniques such as electronic noses, tongues and eyes. This combination of the collected data is called data fusion and it provides complementary information from different sources that must be treated by multivariate pattern recognition techniques. There are three levels of data fusion, namely low-, mid- and high-level. Low- and mid-level data fusion approaches are the most commonly applied. While in low-level fusion the original data matrices are simply concatenated after applying proper pre-processing or variable selection techniques, in mid-level fusion a feature extraction procedure is previously applied to each data matrix to then concatenate the resulting features (i.e. principal component analysis (PCA) scores).

In this study, three instrumental techniques, namely headspace-mass spectrometry (HS-MS or MS), Fourier Transform mid-infrared spectroscopy (FT-MIR or MIR) and UV-visible spectrophotometry (UV-Vis) have been used as an electronic panel to predict the intensity scores of the main sensory attributes of olive oils evaluated by a human taste panel. Low- and mid-level data fusion strategies were applied in order to improve the predictions obtained by modeling each technique individually. Partial least squares (PLS) regression was selected to predict the final intensities for each attribute. Six positive attributes (fruity, bitter, pungent, grassy, sweet and astringent) and four off-fravors (musty, fusty, winey-vinegary and rancid) intensities were assessed from olive oil samples from Catalonia analyzed during four harvests.

2. Materials and methods

2.1. Olive oil samples

Ten sensory attributes, six positive (fruity, bitter, pungent, grassy, sweet and astringent) and four off-fravors (musty, fusty, winey-vinegary and rancid) were provided by the 'Official Taste Panel of Virgin Olive Oil in Catalonia' in Reus (Catalonia, Spain) for 343 olive oil samples during four consecutive harvests (2010–2014). Samples were stored in dark bottles at -20ºC under nitrogen atmosphere until instrumental analyses. All samples were analyzed within 3 months after sensory analysis. The official panel worked with confidential-codified samples and only information about the sensory panel results was provided. Based on the taste panel sensory results 169 samples were graded as EVOO, 129 as VOO and 45 as LOO.

2.2. Sensory analysis

Sensory analysis was carried out by the official panel following the official method of the Olive Oil Council (COI/T20/Doc15) and within the framework of ECC regulation 640/2008. The panel is also accredited according to the ISO17025 norm. 15 ml of each sample were tasted in a normalized blue-colored cup to mask the color differences. The temperature of the oils was kept at 28 ± 2ºC. The positive scored attributes were fruitiness, bitterness, pungency, grassy green, astringent, sweet and apple. The scored sensory defective attributes were musty, winey-vinegary, fusty, rancid and metallic. The samples tested did not show the metallic and apple attributes, and for this reason these descriptors were not studied. Descriptors were evaluated on a continuous, unlabeled, intensity scale (10 cm), and then transformed into numeric variables between 0 and 10. Each sample was tested by 8 to 10 panelists and the median value was provided for the predominant attributes.

2.3. Instrumental analysis

Headspace-mass spectrometry based electronic nose (MS). Five milligrams of olive oil were weighted and placed into a 20ml vial closed with a PTFE/silicone septum. Solid phase micro extraction was performed into the sample headspace (HS-SPME) with a 50/30 μm divinylbenzene/carboxen/ polydimethylsiloxane (DVB/ CAR/PDMS) fiber. The vial was equilibrated for 30 min at 40ºC before extraction, after that the fiber was exposed to the headspace at 40ºC for 1h under constant and mechanical stirring. After extraction, 5 min of thermal desorption of the fiber was introduced into the GC system. An Agilent 6890N gas chromatograph equipped with a HP5973N Mass Selective Detector was used to analyze the volatile compounds. An HP-5MS column (30m × 0.25mm × 0.25μm) was kept at the suitable temperature to transfer the volatiles to the MS detector avoiding chromatographic separation. Mass spectra were obtained in the electron impact mode (70eV) in a mass range from 50 to 250 amu. Two replicates were measured per sample.

Mid-infrared spectroscopy based electronic tongue (MIR). A FT-MIR Nexus (Thermo Nicolet, USA) spectrometer equipped with deuterated triglycine sulfate (dTGS) detector was used to analyze the non-volatile compounds. Spectra were collected at room temperature over the range 4000 – 600 cm-1, at 4 cm-1 resolution and with 36 scans both for background and samples. A thick film of each oil sample was homogenously placed over the ZnSe crystal ARK multi-bounce (horizontal attenuated total reflectance, HATR) with 12 reflections. Spectral acquisition, instrument control and preliminary file manipulation were carried out with the OMNIC software version 6.2 from Thermo Nicolet. All spectra were compensated to eliminate disturbing H2O and CO2 bands by running a blank with air. Only the informative signals were selected, namely the ranges 3257.3 – 2604.3 cm−1 and 1951.4 – 682.8 cm−1. Three replicates were measured per sample.

Ultra-violet visible spectrophotometry based electronic eye (UV-Vis). A single beam UV-Vis Helios Gamma spectrophotometer (Thermo) equipped with diode array technology was used to determine the color. The radiation source was a combination of a deuterium-discharge and a tungsten lamp for the

ultraviolet and visible wavelength range respectively. The instrument was controlled by the Vision Thermo software installed in a PC. UV-Vis spectra were acquired at room temperature within the wavelength range 300 – 1000 nm at 2 nm resolution. Two replicates per sample were measured in a quartz cuvette with a path length of 10 mm.

2.4. Statistical data analysis

All data processing was conducted using in-house Matlab v. 7.8 routines (Mathworks, MA, USA) and the PLS Toolbox software v. 6.2 (Eigenvector Research, Manson, WA, USA).

Principal component analysis (PCA) was first applied for preliminary data visualization. PCA defines new variables (principal components) as linear combinations of the original variables. PCA of the spectral data matrices was used to check spectral reproducibility and instrumental drift, to detect outliers and to investigate possible trends in the samples due to the harvest year, the day of analysis or the value of the attributes [27]. Spectral variations not related to attributes were removed by orthogonalization. The orthogonalization step yielded a transformed data matrix $\mathbf{X}_{ort} = \mathbf{X}\ (\mathbf{I} - \mathbf{P}\cdot\mathbf{P}^T)$, where **X** is the matrix of spectra in rows, **I** is the identity matrix and **P** is the loadings matrix from PCA for a previously determined number of principal components. Then, partial least squares (PLS) regression was used to predict the sensory descriptor intensities (matrix **Y**) from corrected spectra (matrix $\mathbf{X}_{ort}$).

A repetitive procedure of data pre-processing, variable selection (spectral regions), outlier detection and modeling was carried out. The optimum number of latent variables (PLS factors) was determined by leave-one-out cross-validation (LOO-CV) based on the minimum value of the root mean-square error of cross-validation (RMSECV). The dataset was split into a training set (65% of the samples) and a test set (35% of the samples) to evaluate the performance of the final model and to confirm the results. To avoid the results depending on a particular split, the split process was repeated ten times, and the mean and the standard deviations of ten models were reported. Statistics calculated for the PLS models included the coefficient of determination (R2) and the relative root mean square standard error on prediction (rRMSEp). The rRMSEp was expressed as a percentage to correct for the differences in the intensity ranges used for each descriptor. Outliers potentially disturbing the PLS models were detected using Hotelling's T^2 and Q residual statistical tests at a confidence level of 95%. Samples with both high Hotelling's T^2 and Q-residuals obtained at the ten replicated models were deleted from the dataset [28].

PLS regression models were built for individual instrumental data (MS, MIR and UV-Vis), for two-block fused data (MS+MIR) and for three-block fused data (MS+MIR+UV-Vis), using low- and mid-level data fusion strategies (**Figure 1**).

After pre-processing and variable selection in low-level data fusion the datasets were joined together in a single matrix (XDF) before PLS modeling. Different scaling methods were tested to overcome the differences in scale between datasets, both using the data blocks separately and fused. The final fused

block was then randomly split into training and test sets to build the PLS models. In mid-level data fusion the relevant features extracted from the individual data-blocks were concatenated into a single matrix (TDF), which was then split into training and test sets before model building. Different strategies can be chosen to extract the relevant features, such as PCA scores or PLS scores obtained from the individual matrices. In this study the PLS scores were extracted, as in a previous work they were shown to provide better results than PCA scores [29]. The selection of the optimal number of features (latent variables) to be joined was also determined by LOO-CV using the RMSECV statistic.

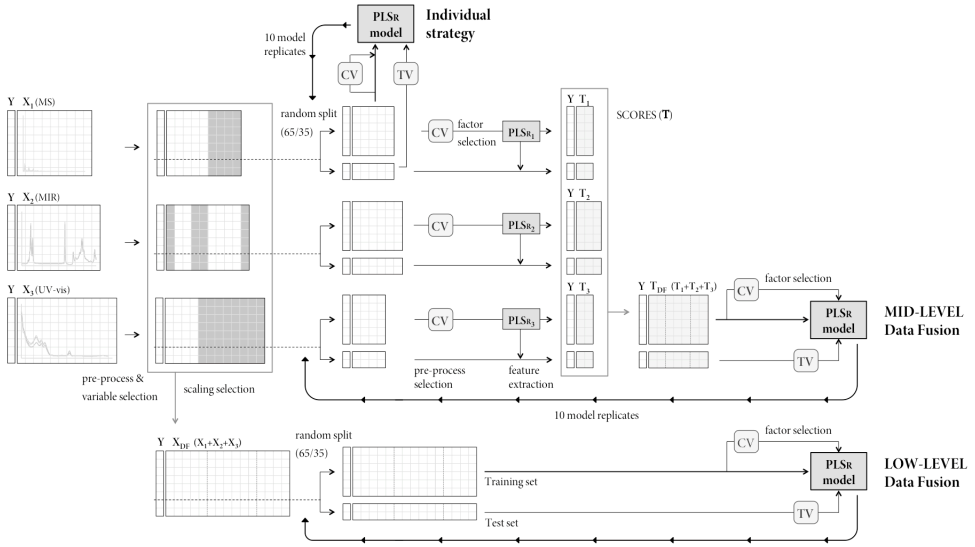


Figure 1. Individual and data fusion strategies followed for PLS models studied. (CV: cross-validation; TV: test-validation)

3. Results and discussion

PCA on the individual datasets showed samples grouped according to harvest year both for MS and MIR. To minimize this effect, each dataset was orthogonalized against the harvest year using the scores on the first principal component in both cases (**Figure 2**). Afterwards, T^2 Hotelling and Q residual statistical tests at a confidence level of 95% were applied for each tested PLS model on the individual datasets to check for outliers. Fourteen samples were considered as outliers and were removed from all the tested PLS models. Then, the optimal pre-processing and variables were selected for all the strategies as those yielding the highest R2 and the lowest RMSEp for the test set. The final prediction results for the optimized conditions are summarized in **Tables 2** and **3** and represented in Pareto Diagrams (**Figure 3**) for each descriptor and each strategy studied, either for individual techniques and data fusion of two- and three-blocks.

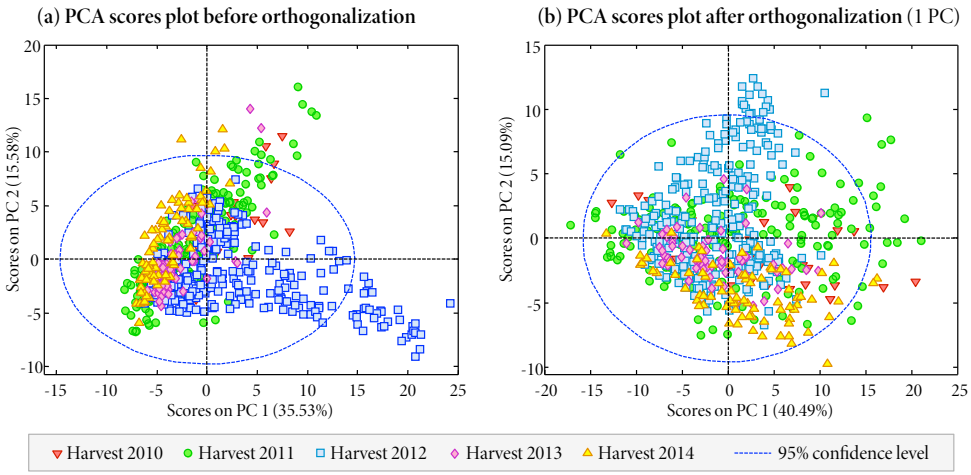


Figure 2. PCA scores plot (PC1 vs. PC2) of MS spectral data before and after orthogonalization to correct the harvest effect.

