UNIVERSITAT ROVIRA I VIRGILI

SENSORY QUALITY CONTROL OF ALCOHOLIC BEVERAGES USING FAST CHEMICAL SENSORS

Luciano Vera Carrasco

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Departament de Química Analítica i Química Orgànica

SENSORY QUALITY CONTROL OF ALCOHOLIC BEVERAGES USING FAST CHEMICAL SENSORS

Doctoral Thesis presented by

Mr **Luciano Alexis Vera Carrasco**

to receive the degree of Doctor with European Mention

by the Rovira i Virgili University

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ROVIRA I VIRGILI

DEPARTAMENT DE QUIMICA ANALITICA
I QUIMICA ORGANICA

C/ Marcel.lí Domingo s/n
Campus Sescelades
43007 Tarragona
Tel. 34 977 55 97 69
Fax 34 977 55 84 46
e-mail: secqaqo@urv.cat

Dr. Olga Busto Busto, Associate Professor of Analytical Chemistry, and Dr. Montserrat Mestres Solé, Agregate Professor of Analytical Chemistry, at the Department of Analytical and Organic Chemistry at the Rovira i Virgili University,

CERTIFY

That the Doctoral Thesis entitled ***SENSORY QUALITY CONTROL OF ALCOHOLIC BEVERAGES USING FAST CHEMICAL SENSORS,*** submitted by Mr **Luciano Alexis Vera Carrasco** 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 September 2010

Dr. Olga Busto Busto

Dr. Montserrat Mestres Solé

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This work constitutes a part of the research developed by the Wine and Food Analytical Chemistry Group of the Universitat Rovira i Virgili. The Ministerio de Educación y Ciencia of Spain (project AGL2007-61550) is fully acknowledged for the financial support given.

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A Paola

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Presentation

Objectives

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By an instinctive and natural act we usually examine a foodstuff to be accepted and consumed. When we ingest any food all the sensory organs are instantly stimulated. These are intrinsic actions that any individual carries out from the childhood, but the personal evaluation of these perceptions is something that depends on many factors [1].

The examination of the organoleptic characteristics of a product through our five senses, based on qualitative and quantitative criteria, is known as sensory analysis. Sensory analysis will lead to discriminations according to the product attributes and also to description of these attributes before making a decision about the acceptance or the rejection of it.

Nowadays, sensory analysis is considered an indispensable tool in food quality control and it is essential to determine if an aliment has the appropriate characteristics to reach the market [2]. Indeed, before the commercialization of a new product or reclassification of a traditional one, the food industry carries out preference tests to the consumers (who can choose from a wide variety of commodities on the market). In this sense, food industries need to guarantee and, when appropriate, to improve the sensorial characteristics of their manufactured goods, since they are related to their assumed properties. Food industries perfectly know that, although these characteristics can be considered only from a strictly regulatory point of view, to get a quality product market studies taking into account the consumer opinion have not to be forgotten.

So, the release of the products to the market will succeed after determining whether they achieve or not the minimal quality requirements. This involves, on one side, compliance with the legal requirements and, on the other side, the manufacturing according to the brand, since this will be the mark of identity of the product itself.

In the food field, the legal fulfilment is usually guaranteed and there are a large number of analytical techniques that support the rigorous compositional inspections that must be

done. However and especially when dealing with alcoholic beverages, the organoleptic interest of the consumers is crucial and the purchase of these beverages is conditioned, almost exclusively, by their sensory preferences. Thus, a proper management of the sensory attributes is a key competitive advantage for the beverage industry. Words like "consistency" and "differentiation" define the keys to succeed and, in many cases, the expert taste panels are responsible of that.

Although the chemical nature of beverages, as in any other food, is rather complex, there are distinctive attributes that allow differentiating one from each other. The sensory expert panels carrying out quality controls evaluate, principally, the sensory attributes related to the sight, smell and taste, with the aim of ensuring that the brand organoleptic profiles are uniform and satisfy the consumer preferences.

The sensory attributes are decisive to establish the authenticity and to detect possible defects of the products and, therefore, they are also crucial when determining their economic value in the market. The sensory panels that evaluate these attributes for quality control are continuously trained and validated to provide an objective assessment. However, the use of these panels implies long time of analysis and high training costs, and they are conditioned mainly by the subjectivity of the responses, sensory saturation and mood and health of the experts involved [3].

This is the main reason why during the last decades it has increased the interest in developing fast techniques of analysis capable both of imitating the human sensory senses and processing the information (chemical and sensorial) as quickly as possible. In that way, the panels have a very valuable support tool for their work that avoids the drawbacks abovementioned. These techniques, known as artificial sensors, operate in a way similar to the human senses, since they are able to distinguish sensory parameters from the signals produced by specific chemical species in the suitable detection systems. Then, these signals are collected and processed by data recognition techniques in order to provide an interpretation based on the differences and similarities of the products tested.

The first publications related to instruments capable of providing sensory information appeared in the 1980s. The main purpose of these investigations was to develop technologies that could imitate the smell and taste senses. The terms "Electronic Nose"

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(e-nose) and "Electronic Tongue" (e-tongue) were recurrent in many published works related to the assessment of food organoleptic profiles [4, 5]. Both techniques recognize the sensory sensations (odour and taste) from the signal that comes from the detection system and that has been processed by pattern recognition techniques. Both e-noses and e-tongues allow comparing the sensory characteristics of similar substances and classifying them according to their nature, origin, making, variety, etc. Moreover, both techniques allow processing samples with a minimum treatment, thus providing analytical information in a fast and realistic way.

The most common type of electronic noses and tongues is based on the interaction between compounds related to aroma and taste with a series of gas and liquid sensors, respectively. The instrumental signals, which can be considered as a "fingerprint" of the samples analyzed, are generated by changes in the physicochemical properties of the sensors.

More recently, other types of artificial sensors based on consolidated spectral techniques have also been used for the same purpose. These sensors are the ones that have been used in this doctoral thesis, specifically the electronic nose based on Mass Spectrometry (MS e-nose) and the electronic tongue based on Mid Infrared Spectroscopy (FT-MIR e-tongue).

One of the most important advantages of this kind of sensors is that, from the registered spectra, chemical information is available. In the MS e-nose, the MS is coupled to a headspace autosampler (HS-MS) and all the compounds present in the volatile fraction of the sample (headspace) are transported directly to the MS ionization chamber, where they are ionized and fragmented. The abundance of each fragmented ion is represented in a mass spectrum providing complete information about the chemical composition of the sample.

In the FT-MIR e-tongue, all the compounds of the liquid analysed absorb mid-infrared energy. The absorption intensity of each compound is registered in the range 400-4000 cm^{-1} (mid-infrared region) and represented in a spectrum, which can be chemically interpreted.

The data obtained from these instruments is collected in a data matrix where the rows represent the samples analysed and the columns represent the measured instrumental variables, m/z ratios in MS and wavenumbers in case of FT-IR. Since these data represent a multivariate system they have to be treated with chemometric techniques to extract the relevant information.

Chemometric techniques allow comparing the responses obtained when analysing the samples and finding similarities or differences between them. Moreover, it is possible, by means of suitable multivariate regression methods, to correlate the sensor signals with sensory attributes related to aroma, flavour and/or taste defined by a specialized tasting panel.

Also, by selecting the more determinant variables with suitable variable selection techniques, additional information can be obtained about the chemical causes of the similarity or differences between samples or about those chemical compounds that are better correlated with a given attribute. In this way, an instrumental and objective organoleptic profile can be established for the food products tested, thus allowing their assigned to a specific origin or quality. Both sensors, together with suitable chemometric tools, will support the work of the conventional sensory panels, helping the brands and quality control organisms and, ultimately, benefiting the consumers.

An interesting issue for a complete instrumental sensory study is data fusion. With data fusion, the information coming from different instrumental techniques (i.e. MS e-nose, FT-IR e-tongue, UV-Vis e-eye) is collected in a single data matrix, providing information about an overall sensory characteristics of the product.

In the present Doctoral Thesis, two chemical artificial sensors (MS e-nose and FT-IR e-tongue) have been applied to the analysis of alcoholic beverages, with the aim of developing new strategies to test the authenticity of these products, from a sensory point of view. The work here presented intends to be an advance towards the development of an electronic taster through the fusion of several chemical sensors: an electronic nose, an electronic tongue and, later on, an electronic eye.

To achieve this global objective, several sub-objectives were defined:

1) To test the applicability of the HS-MS system to the sensory analysis of alcoholic beverages.

2) To evaluate if the FT-IR technique can be used as an electronic tongue for the sensory analysis of alcoholic beverages.

3) To investigate new strategies, based on chemometric techniques, to widen and improve the capacity of analysis of the e-nose and the e-tongue in the field of sensory analysis.

4) To merge the spectral data coming from several spectral techniques (MS e-nose, FT-IR e-tongue and Ultra-Violet Visible e-eye) with suitable data fusion techniques to improve the results in the sensory analysis of food samples.

Structure of the Doctoral Thesis

The Doctoral Thesis here presented is structured in five chapters. Each chapter contains the information listed below:

Chapter 1, "***Chemosensory Analysis"***. It is divided in three parts. The first explains the theoretical aspects referred to the operation of the human senses involved in the sensory analysis of alcoholic beverages, especially in wines and beers, and their relationship with the artificial sensors. The second part introduces some of the most important instrumental techniques used for the same purpose. The third part includes the chemometric techniques of data processing used in this thesis.

Chapter 2, ***"Electronic Nose"***. This chapter describes, in its first part, the MS e-nose from a technical point of view. Its advantages and disadvantages are shown with respect to other types of e-noses and we justify why it was chosen for doing the experimental part related to volatiles. The second part includes the scientific articles derived from the MS e-nose investigations carried out in this doctoral thesis, preceded by a review about the use of electronic noses in quality control in alcoholic beverages. The experimental

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section starts with a method to verify the feasibility of the calibration transfer technique in *HS-MS* wine analysis, followed by a study performed with the MS e-nose about the characterization of beers according their aromatic composition.

Chapter 3, ***"Electronic Tongue"***. This chapter is divided in two parts. The first presents a description of the FT-IR instrument, its advantages and disadvantages and the reasons of their use in this doctoral thesis. The second part start with a review about the *state of the art* of the electronic tongues in the quality control of alcoholic beverages followed by the application of the FT-IR e-tongue technique to predict the tannin amount attribute in red wines.

Chapter 4, ***"Instrumental Data Fusion in Sensory Analysis"***, deals with the fusion of the data obtained from the MS e-nose, the FT-IR e-tongue and UV-visible e-eye in beer analysis. The chapter presents a work where the three techniques have been used to characterize beer samples and improve the performance of each single instrument through LDA. This study points to future trends of the research that should be done in this field.

The report ends with the "***Conclusions"*** of the studies developed, that are included in ***Chapter 5***.

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Chapter 1

Chemosensory Analysis

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The senses allow having a real perception of everything that surrounds us. Humans have five senses (sight, hearing, touch, smell and taste) to receive the information needed to act. The individual or complementary use of each of them will depend on the situation encountered. For example, smell, touch, sight and even hearing come into play while we are eating.

Sensory evaluation only occurs if there is a specific interaction between human perceptions and the food products consumed. It is defined as a scientific discipline used to evoke, to measure, to analyze and to interpret reactions to those properties as they are perceived by the senses [1]. Therefore, this discipline is quantitative and very useful for describing profiles or specific characteristics of a product, but also for quality control monitoring. This is achieved by checking regular samples against specifications, which allows detecting differences between products from different batches, runs, treatments, and so on.

In liquid samples and, more precisely, in alcoholic beverages, the sensory perception mainly implies sight, smell and taste. So, the flavour is one of the main attributes that have an influence on the overall acceptability of this kind of products. Flavour is a wide and complex term that has generated much discussion between a significant number of researchers, both in academic and industrial circles. Commonly, flavour is considered as a combined perception of odour and taste; however, other authors consider it as a comprehensive response, which involves not only the olfactory and gustative systems, but also the somatosensory, visual and auditory systems [2].

When an alcoholic beverage (or a beverage in general) is analyzed by our senses, each sense is stimulated and the information generated is guided to the brain where it is interpreted. For example, the colour of a wine is perceived when the visual light wavelengths reach the human retina, whose cells send a signal via the optical nerve to the brain. Regarding to the flavour, as a combined perception of taste and odour, the sensory

activation is due to chemical compounds that can be either volatile or non-volatile. Taste is perceived by the taste buds located on the tongue and other parts of the mouth, and mainly describe the perception of the attributes sweet, sour/acid, salt, bitter, astringent, metallic, hot, cooling and umami. These sensations are produced by chemical species dissolved in the liquid matrix of the beverage. On the other hand, the smell detects some odorous molecules in the air above the product that stimulate the olfactory receptors at the top of the nasal cavity [1]. These substances are volatiles and can be detected before the consumption of a product (orthonasal perception) but also during drinking as they pass to the nasal cavity from the mouth, through the posterior napes into the nasal cavity (retronasal perception).

The human sensory system is extremely important in food control. This is because, usually, food quality control is carried out by sensory panels that assess the flavour, providing results that can ensure the success of new launches of products and services [3]. Their contribution is so important that the sense of smell is considered a determinant part of neuromarketing because it is a powerful element to attract consumer's subconscious.

However, the use of sensory panels suffers from some drawbacks. The usual sensory analyses carried out are expensive, if rigorous, and too cumbersome to be introduced as a routine procedure [4]. Moreover, they are time-consuming, discrepancies may occur due to human fatigue or stress, and they cannot be used for on-line monitoring [5].

For this reason, the development of alternative instrumental techniques or devices able to overcome the limitations of the human senses has become an attractive topic for the food industry. "Artificial sensors" is the generic name of these instruments and their applications are increasingly widespread, especially in the quality control field. The development of these instruments is based on their ability to mimic the human sensory response, which is strongly dependent on the many biological mechanisms involved from the stimulus to their corresponding interpretation, providing rapid sensory information. Instruments were thus designed on the basis of how the human system operates.

In a few words, the process consists of stimulating the sensory receptors and sending the corresponding signals (sensory information) to the brain, where these are transformed

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into a unified sensory experience that is associated to a previous one. In this way, we can interpret what type of substance is being evaluated. Likewise, the artificial sensors collect the responses through their detection system (equipped with different sorts of sensors), which are then treated with suitable chemometric tools, to extract the relevant information and give an interpretation (figure 1.1).

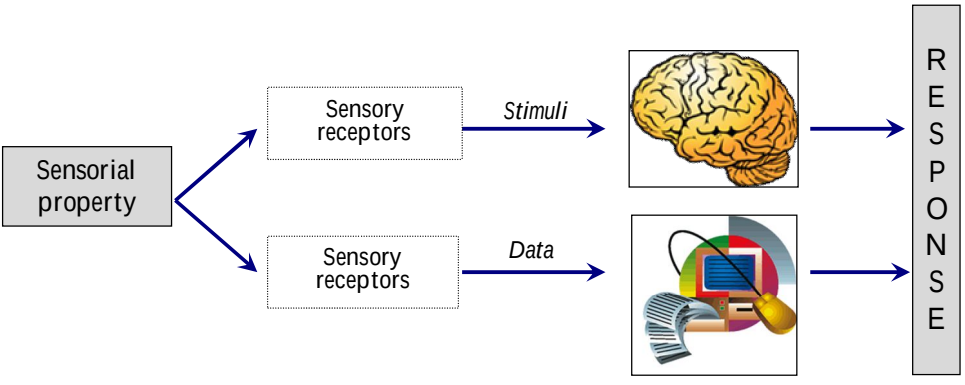

Figure 1.1. Analogy between biological and artificial sensory systems.

As it has already been explained, gustatory and olfactory systems are very important to define the quality of wines and beers. For this reason, in this Doctoral Thesis we have applied both an electronic tongue and an electronic nose to determine some properties of these beverages.

However, prior to present these applications and the results obtained, in this chapter we refer to the human sensory analysis of wines and beers, with special attention to gustatory and olfactory perceptions, and to the sensory instrumental methods of analysis also related to these mouth-feel and nose perceptions. Moreover, since neither e-tongue nor e-nose technologies can be understood without the application of suitable pattern recognition methods, an introduction to these techniques is also included at the end of the chapter.

1.1. Chemistry of the sensory perceptions in wine and beer

The complex chemical nature of alcoholic beverages especially referred to wines and beers induce to an active participation of the sense organs involved mainly in the flavour and colour perception. Below there is a brief description of this perception and the participation of the senses in the sensations originated when wines or beers are tasted.

1.1.1. Visual perceptions

Visual perceptions are the first information that we obtain from the product. The visual appearance reveals much about the quality, style, condition and even possible defects of the product. In beers and wines, the utility of the visual perception is mainly focused on the colour evaluation, although other properties like clarity, brightness or turbidity can also be visually evaluated.

Colour

The colour itself does not exist, but in the mind of the observer. The colour sensation is prompted in the eyes retina by light waves comprising the range of the wavelength of the visible spectrum from 380 to 760 nm. The cells sensible to the colours are the cones and rods. The cones, located in the centre of the retina, respond to a relatively narrow range of wavelengths, with sensitivity peaks in the blue, green and red absorption regions. So, the perceived colour is a result of the stimulation of these three colours. The rods operate in conditions of light illumination, and can be sensitive to the primary impression in black and white [6], which is associated to the perception of brightness and clarity. Once the signal is received, it is conducted by the optic nerve by means of electrical impulses to the primary visual cortex, which is located at the back of the brain. In the visual cortex a high-level processing of the visual image takes place (figure 1.2).

In alcoholic beverages, and liquids in general, the visual attributes depend on the behaviour of their compounds when absorbing, transmitting, reflecting or dispersing the visible radiation. So, the pigments present in the liquid matrix will directly affect the

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intensity and purity of the perceived colour in such way that, the wider the received spectra, the lesser purity of the colour perceived [7].

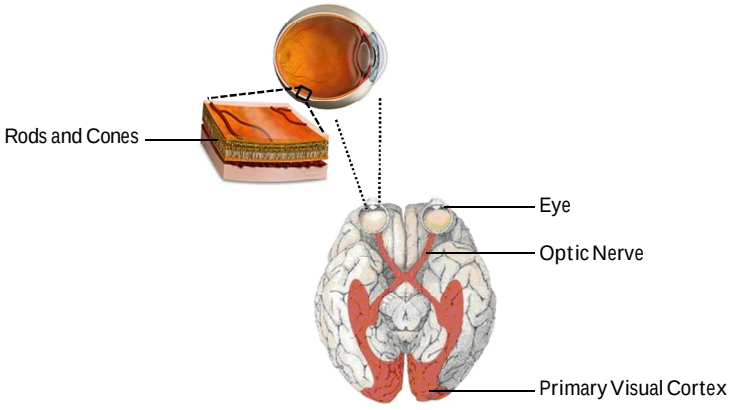

Figure 1.2. Representation of the image generation in the visual system.

In red wines, the compounds responsible of colour are the anthocyanidins, which are always found in their glycosidic form, called anthocyanins [8]. The original grape anthocyanins are responsible of the initial purple-red colour of young red wines and these are displaced progressively by more stable pigments, which are responsible for the brick-red colour of the aged wines [9].

In beers, the colour is a critical parameter for the consumers since it allows the immediate classification of the Lager, Ale and Stout types. Therefore, it requires a careful control. The colour formation in beers, which is mainly attributed to a pigment called melanoidin, is produced during the malting and the production of the brewing must. The oxidized polyphenols and even traces of metals are also considered as responsible of the colour [10].

The colour in beers, and especially in wines, can vary substantially and provide clues about varieties, regions and climates, among other characteristics. Indeed, it is well known that the deep colour in wines can give indications of its structure and ageing. In any case, it is obvious that the colour of these alcoholic beverages will affect their quality perception.

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1.1.2. Olfactory perceptions

The olfactory perception is based on chemical interactions between aromatic volatile compounds and the olfactory receptors located in the nasal cavity. In the olfactory receptors there are about 10 to 20 million of specialized cells, which respond to the aromatic compounds. The receptors send signals to the corresponding halves of the olfactory bulb, located directly above, at the base of the brain [7]. The olfactory bulb collects the information received by the receptor cells in the nose before being definitively sent to other brain regions where the information is interpreted (figure 1.3).

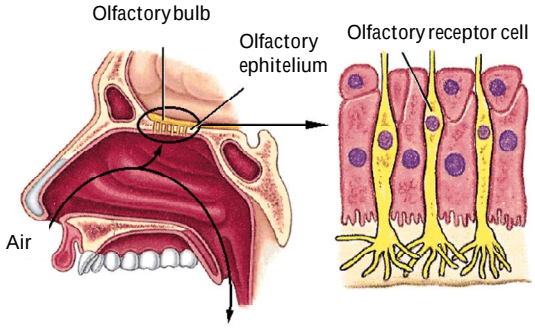

Figure 1.3. Location of important structures of the olfactory system.