General overview. Pareto diagrams in **Figure 3** (positive (a) and negative (b) attributes) represent relative errors (%rRMSEp) versus determination coefficients (R^2). In Pareto diagrams the best prediction models correspond to strategies with the lowest RMSEp and the highest correlations (defined by R^2) that are found at the top-left part of the diagrams. Notice that for the fruity attribute (**Figure 3**a, inverted-triangles) the prediction errors (%rRMSEp) were much lower for data fusion strategies than for the individual techniques. This improvement on the PLS prediction results when data fusion was applied was a general tendency for all the descriptors, reaching R^2 values as high as 0.6 and rRMSEp as low as 14% in the case of fruity, musty and fusty models. Otherwise, the worst PLS prediction results were obtained by the data provided by individual techniques, reaching R^2 values lower than 0.4 and rRMSEp errors higher than 18% in the case of winey, fusty and astringency. However, the models for the individual techniques differ depending on the instrument, with a general trend on obtaining better predictions using MS data and worse predictions using UV-Vis data. The non-specificity of single instrumental techniques to explain certain sensory descriptors was coherent when, for example, taste-related (MIR) attributes like pungency were poorly modeled by the UV-Vis data (**Figure 3a** – squares). But, in some cases, this apparent instrumental non-specificity was useful to complement the information from other techniques and to improve the prediction results when data fusion strategies were applied (i.e. addition of UV-Vis data to MS and MIR (3LL/3ML) to model pungency). This trend was remarkable for the pungent and astringent models and, although less strong for fruity, fusty and rancid, better prediction abilities were observed compared to individual data. Consequently, prediction abilities obtained with PLS models built for individual techniques (**Table 2**) and fused data (**Table 3**) were first studied separately. In both cases relative predictive errors obtained by cross-validation (RMSEcv) and test-set validation (RMSEp) were similar, ensuring that PLS models are not overfitted.

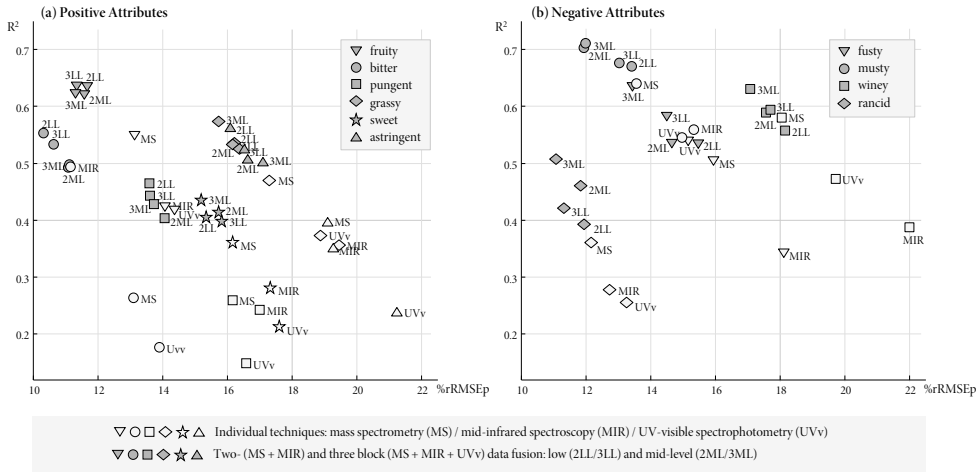


Figure 3. Pareto Diagrams with the averages of %rRMSEp versus R^2 for all the strategies, for the positive (a) and negative (b) attributes. Symbols were light-colored for each of the individual technique: mass spectrometry (MS), mid-infrared spectroscopy (MIR) and UV-Visible spectrophotometry (UV-Vis); and dark-colored for the data fusion strategies using two- (MS + MIR) and three block (MS + MIR + UV-vis) of data: low (2LL/3LL) and mid-level (2ML/3ML).

Individual strategies. **Table 2** shows the optimal PLS models obtained for each descriptor using the three instrumental techniques modeled individually (best individual data highlighted and bolded). The best predicted descriptors were fruity, musty and bitter. These yielded correlations higher than 0.5 (R^2) and errors around 13% (rRMSEp) for both fruity and musty using MS and close to 11% for bitterness using MIR. In general, MS yielded the best predictions for almost all the descriptors. This can be justified due to the importance of the aromatic composition to describe most of the sensory characteristics. Basically fruitiness, sweetness, grassy and all the off-fravors are perceived through the stimulation of the olfactive system by characteristic volatile compounds [13,14], which can be detected by MS. This MS spectral fingerprint includes so much information about the sample composition that even pungent and astringent notes (usually related to tasting perceptions) were similarly explained by electronic nose (MS) and tongue (MIR) responses, with no significant differences. However, MS data did not correlate bitter and fusty intensities as well as other individual techniques. While bitterness was better explained by the MIR data, rising from correlations around 0.2-0.3 to 0.5 R^2, fustiness was slightly better predicted by UV-Vis data with similar correlations than MS (0.5 R^2) but lower errors downing from 16% to 15% rRMSEp. These results are consistent with the literature in the case of the bitter responses, which are strongly related to electronic tongue (MIR) signals due to the stimulation of the gustative sense by characteristic non-volatile compounds from the olive oil phenolic fraction (sercoridoid derivatives) [11,23]. Oppositely, fustiness that is described as a muddy-sediment off-fravor originated by olives in piles during long storage time [8] should be basically explained by the volatiles detected with MS. Although it was correctly explained by MS, fustiness was better predicted by UV-Vis data. This fact can

be associated to the alteration of the color of the final product caused by degradation processes suffered by the stored olives.

Table 2. Prediction results* of one-block strategies (individual techniques) for each descriptor

Descriptor		Intensity range	Technique	LVs	rRMSEcv (%)		rRMSEp (%)		R^2	
					mean	S.D.	mean	S.D.	mean	S.D.
Attributes	Fruity	0.0 - 6.8	MS	7	12,5	0,4	13.1	1.1	0.55	0.05
			MIR	8	14,1	1,2	14.0	1.4	0.42	0.12
			UV-Vis	7	14,6	0,6	14.3	1.0	0.42	0.05
	Bitter	1.0 - 7.0	MS	6	12,5	0,4	13.1	0.8	0.27	0.06
			MIR	13	13,1	1,2	11.1	0.7	0.50	0.05
			UV-Vis	3	14,6	0,6	13.9	0.8	0.18	0.05
	Pungent	2.3 - 6.2	MS	6	15,7	0,4	16.1	1.3	0.26	0.08
			MIR	8	17,1	0,9	17.0	0.9	0.24	0.08
			UV-Vis	7	16,5	0,6	16.6	1.1	0.15	0.04
	Grassy	0.0 - 4.8	MS	5	18,0	0,6	17.2	0.9	0.47	0.06
			MIR	12	18,6	1,6	19.4	2.1	0.36	0.13
			UV-Vis	7	19,6	0,9	18.8	2.0	0.38	0.09
	Sweet	3.5 - 5.5	MS	6	16,5	0,4	16.2	1.0	0.36	0.06
			MIR	8	17,5	0,7	17.4	1.5	0.28	0.07
			UV-Vis	3	18,4	0,6	17.6	1.2	0.21	0.06
	Astringent	0.0 - 4.1	MS	6	19,1	0,4	19.0	1.1	0.40	0.07
			MIR	8	20,2	2,7	19.2	1.8	0.35	0.12
			UV-Vis	8	21,2	1,0	21.2	1.3	0.24	0.07
Defects (off-flavours)	Fusty	0.0 - 6.3	MS	4	15,7	1,3	15.8	0.1	0.51	0.06
			MIR	10	18,0	1,5	18.0	0.1	0.35	0.09
			UV-Vis	10	15,9	1,2	15.1	0.1	0.54	0.09
	Musty	0.0 - 6.9	MS	4	13,9	0,8	13.5	0.1	0.64	0.06
			MIR	13	15,0	0,6	15.3	0.1	0.56	0.12
			UV-Vis	8	14,9	0,4	14.9	0.1	0.54	0.03
	Winey	0.0 - 3.9	MS	4	17,5	0,8	18.1	0.1	0.58	0.06
			MIR	6	22,2	1,1	22.0	0.1	0.39	0.08
			UV-Vis	10	20,0	1,0	19.7	0.1	0.48	0.07
	Rancid	0.0 - 6.7	MS	4	12,9	0,7	12.2	0.1	0.36	0.07
			MIR	5	13,1	1,2	12.7	0.1	0.28	0.11
			UV-Vis	3	13,9	0,7	13.3	0.1	0.26	0.09

* Results expressed as the mean and standard deviation (S.D) of the 10 PLS model repetitions
rRMSEcv: relative root mean squares error of cross-validation; rRMSEp: relative root mean squares error of prediction;
LVs: number of latent variables; R^2: determination coefficient
MS: Mass-spectrometry; MIR: Mid-Infrared spectroscopy; UV-Vis: UV-Visible spectrophotometry

The model predictions for the individual techniques were consistent with the initial idea of using specific instrumental techniques to simulate certain sensory responses, confirming the use of MS, MIR and UV-Vis as electronic senses to predict descriptors based on olfactory, gustatory and visual perceptions, respectively.

In the same way, it has been proved the non-specificity of some instrumental data to correlate certain sensory perceptions, especially UV-Vis responses that are related to the visual information that is not used by the taste panel to define and score olive oil sensory descriptors. Despite the apparent non-existent correlation between these instrumental responses some descriptors, when their information is combined with other instrumental responses by data fusion it can become a valuable input to enhance the overall results. In this study, the MS suitability to explain most of the descriptors was complemented

by the non-volatile information provided by the MIR spectra, called two-block data fusion. Moreover, UV-Vis spectra were also used to contribute with additional information to form a three-block of data matrices (MS + MIR + UV-Vis) (**Table 2**).

Data fusion strategies. As mentioned above, although PLS models obtained by data fusion were better than the models built using single data, in most of the cases the differences between the combination of two- (MS + MIR) or three-blocks (MS + MIR + UV-Vis) of data were not sufficient to justify the use of an additional UV-Vis technique. Only the descriptor of fustiness achieved improved predictions by merging three-blocks of data using mid-level of abstraction, improving correlations from 0.54 to 0.64 (R^2) and lowering errors from 15% to near 13%. Notice that this off-fravor was the only one better explained by the UV-Vis spectral fingerprint and, as a consequence, this data-block should have an important contribution to the data fusion models, where the data fusion of MS and MIR did not even enhance the individual results.

For the rest of the descriptors data fusion of MS and MIR spectral fingerprints were enough to improve the PLS predictive models. Bitter, pungent, grassy, sweet and astringent notes achieved lower errors and higher correlations using a simple concatenation with low-level data fusion (2LL). Mid-level of abstraction was a better option to fuse MS and MIR data for fruity, musty, winey and rancid models. These optimal data combinations selected for each positive and negative olive oil descriptor (highlighted and bolded in **Table 3**) were compared to determine the best modeled descriptors in **Figure 4**. The best prediction results are represented with dark bars that correspond to error values (%rRMSEp) and light bars that correspond to the correlation ability defined with the coefficient of determination (R^2).

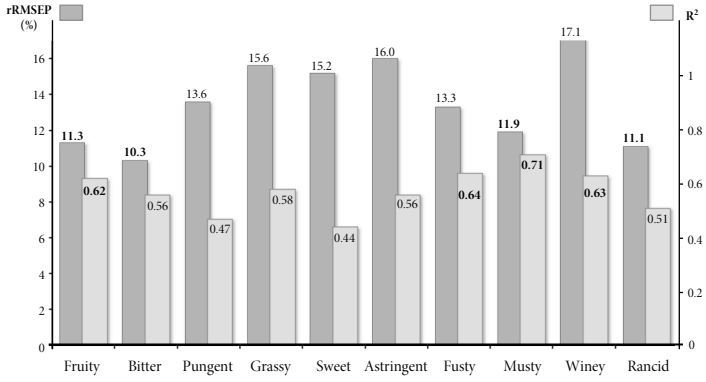


Figure 4. Relative rRMSEp and correlation (R^2) for each descriptor considering the best data fusion strategy to predict their intensities.

Table 3. Prediction results* of 2- and 3-block strategies (low- and mid- level data fusion) for each descriptor.

Descriptor		Data Fusion		LVs	rRMSEcv (%)		rRMSEp (%)		R^2	
		Strategy	Level		mean	S.D.	mean	S.D.	mean	S.D.
Attributes	Fruity	2-blocks	LL-DF	7	11,7	0,4	11.7	0.5	0.63	0.04
			ML-DF	1	9,6	0,7	11.6	1.0	0.62	0.07
		3-blocks	LL-DF	9	11,8	0,4	11.4	0.9	0.63	0.06
			ML-DF	1	9,9	0,4	11.3	0.6	0.62	0.09
	Bitter	2-blocks	LL-DF	8	10,8	0,4	10.3	0.6	0.56	0.06
			ML-DF	2	9,7	0,4	11.1	0.5	0.50	0.08
		3-blocks	LL-DF	11	11,0	0,5	10.6	0.5	0.54	0.06
			ML-DF	3	9,6	0,4	11.1	0.7	0.50	0.05
	Pungent	2-blocks	LL-DF	8	13,3	0,6	13.6	1.0	0.47	0.07
			ML-DF	1	12,2	0,8	14.0	0.7	0.41	0.06
		3-blocks	LL-DF	9	13,9	0,7	13.6	0.8	0.45	0.06
			ML-DF	3	12,0	0,6	13.7	0.8	0.43	0.05
	Grassy	2-blocks	LL-DF	8	16,8	0,8	16.1	1.4	0.54	0.09
			ML-DF	2	14,7	0,7	16.1	1.3	0.54	0.07
		3-blocks	LL-DF	10	17,2	0,6	16.3	1.0	0.53	0.06
			ML-DF	1	14,6	0,6	15.6	1.1	0.58	0.06
	Sweet	2-blocks	LL-DF	6	15,7	0,4	15.3	0.5	0.41	0.05
			ML-DF	2	14,3	0,7	15.7	0.5	0.42	0.04
		3-blocks	LL-DF	9	16,0	0,5	15.8	0.5	0.40	0.04
			ML-DF	3	14,2	0,4	15.2	0.6	0.44	0.07
	Astringent	2-blocks	LL-DF	8	16,4	0,5	16.0	0.7	0.56	0.05
			ML-DF	1	14,9	1,2	16.6	1.5	0.51	0.08
		3-blocks	LL-DF	11	16,8	0,5	16.5	0.8	0.53	0.03
			ML-DF	3	15,3	1,2	17.0	1.0	0.50	0.06
Defects (off-flavours)	Fusty	2-blocks	LL-DF	6	15,3	0,7	15.4	0.08	0.54	0.04
			ML-DF	1	14,8	0,8	14.6	0.10	0.54	0.10
		3-blocks	LL-DF	5	14,7	1,3	14.4	0.12	0.59	0.05
			ML-DF	1	13,6	0,5	13.3	0.09	0.64	0.05
	Musty	2-blocks	LL-DF	8	13,2	1,0	13.4	0.10	0.67	0.07
			ML-DF	1	11,5	1,0	11.9	0.08	0.71	0.03
		3-blocks	LL-DF	8	13,3	0,7	13.0	0.08	0.68	0.05
			ML-DF	1	11,1	0,7	12.0	0.07	0.71	0.06
	Winey	2-blocks	LL-DF	6	18,4	0,9	18.2	0.06	0.56	0.07
			ML-DF	5	16,2	0,6	17.6	0.04	0.59	0.05
		3-blocks	LL-DF	7	18,2	0,7	17.7	0.05	0.60	0.06
			ML-DF	1	16,8	0,7	17.1	0.05	0.63	0.06
	Rancid	2-blocks	LL-DF	6	12,2	0,9	11.9	0.10	0.39	0.10
			ML-DF	2	10,9	0,9	11.8	0.11	0.46	0.09
		3-blocks	LL-DF	7	12,4	0,9	11.4	0.10	0.42	0.09
			ML-DF	3	10,5	0,9	11.1	0.09	0.51	0.07

* Results expressed as the mean and standard deviation (S.D) of the 10 PLS model iterations
rRMSEcv: relative root mean squares error of cross-validation
rRMSEp: relative root mean squares error of prediction;
LVs: number of latent variables; R^2: determination coefficient
2-blocks: MS+MIR; 3-blocks: MS+MIR+UV-Vis; LL-DF: low-level data fusion; ML-DF: mid-level data fusion

The sensory descriptors with the lowest PLS errors (RMSEp) were bitter (10.3%), fruity (11.6%), rancid (11.8%) and musty (11.9%), using mid-level fusion of MS and MIR data, and also fusty (13.3%) and pungent (13.6%) using mid-level fusion of three-blocks (3ML) and low-level fusion of two-blocks (2LL), respectively. Of these models, musty (0.71), fusty (0.64), fruity (0.62) and bitter (0.56) achieved the highest correlation values (R^2). Pungent and rancid attributes were not well predicted, with R^2 values below 0.5. Pungent and sweet attributes showed low correlation values because the limited range of

intensities provided by the taste panel: 2.3-6.2 and 3.5-5.5 for pungency and sweetness, respectively. When the ranges of the reference values are too small, the intensity values required to calibrate the model are not representative of the whole variability of the attribute to be modeled. Grassy, astringency and winey attributes, with short intensity ranges, were also not well predicted, with errors higher than 16%. A similar problem occurred with the models for rancidity where, despite the wider range of intensity scores (0.0-6.7), there were only 10% of the samples with rancidity detected (scores > 0). As a consequence, the sensory data used to build models for rancidity did not represent the variability required to build suitable PLS models.

The best prediction models were obtained for descriptors of paramount importance when categorizing olive oil grades, such as fruitiness, mustiness and fustiness. This is because, as previously mentioned, the olive oil commercial category only relies on determining the presence of fruity notes and on evaluating the intensity of the sensory defects, such as musty, fusty, winey and rancid.

4. Conclusions

The suitability of instrumental sensory techniques simulating the human sensory responses obtained by a taste panel was studied. Despite the difficulty to obtain suitable prediction models for all the sensory descriptors, this study showed relevant results to further investigate in this field. The advantages of applying data fusion strategies and use complementary sensory instrumental information (electronic panel), analogously as a human taste panel does, has been demonstrated. Prediction of several sensory descriptors' intensities was performed using data fusion approaches, applied at different levels of abstraction, being able to enhance the prediction results obtained for single techniques. The best prediction abilities were obtained for four descriptors: fruitiness, fustiness, mustiness and bitterness. All of them were determined coupling MS and MIR instrumental responses working as electronic nose and tongue, respectively.

Also, specificity of the instrumental data aimed on different sensory aspect was proved, which correlated most of the smell-related descriptors to an electronic nose based on MS and most of taste-related descriptors to an electronic tongue based on MIR. Moreover, the usefulness of the visual information (color hided in the human panel) was demonstrated for all the descriptors, except for the fusty defect. However, some aspects have to be considered to understand the limitations of this study. On one side, although the human taste panel provided sensory data following an official method, these experimental data was subjected to experimental restrictions associated to these techniques. On the other side, sensory data should include more variability with wider intensity ranges and a number of samples that represent the descriptor sufficiently.

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Chapter 4. Wine Sensory Analysis

4.1. Introduction

Wine is an alcoholic beverage exclusively made from fermented fresh grapes or grape must. Similarly as olive oil, and thanks to its health benefits and organoleptic characteristics, wine is a highly appreciated product, becoming an indispensable commodity in the Mediterranean basin. World production is mainly focused in this region, being Italy the biggest producer, followed by France and Spain in 2015. Despite other countries like United States, Argentine, Chile and Australia are also great producers [1], the European Union is the world-leading producer of wine, with 45% of world vineyards, 65% of production and 57% of global consumption [2]. In a competitive wine market and after ten years of decline, Spanish wine consumption has recently increased (since 2014), mainly due to the major consumption of wines with Protected Designations of Origin (PDO). In fact, Spain has the largest vineyards extension and, because of its diverse climate conditions, it has around 250 different cultivars and 70 PDOs [2,3].

In order to achieve higher profits, wine production is commonly exposed to fraudulent practices such as addition of cheaper products or other chemicals to compensate wine defects [4-6]. For this reason, specific and continuous control programs are required to maintain the quality, by protecting the consumers from unintentional or voluntary adulteration incidents. Some international institutions have implemented mandatory regulations to minimize fraud and malpractices, ensuring economical value and market wine position. The European Union has a set of rigorous legal guidelines and a strong organizational culture towards wine quality control. In particular, the International Organization of Vine and Wine (OIV) contributes to the harmonization of the existing practices and standards with guidelines and analytical methods that become ipso facto binding within the EU [7]. The OIV provides the analytical assessment to assure quality (wine composition and additive detection) and traceability (labeling and record keeping) [5,6,8-11], as well as the assistance to other organizations, such as the European Office for Wine, Alcohol and Spirit Drinks (BEVABS) [2], to ensure the correct implementation of EU wine quality legislation. Since the introduction of the Common Market Organization (CMO), the current wine legislation (2006 [12], 2007 [13] and 2008 wine reform [14]) was modified in 2013 (CAP reform [15,16]) simplifying the market rules and enhancing reputation of European wines linked to their geographical origin and varietal

identification. Unfortunately, those regulations, good manufacturing practices and traceability procedures are not enough to guarantee the quality and authenticity of a given product, mainly due to the lack of accurate reference materials and standards that can be used to ensure proof-of-identity of wine [5].

4.1.1. Quality of the wine

The concept of wine quality is difficult to define because it has changed over time and cultures [17-19]. It is influenced by several factors, such as grape variety, vintage, winemaking process [20-21], region or vineyard management, which determine the wine composition and its organoleptic characteristics [22]. Moreover, it has to be taken into account that the perception of wine quality is basically subjective, being clearly distinguished into its intrinsic and extrinsic characteristics [19,23].

Wine intrinsic or product-related quality is associated to the wine itself, together with its physical and organoleptic properties. In general, intrinsic quality assessment is developed by winemakers or wine experts, who define specific attributes for each product [24]. These attributes are defined by the hedonic drinking experience, referred mainly to visual characteristics, along with those of bouquet (aroma) and palate (taste and mouthfeel). Appearance and color are the first characteristics perceived, revealing information about age, condition, body or possible defects [25]. Bouquet or aroma is one of the most important elements that define the wine character, with perceived intense and complex aromatic notes for high valued wines [18]. Wine palate sensations indicate both tasting descriptors, like sweetness, acidity and bitterness, and tactile or mouthfeel attributes, like astringency, velvety or viscosity [26]. Other relevant intrinsic attributes are also determined by origin, variety, age, tipicity, potential or complexity of the wines [19,27].

Wine extrinsic quality is related to consumer preferences [19]. Most consumers are not experts and usually cannot taste wine before purchasing it, so they have to rely on wine extrinsic cues for inferring quality [28]. These extrinsic cues are not specific to each product and are defined as general indicators like the country-of-origin or the image of the wine. The former is associated to a certain local area, region or country, which influences the quality perception by evoking traditions or cultures. The other factors that influence consumers'

decisions are associated to the general image of the wine, including bottling, labeling (type of wine or appellation, back label information, brand [27], label aesthetic), presence/absence of awards, price and advertising [22,28].

4.1.2. Wine quality assessment

Notwithstanding the capability of wine producers to control the process to get a suitable final product, additional tools are required to evaluate wine quality in order to guarantee quality standards [18], control the wine production process and ensure authenticity [24,29]. The composition of the wine depends on the variables that affect both viticultural and oenological stages, so it is necessary to define control parameters all along the process to maintain the quality of the product [18,30]. Additionally, when dealing with PDO wines, the control should ensure their geographical origin [18,24] and that these wines meet the requirements that each PDO dictates for guaranteeing their quality [3,31]

However, there are many other frauds that should be detected, most of them based on increasing the wine value illegally to achieve higher market prices [5]. For example, the non-authorized addition of sugar or concentrated grape must before or during fermentation to increase the natural ethanol content [4], the blending with, or replacement by, wines of lesser quality [4,5,11], the dilution of wines with water, the addition of coloring and fravoring substances or the mislabeling (misrepresenting cultivar or geographical origin) [5,11] are common illegal practices. With this regard, physico-chemical analyses are fundamental for the achievement and maintenance of high wine quality standards. Some organizations are focused on defining proper tools to address these problems and assuring wine quality. The European Union, through the OIV, BEVABS and accredited laboratories, has developed and maintained official databases that define wine parameters to establish the 'history' of wine samples. The parameters include from the common physico-chemical properties determined by classical methods to other useful information such as geographical origin, year of production, type of grape, winemaking process, soil composition and weather conditions [2,32-36].

As olive oil, wine primary quality is defined by several parameters, which are included into its composition or its organoleptic characteristics, which can be assessed by physico-chemical and sensory evaluations, respectively.