The aroma is perhaps the most complex sensation, very difficult to be described. The perceived aroma not only arrives by the nasal path but also by the retronasal one when the liquid (or product) is ingested. While the stimuli available to create taste sensations are limited, there are hundreds of volatile compounds in wines and beers that can contribute to aroma perception, depending on their concentration and sensory threshold. They belong to very different chemical families like acids, alcohols, aldehydes, ketones, acetals, esters, sulphur compounds or terpenes, among others. Because of this complexity, it is difficult to characterize wine and beer aroma in a few well-defined components. The situation is further complicated if we consider that many compounds contribute to the aroma both synergistically and antagonistically.

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In wine, the major chemical constituents generate gustatory rather than olfactory influences. In contrast, minor or trace volatile constituents produce a wine's distinctive aromatic characteristic. Acids, alcohols, esters, aldehydes and ketones, sulphur compounds, hydrocarbons derivatives, lactones, terpenes, phenolic and pyrazines contribute to the aroma, whose origins are in the grape, the alcoholic fermentation or the aging, giving rise to primary, secondary and tertiary aroma, respectively.

The main wine acids (tartaric and malic) are non-volatile. However, important aromatic volatile acids are present, as the acetic, butyric and propanoic acids. Higher alcohols, such as 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol, are the most significantly aromatic alcohols. Acetaldehyde is also an important component, because it constitutes more than 90% of the aldehydes present in wines. Finally, fragrances of flowers, fruit, seeds, leaves, woods and root are attributed to terpenes [7].

Esters constitute one of the most important chemical families contributing to the wine aroma, together with fusel alcohols. They are regarded as especially important to wine flavour, and they usually are secondary aromas, arising from the fermentation, and sometimes tertiary aromas arising from ageing, where alcohol-acid rearrangements can occur [11]. More than 160 esters have been distinguished. Most of these compounds are present at concentrations below the limit of human perception. However, those that can be perceived are important for the flavour profile in beers and wines, because they are responsible of the fruity aroma.

In beers, the main groups of compounds related to the aroma are the esters, alcohols, vicinal diketones, sulphur compounds and hop aroma, principally. The esters are the main group of fermentation-derived flavour-active components. They contribute to fruity, floral and solvent-like flavours. The most important esters in beers are ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl caproate and 2-phenylethyl acetate [12]. The alcohols also contribute to the flavour of beers and, particularly, play an important role in the flavour perception of other beer compounds because of their synergistic effect.

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1.1.2. Gustatory perceptions

The word taste comes from the word "tasten" used in the English language in the Middle Ages and means "to examine by means of touch or taste" [13]. These perceptions, detected once the product has been introduced into the mouth, are produced by a pair of chemoreceptors: the specialized receptor neurons and the free nerve endings scattered throughout the oral cavity. The first, grouped in cavities within taste buds, are responsible of generating the perceptions sweet, sour, bitter and salty. The second generate the mouth-feel (tactile) perceptions of astringency, touch, burning, viscosity, temperature, body, prickling and pain [7].

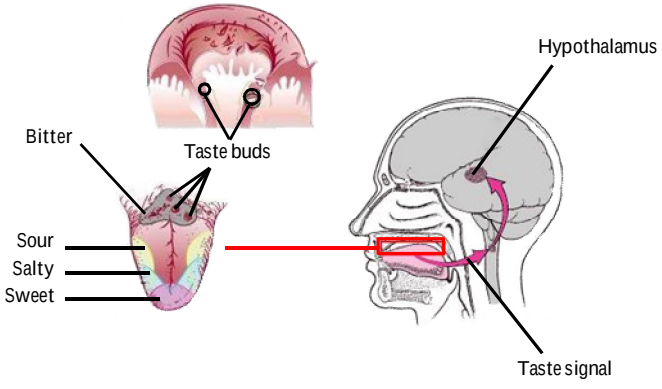

Figure 1.4. Diagram of the functioning of the gustatory system.

The tongue is the main sensorial organ to detect the taste. It is covered by a membrane containing the taste buds (figure 1.4). About two thirds of the mouth taste buds are located on the tongue, where they are found on the sides of raised growths called papillae [7]. These structures are involved in detecting the four principal elements of taste perception: sweet, sour, bitter and salty, as it has been aforementioned. The four perceptions are perceived in specific areas of the buccal cavity (figure 1.4). Some sensations such as bitterness, sweetness and saltiness are detected by receptive areas on the tongue. However, it has to be pointed out that all the other so-called "tastes", like

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strawberry, mint, lemon, orange and so on, are detected by nasal receptors and not by the tongue [14].

The transmission mechanism of the gustative sensation is activated when the stimuli produced by a chemical substance reaches the receptor cells, which individually or in a combined manner are connected to a nervous fibre. This fibre transmits the sensation to the hypothalamus in the brain, evoking emotional responses to the primary stimuli [15]. Each basic sensation is perceived when a compound or a group of them are introduced in the mouth. The tongue responds to a large number of chemicals, but does not distinguish between individual compounds; instead, clusters them according to a specific gustative group.

Sweetness

The sweetness in wines or beers is hard to judge. The perception threshold between persons can be different and the perception itself may also be ambiguous. In some countries, like United States, sweet wines are frequently associated with low-quality wines [16], but not in others, as the well-known French Sauternes exemplifies. Chemically, sweetness is attributed to the molecules of glucose and fructose in wines and fructose, sucrose, glucose and maltose in beers. A characteristic of the sweetness sensation is that it is often associated to viscosity. So, in the case of beers, the "sweet" molecules contribute to "body" or unctuous sensation because they increase its viscosity. The glycerol in wines, also involved in the viscosity property, improves the sensation of sweetness (together with the ethanol).

Sourness

Another component of the gustatory sensation, sourness, is very important in alcoholic beverages. For example, sourness in wine contributes directly or indirectly to its quality. So, if a wine has insufficient acidity, it is considered a "flat" wine. Moreover, the acidity helps to the expression of the red colour in red wines and is related to the pH (ranging from 2.9 to 4.2), which affects the overall beverage chemistry [11]. If the acidity is high, it creates a sour sensation; otherwise, the sensation is smooth. This perception is mostly caused by the acids. Sourness in wines is mainly due to tartaric, malic, citric, lactic and

succinic acids. In beers, where the pH varies from 3.8 to 4.3 depending on the yeast used in their elaboration, sourness is due to the presence of acetic, lactic, pyruvic and succinic acids.

Bitterness

The perception of this sensation is detected on the posterior side of the tongue, being sometimes masked by sweetness. Bitterness is an important property in red wines, where often it is the last main sapid sensation to be detected. In white and red wines, the flavan-3-ols and their by-products are the main responsible of bitterness when their content is high and above the threshold levels [11]. However, in wines, bitterness is often linked to tannins, although this is not completely exact because tannins produce in the tongue an astringency impression rather a tactile sensation.

In beers, bitterness is also a very important characteristic. It is largely attributable to a group of compounds called iso-α-acids and originated from the hop. These iso-α-acids are in fact a mixture of closely-related compounds: three pairs of *cis* and *trans* isomers, each pair deriving from a single α-acid. The typical concentration levels are 10-60 mg/L and their detection threshold is around 5-6 mg/L [10]. This high content highlights the importance of bitterness in the beer flavour.

Salty

Although this taste is not used to describe wines and beers, some salts contribute to taste. The salts present in wine and beer matrices are dissociated in positive and negative ions, being K^+, Ca^{2+}, Cl^- and bitartrates the most common. The salty perception is directly related to the tendency of the salts to ionize. Because the major salts in wine have large organic anions (i.e., tartrates and bitartrates), and dissociate poorly at the pH values of wine, their common cations (K^+ and Ca^{2+}) do not actively stimulate salt receptors. In addition, the relative concentration of Na^+, the primary cation inducing saltiness in wine, is limited, which explains the relative absence of salty sensation in wine [17]. In beers, Na^+ and K^+ ions can contribute to a salty taste at concentrations higher than 150-200 mg/L and 500 mg/L respectively.

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1.2. Instrumental sensory analysis in alcoholic beverages

Traditionally, in food and beverage quality control sensory analysis has been carried out by the human sense organs because, for centuries, they were the only instruments available. However, the advances in physics, chemistry and biology and the growing demands of the food processing industry have accelerated the development of new instruments to minimize reliance on panel tests for routine evaluation of products. Nowadays, different instrumental methods have been applied to analyze and/or evaluate parameters related to the sensory characteristics of food and beverages. The applications of these instruments are based on the assumption that there is a relationship between physical/chemical measurements and the perceived sensory properties. The development of these instrumental methods has become of great interest because they can almost mimic the human sensory senses.

1.2.1. Instrumental measurement of colour

As already said, the human eye creates a perception based on the response given by the combination of many neurons in the retina, which respond to the blue, green and red signals that brain converts into a colour. This means that the eye does not respond to a large spectrum of light intensities, so it is quite difficult to correlate the spectral absorbance and the perceived colour. However, the development of instruments capable of analyzing the colour of alcoholic beverages has been improved over time. The use of spectroscopy, mainly in the visible spectral region, has replaced the historical and simple methods based on the direct visual comparison between a sample and a system of standard colours. Nowadays, it is possible to find different methodologies to measure the colour of a product.

Perhaps the most used way to evaluate colour is the one based on the official method called CIE (*Commission Internationale de l'Éclairage*). This method involves measuring light transmission at 445, 550 and 625 nm (and occasionally at 495 nm) wavelengths, that are called tristimulus coordinates. From these values, a function that tries to imitate the response of the cones [10] is generated, representing the colour into three further

uncorrelated parameters L (a measure of lightness), A (a measure of redness) and B (a measure of yellowness) (CIELAB) in a three-dimensional space [18]. In wineries, the conventional method to measure the amount of colour in wines, in order to control its quality, is the Glories method [19]. By means of this method, colour intensity, tonality and different percentages of colour (measuring at 420, 520 and also 620 nm, to include the blue of young wines) are determined [20].

Other spectroscopic applications perform measurements not only at a single wavelength but in a range of wavelengths. In that sense, the use of a UV-Visible Spectrophotometer (approx. 400 to 800 nm) is very suitable because the information obtained by analyzing the colour in that way may be related to many different chemical species. Thus, for example, the colour in beers and wines is produced, as it has been already said, by melanoidins and anthocyanidins, respectively, which absorb in the visible spectral region. The information obtained from the continuous spectrum, where the absorbances in the whole range of wavelengths is collected, allows, for a set of samples, to obtain a data matrix whose information can be processed and interpreted by means of suitable chemometric techniques [21-23]. Indeed, by means of multivariate calibration models, it is possible to classify, characterize and even quantify the colour intensity of food samples. This allows the UV-Visible Spectrophotometer acting as an electronic eye (e-eye).

1.2.2. Instrumental measurement of aroma

The most common method to analyze the chemical composition of volatiles is gas chromatography, especially when coupled to a mass spectrometer (GC-MS). However, not all the volatiles are odour-active, and for those that have this property, their detection by the human nose is variable. So, some aromatic compounds may be present at concentrations too low to be detectable by GC systems but this low concentration can be enough to be detected by the human nose [24].

A part from the sensory analysis, the main technique to analyse aroma compounds is gas chromatography olfactometry (GCO), a powerful technique in food aroma characterization. It uses the human nose as a chromatographic detector in parallel with a conventional one, such as a Flame Ionization Detector (FID) or a Mass Spectrometric

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Detector (MSD). This technique is the only one that allows distinguishing the odour-active compounds within the whole range of volatiles present in a particular product.

The first devices capable of evaluating the aromatic quality of foods were developed about 30 years ago, These instruments, called electronic noses (e-noses), traditionally involved the use of an array of chemical sensors with broadly overlapping specificities and an appropriate pattern recognition system capable of recognising simple or complex odours [24]. The volatile compounds of the samples interact with the sensors, producing electrical signals for each sample. These data is collected in a data matrix, which is then treated with multivariate analysis techniques to extract the relevant information.

Later on, the e-nose based on mass spectrometry (MS e-nose) was developed. Among other characteristics that will be extensively explained in the following chapter, one of the most important differences between an Ms e-nose and the ones previously described is that MS devices allow obtaining chemical information from the samples analysed, whose molecules have been fragmented in the MS. This information allows interpreting the causes of the differences between samples or studying the aromatic characteristics from the fragmentations of the main odour-active molecules of the samples.

Because of the ability to analyse the volatiles, even those associated to the aroma, this type of e-nose has been also used in conjunction with trained panellists to measure aroma properties through the elaboration of a multivariate calibration model [25].

1.2.3. Instrumental measurement of taste

The first instrumental systems to imitate the taste appeared in the mid 80's. They consisted of a series of potentiometric sensors whose function was basically to differentiate liquids through the signals obtained using sophisticated techniques of data processing. Nowadays, these devices, called electronic tongues (e-tongues), are considered analytical instruments that can artificially reproduce the human taste sensation [26]. They consist of an array of 20 (on average) chemical sensors coupled to chemometric processing tools. The main types of electronic tongues commercially available are based on potentiometric and voltammetric techniques. There are many

studies and applications referred to the use of e-tongues in wine and beer analysis (see chapter 3), mainly related to differentiation, classification and characterization of samples based on the components responsible for taste [27].

Another type of electronic tongue, based on infrared spectroscopy (IR), is presented as a potential alternative to those more traditional systems. In this technique, the absorbance of the compounds in the sample in the infrared region is measured. The principal characteristics of this type of sensor are its speed, reproducibility and ability to provide structural information of a large number of compounds, including those related to taste. Moreover, the absorption spectra collected can be processed with suitable chemometric techniques to characterize or differentiate the samples (alcoholic beverages) depending on the chemical compounds involved in their sweetness, bitterness, acidity and saltiness.

An interesting application of the e-tongue is its potential use as an artificial taster, through multivariate calibration models built by correlating the instrumental response and the evaluations given by a trained sensory panel for a set of samples. However, the main difficulties lie in obtaining a highly trained panel of tasters capable of providing accurate and precise evaluations and having a representative system of samples to calibrate the instrumental system.

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1.3. Chemometrics and data processing

The amount of data collected using artificial sensors is frequently very large, and these data can only be processed by means of multivariate methods of analysis. The original signals obtained from the instrument are collected in a matrix X_{nxm} of *n* samples and *m* variables that correspond to the registered values by the electrochemical or spectrometric/spectroscopic sensors.

When the aroma is analyzed in an alcoholic beverage, about 10^7 biological sensors are stimulated to send signals to the brain where the interpretation based on a reduced number of descriptors is carried out. In artificial e-tongues and e-noses, the signals are provided by the systems of liquid or gas sensors (around 30 maximum) or by the spectral measurements (that correspond to hundreds of sensors). These signals are processed and the dimensionality reduced to a few variables that can be more easily interpreted. So, data processing, which tries to emulate the operation of the brain, represents the last stage of the sensorial process.

The multivariate methods and general tools related to data processing are included in the scientific discipline called Chemometrics. The International Chemometrics Society (ICS) offers the following definition: *Chemometrics is the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods*. Chemometrics spans a wide area of different methods which can be applied in chemistry. There are techniques for collecting good data (optimization of experimental parameters, design of experiments, calibration, signal processing) and for getting information from these data (statistics, pattern recognition, modelling). A summary of the main chemometric tools used during the development of this thesis is detailed below.

1.3.1. Data pre-treatment

Data pre-treatment is the first processing step applied to the raw data and it is needed to remove irrelevant variation from the data that would hamper a correct interpretation of the results. In this Doctoral Thesis, the data pre-treatments used have been:

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- *Centering*, which removes systematic location differences between variables. It consists on subtracting the mean of each variable from every individual value of that variable. The resulting data have zero mean. Centering removes the dependence of the signal averages, but retains the magnitude of its variations. Centering means that the average has been subtracted from every value in a given variable.
- *Autoscaling*. It implies that each variable is first centred, and then divided by its standard deviation. The resulting data has zero mean and standard deviation of one. Autoscaling makes variables of different scales comparable.
- *Row autoscaling or standard normal variate (SNV)*, that corrects the baseline shift by dividing the row centred by its standard deviation.
- *Row profile*, which divides each variable of the sample for the sum of all variables for that sample. It is useful to avoid differences caused by the instruments between the first and the last analysis.
- *Smoothing*, that attempts to reduce random noise of the instrumental signal. The most common technique for smoothing is Savitzky-Golay [28], which is based on adjusting a polynomial of appropriate degree to a small range of signal, thus removing part of the noise.
- *Derivatives*. It emphasizes the differences found in each spectrum. The application of the derivative must be preceded by smoothing to avoid accentuating the noise.

1.3.2. Pattern recognition techniques

Pattern recognition techniques are divided into unsupervised and supervised. The first refer to situations with little or no prior information about groups or classes and the objective is to identify trends of the samples to be grouped. Supervised techniques are used to build classification rules of a series of specified subgroups. So, new unknown samples can be assigned to more probable subgroups [29].

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Unsupervised

The most used technique is principal component analysis (PCA). PCA is a linear transformation that converts the original data to a new coordinate system such that the new set of variables, the principal components (PCs) -linear functions of the original variables- are uncorrelated, and the greatest variance by any projection of the data comes to lie on the first coordinate, the second greatest variance on the second coordinate, and so on. A graphical representation of PCA shows the maximum variability among samples.

Supervised

Linear discriminant analysis (LDA) is a supervised technique that classifies each sample (object) in a specific class, based on a training set of samples whose class is known a priori. There are two approaches to derive a rule for discrimination between groups: the Bayesian and the Fisher approach. *Bayesian*-LDA is based on the hypothesis that the data in the classes follow a normal distribution, being their dispersion described by the same covariance matrix and differing only in the position of their centroid. The classification rule is based on linear discriminant scores, which are directly derived from plugging the density of the multivariate normal distribution into the Bayes equation for *a posteriori* probabilities [30]. A sample (object) is classified in the class for which it has the highest probability. On the other hand, *Fisher*-LDA searches for directions, called canonical variables, which achieve a maximum separation between classes. The first canonical variable is the direction of maximum ratio between inter-class and intra-class variance. Samples and variables can be projected onto the canonical variables and the resulting bi-plot allows establishing relationships between samples and variables.

1.3.3. Multivariate calibration

Sometimes the objective is to relate the instrumental signals coming from the artificial sensors with a certain property of interest (i.e., sensory attribute). This is possible through the use of multivariate calibration techniques. The most usual multivariate calibration technique is partial least squares (PLS) regression. In PLS regression, the spectral dimensionality is reduced to a few orthogonal latent variables, or factors. These

factors are selected to maximize the correlation between the spectra and the properties of interest [31]. A calibration model is built with a set of training samples, validated and finally used to predict the value of the property of new samples.

1.3.4. Variable selection

One of the most common problems in pattern recognition in the presence of noisy input variables, that is, variables containing little or no information related to the problem at hand (classification, prediction). Such variables can worsen the classification or prediction ability of the models. The aim of the variable selection strategies is to find and remove those irrelevant variables. The strategies used in this thesis include discrete variable selection techniques, such as iterative predictor weighting (IPW-PLS), SELECT and uninformative variable elimination (UVE-PLS), and continuous variable selection techniques, such as moving windows PLS (mw-PLS), tree interval PLS (ti-PLS) and main interval PLS (mi-PLS), where intervals of variables are selected.

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Chapter 2

Electronic Nose

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Olfaction is the capacity to detect and identify odours by means of a complex system that include our nose and brain. When we smell something, we are detecting volatile compounds of various chemical structures and molecular sizes at different concentrations. So, the nose acts as a chemodetector that continuously monitors changes in the aroma composition [1]. This sensory system is the most complex because of its great sensitivity and wide range of different perceptions, and enables to detect and discriminate, nearly instantly, hundreds of low molecular mass organic compounds, which we commonly call odours or aroma.

In food, aroma is a fundamental parameter to determine its quality. Usually, for food quality control, the aroma analysis is carried out by experimented sensory panels that are rigorously trained. However, the inherent complexity of the olfactory process, the physical and psychological disposition of tasters and their subjectivity in the evaluation of the products have led to a increasing interest in obtaining instrumental tools that can support this important area of the Analytical Chemistry.

Inspired by the human olfactory system, the electronic nose (e-nose) was developed for the analysis of aromas. The operation principle of this instrument is the detection of volatile substances by means of a sensor array that sends the data collected to a pattern recognition system that finally provides an aromatic interpretation.

The most common types of e-noses have a detection system with an array of gas sensors that collect the electrochemical signals produced when the volatile substances reach them. The main characteristics of these instruments, commercially available since the beginning of the 1990s, are their low cost, portability, fast detection and high sensitivity.

Another and more recent type of e-nose is based on mass spectrometry (MS). As it has been introduced in the previous chapter, in this type of aroma sensor the mass spectrometer is used as the detection system (MS e-nose). The main advantage of the MS

e-nose is that the fragmentation of the volatile compounds (among which there are the ones responsible of the aroma) in the ionization chamber of the mass spectrometer, provides chemical information of the sample. This information is obtained when the *a priori* known fragmentation pattern of the chemical compounds of the volatile fraction of the sample is related to the abundances of the m/z fragments registered by the instrument.

When analyzing alcoholic beverages with the conventional gas-sensor e-noses, the presence of ethanol, the most abundant component in alcoholic beverages, masks the signal produced by other important volatile compounds, which are found in much lower concentrations. This interference poses an important problem that usually implies the use of sample pretreatments, with the subsequent increase of the time of analysis. In MS e-noses, such pretreatments are not necessary because the interference of the ethanol can be skipped by instrumentally avoiding the spectra fragments resulting from the ethanol ionization.