4.1.2.1. Physico-chemical evaluation

The EU Commission Regulation lists analytical methodologies to characterize both grape and wine parameters [34,37], including alcohol content (% vol), relative density, dry extract, pH, color intensity, sugars (sucrose, glucose, fructose), volatile and total acidity, specific acids (tartaric, citric, lactic and malic acids), total phenolic index, free sulphur dioxide, carbon dioxide, and other substances [27,31].

4.1.2.2. Sensory evaluation

The information obtained by organoleptic assessment is essential to ensure the final consumer acceptance [38]. Sensory parameters are not as regulated and rigorous as in olive oil, mainly because of the high variability of wine types, regions and varieties. As a consequence, the sensory evaluations are very diverse and fitted to each particular case. But as physico-chemical analyses, sensory tests must be conducted under standardized and controlled conditions in order to minimize possible bias affecting human or instrumental sensory responses [39,40].

▸ Human sensory taste panel

As described in Chapter 1, there are several methods to estimate sensory descriptors. Wine sensory analyses are mainly carried out through two different methodologies: discrimination and descriptive analysis. In both cases, the judgment of expert panelists is taken as a reference. While discrimination analyses intend to find similarities and/or differences between samples, descriptive analyses seek to evaluate both quantitative and qualitative sensory characteristics to obtain wine sensory profiles [40-45].

Descriptive wine sensory panels have the same key components as other taste panels: a suitable tasting room, a specific wine vocabulary that mainly depends on the wine variety and appellation, a selection of a group of trained panelists or assessors with an appropriate sensitivity and a well-defined analysis procedure. The common procedures indicate that each assessor must first check the visual appearance of the samples, then smell and finally taste the wine samples, but in each case the sensory descriptors and/or their intensity evaluations have to be clearly specified in advance. The scores of these evaluations are written down on a profile sheet (**Figure 4.1**).

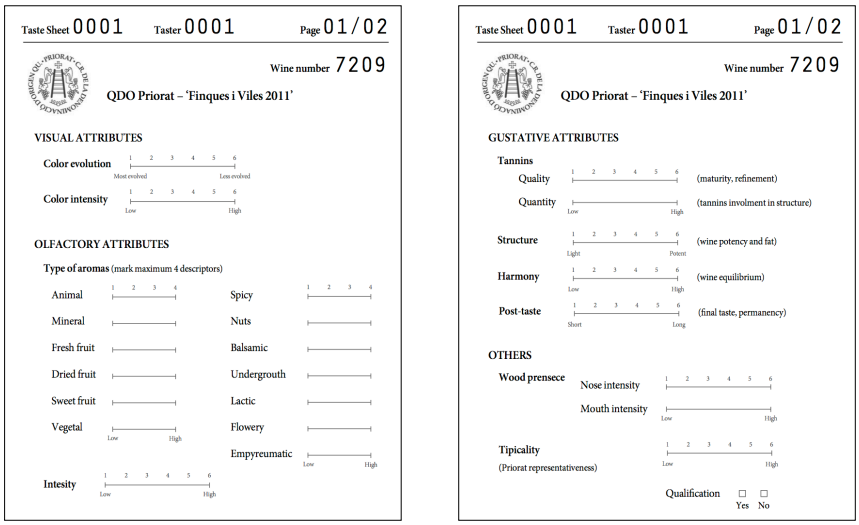


Figure 4.1. Example of a profile sheet used by the 'Parc Tecnològic del Vi' in Falset (Catalonia) for typical wines from the Priorat region.

Sensory descriptors

Usually descriptors (both positive and negative) are grouped into four main sensory groups: visual, olfactory (aromatic notes), gustatory and others. Wine is firstly perceived by vision, where color intensity and appearance aspects like clarity, viscosity, spritz (effervescence) and tears are evaluated. Then, the perception of the different aromas produced by the volatile fraction is evaluated with the olfactory system. These are commonly organized into groups, in what is called the *Wine Aroma Wheel* that contains fruity, floral, vegetal or smoky aromas, among others. Finally, wine is taken into the mouth and two different sensations are perceived: the retronasal aroma (in the nasal cavity) and taste and mouthfeel (in the oral cavity). The first is categorized as previously described by the aroma. Taste generates five gustatory sensations (sweet, umami, bitter, sour, salty), and mouthfeel gives rise to textural sensations of astringency (related to tannin presence), dryness, viscosity, heat, coolness, prickling, and pain. It has to be noted that, apart from these well-defined descriptors, it is also very common to describe other much more subjective wine descriptors, like structure, harmony or tipicity [46,47].

Apart from the standardized descriptive taste panels there are other alternative methods for wine sensory analysis, such as free profiling, free sorting task, projective mapping [23] and

Napping [41], among others. Regardless of the sensory method applied, there are many limitations that should be considered when dealing with human sensory evaluations (as previously mentioned): subjectiveness of assessors, propensity to individual preferences, high between-day and between-panelist variability, human fatigue and high costs of maintenance [27,29,41,48]. Thus, more objective and automatable techniques are demanded to overcome these problems and replace or support human sensory evaluations in a faster and more objective way.

▸ Instrumental analysis

The alternative techniques developed to correlate sensory characteristics with instrumental variables constitute what is called 'instrumental sensometry', involving simple and rapid techniques together with chemometric analysis [25,27,29]. There are two types of instrumental sensometry techniques: classical (or targeted) approaches and non-targeted approaches (as mentioned in previous chapters) .

Classical (targeted) methodologies

Classical approaches are based on target analysis where specific chemical compounds are directly related to sensory attributes. The objective of these methods is to understand the influence of the different wine components over the sensory properties and quality of wine [20,21,23,27,44]. Basically, targeted approaches assess quality working as reference methods to satisfy official regulatory requirements (dictated by OIV or the Association of Official Analytical Chemists (AOAC)), to determine authenticity, to detect frauds or to identify specific (marker) components to predict wine sensory descriptors [25]. As wine is a very complex mixture of components, many analytical parameters can be determined [6,11,29,49].

Regarding aroma components (volatiles), these have been usually extracted, isolated, identified and quantified with GC coupled to MS or other detection systems, and even sensory described if coupled to an olfactometric port (GC-O). These techniques provide a complete chemical profile of the wine volatile fraction [50-52]. Compounds responsible of taste perceptions are usually non-volatile, such as phenolic compounds, acids, sugars, salts and proteins. The wine phenolic profile plays an important role in wine sensory characteristics, being responsible of bitterness, astringency, color and some aromatic notes [6][53]. The

determination of phenolic compounds can be performed individually by HPLC-MS or NMR spectroscopy, or including all of them (total phenolic content) by colorimetry (Folin-Ciocalteu), titration (Löwenthal method), spectrophotometric or gel permeation chromatography (GPC) [6,23,25]. Objective wine color measurement is essential, being the result of a mixture of several components like monomeric anthocyanins and polymeric pigments combined with non-colored phenolics by co-pigmentation. Different official methods based on bisulphite blenching with anthocyanins are used, such as Ribéreau-Gayon and Stonestreet, Somers and Evans, Bakker or Harbertson assays using spectrophotometric measures or CIE L^*a^*b color space measures [25,54].

However, despite the high precision and selectivity of these classical approaches, they are based on univariate measurements of individual components that are then correlated to the sensory descriptors. This approach does not consider the complex combinations between different sensory perceptions [29,50] and different compounds [17,20,23,52], hence resulting in time-consuming/expensive analyses, tedious sample preparation steps and the need of very skilled personnel [11,21,48]. Therefore, the use of non-targeted techniques is required, analyzing the sample in its entirety in order to untangle wine constituent interactions and understand whole matrix combined effects.

Non-targeted methodologies

Non-targeted approaches consider the signal coming from the whole sample including interferences and interactions [18] and allow correlating sensory data with instrumental data by means of multivariate models. As described in Chapter 1, instrumental data are mainly obtained by indirect methods using spectroscopic, spectrometric and sensor techniques that provide a unique chemical fingerprint of each sample and without identification, full separation or quantification of the single compounds [5,11,25,29].

Among the different non-targeted approaches, NIR and MIR spectroscopies have been widely applied as quantitative methods to predict several oenological parameters such as alcoholic degree, density and specific gravity, total acidity, pH, volatile acidity, glycerol, total polyphenol index or reducing sugars [55-57]. Although these parameters may be associated to sensory descriptors (e.g. total acidity with sensory acidity), these work as targeted approaches, limiting the possibility to use the whole spectroscopic information.

As mentioned in previous chapters, the first attempts to find correlations between sensory descriptors and instrumental signals were performed with multisensor systems. There are several available commercial systems in the market based on gas sensor arrays (e-noses) [58-61]; and electrochemical sensors (e-tongues) [25,62-66]. However, due to the limited sensitivity of these sensors to certain compounds and the problems with ethanol interferences, these techniques have been frequently replaced by new spectral-based techniques. MS coupled to a headspace sampling system is an example of electronic nose, which provides a mass spectral fingerprint of the wine volatile fraction [4,29,58,67-69]. In the case of electronic tongues, infrared spectroscopy (mainly MIR) provides a compositional fingerprint that can be related to taste attributes [25,70-73]. Finally, regarding wine color description, the spectra collected from UV-vis spectrophotometry can be related to several chemicals associated to different colors (mainly anthocyanins or melanoidins) and can replace traditional methods based on a system of standard colors [25,74-77]. In all cases, to correlate these spectral responses with the perceived sensory properties, the use of proper multivariate analysis techniques becomes essential.

Despite the good results and rich information obtained from individual spectroscopic or spectrometric instruments, the possibility to combine information from several instrumental sources may provide a more reliable and accurate system to emulate human sensory responses as an 'electronic panel'. This is the reason why there is an increasing development of different data fusion strategies. For example, the data coming from the analysis of physico-chemical parameters, spectra, chromatograms or sensor signals can be analyzed jointly from an extended data set using suitable pre-processing and variable selection techniques prior to data fusion. It is very common to fuse data from similar techniques like NIR and MIR spectroscopies [78], but the simultaneous use of the data provided by electronic noses, tongues and/or eyes increase the amount of information extracted from a sample. This is shown in some studies found in the literature that have applied data fusion techniques to predict wine sensory attributes [21,79-86].

4.2. Electronic Panel

As explained in Chapter 3 (Section 3.4), among the different possible groupings of analytical instruments to setup an electronic panel, in this Thesis a combination of FT-IR spectroscopy (e-tongue), HS-MS (e-nose) and UV-visible spectrophotometry (e-eye) has been chosen. Once the wine samples were analyzed by the three techniques, the responses obtained were processed through data fusion techniques to predict the intensity of sensory attributes that were previously scored by an expert taste panel.

Though experimentals results are provided in this chapter, this is a preliminary study to apply an electronic panel for wine sensory analysis. The material and methods, results and colclusions are shown in a paper format.

4.2.1. Wine sensory analysis

A total of 78 red and aged wine samples were collected from the same vintage (2009) and production area (QDO Priorat). To ensure the representativeness of the samples, these were obtained from 41 different wineries of 12 villages located in the Priorat area (Tarragona, Spain): la Morera de Montsant, Scala Dei, Gratallops, Porrera, Poboleda, Torroja del Priorat, la Vilella Alta, la Vilella Baixa, El Lloar, Bellmunt del Priorat, el Molar and Falset. Wines contained different percentages of several grape varieties, including garnatxa, carinyena, cabernet sauvignon, syrah and merlot.

Wine sensory attributes were evaluated by 42 wine tasting panelists previously trained to evaluate the following 11 general attributes: color evolution and color intensity (visual perception), aroma intensity (olfactory perception), tannin quality, tannin quantity, structure, harmony and post taste (taste perception), nose wood, mouth wood and typicality (other sensations). All of them were scored into a 1 to 6 scale. Moreover, 13 specific aromatic notes were also tested and scored into a 1 to 4 scale. These were animal, mineral, fresh fruit, dry fruit, sweet fruit, vegetal, spicy, nuts, balsamic, undergrowth, lactic, flowery and empyreumatic notes. In all cases, one means the lowest perception and 4 or 6 the maximum perception, except for color evolution that rated oppositely (detailed in **Figure 4.1**). The mean of the resulting scores from the different panelists were calculated to build the models.

4.2.2. Wine instrumental sensory analysis

The sampling techniques used and the operational conditions of the different instruments that constitute the electronic panel are described below.

▶ Electronic nose based on HS-MS spectrometry

The volatile fraction was analyzed by headspace coupled to a mass spectrometry detection system (HP5793N mass selective detector) using the configuration described in **Figure 4.2**. For wine samples, where volatiles are more easily perceptible than for olive oil, a static HS system (G1888 Network Headspace Sampler) was sufficient to extract the aromatic compounds. Therefore, 5 ml of wine were placed into 10 ml vial containing NaCl and closed by a PTFE/silicone septum. The sample was first equilibrated (step 1, **Fig. 4.2**) during 1h under constant stirring at 65ºC to promote the headspace enrichment and to improve the extraction of the volatiles (step 2, **Fig. 4.2**). Then these were conducted to the loop where they were transferred to a 250ºC injection port in splitless mode during 5 min, with the loop and transfer line kept at 80 and 90ºC, respectively.

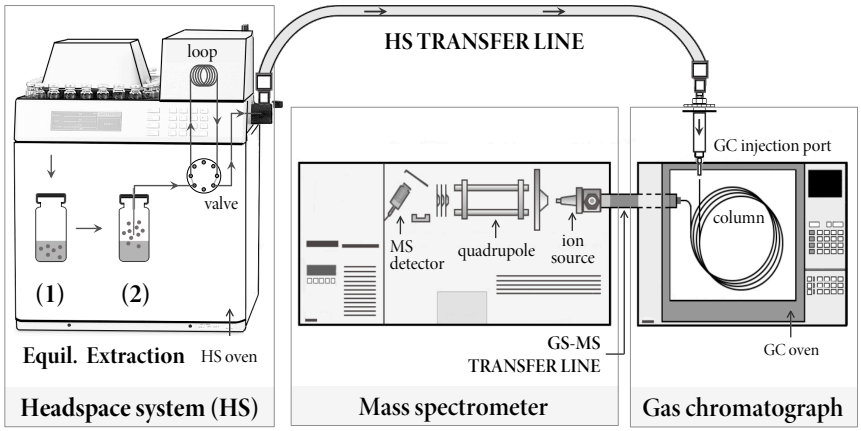


Figure 4.2. Headspace (HS) sampling system and GC-MS detection system used to extract wine volatiles. Structure and description of the main components.

Since the objective was to get a spectrum-fingerprint, no chromatographic separation was necessary; so, the GC column solely acted as a transfer line between injector and detector, avoiding ethanol interferences with a simple separation. In this way, all volatiles reached the

MS system in 5 minutes. Mass spectra were recorded at 70 eV in electron impact mode and in a mass range from 50 to 200 amu. Sample duplicates were analyzed and average spectra were used to build the models.

▸ Electronic tongue based on FT-MIR spectroscopy

The non-volatile wine fraction was analyzed using an FT-MIR Nexus spectrometer. The sampling system used was an autosampler Bacchus TDI (Gavá, Spain) with transmission liquid cells of ZnSe and 0.025 mm width (**Figure 4.3**). Triplicate transmittance spectra were recorded for each sample at 4 cm^{-1} resolution, from 4000 to 400 cm^{-1}, after ultrasound degasification. A water-blank was measured prior to each analysis in order to avoid water absorption bands.

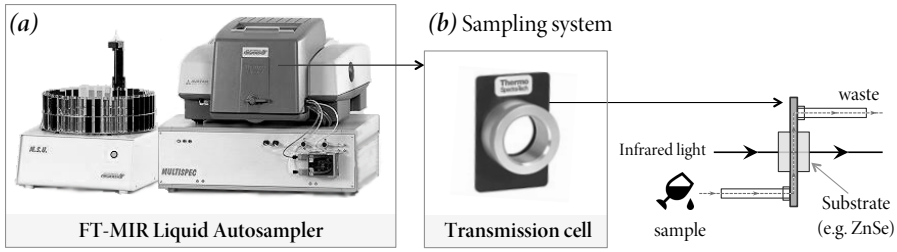


Figure 4.3. Wine adapted FT-MIR autosampler Bacchus TDI (a), IR sampling system and transmittance principle (b).

▸ Electronic eye based on UV-visible spectrophotometry

Color measurements were obtained with a spectrophotometer UV-visible Helλos Gamma (Thermo), equipped with a diode array detection system. The samples were analyzed in duplicate at room temperature with a quartz cuvette of 1 mm pathlength and the spectra were acquired from 250 to 700 nm at 1 nm resolution.

4.2.3. Wine and e-panel

The experimental procedure carried out in this study is shown in **Figure 4.4** and consists on a previous sensory evaluation performed by a wine taste panel followed by the instrumental measurement of the samples. Predictive PLS models were built using the responses of the single instruments and the combined responses of all of them using low- and mid-level data

fusion strategies (procedure described in Chapter 1 (section 1.3.2)). Instrumental responses (spectra) obtained by the different instruments were pre-processed and variable selected in a suitable way before building the final multivariate models.

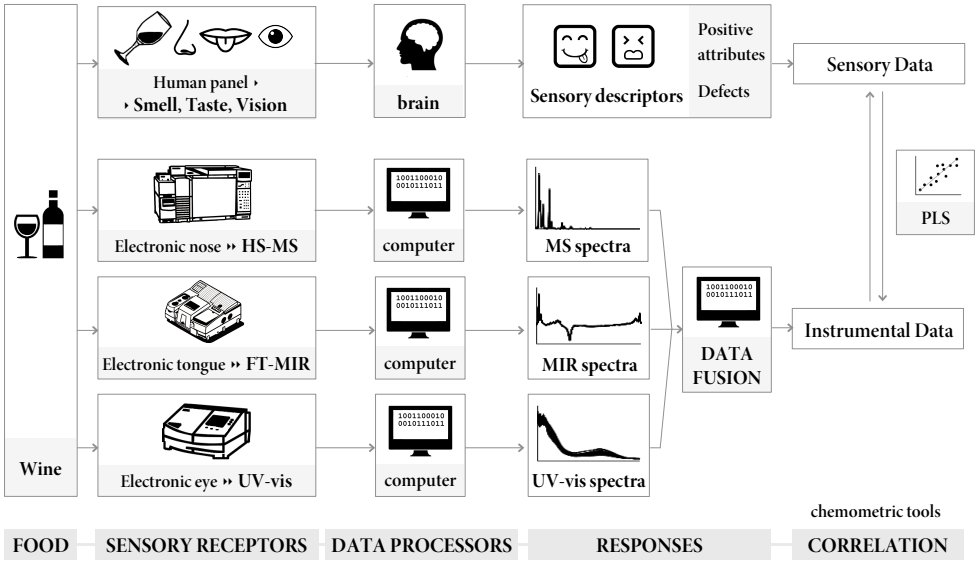


Figure 4.4. Electronic panel system for wine sensory descriptors. Correlation between human sensory analysis and data fusion techniques.

Taste panel responses were statistically processed for all the descriptors; however, one of the main problems of the wine samples analyzed was their wide variability due to their different grape variety and the different percentage of each variety in each sample. As a consequence, the taste panel scores for the wine descriptors had high standard deviations and large score ranges. For this reason, is this study only the descriptors with the lowest score deviations were considered, and being representative of each sense/instrumental response: nose wood (smell), tannin quality (taste) and color evolution (vision).

Regarding instrumental data, these were treated to predict the selected sensory descriptors either individually or combined using low- and mid-level data fusion following the same strategies described in section 3.5 for olive oils. Resulting spectral-fingerprints for all 78 samples are shown in **Figures 4.5**, **4.6** and **4.7**.

Wine mass spectra can be considered representative of the smell-fingerprints of each sample (**Figure 4.5**); however, the elucidation of the compounds responsible of the sensory perception is complex, because no chromatographic separation was performed. For this reason, to get the variables (in this case m/z ratios) that are better correlated with certain sensory descriptors, multivariate techniques like PLS were applied. Afterwards, these m/z were associated to specific volatile compounds present in the wine samples.

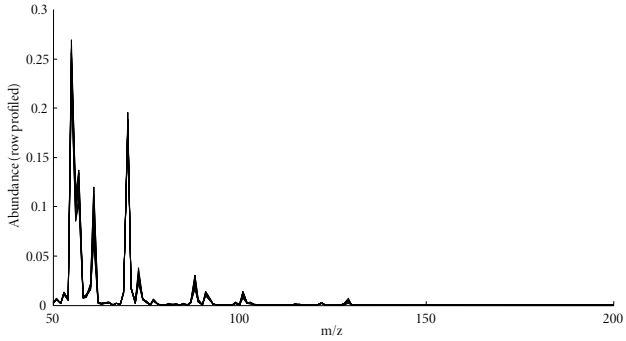


Figure 4.5. Wine spectra obtained by electronic nose based on HS-MS.

A summary of the most common wine volatile substances determined in the literature are shown in **Table 4.1**, together with their main related sensory attributes and the most important MS ions (m/z) obtained by electronic impact (EI) mode.

Of the many different compounds found in wines, the ones that are usually related to the olfactive descriptor considered in this study (nose wood) are some phenolic compounds, terpenes and lactones, together with isoamyl propanoate, capric acid and hexanal. In fact, phenolic compounds like guaiacol, eugenol, isoeugenol, vanillin and methyl/ethyl vanillate, and terpenes like linalool, lonalool oxide, p-cymene and β-sanatol provide wine woody notes, although it has to be noted that they also contribute to other sensory descriptors such as phenolic or floral aroma. Moreover, most of the lactones, even being described with very different descriptors, are very closely related to the overall perception of woody aromatic notes. This is important, because compounds like γ-octalactone, γ-nonalactone, δ-decalactone, whiskylactone and β-damascenone are commonly added to synthetic wines to simulate the woody-like aroma and to train sensory panels [100].

Table 4.1. Summary of the main wine volatile compounds identified in the literature.

Compound	Descriptors	Main MS peaks[1]	CAS number	Ref.
Alcohol				
1-propanol	alcohol, ripe fruit	59	71-23-8	[20,52,87,88]
1-butanol	medicinal, phenolic, fruit	55	71-36-3	[20,52,89,90]
2-methyl-1-propanol	fusel, alcohol, solvent	67	123-51-3	[52,88,89,91]
2-methyl-1-butanol	fusel, alcohol, sweet, fruity	55	137-32-6	[52,89,91]
3-methyl-1-butanol	fusel, alcohol, sweet, fruity	55, 70	123-51-3	[20,52,88,89,91]
1-pentanol	bitter almond, synthetic	55, 70	71-41-0	[88,90,92]
1-hexanol	herbaceous, resin, grass	55, 56	111-27-3	[20,52,89,91]
3-hexen-1-ol	plant, fruity, aromatic, grass	41, 67	928-96-1	[20,52,89]
1-octanol	intense citrus, roses	55, 69, 70, 83	111–87-5	[89,91]
1-octen-3-ol	green, moldy	27, 72	3391-86-4	[87,88,92,93]
benzyl alcohol	flower, sweet, citrus	77, 79, 108	100-51-6	[20,52,89]
2-phenylethanol	flower, rose, honey	91, 92	60-12-8	[20,52,89,91]
2,3-butanediol	fruit	57	513–89-3	[52,91]
glycerol	sweet	61, 73	56-81-5	[88,94]
Esters and acetates				
ethyl acetate	pleasant, rich, sweet	61, 70, 88	141-78-6	[20,88,95]
isobutyl acetate	fruity	56, 61	110-19-0	[96-98]
isoamyl acetate	fruity, banana	55, 70, 87	123-92-2	[52,88,89,96-100]
hexyl acetate	fruity	55, 56, 84	142-92-7	[89,92,96,98,99]
2-phenylethyl acetate	floral, rose, violet	78, 91, 104, 105	103-45-7	[20,52,88,101,102]
ethyl lactate	butter, cream, fruit, lactic	55	97-64-3	[52,89,91]
ethyl propionate	fruity	57, 102	105-37-3	[52,87]
isoamyl propanoate	woody/tobacco, green pepper	55, 61, 70, 73	105-68-0	[87]
ethyl 3-hydroxybutyrate	fruity	117	5405-41-4	[20,52,89]
diethyl hydroxybutyrate	fruity, sugar, sweet	71, 89, 117	626-11-9	[52,89]
ethyl butyrate	floral, fruity, banana, pineapple	60, 71, 88	105-54-4	[20,52,87,89,96,97]
ethyl 2-methylbutyrate	fruity	57, 102, 74	7452-79-1	[87,88,91]
ethyl isovalerate	fruity, apple, berry	57	108-64-5	[52,89,101]
ethyl caproate	fruity, green apple, banana	55, 88, 99	123–66-0	[20,52,88,91]
ethyl caprylate	ripe fruits, pear, pineapple	55, 88	106–32-1	[20,52,89,99,103]
ethyl caprate	grapes	88, 101, 155, 157	110–38-3	[20,88,91,102-104]
ethyl laurate	sweet, waxy, fruity, apple	88, 101, 157, 183	106–33-2	[87,89,91]
ethyl myristate	sweet, waxy	88, 89, 101, 157	124-06-1	[87,89]
monoethyl succinate	chocolate, floral spicy	55, 73, 101, 128	1070-34-4	[52,105]
diethyl succinate	spicy, wine	101, 129	123-25-1	[20,52,89]
Acids				
acetic acid	vinegar, acid, fatty	60	64-19-7	[52,87,88,94,104]
isobutyric acid	fatty-rancid	55	79-31-2	[52,89,91]
butyric acid	fatty-rancid, cheesy, sweaty	60, 73, 89	107-92-6	[20,52,89,94,103]
2-methyl butyric acid	fatty-rancid, cheesy	57, 74, 87	116-53-0	[20,52,89]
isovaleric acid	fatty-rancid, cheesy	60, 87	503-74-2	[20,52,89]
caproic acid	rancid, green, grass, fruity	60, 73, 87	142-62-1	[20,52,89,103]
caprylic acid	fatty acid, dry, dairy, candy	55, 60, 73, 101	124-07-2	[20,52,89,91]
capric acid	fatty acid, dry, woody, rancid	57, 60, 73, 87, 129	334-48-5	[20,52,89,94,103]
benzoic acid	floral, balsamic	51, 77, 105, 122	65-85-0	[52,89,103]
Phenols				
guaiacol	phenolic, smoky, woody	81, 109, 124	90-05-1	[52,100, 106,107]
4-ethylguaiacol	phenolic, leather-like, toasted	137, 152	2785-89-9	[89,91,99,100]
4-vinylguaiacol	clove, phenolic, spicy	77, 107, 135, 150	7786-61-0	[20,52,99,100,106]
m-cresol	phenolic, spicy, leather-like	77, 79, 107, 108	108-39-4	[89,103]