A quite common instrument in analytical laboratories, the gas chromatograph-mass spectrometer (GC-MS), can be used as an electronic nose. The only requirement for that is the use of an uncoated and deactivated capillary column as the transfer line between the GC injector and the mass spectrometer. In this way, there is not chromatographic separation; the analytes are not retained in the column and reach the mass detector all at once [2]. When a set of samples is analyzed, the use of a Headspace (HS) autosampler is very helpful, because it allows automating the sampling process. So, the coupling of these techniques has given rise to the so-called HS-MS e-nose system (or simply MS e-nose).

Despite the advantages of MS e-noses, they present two main drawbacks. The first concerns their non-portability, which does not allow "in situ" analysis. The second is their instrumental signal instability over time, which hampers the construction of stable calibration models [3].

In this second chapter we present the studies performed in the application of the electronic nose based on mass spectrometry (MS e-nose) in wine and beer analysis. A brief introduction of the performance of the HS-MS e-nose device is also included.

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2.1. Introduction

The development of fast analytical procedures to determine the quality of food products, in good accordance with sensory panel data, has been an important challenge for food science in the last decade. The continuous advances in aroma analysis since the appearance of the first electronic noses, allow assuming the consolidation of these instruments as a powerful support, or even an alternative, to the usual sensory panels.

As it has been previously stated, the conventional electronic noses comprise an array of electronic chemical sensors with broadly overlapping specificities and appropriate pattern recognition systems capable of recognising simple or complex odours. Their performance is based on the analysis of the cross-reactivity of an array of solid-state gas sensors where products with similar aroma, generally, result in similar sensor response patterns (similar "fingerprints") [4].

About two decades ago, a new generation of e-noses based on mass fingerprints was introduced. In this new instrument, volatile compounds are introduced into the ionization chamber of a gas chromatographic mass spectrometer (GC-MS) without prior separation. The final response, after the ionization and fragmentation of all volatiles, is represented in a mass spectrum where the abundance of each ionized fragment (m/z) is visualized.

2.1.1. Description

As occurs with the human nose, the operation of an electronic nose begins with "sniffing" and conveying the volatile components of the samples to the detection system. There are many ways of introducing volatiles into the instrumental system, being the most used the Static Headspace (SHS) sampling because its performance is very similar to the human sense. Indeed, this technique does not require sample treatment, does not imply concentration of the analytes and allows easily analyzing the same compounds that reach the human nose when evaluating a product, i.e., only the volatile compounds [2].

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There are other sampling techniques that can also be applied to the analysis of volatiles, such as the Dynamic Headspace (DHS) sampling, where the volatiles are purged by a stream of inert gas and trapped onto an adsorbent [5], or the Solid Phase Microextraction (SPME) and the Stir Bar Extraction (SBSE) techniques, where volatiles are trapped in different kinds of adsorbents that coat a fused-silica fibre (SPME) or a magnetic stirring bar (SBSE). Nevertheless, because of its simplicity and speed, all the analyses carried out with the HS-MS e-nose in this Doctoral Thesis were performed with the SHS technique.

In conventional e-noses, the volatiles interact with an array of gas sensors inducing a reversible physical and/or chemical change in the sensing material that constitutes the sensors [6]. These changes are transduced into electrical signals, which are preprocessed and conditioned before identification by a pattern recognition system. In MS e-nose systems, the volatiles are fragmented and a mass spectrum generated where the abundance of each fragmented ion is represented. In both cases, the set of signals contains information about the volatile composition of the sample and can be considered as a "fingerprint" of the sample analyzed.

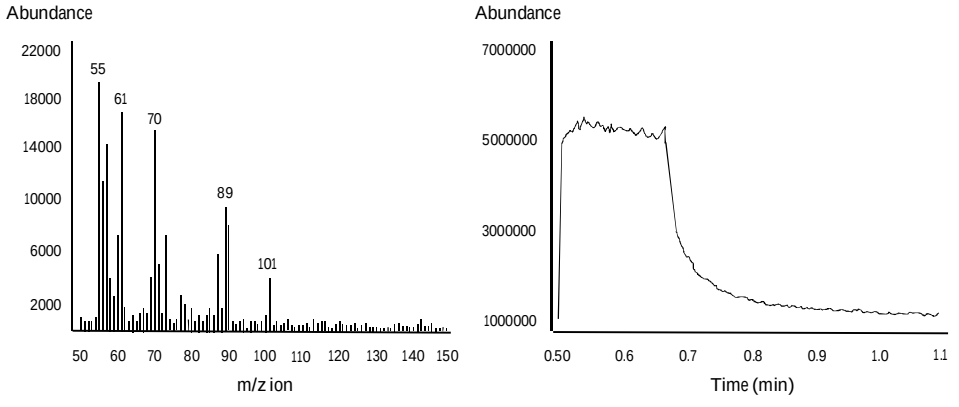

Figure 2.1. Signals obtained by analysing wine with the MS e-nose: Abundance vs time (left), Global mass spectrum (right).

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Figure 2.1 shows the two signals obtained when analyzing a wine sample with an MS e-nose. The figure on the left shows the abundance (sum of all m/z ions detected) vs the time of analysis. As it can be seen, the analysis of a complex sample such as wine, takes only one minute. In the figure on the right, the global mass spectrum of the sample is represented. Since this spectrum contains all the information about the fragmentation of all the compounds that constitute the sample, this will be different for each sample so it is considered to be the "fingerprint" of the sample.

Figure 2.2 schematically shows the HS-MS e-nose used in this Doctoral Thesis. It consists on a static headspace sampler and a gas chromatograph equipped with a quadrupole mass spectrometer detector (GC-MS). The data generated are evaluated with a chemometric system for pattern recognition.

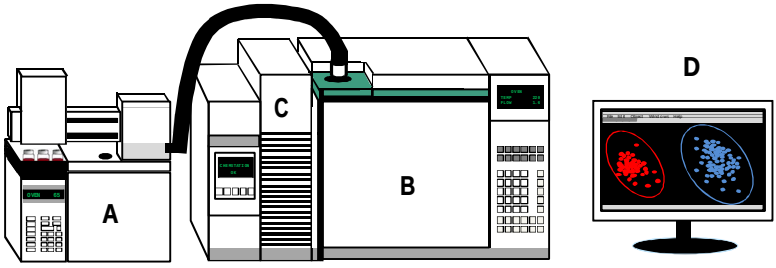

Figure 2.2. HS-MS e-nose scheme: (from left to right): A) Headspace Autosampler (HS); B) Gas Chromatograph (GC): C) Mass Spectrometer (MS); D) pattern recognition system.

Headspace Autosampler. The autosampler allows taking a prefixed volume of the headspace over the sample contained in a tightly capped vial and introducing the volatiles into the GC-MS system. First, a sample set is placed in the carrousel. In consecutive order, each individual sample is pushed by the mechanical arm into the oven, where it is thermostatized to a determined temperature, for a well-defined time, to reach the chemical equilibrium between the liquid and gas phases. Then, the headspace is pressurized and vented until the loop is filled. Afterwards, the content of the sample loop

is transferred by the carrier gas over the transfer line to the gas chromatograph injection port (figure 2.3).

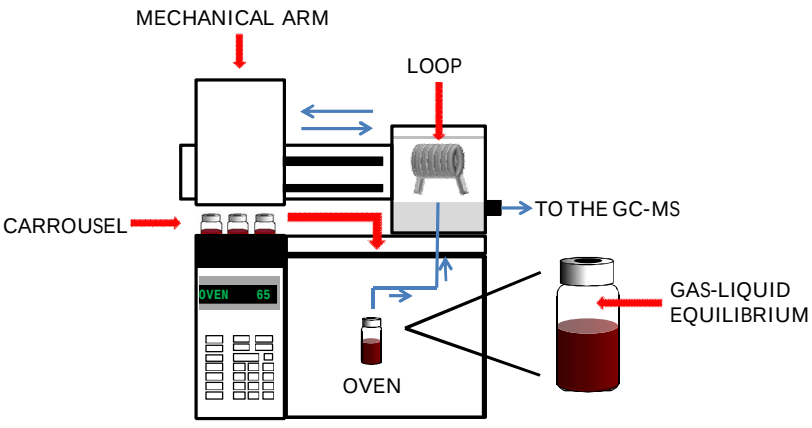

Figure 2.3. Structure and performance of the Headspace Autosampler.

GC-MS system. The volatiles transferred from the Headspace Autosampler are introduced into the ionization chamber of the MS without chromatographic separation (figure 2.4). The role of the GC is only to transfer the volatiles to the MS so we substituted the capillary chromatographic column by a short capillary column uncoated and deactivated, usually called retention gap. Once inside the ionization chamber, the molecules are ionized and sent, by means of a low accelerating potential, to the quadrupole mass analyzer (QMA). The QMA is formed by four cylindrical metallic rods, which create a magnetic standing field that allows the access only of ions of one particular m/z at a time. The quadrupole can operate in two modes: full scan of a selected mass range and Selective Ion Monitoring (SIM). In the latter mode, sensitivity for a single analyte is enhanced by monitoring only a few selected m/z ions [7]. On entering the ion detector, the ions are deflected onto a cascade plate where the signal is multiplied and then sent to the data system as an ion current m/z versus time [8].

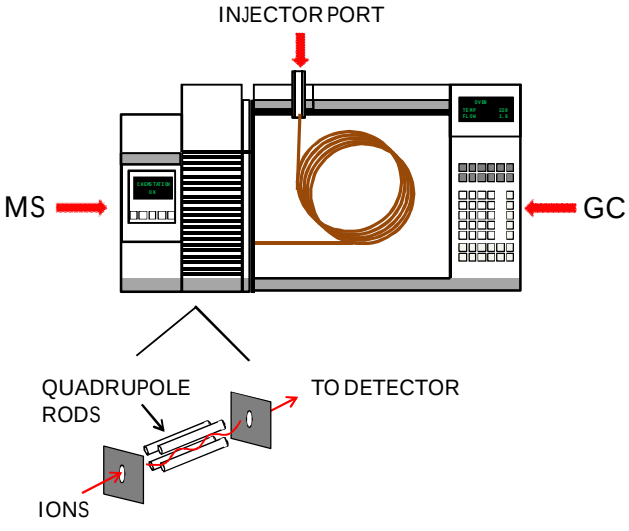

Figure 2.4. Mechanism of the GC-MS when used as electronic nose.

Each fragmentation ion (m/z ratio) in the mass spectrum obtained acts as a "sensor" and its abundance is equivalent to the sensor signal. So, the number of sensors in MS-based e-noses will vary depending on the m/z ratio range considered.

Data analysis system. The signal generated by the MS is processed by using pattern recognition techniques. The objective of this last step of the e-nose analysis, which uses chemometric software, is to interpret, by means of calibration/classification models or others strategies, the signals obtained and, in this way, to emulate the brain. So, using suitable chemometric tools it is possible to differentiate, to quantify (by means of calibration models) and even to characterize the different samples analysed.

2.1.2. About this chapter

Some years ago, the MS e-nose was already successfully applied to the analysis of alcoholic beverages in our research group [9-11]. These applications, as well as many other found in the literature [12-20], have allowed considering this instrument as a well-established technique in this particular field. Moreover, the increasing demand for fast analyses in the food industry encouraged us to investigate the possibility of implementing this instrument as an artificial taster of alcoholic beverages.

This implies the calibration of the instrument versus the responses of a properly trained panel. However, to achieve this goal, we first had to solve the problem that implied the instability of the MS signal, a problem already detected in previous studies. This instability makes it difficult to get durable calibration models so it was necessary to plane a reliable strategy. We carried out different experiments in order to evaluate the suitability of the calibration transfer technique by using a stable sample. In this way, we could ensure that the changes observed on the response of this sample over the time were due only to the equipment and, therefore, the calibration transfer only considered this source of variation.

The next step consisted on studying new strategies based on chemometric tools to get as much information as possible from the collected signal. This led to characterization studies, from the chemical information provided by the MS e-nose. In this way, the potential applicability of the HS-MS e-nose to the field of alcoholic beverages increases substantially.

In the following pages, we present the results obtained when applying the MS e-nose to the analysis of wines and beers, which have resulted in two papers published in two prestigious journals of analytical chemistry. Prior to that, we include a review about the *state of the art* of the applications of e-noses in alcoholic beverage analysis, which has also been published in a relevant technical journal related to beers.

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2.2. Electronic Noses in the Quality Control of Alcoholic Beverages

L Vera, MP Marti, R Boqué, M Mestres, O Busto, J Guasch

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The applications of electronic noses in the field of food and beverages have awakened the interest in researchers and in the food industry. The many applications of the various types of electronic noses range from the samples' differentiation, which was the main objective of the first studies, to the most current ones among which we can highlight those that try to correlate sensory and instrumental responses.

The following review refers to the evolution of electronic nose instruments in the last years and the main applications, to date, in the analysis of alcoholic beverages.

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Electronic noses in the quality control of alcoholic beverages

L Vera[1], MP Martí[1], R Boqué[2], M Mestres[1], O Busto[1], J Guasch[1]

[1] Grup de Química Analítica Enològica i dels Aliments. Unitat d´Enologia (XaRTA).
[2] Grup de Quimiometría, Qualimetria i Nanosensors
Department of Analytical Chemistry and Organic Chemistry
Universitat Rovira i Virgili, Campus Sescelades. Marcel·lí Domingo, s/n 43007
Tarragona

Abstract

In the last 25 years, research into electronic noses has been constant. The main purpose of this research is to achieve an instrument that can differentiate samples according to their global volatile composition, quickly and objectively, so that product quality in industry can be ensured and controlled. The electronic noses developed so far can be classified into two groups on the basis of their detection systems: classical instruments, which are based on solid state gas sensors, and new instruments, which are based on mass spectrometry (MS). In general, MS-based instruments have the advantage over the classical instruments in aspects such as stability, sensitivity and versatility. Furthermore, in the analysis of alcoholic beverages, the advantages of MS-based instruments are even more evident because the high ethanol content of the samples interferes with the solid state gas sensors.

This article presents an overview of electronic nose applications in the field of alcoholic beverages. The electronic noses that have been developed are described and their advantages and disadvantages are discussed.

Introduction

Food aroma is composed in most cases of complex mixtures of hundreds of volatile organic compounds, with different sensory and chemical properties. Subtle differences in the relative amounts of these compounds can often determine the smell of the product. However, not all the volatile compounds contribute to the aroma. The contribution of each compound depends on its concentration and its sensory threshold (the minimum concentration that can be perceived by the human nose). The ratio between these two parameters is defined as the odor activity value (OAV). When the OAV of a compound is greater than one, it will contribute to food aroma [1].

The human nose is still the most commonly used "instrument" in many industries for evaluating the quality of food odor. It is certainly the most appropriate because the complexity of most food aromas means that they are very difficult to characterize with conventional flavor analysis techniques such as gas chromatography (GC) or gas chromatography olfactometry (GCO) [2]. However, sensory analysis by a panel of experts is a costly process for industries because it requires trained people who can only work for relatively short periods of time. There are also other problems such as the subjectivity of human response to odors and the variability between individuals.

Consequently, there is an enormous demand for an instrument that can overcome the problems of human sensory panels. The earliest publications about instruments for odor assessment appeared at the beginning of the eighties [3]. The main purpose of the new research was to develop an instrument that could mimic the human sense of smell and provide rapid sensory information (differences and similarities among samples and presence of aromatic defects).

Thus, instruments were designed on the basis of how the human olfactory system operates. When we smell something, we breathe the volatile compounds into the nose, where they interact with receptor proteins in the cell membrane. Then, it is thought that a chain of molecular events encodes most of the information about the quality and concentration of each odorous compound in the output signals of the olfactory receptor cells. Finally, this olfactory information is sent to the brain, where it is transformed into a unified sensory experience that is associated with previous experiences [4]. It is

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surprising that the sensitivity of the human nose does not come from the binding constants of the receptors in the primary olfactory-receptor cells. It is the amplification processes and the subsequent neural processing (which occurs in the brain) that are responsible for noise reduction, drift elimination, sensitivity enhancement by approximately three orders of magnitude and discrimination between thousands of odors [5]. Likewise, in the instruments developed the volatile compounds interact with an array of gas sensors. Each gas sensor responds more selectively to a certain group of chemical compounds but also shows a broad and overlapping response to the others (cross-selectivity). In this way, a small number of gas sensors (usually 8 to 32) can respond to a variety of different complex odors and there is no need to have one specific sensor for each individual compound. In a subsequent step, the data are processed with chemometric techniques in order to compare and/or classify the samples according to their volatile composition. This technique does not provide information on the amounts of the individual aroma compounds; rather it makes a global and qualitative estimation of the aroma profile. In this respect, it is similar to human olfactory perception.

"Electronic nose" (e-nose) is the most commonly used term in the literature to refer to this type of instrument, although other terms such as artificial nose, mechanical nose, odor-sensing system or electronic olfactometry are also used. Gardner and Bartlett [2] defined the e-nose as "an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognizing simple or complex odors". Although the definition was appropriate at that time, nowadays it does not include all the e-nose systems that are available on the market.

At the end of the nineties, a new type of e-nose based on mass spectrometry (MS) was developed [6]. Even though some researchers do not consider that the MS-based system is an e-nose [7], the purpose of this new instrument, like the classical ones, is to differentiate and subsequently classify samples according to their volatile composition in a fast and simple way.

This paper presents the state of the art of the various e-noses, with particular reference to the field of food analysis, and gives an overview of the applications developed in the quality control of alcoholic beverages.

Instrumental aspects

The general principle of the e-nose technique is that the volatile compounds in the headspace of the sample are introduced into a detection system. Then, for each sample analyzed, the detector generates a set of signals that contains the information about the volatile composition of the sample. This set of signals is like a "fingerprint" of the sample analyzed. When several samples are analyzed, a data matrix is generated that is subsequently treated with chemometric techniques so that the samples can be compared on the basis of their volatile composition, and classified or discriminated depending on their origin, variety, purity, ripeness, aging, or any other property of interest. When these statistical techniques are used it should be borne in mind that results will only be reliable if a significant number of samples of the different categories of the property of interest are analyzed. This, however, is not a drawback, because e-noses are simple to use and provide fast responses [7].

Therefore, an e-nose is a union of three elements: the sample handling system, the detection system and, last but by no means least, the data analysis system. In this section, we describe the underlying techniques on which each of these three elements are based. We also explain their advantages and disadvantages with particular reference to the field of food analysis.

Sample handling

As it has been mentioned, speed and simplicity are two important characteristics of e-noses. Depending on the technique chosen to extract the volatile compounds of the sample, sample handling may therefore be a critical step. Static headspace (SHS) is the most common technique (see tables 1 and 2) because it is very simple to use. Sample temperature, equilibration time, vial size and sample quantity are the main parameters that have to be optimized. Because of the poor repeatability of manual headspace injection, it is recommended that an automatic headspace sampler be used. In some applications a vapor-flow system [8-10] has been used, which has provided better control than manual headspace injection of the operating temperature and the amount of analyte that is introduced into the detector.

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For some applications, the SHS technique has the drawback of low sensitivity because the volatile compounds are not preconcentrated. To increase the sensitivity, the dynamic headspace (DHS) technique has been used in some applications [11-13]. Although DHS improves the sensitivity of the system, it introduces a supplementary step in the method, which increases the time of analysis. Moreover, analytical artifacts (memory effects, bleeding or irreversible adsorption) are generated in some cases.

Another extraction technique is solid-phase microextraction (SPME) [11, 14-16]. This technique has a considerable concentration capacity and it is very simple because, unlike DHS, it does not require especial equipment [17]. Even though it has been developed only recently, stir bar sorptive extraction (SBSE) is a promising extraction technique when a very high sensitivity is required [18].

Although, a priori, any sampling headspace technique can be used as the sample handling part of an e-nose, the choice must be made with care and has to take into account the type of sample and the method specifications required [20, 21]. Moreover, it has to be noted that the use of sample concentration systems decreases the speed of the analysis, which is the main advantage of an e-nose.

Detection system

The detection system is the main part of an e-nose. So, e-noses can be classified into two groups according to the detection system: e-noses based on gas sensors and e-noses based on mass spectrometry. In this section, these two technologies are described and their advantages and disadvantages are discussed.

Gas Sesnsors

As it has been explained above, gas-sensor e-noses operate on the basis of physical and/or chemical interactions of the volatile compounds with an array of solid state gas sensors, each one having a partial specificity. Ideally, the sensors should fulfil a number of requirements such as broad selectivity, high sensitivity, robustness, stability, rapid response and rapid recovery times. Moreover, if they are used in poorly controlled

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environments, they will be required to have a low sensitivity to environmental variables such as temperature and humidity. Although no single technology fulfils all these requirements at the moment, a range of sensor technologies has been reported for e-nose applications [22].