Table 4.1. *Continued.*

Compound	Descriptors	Main MS peaks[1]	CAS number	Ref.
4-ethylphenol	must, phenolic, leather-like	77, 107, 122	123-07-9	[20,23,52,89,103]
4-vinylphenol	sweet, spicy, pharmaceutical	120	2628-17-3	[89,101,103,105]
syringol	phenolic	111, 139, 154	91-10-1	[52,89]
eugenol	spicy, woody	77, 103, 149, 164	97-53-0	[23,89,99,107,108]
isoeugenol	woody	77, 103, 149, 164	5932-68-3	[89,104,107]
vanillin	vanilla, woody, phenolic	81, 109, 151, 152	121-33-5	[98-100,108,109]
methyl vanillate	vanilla, woody	-	3943-74-6	[23,52,103,108]
ethyl vanillate	vanilla, chocolate, woody	123, 151, 152	617-05-0	[23,52,89,103]
acetovanillone	caramel, vanilla	123, 151, 152, 166	498-02-2	[20,52,89,108,109]
Aldehydes				
acetaldehyde	visual, oxidation	<50	75-07-0	[87,91,94,97,100]
benzaldehyde	bitter almond, burnt sugar	77, 105, 106	100-52-7	[20,52,89]
benzenacetaldehyde	honey, floral rose, chocolate	65, 91, 92, 120	122-78-1	[25,89]
hexanal	green. woody	56, 57, 67, 72	66-25-1	[87,91,98,108]
2-furfural	caramel	95, 96	98-01-1	[52,89,91,106]
5-methyl furfural	sweet, caramel	53, 109, 110	620-02-0	[89,91]
Terpenes				
α-terpineol	floral, oil, anise, mint	59, 93, 121, 136	10482-56-1	[20,52,89,91,106]
terpinen-4-ol	flowers	71, 91, 93, 111	562-74-3	[89,91]
β-citronellol	rose	67, 69, 81, 82, 95	106-22-9	[20,52,89]
linalool	flower, fruity, woody	71, 93, 121, 136	78-70-6	[20,25,87,102,106]
linalool oxide	woody, floral, herb	59, 93, 94, 111	5989–33-3	[89,91,95]
nerol	flower, grass	67, 69, 93	106-25-2	[20,52,89,91]
geraniol	rose, geranium	67, 69, 93	106-24-1	[20,52,89,91,106]
E,E-farnesol	muguet (flower)	69, 81, 161	106-28-5	[20,52,89]
Z-4-carene	-	93, 121, 136	29050-33-7	[91]
p-cymene	woody, spicy	91, 119, 134	99-87-6	[91]
β-santalol	woody, sweet	93, 94, 121	11031-45-1	[91]
Sulphur compounds				
methionol	potato, baked cabbage	61, 88, 105, 106	505-10-2	[20,52,89,101,103]
methional	vegetable	61, 76, 104	3268-49-3	[89,101,104]
2-methyl-3-furanthiol	meat, fish, metallic	85, 113, 114	28588-74-1	[97,101]
3-thiohexanol	sulfurous, fruity, tropical	55, 57	51755-83-0	[97,101]
ethanethiol	off-fravor	62	75-08-1	[99,100]
dimethyl sulfide	vegetable	61, 62	75-18-3	[45,104]
Carbonyl compounds, lactones and enolones				
γ-butyrolactone	sweet, butter, empyreumatic	56	96-48-0	[52,89-91,98,103]
γ-caprolactone	swwet, creamy, tobacco	56, 57, 70, 85	695-06-7	[89]
γ-octalactone	woody	85	104-50-7	[99,100,103]
γ-nonalactone	woody, over-ripe fruit	57, 85, 157	104-61-0	[87,100,103,106]
δ-decalactone	woody, spicy	57, 85, 99	706-14-9	[89,91,97,100,103]
pantolactone	liquorice, coconut	57, 71	599-04-2	[20,89]
E-whiskylactone	woody, coconut, toasted	69, 71, 87, 99	39638-67-0	[23,25,89,99,107]
cis-whiskylactone	woody	69, 71, 87, 99	55013-32-6	[52]
β-damascenone	woody, sweet, fruity, earthy	69, 121	23726-93-4	[25,99,103]
sotolone	sweet, caramel, sugar, coffee	55, 83, 128	286644-35-9	[89,97]
furaneol	sweet, pineapple	57, 85, 128	3658-77-3	[97,101,104,107]
homofuraneol	bready, sweet, caramel	142	27538-10-9	[97,101,104]
acetoin	buttery, lactic, flowery	88	513-86-0	[52,89,91,103]

[1] Only m/z higher than 50 (acquired range by MS based e-nose between 50-200)

As with mass spectral data, **mid-infrared spectra** also provide fingerprints of each analyzed sample (**Figure 4.6**), though in this case the signals obtained are due to the presence of non-volatile substances that can be connected to gustative perceptions. Wine IR spectra are complex, so it becomes necessary to select absorptions at specific frequencies (cm^{-1}) or blocks of frequencies to find the relationships with sensory descriptors.

The most representative wine absorption bands, molecular vibrations, functional groups and substances associated are described in **Table 4.2** and highlighted in **Figure 4.6** [70,71, 110,111].

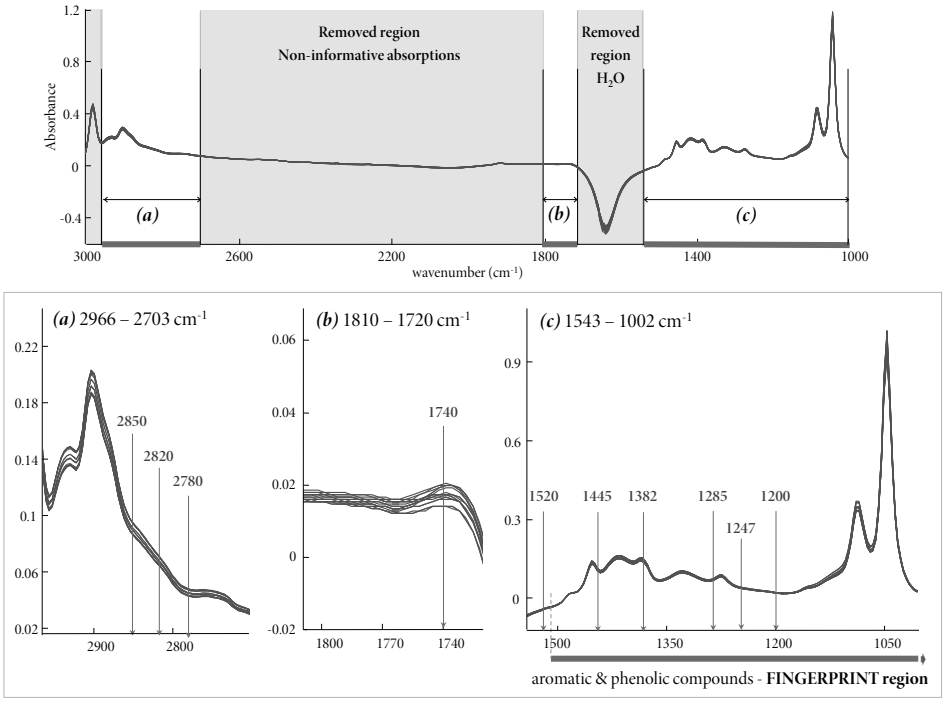


Figure 4.6. Wine spectra obtained by electronic tongue based on FT-MIR. Removed regions (grey) at 1543-1716 cm^{-1} (water) and 1812-2699 cm^{-1}.

Due the high complexity of the MIR wine spectrum, in this study it was divided into three principal regions (**Figure 4.6 a, b, c**) and the zones with not useful information were removed. For example, the highest frequencies (> 2970 cm^{-1}) do not provide valuable information because they describe the stretching O-H vibrations from water and hydroxylated molecules,

or the regions between 2699 and 1812 and 1716 and 1543 cm^{-1}, which contain non-informative and strong absorption bands, respectively [110,111].

Table 4.2. Molecular vibrations and compounds associated to wine absorption bands from MIR spectra.

Frequency (cm^{-1})	Functional groups	Molecular vibrations	Groups ⇢ Compounds
2969–2699	⇢ grape tannins		
2970–2845	C-H	stretching, $\nu_{asym/sym}$	aliphatic methyl (–CH_3), methylene (-CH_2-) & methyne (>CH-) ⇢ fatty acids, polyols (glycerol), free phenolic acids, catechins, cholesterol, carotenoid pigments
2850–2815	C-H	stretching, ν	special methyl (O–CH_3) ⇢ methoxy, methyl ether
2820–2780	C-H	stretching, ν	special methyl (N–CH_3) ⇢ methylamino
2000–1660	C=C	overtones	aromatic combination bands
1812–1716	-	-	⇢ **grape tannins**
1740	C=O	stretching, ν	carbonyl groups ⇢ galloyl unit on epicatechin gallate
1718	C=O	stretching, ν	carbonyl group ⇢ esters & fravors ⇢ **oak tannins** (hydrolyzable)
1700	C=O	stretching, ν	carbonyl groups ⇢ organic acid
Fingerprint region (1577 - 933 cm^{-1})			
1577-1060	-	-	⇢ **grape tannins**
1510–1450	C=C–C	stretching, ν	aromatic ring ⇢ aromatic compounds ⇢ **polyphenols**
1520, 1445	-	-	⇢ **grape tannins** (strong bands)
1485–1445	C-H	bending	aliphatic methylene (-CH_2-)
1470–1430	C-H	bending $_{asym/sym}$	aliphatic methyl (–CH_3)
1450-1410	C-O	stretching, ν_{sym}	-
1407	COO-	stretching, ν_{asym}	carboxyl ion (COO-) ⇢ carboxylic acid, ester, carbonyls
1410–1310	O-H	bending, $\delta_{in\text{-}plane}$	hydroxy group ⇢ phenol & alcohols
1395–1385	C-H	multiplet	aliphatic methyl (–CH_3) ⇢ trimethyl or "tert-butyl"
1385–1380	C-H	doublet	aliphatic methyl (–CH_3) ⇢ gem-dimethyl or "iso"
1382	O-H	bending, $\delta_{in\text{-}plane}$	hydroxy group ⇢ **polyphenols**
1380–1370	C-H	bending $_{asym/sym}$	aliphatic methyl (–CH_3)
1370–1365	C-H	doublet	aliphatic methyl (–CH_3) ⇢ gem-dimethyl or "iso"
1365	C-H	multiplet	aliphatic methyl (–CH_3) ⇢ trimethyl or "tert-butyl"
1350	S=O	stretching, ν	sulfonates groups
1350	C-H	bending, $\delta_{in\text{-}plane}$ bending, $\omega_{wagging}$	methyne (>CH-) & methylene (-CH_2-)
1350-1000	C-C	stretching, ν	skeletal vibrations
1340-1160	C-OH	stretching, ν	hydroxy group ⇢ **phenol**
1350–1330	C-H	bending, $\delta_{in\text{-}plane}$	methyne (>CH-)
1310–1290	C–H	bending, $\delta_{in\text{-}plane}$	vinylidene
1300	S=O	stretching, ν	sulfate groups
1285	C–O	stretching, ν	ethereal C-O & pyran-derived ring structure ⇢ **flavonoid-based tannins** (condensed and hydrolyzable)
1247	C-O	stretching, ν	esters ⇢ **flavonoid type compounds**
1270-1000	C–H	bending, $\delta_{in\text{-}plane}$	aromatic group C-H ⇢ aromatic compounds & sugars
1200	C–O	stretching, ν	carbonyl group phenol ⇢ **oak tannins**
1175	S=O	stretching, ν	sulfonates groups
1150	S=O	stretching, ν	sulfate groups
1100-970	C-O	stretching, ν	carbonyls ⇢ glucose, oligo- & polysaccharides
	O-H	stretching, ν	alcohols ⇢ ethanol
1060–933	-	-	⇢ **grape tannins**
1055-925	C-H	bending, $\delta_{in\text{-}plane}$	methylene (-CH_2-) from cyclohexane ring ⇢ polyphenolic compounds ⇢ **grape tannins**