Year	Sample	Aim of the study	Extraction system	Detection system	Data treatment	Ref.
1991	Whisky	Differentiation	Vapor-flow	QMB	ANN	[8]
1991	Wine, beer, spirits	Differentiation	DHS	MOS	PCA, HCA	[44]
1991	Whisky, cognac	Classification	DHS	MOS	LSA	[44]
1992	Beer	Differentiation	Vapor-flow	MOS	ANN	[9]
1993	Brandy, gin, eau-de-vie	Differentiation	SHS	CP	PCA	[47]
1995	Wine	Differentiation	SHS	MOS	PCA	[38]
1996	Wine	Differentiation	SHS	MOS	ANN	[39]
1998	Beer	Differentiation	Vapor-flow	CP	PCA, DFA, ANN	[10]
2000	Beer	Differentiation	SHS	QMB	PCA	[41]
2000	Wine	Differentiation	SHS	MOS	PCA	[41]
2000	Wine	Differentiation	DHS	CP	PCA	[42]
2001	Coconut liquor	Differentiation	SHS	CP+QMB+MOS	PCA	[40]
2001	Wine	Differentiation	SPME	CP	PCA	[43]
2001	Wine	Differentiation	SPME	CP	PCA	[43]
2002	Wine	Aroma fermentation	SHS	CP	PCA	[45]
2004	Beer	Hops	¿?	MOS	PCA, SOM	[57]
2005	Wine	Off-flavours	SHS	MOS	PCA	[36]
2005	Beer	Hops	SHS	MOS	PCA, SOM	[52]
2006	Wine	Classification	SHS	SAW	PCA, PNN	[53]
2006	Wine	Differentiation	SHS	SAW	PCA, PNN	[54]
2006	Wine	Classification	SHS, DHS	MOS	PCA, PNN	[55]
2006	Wine, vodka, whisky, tequila, beer	Effect of alcohol on the detection of aroma	SHS	MOS	PCA	[35]
2006	Wine	Typical aroma identification	SHS, DHS	MOS	PCA, LDA, PNN	[56]
2007	Wine	GC-MS & Sensory panel correlation	SHS, DHS	MOS	PCA, PLS	[58]
2008	Wine, vodka, whisky, tequila, beer	Identification of alcholic beverages by GC	SHS	MOS	PCA, DFA	[59]
2008	Wine	Discrimination	SHS, DHS	MOS	PCA, PNN	[60]
2009	Beer, wine	Off-flavours	SHS	MOS	PCA, DFA	[61]
2009	Wine	Classification	SHS	MOS	LDA,	[62]
2009	Wine	Panel comparison	DHS	MOS	PCA, PNN	[63]
2010	Wine	Aroma discrimination	DHS	MOS	PCA, PNN	[64]

1 DHS: dynamic headspace; SHS: static headspace; SPME: solid phase microextraction
2 QMB: quartz microbalances; MOS: metal oxide semiconductors; CP: conducting polymers
3 ANN: artificial neural networks; PCA: principal component analysis; HCA: hierarchical cluster analysis; LDA: linear discriminant analysis; DFA: discriminant function analysis; PNN: probabilistic neural network; SOM: Self Organized Maps

Table 1. Main applications of e-noses based on gas sensors to the analysis of alcoholic beverages (chronological order).

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Year	Sample	Type of study	Extraction system	Detection system	Data treatment	Ref.
2002	Whisky	Adulteration	SHS, SBSE	MS	PCA, SIMCA, PCR	[19]
2002	Beer	Differentiation	SPME	MS	PCA	[50]
2003	Wine	TCA determination	SHS	MS (SIM)	PLS	[30]
2003	Wine	Differentiation	SHS	MS	PCA	[50]
2004	Wine	Differentiation	SHS	MS	PCA, SIMCA	[48]
2005	Wine	Classification	SHS	MS	PCA, DPLS, LDA	[65]
2005	Sugar cane spirits	Ageing	SHS	MS	PLS	[49]
2005	Beer	Characterization	SHS	MS	PCA	[66]
2007	Beer	Classification	SHS	MS	PCA, LDA, KNN, DPLS	[67]
2007	Wine	Monitoring yeast degradation	SHS	MS	PCA, PLS, SLDA	[68]
2008	Wine	Aroma prediction	SHS	MS	PCA, PLS	[69]
2010	Wine	Calibration transfer	SHS	MS	PLS	[70]
2010	Wine	*Tempranillo* classification	SHS	MS	PCA, DPLS	[71]
2010	Wine	Wine temperature	SHS	MS	PCA	[72]

[1] SHS: static headspace; SBSE: *stir bar sorptive extraction*; SPME: solid phase microextraction. [2] MS: mass spectrometry; SIM: *selected ion monitoring.* [3] PLS: partial least squares; PCA: principal component analysis; SIMCA: *soft independent modelling of class analogy*; PCR: principal component regression; LDA: linear discriminant analysis; DPLS: discriminant partial least squares; KNN: *K-Nearest Neighbours*

Table 2. Applications of HS-MS systems to the analysis of alcoholic beverages (chronological order).

Current gas sensors can also be divided into two main groups according to the working temperature [23]: sensors that operate at high temperatures (175-450 ºC) and sensors that operate at room temperatures. Of the former, metal-oxide semiconductors (MOS) are probably the most widely used in e-nose applications. They display a high level of sensitivity for a range of organic vapors and provide perhaps the best balance between drift, lifetime and sensitivity [24]. However, an important disadvantage of MOS devices is the logarithmic dependence of the sensor response on the gas concentration. This causes problems in the presence of high concentrations of detectable species (for instance, ethanol in alcoholic beverages). Other potential problems have also been reported when MOS devices are used with food products: for example, the baseline recovery is slow when they are exposed to high molecular weight compounds or they are susceptible to poisoning by sulphur-containing species or by weak acids [24].

Of the sensors that operate at room temperature, conducting polymer sensors (CP) are the ones most commonly used. Their selectivity is generally better than that of MOS

sensors and they are relatively resistant to poisoning. But the polymer response is sensitive to humidity. This is an important drawback when water is a major component of the sample headspace because it can act as a serious interference, even when the conditions are rigorously controlled.

The quartz crystal microbalances (QMB) and the surface acoustic wave transducers (SAW), which are acoustic wave devices, appear to be highly promising. Their sensitivity is at the $\mu g \cdot L^{-1}$ level whereas the sensitivities of MOS and CP sensors are at the $mg \cdot L^{-1}$ level [23]. However, acoustic wave devices, like other technologies, still have several problems that need to be addressed, such as poor batch-to-batch reproducibility during manufacturing and the dependence of the response on temperature [24].

Finally, fiber-optic chemical sensors are small, simple to fabricate, inexpensive and can be made with a wide range of coatings. It should be pointed out that their response is fast. Whereas the response time of other types of gas sensors is of several minutes, the response time of fiber-optic chemical sensors is between 100 ms and 3 s. The lifetime of these sensors, however, is limited by photobleaching processes. Moreover, the optical format also requires relatively sophisticated instrumentation to be used [4].

At first, e-noses tended to be based on an array of gas sensors of the same type, but practical experience has shown that this often does not produce enough information for many real-world problems. Increasingly, the tendency is to combine different types of gas sensors to produce hybrid systems. However, this involves more complex electronics and it is then necessary to normalize or standardize the different sensor outputs [24].

Despite the considerable number of applications that appear in the food analysis literature [4, 23-26], much development is still required before e-noses based on gas sensors can reach their full potential.

Mass Spectrometry

The e-noses based on MS were developed only a few years ago [5]. These new instruments introduce the volatile compounds into the ionization chamber of a mass spectrometer without prior chromatographic separation [16, 27]. The mass spectrum obtained results from the simultaneous ionization and fragmentation of all the volatile

compounds. Each fragment ion (m/z ratio) represents a "pseudo sensor" and its abundance is equivalent to the sensor signal. Therefore, the number of "pseudo sensors" in MS-based e-noses is much larger than in gas sensor-based e-noses. Moreover, by selecting the optimal set of fragment ions (the optimal "sensor array") the instrument can be tailored to particular applications with successful discriminations. The selection of particular fragment ions may be based on knowledge of the headspace composition - analytical chemistry can provide the necessary tools to characterize the samples- or on the results of mathematical feature extraction. The sensitivity and selectivity of the instrument can be improved using the mass spectrometer in the selected ion monitoring (SIM) mode. Another important advantage is that these "pseudo sensors" contain chemical information of the sample. Then, information about what types of compounds are responsible for the differences between samples can be obtained from the ion fragmentation patterns. These results can then be compared directly with the results obtained from conventional GC-MS instruments. Furthermore, more specific information about the molecular origin of the fragment ions can be obtained by using an interface that permits a soft ionization of the molecules at atmospheric pressure instead of electronic impact ionization at 70 eV, which is the commonest type of ionization, in association with a time-of-flight (TOF) analyzer [18]. Results can be similar with chemical ionization, which does not fragment the analytes so much. In some cases, these strategies improve the classification or prediction results [28]. Alternatively, the quality of spectral fingerprints can be enhanced by modifying the ionization energy level in an electronic impact configuration. It has been shown that, in general, the total abundance of the mass fragments and the discriminating power of the mass spectra increase with the ionization energy level. However, the energy level that yields the best signal-to-noise ratios depends on the food commodity studied [29].

An interesting fact is that a GC-MS instrument can be used as an e-nose by simply using an uncoated deactivated retention gap as a transfer line between the sample handling device and the detector, instead of the chromatographic column. In this way, all the compounds are introduced into the mass spectrometer rapidly and simultaneously, because there is no chromatographic separation. An e-nose configuration can also be achieved by applying strong chromatographic conditions (high temperature and high carrier gas flow) to an analytical capillary column. With this configuration, it is easy to

switch from a GC-MS working as an e-nose to a conventional GC-MS, simply by changing the temperature program and the column gas flow. Another advantage of using a chromatographic column is that a minimum separation can be carried out that might help to uncover minor differences in trace components that would otherwise be masked by other constituents with higher concentrations. This strategy has been shown to improve classification and prediction results [30].

Signal instability is one of the problems of MS-based e-noses that have not been completely solved yet. It causes the mass spectrum of a sample to change when the same sample is analyzed some days later. Many factors contribute to signal instability and their origin can be found in the mass spectrometer itself (e.g. gradual fouling of the ion source, vacuum instability, aging of the ion multiplier, change of a filament, etc). Strategies such as internal standardization have been used to correct for signal instability. This involves dividing the abundance of each fragment ion (mass intensity) by the intensity of one fragment ion of an internal standard that has been added to the samples [15]. The problem of this strategy lies in finding a suitable internal standard, bacause it must have a fragmentation pattern that does not interfere with the sample profile. Furthermore, internal standardization is very difficult in the case of solid samples. A way of correcting mass spectra in solid samples is the standard gas addition technique [31], which introduces a very small quantity of xenon continuously into the source of the mass spectrometer. Then, the abundance of each mass fragment ion in the spectrum of the product analyzed is normalized with respect to the abundance of xenon measured during the analysis.

Sometimes, sensitivity changes are not constant along the mass axis and the signal increases differently for some fragment ions or even decreases for others. In such cases, internal standardization and standard gas addition techniques cannot solve the problem of signal instability and calibration transfer techniques have to be used. Calibration transfer is based on the signal variation observed for a set of reference samples that must be analyzed together with the problem samples [32]. Then, when a new set of samples is analyzed, the mass spectrum of each sample is corrected taking into account the changes in the mass spectra of the reference samples. This requires an additional set of samples to be analyzed at regular intervals, which is not a big drawback because the MS-based e-

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nose is a rapid technique. The main problem is the management of the reference samples (selection, physical and chemical stability, storage), in food analysis, especially in wines, due to their continuous evolution even once bottled. However, this problem has been overcome with the use of synthetic wines. These synthetic standards, prepared with an aromatic matrix similar to that of the wines studied, proved to be a good solution as reference samples because of their stability, reproducibility and representativity [83].

Data Analysis Techniques

The first step in data analysis is to pre-process the signals generated by the gas sensors or the mass spectrometer. This process transforms the data into their most appropriate form, and enhances the data features that are useful in the subsequent steps. The pre-processing steps include normalization, baseline correction, noise reduction or variable weighing, among others [33]. Afterwards, pre-processed data are analyzed by various chemometric techniques, which are available in statistical software packages that are usually included in the instrument.

The chemometric techniques used include unsupervised and supervised pattern recognition (PR) techniques. The former reveal natural groupings of the samples in the data set and also detect outlying samples. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) are the most commonly used unsupervised PR techniques. As an example, figures 1 and 2 show the results of applying PCA and HCA, respectively, to the data we obtained in the analysis of brandies from Jerez of different ages using an MS-based e-nose. In increasing aging, the brandies are Solera, Solera Reserva and Solera Gran Reserva. Figure 1 shows that the brandies cluster according to the aging time. In HCA, the relationships between samples are presented in the form of a hierarchical tree (dendogram) and the length of the branches linking two clusters is related to their similarity: the shorter the branch, the greater the similarity between samples or clusters of samples. Figure 2 shows that the Solera brandies are completely different (similarity 0) from the rest of the samples and that one of the sets of Solera Gran Reserva brandies is more similar to the Solera Reserva than to the other Solera Gran Reserva brandies.

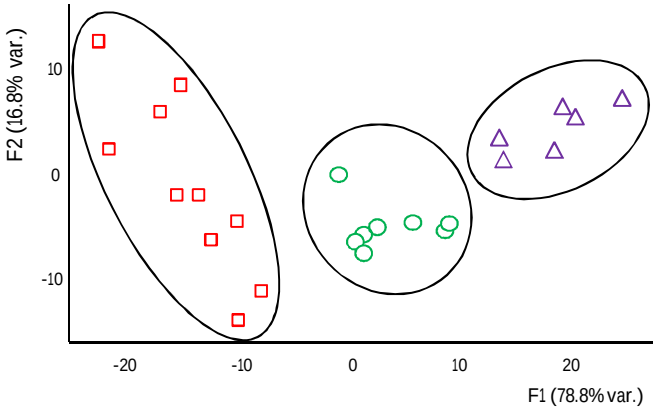

Fig. 1. PCA score plot obtained in the analysis of aged brandies using an HS-MS system. Brandies: Solera (Δ); Solera Reserva (○); Solera Gran Reserva (□).

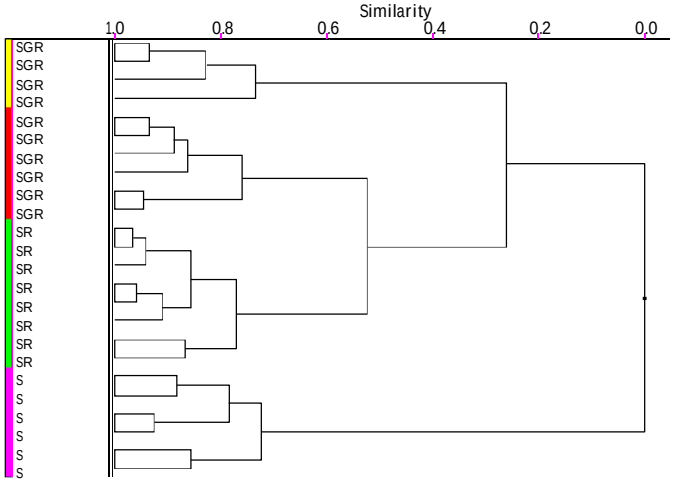

Fig. 2. HCA dendogram obtained in the analysis of aged brandies using an HS-MS system. Brandies: Solera (S); Solera Reserva (SR); Solera Gran Reserva (SGR).

In the supervised PR techniques the class membership is known in advance. The aim of these techniques is to build a model to discriminate between several predefined classes or to assign an "unknown" sample to a given predefined class. Soft independent modeling of class analogy (SIMCA), K-nearest neighbours (KNN), linear discriminant analysis (LDA)

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or discriminant function analysis (DFA) are the supervised PR techniques most commonly used in food applications. Table 3 shows the results obtained when the SIMCA technique was applied to the brandies data set. The interclass distance of a SIMCA model is a useful measure of class separation. Therefore, taking into account that an interclass distance above 3 indicates a good differentiation between classes [28], it can be concluded that the SIMCA model is able to differentiate brandies with different aging times.

Classes	Distances between classes
Solera/Solera Reserva	3.4
Solera/Solera Gran Reserva	11.5
Solera Reserva/Solera Gran Reserva	6.0

Table 3. Results of tye SIMCA model for the brandies with diferent aging times.

If the aim of the analysis is to predict a property (for instance, analyte concentrations or aging times) multivariate regression methods, such as principal component regression (PCR) or partial least-squalres (PLS) regression, have to be used.

When the purpose of the analysis is to predict a continuous property (e.g. analyte concentration or aging time), regression methods, such as partial least squares (PLS) or principal component regression (PCR), are required.

Finally, when the instrumental responses recorded are linear, as in MS-based e-noses, all the aforementioned statistical methods provide very good results. However, in gas sensor e-noses, instrumental responses are essentially non-linear and, in those cases, non-linear chemometric methods, such as artificial neural networks (ANN), provide better results [23].

Mass spectrometry vs gas sensors in the analysis of alcoholic beverages

As has been shown in the previous section, MS-based e-noses have several advantages over the classical gas sensor-based e-noses. In the analysis of alcoholic beverages, MS-based e-noses have an additional advantage, because ethanol interferes with gas sensors but not with mass spectrometers. This section gives an overview of the applications developed in the analysis of alcoholic beverages [34] and discusses the potential of the technique in this field.

Gas Sensors

Food analysis is probably the field with the greatest number of applications using e-noses. However, in alcoholic beverage research, few studies have been made with this type of instrumentation, mainly because of the problems that ethanol causes in gas sensors. In many cases, the high ethanol content of alcoholic beverages saturates the gas sensors and masks the response of the sensor array to other volatile compounds [35, 36]. Consequently, the samples may be differentiated on the basis of variations in their ethanol content instead of variations in the contents of the volatile compounds that are responsible for the aroma. As an example of this, a hybrid e-nose combining three different types of sensors (MOS, CP and QMB) was used to show that it is impossible to distinguish a reference solution with an ethanol content of 12% from the same solution that also has 1 g/L of acetic acid or 300 mg/L of ethyl acetate [37]. It is well known that a wine with these concentrations of acetic acid and ethyl acetate -indicating a high volatile acidity surely caused by a bad storage of the product- is unacceptable.

Table 1 shows the main applications of e-noses based on gas sensors to the analysis of alcoholic beverages. Some studies did not take the ethanol content of the samples into account [8-10, 38-40] and, as mentioned above, this may distort the interpretation of the results. Several strategies have been developed to decrease the ethanol content of the samples and to increase the concentration of the other volatile compounds before the analysis with an e-nose. Heberle et al. [41] proposed to use a chromatographic column between the headspace sampler and the sensor array to separate the ethanol from the other volatile compounds. Other strategies are the use of extraction and concentration techniques, such as DHS and SPME, instead of the classical SHS [42-44]. It has been

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shown that these two techniques decrease the ethanol and water content of wine extracts and also increase the concentration of the other volatile compounds. This can be observed in figure 3, which shows the results of the PCA carried out on the data obtained from analyzing three different wines, water and an ethanolic solution (15% v/v) using an e-nose based on conducting polymer gas sensors [42]. The results shown in figure 3a were obtained using the SHS as the sample handling technique. It can be observed that the sensors were only capable of distinguishing the water vapor from the rest of the samples. Instead, when the DHS technique was used (figure 3b) the separation of the clusters was enhanced and the different samples could be discriminated.

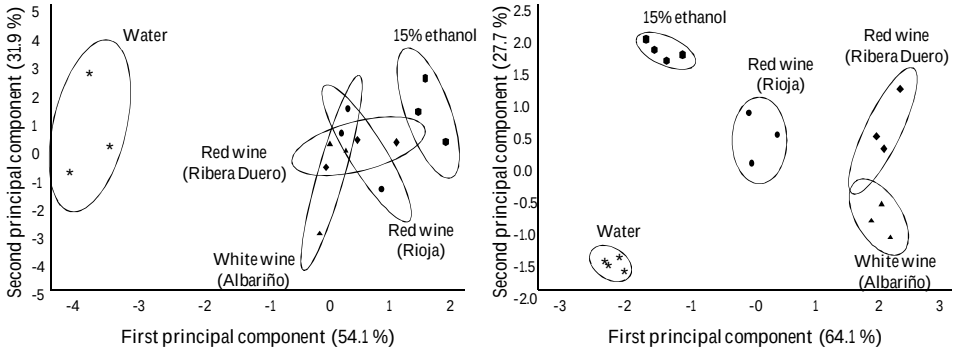

Fig. 3a and 3b. PCA score plot obtained from the analysis of three wines (Ribera del Duero red, Rioja red and Albariño white), water and an ethanolic solution (15% v/v) using an e-nose based on CP gas sensors [42]. (a) SHS sample treatment. (b) DHS sample treatment.

A pervaporation technique was used by Pinheiro *et al.* [44] to monitor aroma production during wine-must fermentation. In this work the aroma compounds were enriched relative to ethanol and, consequently, sample discrimination was improved. In this selective membrane separation process, the aroma compounds are concentrated in the permeate because the membrane has a greater affinity for these compounds than for ethanol and even less for water [37]. In a wine classification study using e-noses based on

MOS sensors, Fort *et al.* [46] applied the same separation technique to water solutions spiked with ethanol and other volatile compounds.