To study the taste descriptor selected (tannin quality), the spectral signals associated to tannin compounds had to be identified. This is a complicated task due to the relative small responses of these compounds and the combination of absorptions in the same spectral region in which ethanol, organic acids and other polyphenols absorb too. The most characteristic signals of grape tannins are two major peaks at 1520 and 1445 cm^{-1} located at the same region where aromatic ring C=C-C stretching vibrations absorb, and a band at 1285 cm^{-1} corresponding to C-O stretching deformation of the pyran-derived fragment of flavonoid based tannins. The most characteristic region, called *fingerprint region* (1577-933 cm^{-1}), contains specific information of the wine samples. In this case, there are several peaks, corresponding to stretching and bending vibrations of phenols (O-H) and aromatic compounds (C-H) (**Table 4.2**) [70,71,110,111].

Finally, wine **UV-visible spectra** offer color-fingerprints that can be related to color descriptors (**Figure 4.7**). It has to be considered that red wine contains multiple colored compounds, but the information provided from specific chemical compounds is not sufficient to predict the wine color. As a result, the combined and unspecified data provided by the whole UV-visible spectrum should contribute to the wine color explanation.

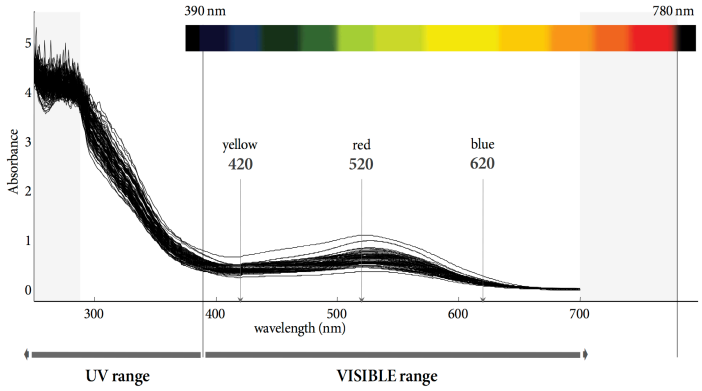


Figure 4.7. Wine spectra obtained by electronic eye based on UV-visible spectrophotometry. Removed region (grey) at 200-290 nm (instrumental noise).

The wine color evolution is inevitable, showing different colorations (and spectral responses) depending on the wine age. For example, young red wines are usually intensive reddish with violet tones, with a red spectral maximum at 520 nm together with other smaller bands at 420

and 620 nm from yellow and blue absorptions, respectively. Through the years, wines tend to evolve and reduce the red tones and increase the yellow components from rusted color (5 years) to brownish tones (20 years). This color-time evolution is determined by their chemical composition, especially of wine phenolic compounds. Non-flavonoid phenolic compounds do not directly contribute to the wine color, but act as co-pigments that module the wine color. The main responsible compounds of wine coloration are the flavonoid phenols, such as flavonols, anthocyanins and flavan-3-ols (catechin, condensed tannins and proanthocyanidins). In particular, there is a strong contribution of anthocyanins to the final reddish-blue color and flavonols to the yellow tones [25,54,76,77,112,113]. However, it has to be pointed out that the presence of non-flavonoid phenolic compounds is also crucial because these are the most important co-pigments that module the wine color.

Although those individual instrumental responses have a direct relationship with specific sensory descriptors, these are also influenced by other perceptions detected with other senses. For example, color evolution is an attribute that the taste panel evaluates visually and that can be easily related to electronic eye responses. However, this attribute is highly associated to colored compounds like tannins, which can be also detected by an electronic tongue. So, the combination of the different instrumental responses, working as an electronic panel, may provide complementary information to improve the final prediction of sensory attributes.

4.2.3. Results and discussion

For both individual and data fusion strategies, different pre-processing and variable selection combinations were tested depending on the sensory parameter to be modeled. Data fusion was applied at two levels: low-level, where data matrices were simply concatenated prior to PLS model building; and mid-level, where PLS models were built for each individual data block to then join characteristic features (latent variables) to build the final PLS model.

The prediction criteria to select optimal PLS models were the same as described in previous chapters. The number of latent variables was chosen with leave-one-out cross-validation using a random subset of the samples (70% of calibration set) and considering the lowest root mean squares error (RMSECV). The remaining 30% of the samples were used for external validation (test set) to obtain the final prediction parameters: root mean square error of

prediction (RMSEP) and determination coefficient (R^2p). The final results obtained by the different strategies for each studied descriptor are shown in **Table 4.3.**

All data fusion strategies improved the prediction results obtained by individual techniques. In order to simplify these results a Pareto diagram was built (**Figure 4.8**) with the RMSEP plotted against the determination coefficients (R^2p).

Table 4.3. Test-validation PLS results of individual and data fusion strategy for each descriptor studied (*). Bold and highlighted techniques are the best strategies selected for each descriptor.

Descriptor	Strategy	Technique	Data fusion level	RMSEP		R2p	
				mean	S.D.	mean	S.D.
Nose wood	Individual	MS	-	0.26	0.04	0.38	0.17
		MIR	-	0.34	0.06	0.05	0.05
		UV-vis	-	0.30	0.01	0.19	0.10
	2-blocks DF	MS + UV-vis	Low-level	0.25	0.02	0.53	0.15
		MS + UV-vis	**Mid-level**	**0.25**	0.04	**0.56**	0.15
	3-blocks DF	MS + MIR + UV-vis	Low-level	0.25	0.04	0.42	0.18
		MS + MIR + UV-vis	Mid-level	0.24	0.04	0.56	0.11
Tannin quality	Individual	MS	-	0.24	0.03	0.23	0.15
		MIR	-	0.22	0.03	0.42	0.13
		UV-vis	-	0.25	0.03	0.23	0.10
	2-blocks DF	**MIR + UV-vis**	**Low-level**	**0.16**	0.02	**0.70**	0.08
		MIR + UV-vis	Mid-level	0.16	0.04	0.65	0.17
	3-blocks DF	MS + MIR + UV-vis	Low-level	0.16	0.03	0.70	0.07
		MS + MIR + UV-vis	Mid-level	0.15	0.03	0.72	0.11
Color evolution	Individual	MS	-	0.28	0.05	0.23	0.12
		MIR	-	0.27	0.04	0.38	0.20
		UV-vis	-	0.15	0.01	0.79	0.05
	2-blocks DF	MIR + UV-vis	Low-level	0.14	0.02	0.83	0.05
		MIR + UV-vis	**Mid-level**	**0.13**	0.02	**0.86**	0.03
	3-blocks DF	MS + MIR + UV-vis	Low-level	0.13	0.02	0.85	0.05
		MS + MIR + UV-vis	Mid-level	0.13	0.02	0.84	0.05

(*) Results indicated as a percentage and presented as mean (standard deviation) of the 10 models.
MS: headspace/mass spectrometry, MIR: mid-infrared spectroscopy, UV-vis: UV-vis spectroscopy

When the individual techniques are only considered, after optimal pre-processing and spectral region selection, the obtained prediction results confirmed the correlation between each sensory descriptor with the corresponding sensory device (electronic nose, tongue or eye). So, the most suitable single techniques to predict specific descriptors were: HS-MS (e-nose) data to predict aromatic attributes like *nose wood* (RMSEP = 0.26), FT-MIR (e-tongue) data to predict tasting sensations due to *tannin quality* (RMSEP = 0.22), and UV-visible (e-eye) data to predict visual descriptors like color evolution. Once the optimal spectral regions were selected, the variance in projection (VIP) scores for the different variables were studied

to understand the chemical information provided by the different sources. Nose woody notes were predicted by the HS-MS spectral region between 50 and 150 amu and were mainly described by the VIP variables m/z 110, 95, 81, 82, 109 and 59. These m/z values were related to woody descriptive compounds such as guaiacol, vainillin and linalool oxide and other substances that describe other sensory properties (5-methyl furfural, 2-furfural, β-citronellol, α-terpineol and E,E-farnesol) as reported in **Table 4.1** [52, 89, 95, 99, 100]. Correspondingly, the main FT-MIR region to predict tannin quality ranged from 1290 to 1100 cm^{-1}, coinciding with the IR wine fingerprint region. The variables with the highest VIP scores were 1126 and 1145 cm^{-1}, from a characteristic region where carbonyl and alcohol stretching vibrations ($\nu_{C\text{-}O}$ and $\nu_{H\text{-}O}$) occur due to the presence of grape tannins. Also, high VIP values were obtained at 1240, 1290 and 1207 cm^{-1}, corresponding to C-O stretching vibrations of flavonoid-type and oak tannins (**Table 4.2**) [70, 71, 110, 111]. Finally, color evolution achieved the best prediction using the UV-vis spectral range from 291 to 700 nm with high VIP scores from variables between 540 and 600 nm. As previously mentioned, wine color evolves from red tones (young wines) to brownish tones (aged wines), and these colors absorb at this region, with maximum absorbances at 520, 420 and 620 nm, for red, yellow and blue, respectively [54, 77, 113]. However, except for color evolution, results were not as good as the ones provided by the human taste panel, where errors (estimated as weighted standard deviations) were around 0.15.

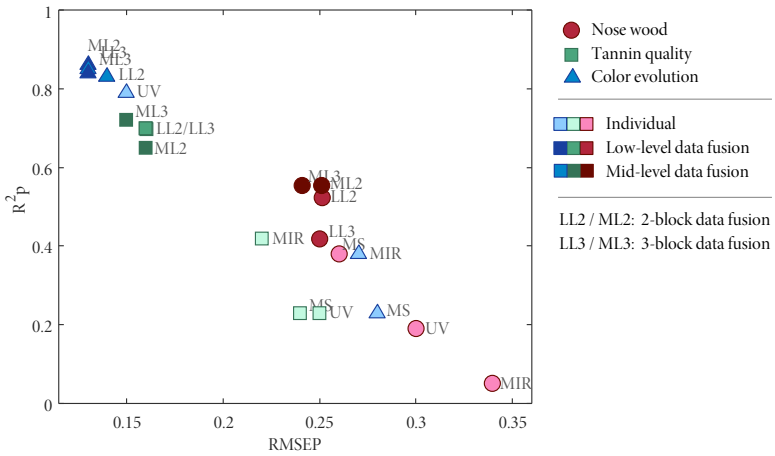


Figure 4.8. Pareto Diagram of the obtained final results to predict wine sensory descriptors. Data fusion results are represented with darker filled symbols and individual strategies by lighter filled symbols.

In order to enhance the predictions, the spectra from the three instruments were combined through different data fusion strategies. Data fusion improved the predictions for all the descriptors, lowering errors (RMSEP) and enhancing correlations (higher R^2p). Results are shown at the upper-left side of the **Figure 4.8**.

The nose wood descriptor was clearly more correlated individually by MS, followed by UV-vis. Consequently, the data fusion of both techniques, using either low- or mid-level of abstraction, notably improved the model correlation (from 0.38 to 0.56) without a significant variation of the predictive errors (around RMSEP = 0.25). When MIR data were added, in a three-block data fusion, predictions did not improve nor decreased. This is because the information provided by the MIR spectra is not related to a smell-descriptor like nose wood as it is the information of the MS spectra. However, in an unexpected way UV-Vis spectra seem to provide information related to this descriptor.