Although all these strategies improve the results of analyzing alcoholic beverages with e-noses based on gas sensors, the speed and the simplicity, two of the main advantages of the e-nose systems, are compromised. Slater et al. [47] avoided the ethanol problem by making the ethanol concentration the same in all the samples. However, if this strategy is to be successfully applied the ethanol content of each sample must be known.

Mass Spectrometry

The most recently developed MS-based e-noses are promising instruments for the analysis of alcoholic beverages because, unlike what happens in gas sensors, ethanol does not cause saturation problems in the mass spectrometer. However, the ethanol fragment ions are much more abundant than the fragment ions of the other volatile compounds. As a consequence, when the chemometric analysis is performed, samples may be differentiated by their ethanol content alone. This problem can easily be solved if the fragment ions corresponding to ethanol are not included in the fragment ion range selected for the mass spectrometric analysis.

Table 2 shows the applications developed using this new generation of instruments in the field of alcoholic beverages. An e-nose consisting of an SHS autosampler and a mass spectrometer was successfully applied to differentiate and classify wines according to various oenological parameters [48]. Figure 4 shows the results of the PCA carried out on the data obtained from analyzing different wines. It can be observed that wines cluster according to their origin. It should be pointed out that a considerable number of wines were analyzed during these studies (table 2). This was possible because the total run-time analysis of a sample when an MS-based e-nose is used is around 5 minutes. Such a short time makes it possible to analyze the high numbers of samples necessary for obtaining reliable results in the chemometric data analysis.

MS-based e-noses have also been shown to be suitable for making quantitative analyses of alcoholic beverages. A multivariate calibration was carried out to determine the concentration of 2,4,6-trichloroanisole (TCA) in wines [30], This compound is the main

cause of a wine off-flavor known as "cork taint". The method developed made it possible to carry out a fast screening of the TCA content in wines. The high sensitivity of the technique when the detector is used in SIM mode enabled the concentration to be determined at ultratrace (sub μg/L) levels with suitable performance parameters (calibration and prediction errors of around 0.44 μg/L and 0.74 μg/L, respectively).

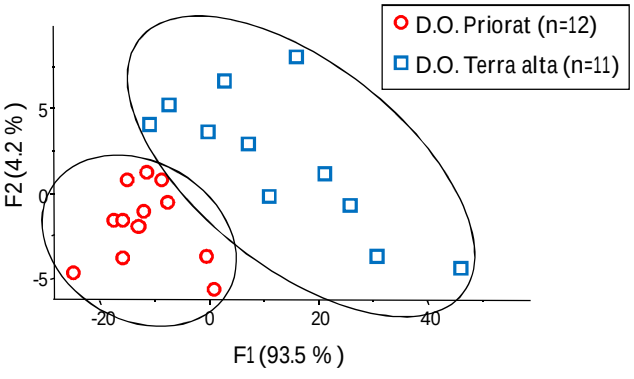

Fig. 4. PCA score plot obtained in the analysis of Priorat (○) and Terra Alta (□) wines using an HS-MS system [48].

Another quantitative study was carried out with Cuban sugar cane spirits to determine the aging time of the samples in oak barrels [43]. Figure 5 shows that there was a good fit between the aging months predicted by the PLS model and the actual aging months of the samples. The sugar cane spirits were also sensory evaluated by a panel of trained judges. The correlation between the sensory data and the MS signal was good and this made it possible to accurately predict the panelists scores [49].

Since MS-based e-noses have been developed recently, few studies on alcoholic beverages have been carried out yet. However, these studies show that the technique has a considerable potential in this field. The simplicity and speed of analyzing complex samples are important advantages over the classical chromatographic techniques and also over sensory analysis in quality control applications.

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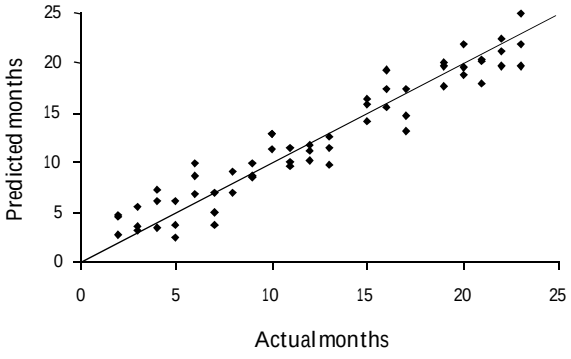

Fig. 5. Aging time of the sugar cane spirits predicted with a PLS model *vs* actual aging time of the samples [49].

Mass Spectrometry vs Gas Sensors

There is no doubt that MS-based systems have considerable advantages over the commonly used gas sensor arrays, in terms of adaptability and sensitivity. With regard to adaptability, when a classical e-nose is purchased the number and type of gas sensors that have to be incorporated must be specified, taking into account the possible applications that will be carried out with the instrument. With mass spectrometry this is unnecessary and the instrument can be tailored to particular applications simply by selecting the optimum set of fragment ions. In fact, as it has been explained above, any conventional GC-MS can be used as an e-nose device. An optimal instrument configuration also allows the same instrument to be used as a rapid screening tool (e-nose) and also as a research tool for revealing further chemical information about doubtful samples. Therefore, an MS-based e-nose can be used to determine not only if one sample is different from another, but also why it is different.

Sensitivity often prevents gas sensor-based e-noses from being used in the field of alcoholic beverages because gas sensors are often subject to interferences from the water and ethanol in the sample, which reduces the sensitivity to other constituents. Furthermore, the sensors' signal often takes a long time to recover before it can analyze the next sample, which involves a very long real run time analysis for each sample.

Long term stability -which is of major importance in routine measurements-, is, in general, an unresolved problem in e-nose devices. Gas sensors are known to be subject to short- and long-term drift due to factors such as changes in relative humidity, temperature, etc. In addition, individual sensors have to be replaced periodically, which contributes to the instability of the technique and involves carrying out instrument calibrations continually. Even though the problem of signal stability is less pronounced in MS-based e-noses, it is still important in the long term. As it has been discussed above, various strategies (internal standardization, standard gas addition and calibration transfer) have been developed to solve this problem. However, considerable research is still necessary, mainly to model sensitivity changes that affect each fragment ion in a different way. Calibration transfer techniques seem to be the most suitable solution to this problem even though they involve additional work. Perhaps the main drawback of the MS-based e-nose is its price: it is much more expensive than gas sensor-based instruments.

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2.3. Use of synthetic wine for models transfer in wine analysis by HS-MS e-nose

L Vera, R Boqué, M Mestres, O Busto, J Guasch

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The new generation of electronic noses (MS e-nose) is known by their sensibility, versatility and the ability to obtain chemical information of the sample when relating the relevant m/z ions obtained in the analyses with the typical fragments of volatile chemical compounds of the sample. However, in addition to the disadvantage that implies its difficult, if not impossible, portability, this technique presents another problem much more important: the signal instability over time. This instability means that the response of the e-nose (i.e. the mass spectra of the sample) will be different depending on the moment in which the analysis is carried out. This variable behaviour of the equipment makes it complicated that the calibration models work successfully. The problem of instability can be due to different causes such loop HS autosampler contamination, the fouling of the MS ion source, the MS vacuum instability or the aging of the MS ion multiplier.

Among the different strategies to solve the signal instability problem, the calibration transfer appears as one of the most satisfactory. However, this strategy requires the use of stable transfer samples with a similar matrix to the sample set of calibration. This requirement is difficult to reach when dealing with wines because of their intrinsic instability produced by their constant evolution.

The following work refers to the application of the calibration transfer method to the analysis of wines that proposes a good way to solve the problem that the wine evolution poses. To get a suitable sample to be used as transfer set, this sample had to be as stable as possible and had to have a similar matrix to the analyzed one. Taking into account these two requirements, we proposed the use of synthetic wines elaborated with a hydroalcoholic solution that contained the most important volatile compounds.

The second difficulty was related to the selection of standards (compounds) to add to the wine with the purpose of elaborate calibration models capable of predicting the concentration of these standards in other wines. These standards had to meet two main conditions: 1) belonging to groups of compounds found in wine to guarantee the applicability of the calibration transfer and 2) its main fragmented ions belong to the range between 50 to 150 m/z ion (range considered in this study). Based on these requirements, the compounds chosen finally were ethyl hexanoate, isoamyl acetate and 2-methylbutanol (ethylic ester, acetate, and alcohol respectively).

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Use of synthetic wine for models transfer in wine analysis by HS-MS e-nose

L Vera[1], M Mestres[1], R Boqué[2], O Busto[1], J Guasch[1]

[1] Analytical Chemistry of Wine and Food Research Group
[2] Chemometrics, Qualimetrics and Nanosensors Research Group
Departament de Química Analítica i Química Orgànica, Unitat d'Enologia del XaRTA. Facultat d'Enologia de Tarragona. Universitat Rovira i Virgili, C/ Marcel·lí Domingo, s/n 43007 Tarragona, Catalonia, Spain

Abstract

A method to verify the feasibility of the calibration transfer technique in HS-MS wine analysis is proposed. PLS multivariate calibration models, whose elaboration and prediction quality are fully discussed, have been built for the quantitative determination of three of the most common volatiles found in wine aroma: ethyl hexanoate (EH), isoamyl acetate (IA) and 2-methyl butanol (MB). The method involves the use of a fortified synthetic wine to overcome the instability drawbacks related to the natural evolution of real wines. The results showed a decrease of the prediction error through the time for the three compounds when the calibration transfer is applied.

Keywords: Headspace-Mass Spectrometry, e-nose, calibration transfer, volatile compounds, synthetic wine, wine

Introduction

Aroma is a fundamental parameter to define the quality of wines. It is composed by hundreds of volatile chemical compounds which belong to very heterogeneous groups such as alcohols, esters, acids, aldehydes, ketones, etc. and that are present at very different concentration levels (from several mg/L to a few ng/L). Due to this complexity, the most widely used technique to determine the aroma composition has been the conventional gas chromatography (GC) [1], which, although slow, is very effective in this kind of analysis, especially when it is coupled to a mass detector. However, to evaluate the aroma properties, sensory analysis becomes imperative, although it is time consuming and requires a trained panel of tasters, which implies subjectivity on the response and variability between individuals [2]. To overcome these limitations and obtain fast and objective aroma information, in the last 25 years new techniques have been studied, such as electronic noses, whose main advantage over other techniques is that they give analytical responses in a few minutes and, as in GC methods, from the overall volatile composition of the samples [2-11].

The most common type of electronic noses is the one based on the interaction of compounds of the headspace with a series of gas sensors whose physic-chemical properties determine the instrumental response, giving a "fingerprint" of the samples analysed that can be used, by means of suitable chemometric tools, to characterize and classify wines. However, when dealing with spirits, these gas sensors are not suitable enough because ethanol, due to its predominance in the headspace, overlaps the response of other volatiles. For this reason, some sample pretreatments must be carried out [12], which slow down the analysis. In addition, sensor passivations cause a great variability on the responses and, as a consequence, the reliability of the models must be continuously checked.

At the end of the 90's, a new type of e-noses appeared, based on coupling the headspace (HS) with a mass spectrometry (MS) technique. In HS-MS systems, the volatile compounds in the headspace of the sample are injected directly in the ionization chamber of the mass spectrometer, where they are fragmented. The result is a global mass spectrum for each sample analysed that constitutes, as in gas sensor e-noses, a sample fingerprint. When dealing with HS-MS e-noses, no sample pretreatment is needed

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because the interference of the ethanol can be skipped by instrumentally avoiding the spectra fragments resulting from the ethanol ionization. Furthermore, the run times are usually very fast (1-5 min/sample), mainly when an autosampler is used. Many applications of HS-MS e-noses can be found in literature, especially related to wine characterizations [13-18].

However, this technology (also called chemical sensor), exhibits a great problem of instability due to loop HS autosampler contaminations and to the MS itself, as a result of the fouling of the ion source, the vacuum instability or the aging of the ion multiplier [24]. If these effects are not corrected, a high irreproducibility, a gradual drift and a loss of sensitivity [19, 20] eventually lead to unsuitable calibration models.

In order to solve the signal instability problems of the HS-MS e-nose, different strategies have been proposed: a) addition of He-Xe [21] to normalize the abundances of each ionic fragment with respect to ^{129}Xe; b) use of an internal standard [22] to rate each mass intensity to the intensity of the fragment corresponding to the internal standard; c) and calibration transfer [23], in which transfer samples (samples of known properties) are processed together with the samples used to build the calibration models.

The principal advantage of the calibration transfer method is that it allows the correction of the signal variations in an independent way in each mass-to-charge ratio (m/z). This overcomes the problem when a signal increases for a fragment and decreases for another one. Moreover, the analysis of an additional sample set is not a problem for a fast technique like HS-MS and it avoids the interference caused by the addition of a standard to the samples. These advantages have favored the use of the transfer calibration method in many applications [24-29].

However, in the HS-MS e-nose calibration step, one of the most important drawbacks found when preparing the wine aroma transfer samples is that these samples are intrinsically unstable and usually irreproducible, since wines evolve continuously, even being bottled. So, to get reliable results it is important to have a standardized method for preparing and using these standards.

In the present work we propose the use of synthetic wines as transfer samples. To verify the reliability of the calibration transfer, the methodology has been applied to the quantification of three of the most characteristic volatile compounds in wine aroma.

Material and methods

Instrumental

All analyses were performed with an HS-MS e-nose comprising an HP 7694 static headspace sampler, an HP 6890 gas chromatograph and an HP 5973 quadrupole mass spectrometer from Hewlett-Packard (Waldbronn, Germany). With this setup, the function of the gas chromatograph was to transfer the volatiles to the MS and not to chromatographically resolve the peaks. So, the analytical column (HP-5MS) was always used in temperature conditions that guarantee the total sample transference in less than 5 min. The softwares used for data collection and analysis were Pirouette 4.0 from Infometrix, Inc. (Woodinville, WA, USA) and PARVUS [30].

Samples and standards

To build and validate the different models, we selected three wines: a red, a rosé and a white wine, all of them with neutral aroma, that is, wines with no predominant note. All the samples were stored under nitrogen atmosphere, in darkness and at 4 ^{o}C to guarantee their stability.

For the calibration transfer, we elaborated different five synthetic wines by diluting 3.5 g of tartaric acid and 120 mL of ethanol in a suitable amount of Milli-Q quality water to give 1L of solution and adjusting the pH to 3.5. Moreover, to obtain a matrix as similar as possible to a real wine sample, we added 25 of the main wine volatiles at different concentrations inside the usual range of concentration of these compounds in real wine [31] (Table 1). These samples were also stored in darkness, under nitrogen atmosphere and at 4 ^{o}C.

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Compound	Range	Wine A	Wine B	Wine C	Wine D	Wine E
Methanol	40-120	70	110	90	100	50
1-Propanol	10-50	15	30	45	25	40
2-Methyl 1-propanol	45-140	60	85	130	105	90
2-Methyl 1-butanol	50-80	55	50	65	60	75
3-Methyl 1-butanol	120-320	140	300	270	160	220
2-Phenylethanol	20-130	90	65	110	55	30
Ethyl butyrate	0.01-4.0	0.9	3.0	2.5	1.5	3.5
Ethyl 2-methylbutyrate	0.05-1.0	0.1	0.6	0.8	0.2	0.1
Ethyl 3-methylbutyrate	0.01-0.04	0.02	0.01	0.04	0.03	0.02
Ethyl hexanoate	0.02-2.0	1.0	1.5	0.5	0.1	0.1
Ethyl octanoate	0.05-3.0	0.5	2.0	2.5	1.5	0.1
Ethyl decanoate	0.0-2.0	0.5	1.5	1.0	1.2	0.01
Ethyl acetate	30-150	130	90	100	45	70
Methyl 2-propyl acetate	0.01-1.0	0.1	0.6	0.5	0.1	0.03
Methyl 3-butyl acetate	0.03-10	2.0	6.0	0.7	8.0	3.0
Hexyl acetate	0.0-0.5	0.4	0.4	0.1	0.1	0.2
Phenylethyl acetate	0.01-2.0	1.0	1.8	0.1	0.5	0.7
Ethyl lactate	10-300	250	160	55	90	200
Acetic acid	50-600	550	420	95	160	310
2-Methyl propanoic acid	1.0-6.0	5.0	2.0	5.5	3.0	4.3
Butyric acid	1.5-4.0	1.9	2.5	2.2	3.5	3.0
3-methylbutyric acid	0.5-5.0	4.0	1.0	3.0	5.0	2.0
Linalool	0.001-0.01	70	110	90	100	50
Ethanal	5.0-100	15	30	45	25	40
Dicacetyl	2.0-3.0	60	85	130	105	90

Table 1. Concentration [mg L^{-1}] of the different chemical added to the 5 synthetic wines and usual range [mg L^{-1}] of these compounds in real wines [30].

The different aroma chemicals (purity>97%) added to the synthetic wine were supplied by Sigma-Aldrich (Madrid, Spain) and Fluka (Madrid, Spain). All the other chemicals and reagents used were of analytical grade.

Stock solutions of the standards chosen for the calibration transfer (ethyl hexanoate (EH), isoamyl acetate (IA) and 2-methyl butanol (MB)) were prepared in ethanol to give final concentrations of 100 μg L^{-1}, 1000 μg L^{-1} and 10000 μg L^{-1}, respectively. Known amounts were added in a randomized way to the different wines to predict them from the HS-MS e-nose calibration model.

Following the procedure described by Pérez Pavón *et al.* [23], three different sample sets were prepared.

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Calibration set: used to build and validate the PLS models (table 2). 2-methyl butanol, ethyl hexanoate and isoamyl acetate were added up to white, red and rosé wines, to prepare the 69 samples -23 of each type- having the usual concentrations found in the literature [31].

White Wine			Rosé Wine			Red Wine		
MB	**EH**	**IA**	**MB**	**EH**	**IA**	**MB**	**EH**	**IA**
53	2.0	1.5	56	1.0	1.8	77	0.7	3.6
58	0.6	5.5	73	1.4	2.8	50	1.4	9.5
64	1.4	4.0	53	2.0	4.0	54	1.9	4.0
73	0.2	9.5	58	1.1	7.0	73	0.2	8.4
80	1.1	2.6	73	1.6	5.5	80	1.6	5.5
56	1.5	8.4	68	1.9	10.0	64	0.6	2.8
50	0.8	3.6	80	1.4	3.6	50	1.4	7.0
73	1.6	7.0	54	0.7	8.4	58	1.1	1.8
54	1.4	1.8	77	0.2	9.5	73	2.0	2.6
68	0.7	2.8	50	1.5	2.6	56	1.0	6.4
77	1.9	10.0	50	0.8	6.4	53	1.5	1.5
50	1.0	6.4	64	0.6	1.5	68	0.8	10.0
58	0.3	8.4	71	0.6	1.8	72	1.58	4.8
63	0.5	4.9	76	1.8	9.6	59	0.7	1.6
78	1.7	9.4	56	0.8	4.6	50	1.1	8.8
68	1.8	5.6	50	1.0	9.5	74	1.2	4.7
50	0.3	2.9	78	0.6	5.6	56	1.0	2.9
80	1.0	9.5	56	1.8	4.7	64	0.6	9.5
58	0.6	1.5	60	1.2	2.9	80	0.3	5.6
74	1.2	4.7	70	0.3	1.5	70	1.8	1.5
54	0.5	8.2	62	0.6	8.2	50	0.6	7.4
75	0.8	7.2	80	0.9	7.2	68	1.5	6.4
60	1.5	6.2	54	1.5	6.0	60	1.6	8.6

Table 2. Concentration (mg L^{-1}) of 2-methylbutanol (MB), ethyl hexanoate (EH) and isoamyl acetate (IA) added to wines used for the elaboration and external validation of model.

The set consisted on two subsets: a calibration subset (18 samples of every type of wine, selected according to the algorithm of Kennard-Stone) and an external validation subset (5 samples of white, red and rosé wines).

Test set: used to evaluate the predictive ability of the model trough time (table 3). Eight samples of each type of wine were prepared as done for the calibration set, and analyzed after one (t_1), three (t_3) and six (t_6) weeks after the model was built.

Transfer set: used to transfer the calibration model through the time. We used 5 synthetic wines (table 1) as transfer samples. These samples were analysed at the beginning of this

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study (t_0), together with the calibration set and also at t_1, t_3 and t_6 together with the test set.