The tannin quality descriptor, that was better predicted individually by MIR, achieved better results by fusing this block with the UV-vis data, with an important error reduction (RMSEP from 0.22 to 0.16) and PLS correlation enhancement (R^2p from 0.42 to 0.70). Moreover, when including MS data on the fusion strategies the quality parameters evaluated showed a slightly improvement. This may be explained by the wide number of compounds considered when working with MS, which may provide taste-related information not determined by the other techniques.

Finally, color evolution achieved better predictions by merging MIR and UV-vis data. A slight error reduction (RMSEP from 0.15 to 0.13) but higher correlations (R^2p from 0.79 to 0.86) were obtained. In this case, the addition of MS data did not enhance the predictive results.

Both tannin quality and color evolution predictions did not greatly improved when fusing MIR and UV-vis matrices. This may be due to the multi-function of the tannin compounds, which have an influence in taste (e.g. bitterness) and color (red/blue color) sensations. From the results obtained it can be observed that the fusion of all three instrumental techniques did not significantly improve the final predictions of the sensory descriptors selected to justify the time-consuming (costs) of the analysis. Thus, final PLS regression models selected for each descriptor were obtained by data fusion of two-blocks of data using low- (tannin quality) and

mid-level (nose wood and color evolution) data fusion. In **Figure 4.9** the measured panel scores (Y measured) are plotted versus the predicted PLS scores (Y predicted).

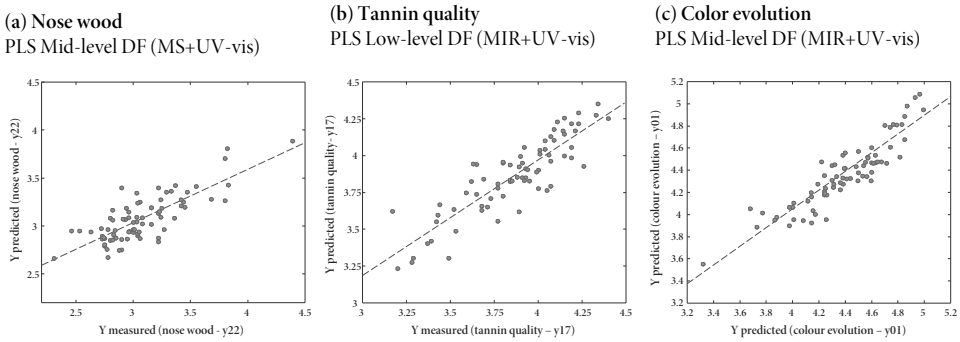


Figure 4.9. Final data fusion PLS models obtained for each wine sensory descriptor studied: nose wood (a), tannin quality (b) and color evolution (c).

4.2.4. Conclusions

This study has shown the ability of sensory instrumental techniques to predict some sensory descriptors perceived with different senses (smell, taste and vision) and described by a human sensory panel. The different descriptors studied were well predicted by using the individual instrumental technique associated to the main human perception required in each case. Moreover, the combination of the different instrumental data through data fusion strategies (electronic panel) allowed complementing the information and enhanced the model predictive abilities.

The good results obtained for "nose wood", "tannin quality" and "color evolution" attributes are encouraging to carry out additional studies to predict additional sensory descriptors. Finally it has to be considered that the results provided by the reference methodology (taste panel) demand intense training programs and accreditation processes to obtain accurate results for such a diverse and complex matrix.

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Chapter 5. Conclusions

In this chapter the main conclusions drawn from different methodologies developed and the research results obtained are summarized. Specific conclusions of each individual study have been commented at the end of each corresponding research paper.

The main goal of this Thesis was the development of novel instrumental methodologies to simulate human sensory responses and this objective has been achieved through the selection and treatment of data from the reference methods (mainly human taste panel), the optimization of the instrumental methodologies for the studied food matrices (almonds, olive oils and wines) and the development of adequate chemometric methodologies to build and validate suitable multivariate classification and regression models.

Therefore, specific conclusions associated to the different chapters are derived from the objectives:

1. *To develop adequate chemometric tools to build the optimal multivariate models relating sensory and instrumental information, both for data collected from single instrumental techniques and for data collected from various techniques by applying different data fusion approaches.*

   In-house programs (algorithms), using the Matlab software, were developed to build and select the optimal multivariate models for the different applications. Data pre-processing, variable selection and validation procedures were programmed for the data obtained by the different instrumental sources. Moreover, data-block pre-processing and feature extraction procedures were also programmed and applied to fused data at different levels of abstraction (low- and mid-level data fusion).

2. *To evaluate a preliminary instrumental sensory technique emulating an electronic tongue based on FT-NIR spectroscopy to discriminate samples by using one single sensory attribute related to taste perception.*

   NIR spectroscopy data were very satisfactorily modeled with discriminant PLS-DA techniques to differentiate the almonds with the negative attribute "bitterness". This new methodology achieved high classification abilities, comparable to the reference method (Raman spectroscopy), providing a simple, robust, fast, non-destructive and economically

attractive alternative. Moreover, this method is highly applicable in the industry, as it is suitable to be implemented for quality control in on-line processes.

3. *To evaluate individual instrumental sensory techniques, such as an electronic nose based on Mass Spectrometry, an electronic tongue based on FT-Mid Infrared spectroscopy and an electronic eye based on UV-visible spectrophotometry, to discriminate olive oil and wine samples depending on the presence or absence of certain sensory attributes or to predict their score intensities.*

   - Data obtained by individual techniques (MS and FT-MIR) were modeled with discriminant PLS-DA to identify musty, winey, fusty and rancid defects in olive oil samples previously analyzed by an official sensory panel.

   - Data obtained by individual techniques (MS, FT-MIR and UV-Visible) were modeled with PLS regression to predict specific olive oil and wine sensory descriptors analyzed by an official sensory panel.

   In both cases, the sensory attributes were acceptably modeled through the optimal pre-processing data and selection of spectral regions (variables). These specific regions also allowed the identification of some specific compounds, which can be correlated with these sensory. These results showed that the developed techniques might be a useful tool to help or complement human taste panels and offer more objective and reliable results.

4. *To combine the data collected from the instrumental sensory techniques described in objective 3 using suitable data fusion strategies (electronic panel), in order to improve the discriminant or predictive models obtained for certain sensory descriptors.*

   The combination of the data obtained by the three different instrumental techniques, headspace-mass spectrometry (HS-MS), mid-infrared spectroscopy (MIR) and UV-visible spectrophotometry (UV-vis) proved to be a useful tool to:

   - Classify olive oil samples according to their category and the presence of certain sensory defects. Data fusion strategies, together with PLS-DA models, improved the discrimination between olive oils for most of the sensory properties studied. The combination of three techniques was considered as the best strategy to discriminate

non-edible olive oils and detect the presence of musty, winey and fusty defects. However, only two techniques (MS + MIR) were necessary to detect rancidity presence and the single use of MS was the best choice to discriminate non-defective oils.

- Predict different olive oil sensory descriptor intensities using PLS regression models and data fusion approaches, which were able to enhance the prediction results obtained with one single technique. The best prediction abilities were obtained for four descriptors: fruitiness, fustiness, rancidity and mustiness. All of them were predicted using a mid-level fusion approach, by coupling all three techniques, except mustiness that only required coupling two instrumental techniques (MS and MIR).

- Predict three wine sensory descriptors described by a human sensory panel: nose wood, tannin quality and color evolution, using data fusion approaches to improve the results obtained from the single instrumental techniques.

These results showed that the data fusión strategies developed might be a useful tool to help or complement human taste panels, offering more objective and reliable results that the models built with single instrumental techniques.



Appendix

A1 List of papers presented by the author of this Thesis.

[**Paper 1**] E. Borràs, J. Ferré, R. Boqué, M. Mestres, L. Aceña, O. Busto, *Analytica Chimica Acta* 891 (2015) 1-14

[**Paper 2**] E. Borràs, J.M. Amigo, F. van den Berg, R. Boqué, O. Busto, *Food Chemistry* 153 (2014) 15-19

[**Paper 3**] E. Borràs, M. Mestres, L. Aceña, O. Busto, J. Ferré, R. Boqué, A. Calvo, *Food Chemistry* 187 (2015) 197-203

[**Paper 4**] E. Borràs, R. Boqué, J. Ferré, M. Mestres, L. Aceña, O. Busto, *Food Research International*, Submitted

[**Paper 5**] E. Borràs, J. Ferré, R. Boqué, M. Mestres, L. Aceña, A. Calvo, O. Busto, *Food Chemistry* 203 (2016) 314-322.

[**Paper 6**] E. Borràs, R. Boqué, J. Ferré, M. Mestres, L. Aceña, O. Busto, *Talanta* (DOI 10.1016/j.talanta.2016.04.040)

A2 Contributions to national and international meetings attended.

Oral communications

Borràs, E.; Amigo, J.M.; van den Berg, F.; Boqué, R.; Busto, O.
Application of Near Infrared and PLS-DA to Classify Almonds with respect to their Bitterness. **VIII Colloquium Chemiometricum Mediterraneum**. Bevagna, Italy (2013)

Borràs, E. ; Ferré, J.; Boqué, R.; Mestres, M.; Aceña, L.; Busto, O.
Prediction of olive oil sensory descriptors using instrumental data fusion and partial least squares (PLS) regression. **VI Chemometrics Workshop for Young Researchers.** Valencia, Spain (2015)

Poster communications

Folcarelli, R.; Boqué, R.; Borràs, E.; Aceña, L.
Detection of adulteration in thermally treated extra virgin olive oils by FT-IR and chemometric analysis. **V Chemometrics Workshop for Young Researchers**. Badajoz, Spain (2013)

Borràs, E.; Fernàndez, S.; Aceña, L.; Mestres, M.; Busto, O.
Instrumental sensometry applied to enological analysis. **I Jornada CEICS d'Enologia**. Tarragona, Spain (2013)

Borràs, E. ; Ferré, J.; Boqué, R.; Mestres, M.; Aceña, L.; Calvo, A.; Busto, O.
Identification of olive oil sensory defects by multivariate analysis of mid infrared spectra. **14 Instrumental Analysis Conferences (JAI)**. Barcelona, Spain (2014)

Borràs, E. ; Ferré, J.; Boqué, R.; Mestres, M.; Aceña, L.; Calvo, A.; Busto, O.
Modeling Olive Oil Sensory Defects with Headspace-Mass Spectrometry and Multivariate Analysis. **Food Analysis Congress**. Barcelona, Spain (2014)

Valls, A.; Borràs, E.; Boqué, R.; Ferré, J.; Brull, A.; Mestres, M.; Aceña, L.; Busto, O.
Modeling wine sensory descriptors using instrumental techniques data fusion. Simulation of an electronic taste panel. **XIII National Congress in Enological Research (GIENOL 2015).** Tarragona, Spain (2015)

Borràs, E. ; Ferré, J.; Boqué, R.; Mestres, M.; Aceña, L.; Calvo, A.; Busto, O.
Identification of sensory defective olive oils with instrumental data fusion strategies and multivariate analysis. **XV Chemometrics in Analytical Chemistry International Conference (CAC)**. Changsha, China (2015)

Folcarelli, R.; Boqué, R.; Borràs, E. ; Aceña, L.
Detection of adulteration in thermally treated extra virgin olive oils by FT-IR and chemometric analysis. **XV Chemometrics in Analytical Chemistry International Conference (CAC)**. Changsha, China (2015)

A3 Research stays and training courses attended.

Research stay

Copenhagen (January – April 2013)

Objective: **Development of a novel NIR methodology to discriminate almond sensory properties using multivariate analysis.**
Supervised by Dr. José Manuel Amigo
Department of Food Sciences, Faculty of Sciences, University of Copenhagen
Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

Training courses

Herramientas Quimiométrics para PAT, June 2012
University of Barcelona, Chemistry Faculty, Diagonal 645, 08028, Barcelona, Spain
Preprocessing of quantitative NMR data for chemometric analysis, March 2013
University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

The main objective of this Doctoral Thesis is the development of new instrumental methodologies to simulate human sensory responses. These instrumental analysis are focused on determining different sensory parameters from typical food and beverages from the Mediterranean region, such as almonds, olive oil and wine.

The studies include the use of responses provided by a human taste panel, the optimization of the analytical procedures for the instrumental techniques and the development of suitable chemometric tools to build the multivariate models. Data fusion strategies have been developed to combine different instrumental data that simulate specific human senses (smell, taste and vision) to work with, what is called, an electronic panel.