	White Wine			Rosé Wine			Red Wine		
	MB	EH	IA	MB	EH	IA	MB	EH	IA
t_1	53	2.0	1.8	56	0.6	2.0	77	0.7	1.8
	54	0.7	5.5	71	1.4	2.6	50	1.4	9.5
	64	1.4	4.0	53	2.0	4.2	69	1.9	2.8
	77	0.2	9.5	58	1.1	7.0	77	0.2	8.4
	80	1.1	2.4	77	1.6	5.5	80	1.6	5.5
	58	1.5	8.4	68	1.8	10.0	72	0.6	4.4
	50	0.5	3.6	80	1.4	3.6	59	1.4	7.0
	73	1.6	6.4	54	0.7	8.4	54	1.1	3.6
t_3	66	1.5	6.8	68	0.7	8.4	80	0.3	6.2
	56	2.0	1.6	50	1.5	6.2	65	1.9	10.0
	63	1.6	4.2	78	1.9	10.0	54	0.6	5.2
	52	0.9	9.0	70	0.4	3.4	66	1.3	2.8
	80	0.4	7.9	57	0.8	9.6	58	1.7	8.6
	56	0.2	3.0	60	1.3	4.6	62	0.6	3.4
	52	0.7	5.5	80	1.2	3.2	55	0.7	9.0
	73	1.0	9.6	54	0.9	7.2	76	1.5	7.4
t_6	56	1.9	3.0	69	0.4	9.6	80	0.5	10.0
	68	0.4	9.6	50	1.1	7.8	18	1.4	3.4
	78	0.9	6.4	78	0.3	8.4	34	1.0	5.2
	51	1.7	3.8	70	2.0	4.9	57	0.4	1.7
	58	0.8	5.5	57	1.5	7.4	92	1.7	9.2
	64	1.4	6.0	60	0.9	3.6	82	1.9	7.1
	75	1.2	8.4	80	1.4	1.6	75	0.7	9.0
	50	0.3	7.0	54	1.6	10.0	64	1.5	6.0

Table 3. Concentration (mg L^{-1}) of 2-methylbutanol, ethyl hexanoate and isoamyl acetate added to wines to verify the predictive capacity of the model through the time (t_1, t_3, t_6).

Analytical procedure

Aliquots of 5.0 ml of wines and calibration samples were placed in 10 mL vials with NaCl (2M). The vials were hermetically capped with PFTE/silicone septum under N_2 atmosphere. All the samples were prepared and analyzed in triplicate.

The samples were kept at 65 ^{o}C for 1 h and under constant stirring in the autosampler. The headspace generated was transferred to the injection port at 90^{o}C. The injection was made at 250^{o}C using an inlet of 1.5 mm i.d. An HP-5MS column (30m x 0.25 mm x 0.25 µm) was used. The oven temperature program was as follows: 70 ^{o}C (1 min), 70 ^{o}C min^{-1}, 180 ^{o}C (2.5 min). The carrier gas was helium with a flow-rate of 1.6 ml min^{-1}. All these

conditions have been previously optimized in our lab [14] and allow the transference of all the compounds to the detector in only 5 minutes. The mass spectra were recorded by electronic impact (EI) ionization at 70 eV with a temperature of 230 ^{o}C in the ion source and 150 ^{o}C in the mass quadrupole. To discard the ethanol interference mass-to-charge ratios lower than 50 m/z were not analysed.

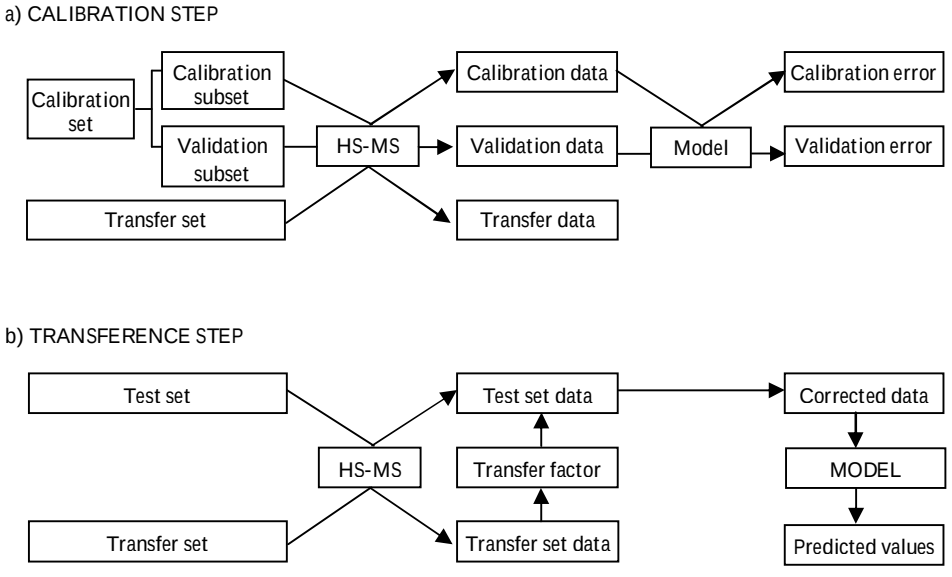

Fig. 1. Diagram of the methodology carried out in the study.

The analytical signal obtained after ionization and fragmentation of each sample (usually registered as the sum of the abundances of all the ions detected during the data-acquisition time), is a row of the measured m/z values. The data matrix of samples (rows) and m/z values (columns) was treated by chemometrics to perform multivariate calibration. Figure 1 shows the methodology used in this study.

Statistical and multivariate analysis

We used the partial least squares (PLS) regression method to build the multivariate calibration models between the m/z values (predictors) and the concentration of MB, HE and AI (responses). A PLS model was built for each response for each wine: 9 different models were obtained. The quality of the models was checked by means of the root mean square error of calibration (RMSEC), the root mean square error of cross validation (RMSECV), and the root mean square error of prediction (RMSEP), calculated as:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{i=n}\left(y_i - \hat{y}_i^C\right)^2}{n - F - 1}} \qquad \text{Eq. 1a}$$

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

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{i=m}\left(y_i^* - \hat{y}_i^P\right)^2}{m}} \qquad \text{Eq. 1c}$$

were y_i is the known concentration value, $\hat{y}_i^C$ and $\hat{y}_i^{CV}$ are the values computed by the model in calibration and predicted by cross validation respectively, n is the number of samples in the calibration set and F is the number of factors of the model selected by the criterion of predictive residual error sum of squares (PRESS) (Eq 1a and 1b). For the external test sets, y_i^* is the known concentration value, $\hat{y}_i^P$ the value predicted by the model and m is the number of samples of the set (Eq 1c). (Eq 1a and 1b)..The models were internally validated by means of cross-validation. With this procedure, a sample is left out and the model is built with the rest of the samples. The sample left out is predicted and the residual kept. The procedure is repeated until all samples have been left out once. Finally, the root mean square error of cross-validation (RMSECV) is calculated using Eq. 1b. Additionally, for each model the residual predictive deviation (RPD) was calculated as the ratio between the RMSECV and the standard deviation of the values of concentration added. An RPD>3 is indicative of a suitable calibration model. On the other hand, a

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parameter that indicates the dispersion of the data with respect to the average is the coefficient of variation (CoV) which is defined as the ratio between the standard deviation and average ($\bar{x}$) of the concentration values added for all the samples in the calibration set. The relative standard deviation, RSD, was used to evaluate the percentage of variation of the RMSE (RMSECV and RMSEP) with respect to the average ($\bar{x}$) of the known concentration values:

$$RSD(\%) = \left(\frac{RMSE}{\bar{x}} \right) \times 100$$

The algorithm used for the calibration transfer was the multiplicative correction algorithm, where the value of the average abundance of each mass-to-charge (m/z) ratio of the test samples, $I(m/z)_P$, is multiplied by the transfer factor R_T. This factor is obtained as the ratio between the average of each m/z intensity of the *p* transfer samples, *I*(m/z), measured at two different times: the time of building the calibration model (t=0) and the time of analysis of the test samples (t= t'). So, the corrected abundance value, $I(m/z)_C$, of each fragment is calculated with the following expression:

$$I_{(m/z)_C} = I_{(m/z)_P} \times R_T \quad , \qquad R_T = \left(\frac{\sum_{1}^{p} I_{(m/z)_0}}{\sum_{1}^{p} I_{(m/z)_{t'}}} \right)$$

Results and Discussion

Under the conditions described above, the analytical signal obtained after the ionization and fragmentation of the volatiles injected in the electronic nose was a global mass spectrum, result of the sum of the abundances of all the ions detected during the data-acquisition time (TIC). These data can be arranged in a *n*x*p* multidimensional matrix, where *n* is the number of samples and *p* the number of m/z ratios.

Calibration model

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As explained above, to build the calibration model we used a set of 23 spiked samples with different amounts of EH, IA and MB for each type of wine (white, red and rosé).

Since many m/z channels do not provide a significant signal (abundance), a variable selection procedure was applied to select the most important mass channels. The standard deviations rank algorithm, provided by Pirouette 4.0, was used for m/z variable selection. The standard deviation for all the samples was computed for each variable and then the first 25 variables with the highest values of standard deviation were retained and included in the new set (figure 2). Moreover, we verified that the m/z ratios of the added compounds were included in this variable selection. In order to avoid differences caused by the instrument between the first and the last injection, the row profile normalization available in PARVUS was applied to the spectra. The row profile algorithm divides each m/z of the sample for the sum of all m/z for that sample.

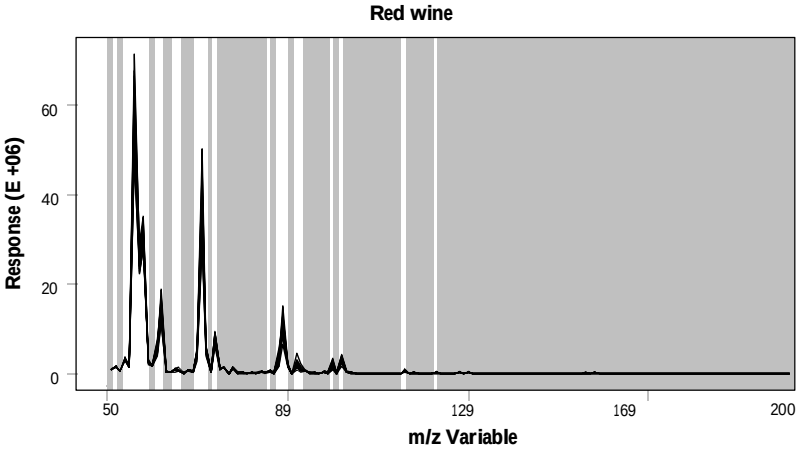

Fig. 2. Selected variables for red wine using the standard deviations rank algorithm.

The analytical performance parameters of the calibration models are summarized in Table 4. In all cases the RPD values are around 3, so the models obtained seem acceptable. It can be observed that the lowest %RSD values are found for MB and the highest for HE. CoV values for EH and IA are similar, and around 3 times higher than for MB.

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Once the calibration models were obtained, the external validation samples were also analyzed and the quality of the predictions was evaluated with the RMSEP (Eq. 1c). These samples showed similar results compared to the cross validation samples.

		CALIBRATION SET					
		FACTOR	RMSEC	RMSECV	CoV	%RSD	RPD
Red	MB	6	2.05	3.13	0.17	4.93	3.50
	EH	7	0.09	0.13	0.47	11.66	4.07
	IA	4	0.45	0.64	0.51	11.80	4.30
Rosé	MB	4	1.89	3.03	0.16	4.82	3.29
	EH	5	0.14	0.21	0.51	18.77	2.70
	IA	4	0.51	0.71	0.52	12.30	4.18
White	MB	4	3.23	4.79	0.17	7.43	2.29
	EH	7	0.11	0.18	0.55	17.74	3.09
	IA	4	0.49	0.75	0.54	13.72	3.94

Table 4. Analytical results for the calibration day (t=0) for the nine models elaborated. The RMSEC and RMSECV values are in mg L^{-1} units.

The accuracy of the models, evaluated with RMSEC and RMSECV, depends mainly on three factors: the concentration of the analyte, the standard deviation of the analyte concentrations in all the samples and the ion abundance (m/z) of the compound added.

Regarding the first factor, it can be observed that the concentration levels of the MB are around 60 times higher than the ones of EH and 12 times higher than the ones of IA. However, the %RSD values for the HE are the highest and for the MB are the lowest. This is a normal behaviour taking into account that the %RSD should be higher for low concentration values.

Also, there are differences in the CoV values (table 4) between the three compounds. The CoV values for MB are lower than the values for EH or IA, which are around 3 times higher. These results indicate that the concentration variability of MB in the different samples is low. This fact would explain the lower RPD values for MB. High CoV values are preferred to obtain better PLS models.

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MB		EH		IA	
m/z	*Rel. abund*	*m/z*	*Rel. abund*	*m/z*	*Rel. abund*
57	**100**	**88**	**100**	**70**	**63.4**
56	**87.8**	60	38.9	**55**	**43.5**
70	**42.4**	99	51.9	73	13.9
55	**28.2**	71	26.1	**61**	**12.8**
-	-	101	25.8	-	-
-	-	**61**	**23.6**	-	-
-	-	73	23.3	-	-
-	-	**70**	**22.9**	-	-

Table 5. Relative abundance of the m/z ions produced in the fragmentation of the compounds added (in bold the m/z channels coincident with the most abundant m/z of wine).

Concerning the ion abundance of the compounds added, it has to be said that the maximal m/z abundances in the wine matrix are produced by the 55, 70, 57, 56, 88 and 61 ions (55, 70 and 57 are the greatest). Table 5 shows the relative abundance of each m/z produced in the fragmentation of each one of the compounds added. The four most abundant m/z ions that provide MB coincide with the four maximal abundances in the wine matrix; and this fact, together with the high concentration values of MB, gives low %RSD values. Among the different m/z ratios obtained for EH, only three of them coincide with the m/z wine ratios (55, 57 and 88) and these do not show high abundances in the wine matrix. This fact, together with the low EH concentration levels, results in a low signal to noise ratio which hampers to obtain an optimal model and posterior good predictions. Regarding IA, there are three m/z ratios coincident with those of the wine and with important abundance. In this case, this coincidence and the high dispersion (CoV values) lead to the best models.

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Transference of the calibration model

To monitor the validity of the model through the time, we used a test set. Together with the test samples, a set of transfer samples were also analyzed. These samples must be very stable, reproducible, and representative [32]. The stability is an important aspect because the changes registered in the signals should only be attributed to instrumental causes. It is also necessary that the transfer samples have a matrix as similar as possible to the real samples, because only in that way the signal changes (m/z intensity) will be comparable to the signal changes of the wine. Thus, once registered the signal changes in the synthetic wines, it will be possible to correct the signal of the test samples. Figure 3 shows the great similarity between the mass spectra of a real wine and a synthetic wine.

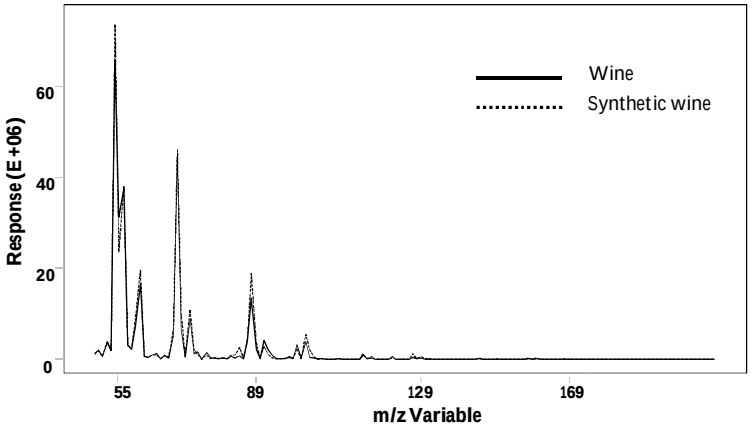

Fig. 3. Comparison between the global mass spectrum of a real wine and a synthetic wine

The first test set was analyzed one week after building the model (t_1) (table 6). The %RSD values were, in general, similar whether the calibration transfer was applied or not. RMSEP values improved for EH and IA and were similar or slightly worse for MB.

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		t_1 prediction set											
		Without calibration transfer						With calibration transfer					
		RMSEP	$\bar{x}$	%RSD	m	c	R^2	RMSEP	$\bar{x}$	%RSD	m	c	R^2
Red	MB	4.13	67.25	6.14	0.88	11.10	0.96	4.13	67.25	6.14	0.93	1.27	0.94
	EH	0.24	1.12	21.74	1.15	-0.04	0.91	0.21	1.12	18.55	1.05	0.03	0.90
	IA	0.53	5.38	9.82	1.03	0.16	0.98	0.44	5.38	8.25	1.02	0.05	0.98
Rosé	MB	6.93	64.63	10.73	0.66	19.40	0.61	9.00	64.63	13.93	0.69	13.40	0.61
	EH	0.34	1.33	25.33	0.86	0.46	0.84	0.22	1.33	16.46	0.81	0.21	0.80
	IA	0.61	5.41	11.34	0.93	0.69	0.96	0.53	5.41	9.86	0.93	0.47	0.96
White	MB	3.97	63.63	6.24	1.08	-2.85	0.94	4.03	63.63	6.33	1.10	-9.27	0.96
	EH	0.24	1.12	21.21	0.96	0.25	0.95	0.21	1.12	18.86	0.86	0.28	0.92
	IA	0.88	5.20	16.89	1.02	0.56	0.95	0.75	5.20	14.50	0.99	0.50	0.95

		t_3 prediction set											
		Without calibration transfer						With calibration transfer					
		RMSEP	$\bar{x}$	%RSD	m	c	R^2	RMSEP	$\bar{x}$	%RSD	m	c	R^2
Red	MB	9.89	64.50	15.33	0.81	21.00	0.78	12.81	64.50	19.86	0.79	25.20	0.75
	EH	1.11	1.07	104.04	0.99	1.11	0.93	0.20	1.07	18.62	0.88	0.14	0.88
	IA	1.48	6.58	22.54	0.82	2.54	0.96	0.65	6.58	9.88	0.80	1.40	0.96
Rosé	MB	13.39	64.63	20.72	0.73	29.70	0.73	8.02	64.63	12.41	0.75	21.80	0.73
	EH	0.83	1.07	77.83	1.13	0.68	0.96	0.18	1.07	16.72	1.05	0.09	0.95
	IA	1.56	6.58	23.68	0.84	2.51	0.96	0.64	6.58	9.74	0.82	1.40	0.96
White	MB	14.27	62.25	22.93	0.89	21.00	0.94	7.95	62.25	12.77	0.87	15.70	0.94
	EH	0.52	1.05	49.53	1.13	0.36	0.96	0.14	1.05	13.51	1.04	-0.11	0.96
	IA	0.70	5.95	11.70	1.04	0.41	1.00	0.18	5.95	3.00	1.03	-0.24	1.00

		t_6 prediction set											
		Without calibration transfer						With calibration transfer					
		RMSEP	$\bar{x}$	%RSD	m	c	R^2	RMSEP	$\bar{x}$	%RSD	m	C	R^2
Red	MB	18.09	62.75	28.82	1.16	7.35	0.99	16.37	62.75	26.09	1.13	7.41	0.99
	EH	1.33	1.14	117.01	1.10	1.19	0.81	0.30	1.14	26.52	0.94	-0.04	0.76
	IA	3.10	6.45	48.11	0.91	3.65	0.97	0.75	6.45	11.70	0.85	1.42	0.96
Rosé	MB	22.87	64.75	35.32	1.01	21.60	0.83	6.98	64.75	10.78	1.10	-1.00	0.87
	EH	1.33	1.15	115.79	1.08	1.23	0.93	0.33	1.15	28.84	0.99	0.27	0.87
	IA	3.57	6.66	53.51	0.99	3.58	0.94	0.80	6.66	12.05	0.91	0.99	0.94
White	MB	28.01	62.50	44.81	1.01	27.10	0.98	10.09	62.50	16.14	0.98	11.40	0.98
	EH	0.67	1.07	62.93	1.20	0.45	0.98	0.10	1.07	9.65	1.07	-0.10	0.98
	IA	2.66	6.21	42.75	1.00	2.62	0.97	0.73	6.21	11.81	0.93	1.08	0.97

Table 6. Results for the prediction samples using the model elaborated at t_0. RMSEP, average ($\bar{x}$), %RSD, slope (m), intercept (c) and correlation coefficient R^2 are showed.

The next analyses were carried out after 3 and 6 weeks, respectively (t_3 and t_6) (table 6). For t_3, most of the predictions improved with the calibration transfer, especially for EH that showed an improvement of 250-450% (in terms of %RSD). After six weeks (t_6), it can be observed a clearly enhancement in all cases because the %RSD values decreased for the three compounds in the three type of wines, especially for EH and IA, where %RSD values decreased by a factor of three.

Figure 4 shows the results of prediction at t_6 without and with application of the calibration transfer. As it can be seen, the use of the model transfer strategy is essential; the prediction results tend to be closer to the line with a slope value of 1, which would imply the total coincidence between the predicted and the actual values.

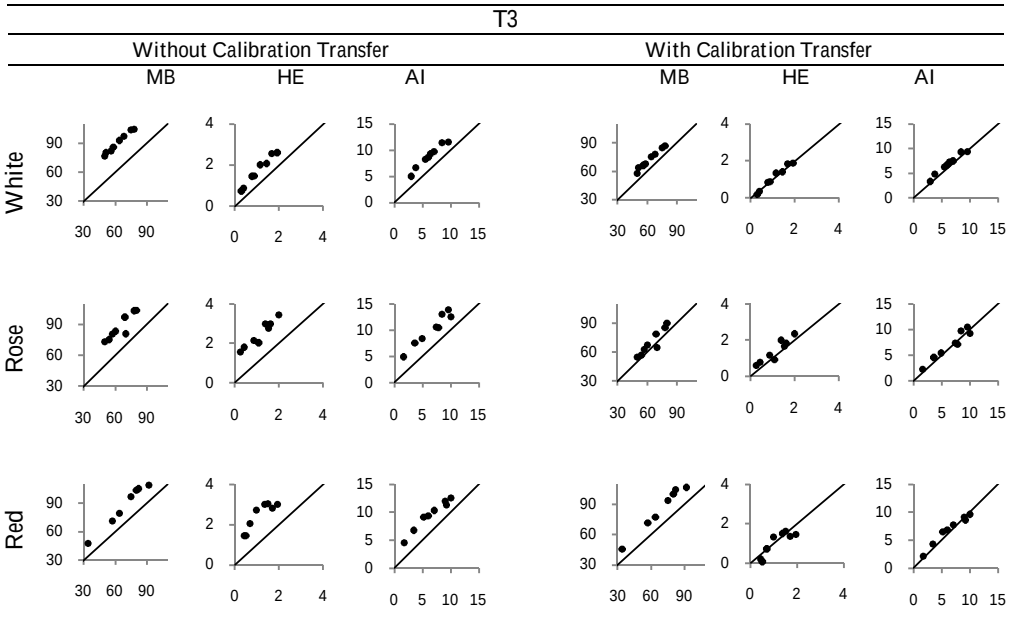

Fig. 4. Graphical results for the prediction samples analyzed six weeks after the elaboration of the model.

Conclusions

The results of this study show that calibration transfer is a suitable tool for the HS-MS e-nose technique when it is applied to the analysis of wine aroma. The use of a synthetic wine, prepared with the most abundant volatile compounds in wines, proves to be a good solution to the instability problem that implies the use of real wines as transfer samples. The signal variations that synthetic wines undergo over time can be considered as representative of the ones found in real wines, thus enabling an accurate correction, which is more evident at long times after the calibration model was built.

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2.4. Aromatic characterization and classification of beer samples by means of an MS e-nose and chemometric tools

L Vera, L Aceña, R Boqué, J Guasch, M Mestres, O Busto

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An important characteristic of the MS e-nose instruments is its ability to provide chemical information of the samples. This information can be obtained through the fragmentations registered which can be assigned to important aromatic compounds present in the sample. To better evaluate the information, it is very helpful a graphic representation but, due to the complexity of the information contained in the spectra, to represent this chemical information is necessary the proper use of the chemometric techniques. Indeed, when using some chemometric techniques it was possible the visualization of the scores and loadings which allowed leading to chemical interpretations.

The great majority of studies with the MS e-nose in alcoholic beverages analysis refer to discrimination/classification, differentiation and quantification of parameters (see section 2.2). However, the following publication refers to the application of the MS e-nose to the study of characterization of sample beers. The beers used in this study, although elaborated in different factories, *a priori* should be the same, since there are commercialized under the same brand. Nevertheless, the results obtained showed a differentiation which could be related to the brewer factory.

The goal of this study was to find the possible causes of the differences from the chemical information. The Fisher Linear Discriminant Analysis (Fisher-LDA) as supervised classification technique was used and the interpretations were made respect the score and loading representation. The fragments (variables) responsible of the discrimination between factories were selected by a variable selection algorithm and, finally, these were related to the chemical compounds.

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Aromatic characterization and classification of beer samples by means of an MS e-nose and chemometric tools

L Vera[1], L Aceña[1], J Guasch[1], R Boqué[2], M Mestres[1], O Busto[1]

[1] Analytical Chemistry of Wine and Food Research Group
[2] Chemometrics, Qualimetrics and Nanosensors Research Group
Department of Analytical Chemistry and Organic Chemistry
Campus Sescelades. Facultat d'Enologia de Tarragona.
Universitat Rovira i Virgili
43007 Tarragona (Spain)

Abstract

An electronic nose based on coupling the headspace (HS) with a mass spectrometer (MS) has been used in this study to classify and characterize a series of beers according to their production site and their chemical composition. With this objective, we analysed 67 beers of a same brand and prepararion process but produced in different factories. The samples were also subjected to sensory evaluation by a panel of experts.

Linear discriminant analysis (LDA) was used as classification technique and Stepwise-LDA based on the Wilk's Lambda criterion was used to select the most discriminating variables. To interpret the aroma characteristics of the beers from the m/z ions obtained, score and loading bi-plots were obtained by applying the canonical variables.

Because the beers analyzed were marketed with the same name and brand, we expected to be working with the same product irrespective of its origin. However, results from both sensory evaluation and use of the e-nose revealed differences between factories.

With the e-nose it was possible to relate these differences to the presence (and abundance) of characteristic ions of different compounds typically found in beer. These results demonstrate that the HS-MS e-nose is not only an aroma sensor capable of classification of, and differentiating between samples but also to provide information about the compounds responsible for this differentiation.

Keywords: MS e-nose, beer, volatile compounds, classification, characterization, LDA

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Introduction

Beer is an alcoholic beverage, not distilled, with a bitter taste, and made by the brewing and fermentation of soluble sugars from malt starch in water and, often, flavoured with hops which add bitterness. Several variants, with a wide range of shades, are known, because of the different production processes and ingredients used. So, every type of beer has specific organoleptic properties encompassing gustative, visual and aroma perceptions.

As for other alcoholic beverages, organoleptic properties should be evaluated for quality control of the breweries because, when a consumer buys a beer of a particular brand, he also expects specific organoleptic characteristics. However, these characteristics are affected by the raw materials, the fermentation of the wort and the technological conditions used for production. Hence, for breweries it is a challenge to obtain always a similar product, especially when that product is produced in different factories.

Although sensory evaluation of beer involves all the senses, the smell and the taste are the most important. The olfactory evaluation of beers is very complex because the aroma results from a complex mixture of several volatile chemical compounds that belong to very heterogeneous groups, for example alcohols, esters, sulphur compounds, ketones, aldehydes, etc. present at very different concentrations (from several mg/L to a few ng/L).

The presence of this variety of volatile chemical substances gives the beers different aroma qualities, which enable their characterization. The alcohols (the principal group of volatile compounds), play an important role in the flavor perception of other beer components due to the synergistic and antagonistic effects that these compounds induce on the aroma perception. Moreover, the higher alcohols are precursors of the more flavor-active esters that contribute to a fruity aroma impression. Indeed, higher alcohols and esters are necessary for the aroma profile of a high-quality beer. Other important chemical groups which should be highlighted include the hop oils, which are associated to bitterness (iso-α-acids) and that provide a unique “hoppy” aroma, and also the sulfur compounds. The later, are not desirable in beer because of their unpleasant odor (cabbage, onion, garlic, rubber, etc), even at low concentrations, because of their low odor thresholds [1].

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On the other hand, it must be taken into account that beer aroma arises not only when sniffing, but also when tasting the beer because, since the mouth is connected to the nose via the retronasal passage, the volatile compounds can also reach the nose receptors [2]. All these complexities hamper the fast and reliable evaluation of beer aroma, needed during quality control, to define the quality, characteristics and authenticity of the beer.

Recently, interest in developing instrumental techniques for rapid aroma analysis has increased. With the development of the "electronic nose" technique it has been possible to obtain analytical responses in a few minutes and from the overall volatile composition of the samples. Indeed, in wine analysis, there are several studies where electronic noses have been successfully applied in classification, discrimination and even correlation/comparison with assessments of sensory panels [3-12].

The electronic nose based on mass detector (HS-MS e-nose, also called chemical sensor) is able to carry out analyses in very short times and with minimum sample preparation. In this instrument the volatile compounds extracted from the headspace of liquid samples are introduced in the ionization chamber of a mass spectrometer, in which they are fragmented. The fragmentation and ionization of all the volatile compounds are recorded as the abundance of the mass to charge ratio (m/z) of each ion in the called mass spectrum. Finally, the mass spectral data obtained for each sample are collected in a data matrix, where the m/z abundances are the columns and the samples are the rows. This enables chemical information to be obtained about the compounds responsible for the differences between the analyzed samples. Final interpretations are possible by use of chemometrics, which enable classification differentiation and characterization of the samples.

The principal advantage of these instruments is that the run times are usually very short (1-5 min/sample), mainly when an autosampler is used. Some applications of HS-MS e-noses related to beer characterization can be found in the literature [13-15]. Although the signals from these instruments are unstable over time, different strategies have been proposed to solve this problem. The calibration transfer method [16] has been reported as a suitable option and it has been successfully applied to wine analysis [17].

In this work, we attempt to achieve two principal objectives. The first was to determine whether the HS-MS e-nose is able to discriminate among beers produced in different factories that a priori should be the same, because they are marketed as the same product; the second was to verify if the HS-MS e-nose can aid identification of the chemical compounds responsible of these differences.

Material and methods

2.1. Sensory analysis

The beer samples were sensorially evaluated by a panel of 10 trained assessors, who performed the sensory analysis considering the aroma as a global impression and only when a very remarkable organoleptic note was detected did they describe that perception. The assessors made the sensory evaluation without knowing the origin or any other information related to the beers (blind tasting).

The evaluation of the aroma profile of the different samples was performed on a discontinuous scale from 1 (very good) to 5 (very bad). The coefficient of variation for sample replicates of the panellists was approximately 15%.

From the sensory results, the beers were classified in four categories: category I included the beers evaluated with values below 2.6; category II included the beers within a scoring range of 2.61-2.79; category III included the beers within a scoring range 2.8-2.99; and finally category IV included the beers with values higher than 3.

2.2. Samples

Sixty-seven lager beer samples of the same brand were obtained from four different factories: 11 from factory A, 15 from factory B, 21 from factory C and 20 from factory D. To ensure the samples were representative, the sampling was conducted over six months and, for each sample, we took, randomly, 3 bottles of freshly bottled beer.

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2.3. Instrumental

Beer analysis was performed in a HS-MS from Hewlett-Packard (Waldbronn, Germany), composed of a HP 7694 static headspace sampler, a HP 6890 gas chromatograph and an HP 5973 quadrupole mass spectrometer with a diffusion pump. Because the function of the gas chromatograph was to transfer the volatiles from the headspace sampler to the MS detector, the apolar analytical column (HP-5MS) was kept at the suitable temperature to guarantee transfer of the volatiles in less than 5 min to the MS and to avoid the chromatographic separation. The softwares used for data collection and analysis were Pirouette 4.0 from Infometrix, Inc. (Woodinville, WA, USA) and PARVUS [18].

2.4. Beer analysis

Before analysis, the beer samples were degassed by ultrasonication for 15 min. Samples (5.0 ml) were then placed in 10 mL vials with NaCl (2M). The vials were hermetically capped with PFTE/silicone septa under N_2 atmosphere. All the samples were prepared and analyzed in triplicate.

To achieve equilibrium of the volatile compounds between the liquid and the headspace, the samples were termostated at 65 ^{o}C for 1 h and under constant stirring in the headspace autosampler. Three millilitres of the headspace generated was then transferred to the injection port through the transfer line at 90 ^{o}C. Injection was in splitless mode for 1.6 min at 250 ^{o}C using an inlet of 1.5 mm i.d. An HP-5MS column (30m x 0.25 mm x 0.25 µm) was used. The oven temperature program that guarantees the transference of the volatiles in the shortest time (less than 5 min) to the MS was: 70 ^{o}C (1 min), 70 ^{o}C min^{-1}, 180 ^{o}C (2.5 min) [19]. The carrier gas was helium with a flow-rate of 1.6 ml min^{-1}. The mass spectra were recorded by electronic impact (EI) ionization at 70 eV with a temperature of 230 ^{o}C in the ion source and 150 ^{o}C in the mass quadrupole. The mass-to-charge ratio range (m/z) used was 50-150. Because the ethanol is the most abundant volatile compound (approx. 5%), ratios lower than 50 m/z were not analysed to avoid interference from ethanol.

2.5. Multivariate analysis

Principal Component Analysis (PCA)

The PCA was used for a preliminary visualization of the 67 beers. PCA reduces the dimension of the data matrix and compresses the information in a few new variables called principal components, which are linear combinations of the original variables. The first principal component, PC1, covers the maximum information direction and is orthogonal (that is, explains complementary information) to PC2. This second PC is orthogonal and explains more information than PC3, and so on. The graphical representation obtained from PCA shows the maximum variability among samples.

Linear Discriminant Analysis

Linear Discriminant Analysis (LDA) was used to discriminate among the beer samples according to their factories. LDA is a class-modeling technique that classifies each sample (object) in a specific class, where the class of each sample is known *a priori* (supervised). To use LDA it is necessary that the number of objects (samples) is less than the number of variables. For spectral measurements (where normally the number of variables is greater than the number of samples) an alternative is the use of the principal components determined by PCA on the original data.

Two approaches, the Bayesian and the Fisher approaches, are used to derive a rule for discrimination between groups.

Bayesian linear-discriminant analysis. This is based on the hypothesis that the data in the classes follow a normal distribution, with their dispersion described by the same covariance matrix and differing only in the position of their centroid. The classification rule is based on linear discriminant scores, which are directly derived from plugging the density of the multivariate normal distribution into the equation for *a posteriori* probabilities [20]. A sample (object) is classified in the class for which it has the highest probability.

Fisher Linear Discriminant Analysis [18]. This is based on obtaining the canonical variables, which are the directions with the maximum discriminant power among classes.

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The canonical variables (CV) are obtained by maximizing the ratio between the between-class variance and the within-class variance, w/p:

$$w = \frac{C}{C-1}\frac{\sum_{c=1}^{C} I_c(\overline{d_c} - \overline{d})^2}{I} \qquad \text{Eq. 1A}$$

$$p = \frac{\sum_{c=1}^{C}\sum_{i=1}^{I_c}\left(d_{ic} - \overline{d_c}\right)^2}{(I-C)} \qquad \text{Eq. 1B}$$

with:

$$\overline{d}_c = \sum_{i=1}^{I_c} d_{ic} \Big/ I_c$$

$$\overline{d} = \sum_{c=1}^{C}\sum_{i=1}^{I_c} d_{ic} \Big/ I$$

where *C* is the number of classes, I is the total number of objects, I_c is the number of objects of class *c* and d_{ic} is a coordinate where the points are projected.

This means that the distances among centroides of each class must be the longest possible compared to the within-class dispersion. So, the first canonical variable will be the direction with the maximal w/p ratio. With this method, it is possible to obtain a visual representation of the data information by overlapping the scores and loading plots (bi-plot).

Variable selection

Additionally, the Stepwise-LDA (SLDA) [18] algorithm was applied to find the variables that best discriminate the factories and to study their potential contribution to the aroma of beers. SLDA was applied using the Wilk's Lambda criterion, which computes the ratio between the determinants of the within-class variance matrix and of the variance matrix

for the whole set of samples. The selected variable is the one that produces the highest decrease of this ratio.

Results and discussion

Figure 1 shows the percentage of beers of each factory that were classified in each one of the four categories according to the sensory score. As it can be observed, factory A differed from the others because of its high percentage of beers classified in category 4 (C4) and also because of the absence of samples classified in C1. Factories C and D showed similar percentages of beers classified in categories C1 and C4. Finally, factory B had most of its beers classified in category 3. From these results it appears that the beers coming from factories C and D had very similar organoleptic properties and that they were somewhat different from the ones of factories A and B. Also, the sensory perception of beers coming from factory A was different to that of beers from factory B.

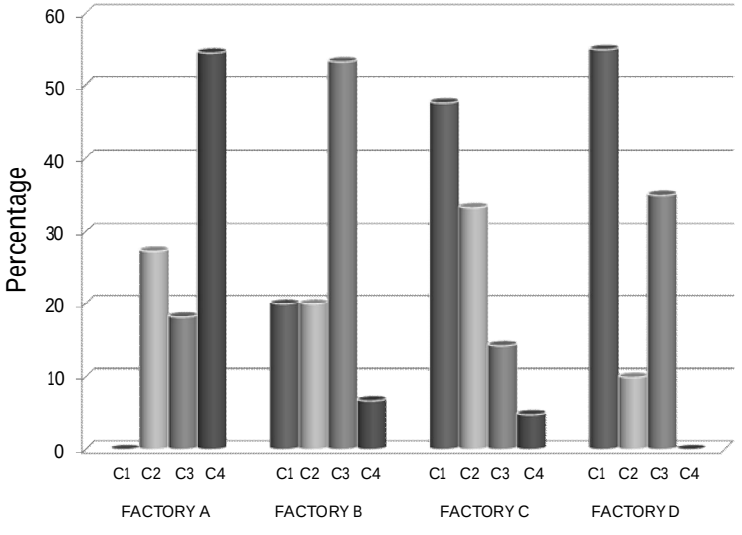

Fig. 1. Percentage of samples of each factory assigned to the 4 quality categories.

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Figure 2 shows the mass spectra of the 67 beers. This information was also collected in a data matrix of 67 samples (rows) and 101 m/z ratios (columns). The range of m/z used in this study was restricted from 50 to 150 to avoid the ethanol influence (m/z=45 and 46) and because the main compounds that contribute to the beer aroma are fragmented in m/z less than 150. The range between 100 and 150 m/z ion has been magnified in figure 1 to show other important m/z ions.

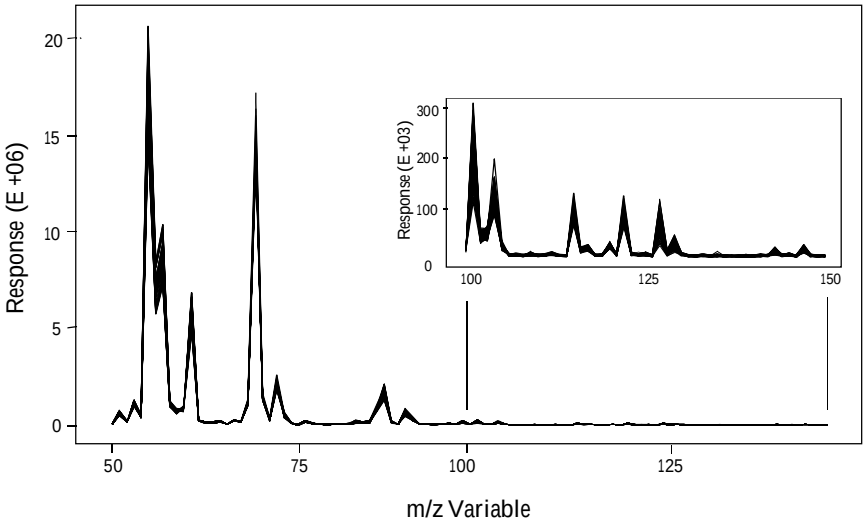

Fig. 2. Spectra of the 67 beers obtained by the analysis by MS e-nose.

In order to minimize the usual signal shift -the analyses were carried out in different days because of the large number of samples-, the collected spectra were normalized. Thus, the data matrix was initially pre-treated with row profile, which divides each m/z abundance of the sample by the sum of all m/z abundances for that sample. Prior to the execution of each algorithm, the data were autoscaled to give importance to those ions with lower abundance values but belonging to important aromatic compounds with low perception thresholds.

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We first applied PCA to the data. The score plot of the two first PCs (37% variance explained) is shown in figure 3. The first PC shows a separation between factories, although the beers of factories C and D are not completely differentiated. This behavior was similar to the one observed with the sensory values provided by the panel of experts (Figure 1).

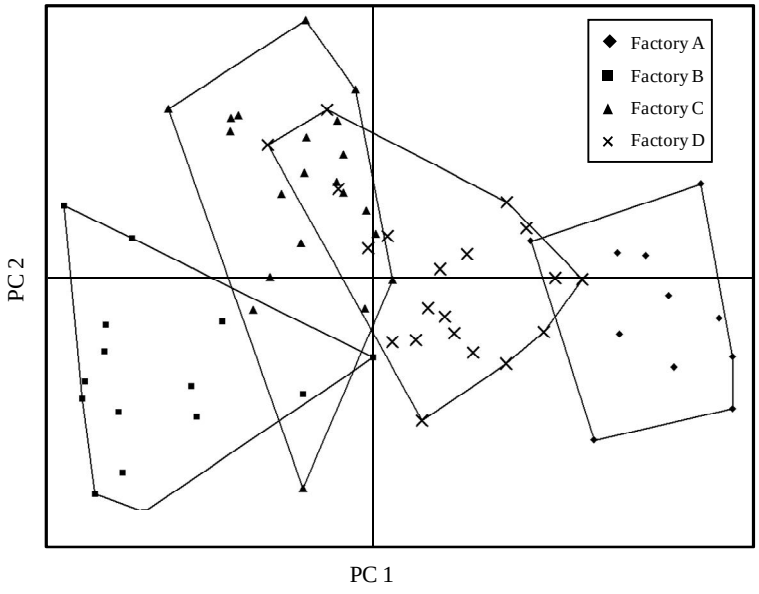

Fig. 3. Score plot on the first two principal components (37%) computed with all variables.

Table 1 and figure 4 show the Bayesian LDA results. As in figure 3, figure 4 shows a clear separation of factories A and B, whereas factories C and D are not separated completely. Due to the high number of collected variables (101), LDA was executed on the first seven principal components, which explain the 66% of the total variance. Table 1 shows the confusion matrix obtained after cross-validation with five cancellation groups. In this process, the original set is splitted in five subsets (or cancellation groups) where a given subset is left out to be used as an evaluation set while the remaining four subsets are used to compute the classification rule. The process is executed until each cancellation group is used as an evaluation set (5 times). The percentages of classification (cross-validation data) in all the factories are greater than 80%. In figure 4, factories A and B are the most

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separated whereas factories C and D although well discriminated are quite close to each other. The first discriminant score discriminates all the classes. The second discriminates factory B from the rest of factories.

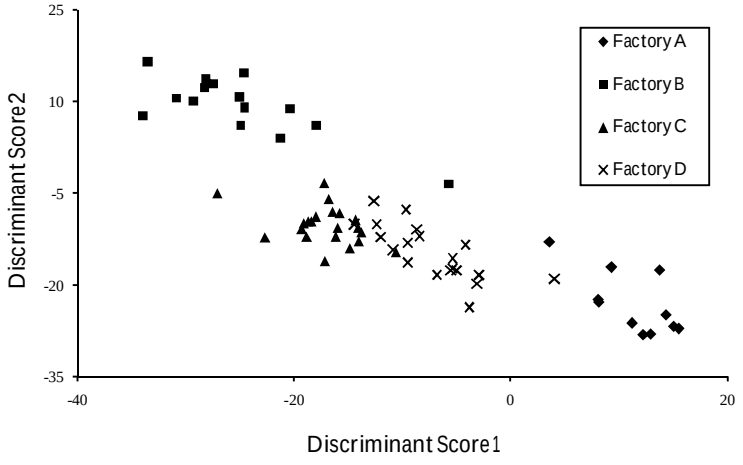

Fig. 4. Score plot of the first two discriminant scores of the Bayes LDA of the 67 sample beers belonging to four different factories (A-D).

This preliminary interpretation of the PCA and LDA results reveals that the four factories show different aromatic characteristics, mainly factory B with respect to factory A.

With the aim of finding the more discriminant variables between factories and studying their contribution to the aroma of beers, the Stepwise-LDA algorithm was used. To select the variables, the Wilk's Lambda criterion was used but the first one (m/z=74) was selected by maximizing the ratio between the total sum of squares and the within-class sum of squares. To obtain the variables by SLDA, the algorithm was cross-validated with five cancellation groups. Finally, the 74, 104, 55, 58, 87, 71 and 61 m/z ions were selected and Fisher LDA was applied.

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LDA PREDICTION MATRIX (CROSS VALIDATION SET)					
True Class	Assigned to class				% Correct
	F1	**F2**	**F3**	**F4**	Classification
F1	10	0	0	1	90.9
F2	0	14	0	1	93.3
F3	0	0	20	1	95.2
F4	1	0	3	16	80.0
TOTAL: 89.6% Classification ability					

Table 1. Classification results of the four classes computed by Bayes LDA (cross-validation data).

Figure 5 shows a bi-plot for the two first canonical variables. The computed between-class (w) and the within-class (p) variances were 0.53 and 0.03, respectively. The Fisher ratio (w/p) value was 17.6. This value indicates that the centroids of the four factories were sufficiently distant one from each other and that the spread within each factory was low.

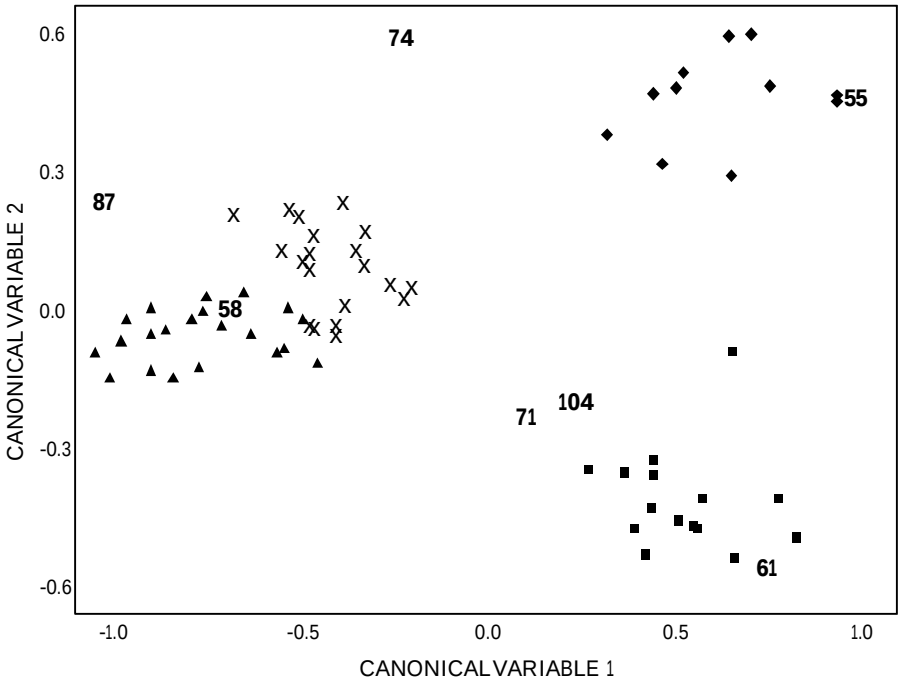

Fig. 5. Bi-plot graph of the canonical variables computed with the first 7 selected variables by SLDA.

The differences between factories can be studied on the basis of the 7 selected ions and their relationship with the most important groups of chemical compounds found in beer (table 2). According to figure 5, the 55 m/z ion, which is important mainly in alcoholic compounds, is highly related to factory A. The 61 m/z ion, located in the region of factory B, is a typical ion related to glycerol and also to sulfur compounds. Others ions related to factory B are the 71 m/z ion, characteristic of linalool and ethyl butyrate, and the 104 m/z ion, which is the most important fragment of phenethyl acetate. Factories C and D are mainly characterized by the 58 and 87 m/z ions. The 58 m/z ion is a fragment of 3-methyl thiopropanol and the 87 m/z ion is characteristic of the chain branching in esters [21].

Esters	*mg/l*	m/z
Ethyl acetate [(a)]	10-60	-
Isobutyl acetate [(a)]	0.01-0.25	-
Isoamyl acetate	0.5-5.0	70
Ethyl caproate	0.1-0.5	**88**,99
Ethyl caprilate	0.1-1.5	**88**
Ethyl caprate	0.01-1.0	**88**
Phenethyl acetate	0.05-2.0	**104**
Nicotinato etilo	1.0-1.5	**106**,78
Ethyl butyrate	0.04-0.2	**71**,88
Ethyl 2-methylbutanoate	0.001-0.015	**57**,102,74
Alcohols		
2-methyl 1-butanol	8-30	**57**,56
2-methyl 1-propanol[(a)]	4-56.6	**-**
3-methyl 1-butanol	30-70	**55**,70
Phenetyl alcohol	8-35	**91**,92
1-octen-3-ol	0.03	**57**
Tyrosol	3-40	**107**
1-pentanol	2-10	55,70
Glycerol	1200-2000	**61**
3-methylthiopropanol	0.05-1.3	**106**,61,58,57

Sulfur compounds	*µg/l*	m/z
Ethylene sulfide	0.3-2.0	**60**,59
Ethanethiol	0-20	**62**
1-propanethiol	0.1-0.2	**76**
Dimethyl sulfide	10-100	**62**
Diethyl sulfide	0.1-1.0	**75**,90,61
Dimethyl disulfide	0.1-3	**94**,79,61
Dimethyl trisulfide	0.01-0.8	**79**,126
Methyl thioacetate	5-20	90
Ethyl thioacetate	0-2	90
3-methyl thiopropanal	20-50	104
Hop oil-derived		
Linalool	1-470	**71**,93
Geraniol	1-90	**69**
A-terpineol	1-75	**59**,93,121
Citronellol	1-90	**69**
Geranyl acetate	35	**69**
Humulene epoxide ii	1.9-270	104
Others (aldehydes, ketones)		
Acetaldehyde [(b)]	1200-24400	-
Diacetyl [(a)]	10-400	-

Table 2. Important chemical compounds commonly found in aroma beers [2, 23]. (a) Non-important mass abundances in 50-150 m/z. (b) Fragmentation below 50 m/z. The bold numbers are referred to the 100% of relative abundance when the molecule is fragmented.

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Figure 5 shows that a reduced number of ions -which can be related to typical fragments of some important aromatic compounds found in beers- are capable of discriminating the same type of beers produced in different factories. However, for a more complete characterization of the beers, the participation of other ions is necessary. For this reason, the first eighteen m/z ions selected by SLDA were used to obtain the Fisher LDA bi-plot represented in figure 6. From this figure, the interpretation of these ions can be studied on the basis of the fragmentation of the different groups of chemical compounds found in beer (table 2), making possible a more complete characterization of the beers produced by each factory.

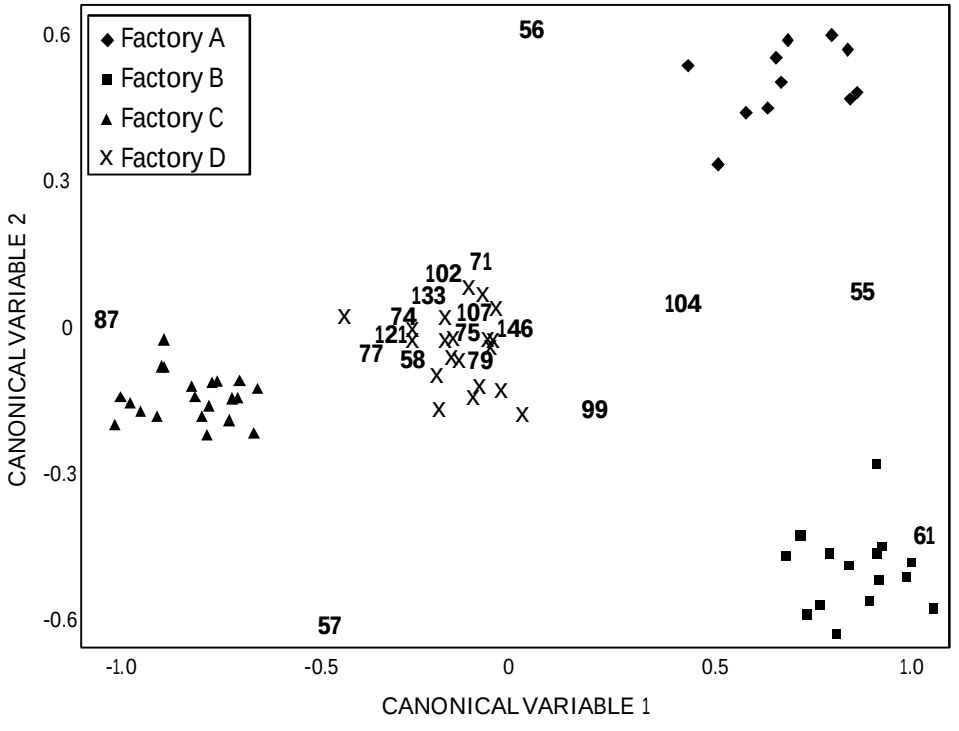

Fig. 6. Bi-plot graph of the canonical variables computed with 18 selected variables obtained by SLDA.

Alcohols. The 55, 56 and 57 m/z ions are characteristic of important alcoholic compounds found in beer such as isoamilic alcohols (3-methyl 1-butanol and 2-methyl 1-butanol), 1-

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pentanol and 1-octen 3-ol, and are highly related to factories A and B (57 m/z ion in the second CV). The 61 m/z ion located in the region of factory B, is a fragment related to the glycerol and 3-methyl thiopropanol. Other important fragments of the 3-methyl thiopropanol and tyrosol, the 58 m/z and 107 respectively, are highly correlated with factory D. The 91 m/z ion, typical of the phenetyl alcohol, an important aromatic compound in beer, does not seem to be a discriminant ion to classify the factories.

Esters. An important fragment is the 104 m/z, which is characteristic in phenetyl acetate and seems to describe, in the first canonical variable, factories A and B principally. The 99 and the 102 m/z ions, fragments related to ethyl caproate and ethyl 2-methylbutanoate, respectively, and the 71 m/z ion, the most important fragment of the ethyl butyrate, are highly correlated with factory D. The 74 m/z ion, located in the region of factory D, is an important fragment of methyl esters, among others, the methyl caprate [22]. The 87 m/z ion is important in the description of factory C. This ion is characteristic of the chain branching in esters, besides being an indicator of oxygen [23]. However, the 88 m/z ion, belonging to the significant flavor-active ethyl esters found in beer (table 2), was not selected. This may indicate that the main ethyl esters do not seem to be important to explain the differences between factories. Finally, the 99, 102 and 71 m/z ions, related to the other esters shown in table 2, describe the beers of factory D mainly.

Sulfur compounds. The dimethyl sulfide and the 3-methyl thiopropanol are present at important concentration levels in beer (mg/L), while the other sulfur compounds are present at levels of μg/L [24]. The main indicators of S in the molecules are the 61 and 75 m/z ions [23]. In 3-methyl thiopropanol, the 61 m/z is an important fragment, which is described by the first CV and is highly related to the beers of factory B. The 62 m/z ion -no selected by SLDA- is the most abundant when the molecules of dimethyl sulfide and ethanethiol are fragmented. The 75 and the 79 m/z ions, located in the region of factory D, are the most important fragment of the diethyl sulfide and dimethyl trisulfide respectively. Other m/z ion, the 104, is characteristic of the 3-methyl thiopropanal. The 61, 79 and 90 m/z ions (the latter not selected by SLDA) are characteristic in other sulfur compounds commonly found in beer. Most of the sulfur compounds mentioned describe mainly to the beers of factory D.

Hop oil-derived and others. The 71 m/z ion describes factory D and is important in linalool, which exhibits floral notes in beer [25]. The 121 m/z ion is a fragment of α-terpineol (table 2), and is related to the samples of factory D. The 104 m/z ion is also a characteristic fragment in humulene epoxide II (herbal flavor in beer).

With regard to the other m/z ions selected by SLDA we found that the 77 m/z ion is assigned to the molecular ion $C_6H_5^+$, which belongs to the aromatic compounds in general [21]. The other m/z ions located in the region of factory D (133 and 146), have a high molecular weight. They may belong to minor compounds in the aroma of beer.

According to figure 6, the beers of factory D seem to be more complex in aroma than the others beers. These beers seem to have a fruity flavor due to esters. Factories A and B are described principally by the typical ions of the alcoholic compounds. A sulfur character, due to 61 m/z ion, seems to be associated to the beers of factory B. Factory C is not clearly characterized.

To corroborate the validity of the interpretation made above, we used the concentrations of some of the most important volatile compounds of beers, quantified in the samples considered. The beers were analyzed by GC-MS [26].

Fisher LDA was applied to the matrix of the concentration values for all the beers studied. Figure 7 shows the bi-plot for the first two CV. The plot shows a quite good discrimination between the four factories. The beers of factory D seem to be more complex in aroma because most of them are related to esters (ethylic esters). This appreciation is similar to that described in figure 6, where factory D showed a correlation with the fragments related to esters. The exception is the isoamyl acetate, which is located in the region of factory C.

The acetaldehyde, not represented in figure 6 (m/z below 50), is located in the region near the samples of factories B and C and the alcohols seem to be related to the samples of factory A principally, as it was described in figure 6. However, the 2-methyl 1-propanol is more related to factory C. The dimethyl sulfide is important in Factory A (first CV) and B (second CV). The latter confirms the interpretation made in figure 6.

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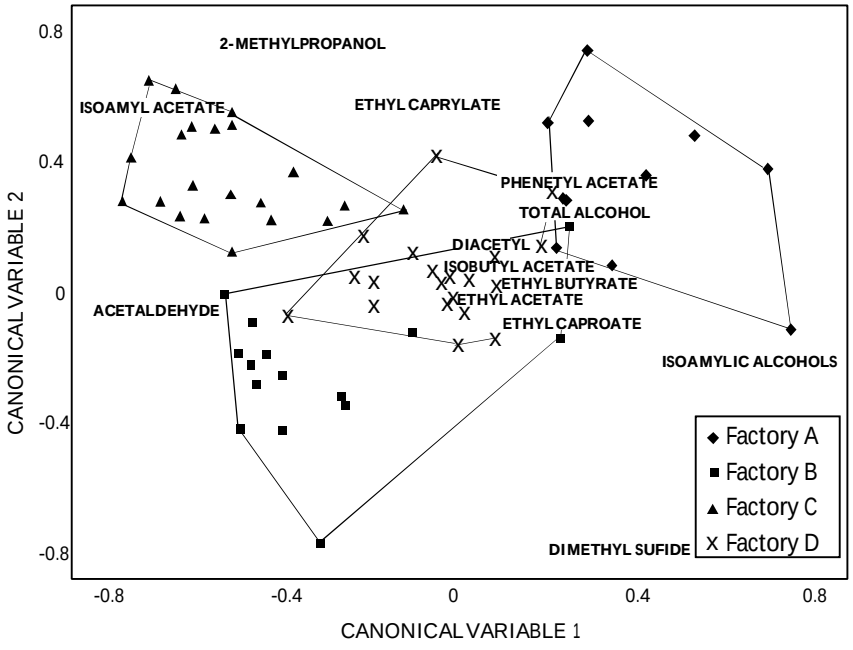

Fig. 7. Bi-plot graph of the canonical variables computed with the measured values.

Although a limited number of volatile compounds of table 2 were found (only 13), figure 7 clearly shows a relation of the samples of factories A and D with alcohol and ester compounds, respectively, as it was discussed in figure 5. However, whereas the behavior of factory A could be sensorially corroborated because most of the samples from this factory were associated to the descriptor "rancid or malty", which is typical of many alcohols, the relationship between the samples from factory D and the typical sensory notes of esters was not clearly found due to the great variety of attributes used to describe the samples belonging to this factory. The beers of factories B and C neither were clearly characterized from the compounds analyzed nor from the sensory results, although an important number of samples of factory B was described with fruity notes, among others, which could be related to the presence of some esters.

Although it is not possible to ensure that the m/z selected belong mainly to a specific compound, the interpretations made are highly probable because the compounds in study are flavor-active and predominant in the volatile matrix of beers. It is also important to emphasize the usefulness of the MS e-nose, not only in the discrimination but also in the

characterization of the samples, since it is possible to obtain chemical information, in the way of fragment ions that can be attributed to certain compounds that may be responsible for the differences found in beers with identical process of elaboration.

Conclusions

In this work we have shown that beers of the same brand but produced in different factories can be discriminated based on their aromatic profile, analyzed by an MS e-nose. Application of Fisher LDA with the 18 more discriminant m/z ions (selected with the Stepwise-LDA algorithm) revealed a relationship between alcoholic volatile compounds, sulfur compounds and ester compounds with factories A, B and D respectively.

The results achieved in this study allow considering the MS-e-nose as a potential aroma sensor because it is capable of discriminating and characterizing the samples according to their predominant aromas with the help of multivariate analysis techniques. Moreover, with the application of variable selection techniques it is possible to obtain information about the possible compounds responsible for the differences found between samples.

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Chapter 3

Electronic Tongue

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An electronic tongue (e-tongue) is an instrument that attempts to imitate the structure of the human gustatory system. It is expected to be able to reproduce its behavior as well. E-tongue traditional instruments are equipped with non-specific sensor arrays, and they produce signals that are not necessarily specific for any particular chemical compound related to taste. When these non-specific sensor arrays interact with the different taste-causing chemical substances, different patterns are obtained, which have to be statistically processed to get suitable information [1].

Nowadays, there are many types of electronic tongues developed since the first devices were commercially available in 1990. The most used employ a wide variety of chemical sensors based on potentiometric, voltammetric, amperometric, impedimetric and/or conductimetric electrochemical principles. However, an emerging type of e-tongue, based on optical measurements by Fourier Transform infrared spectroscopy (FTIR), has been recently applied in food analysis. With this technique, the absorption spectrum of a sample can be attributed to its soluble compounds present on it that, usually, are related to taste attributes. There are three main properties that make this technique an interesting alternative for measuring this kind of compounds: sensitivity, speed and lack of sample pretreatment. Furthermore, with IR spectroscopy chemical information about the composition of the sample can be obtained, because it is based on spectroscopic measures which are characteristic for the different types of compounds. Its main disadvantages are its non-portability and its relative high cost.

In this fourth chapter, a description of the FTIR technique is introduced followed by the *state of art* of the applications of the different types of e-tongues in the quality control of alcoholic beverages. Then, the experimental study related to the application of an FTIR in the analysis of wines to mimic the panel response of the *tannin amount* attribute is included.

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3.1. Introduction

In the last years it has been an increase of applications of Fourier Transform Infrared Spectroscopy (FTIR) to food studies [2], because of its numerous advantages both in qualitative and quantitative analytical determinations.

FTIR is a non-destructive technique that provides structural information about molecular features of a large range of compounds [3]. The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. So, a molecule that is exposed to infrared rays absorbs infrared energy at frequencies that are characteristic of that molecule. For FTIR analysis, a sample spot is subjected to a modulated IR beam. The sample's transmittance and reflectance of the infrared rays at different frequencies are translated into an IR absorption plot that can be considered as a fingerprint of the sample [4]. The resulting FTIR spectral pattern is then analyzed and matched with known signatures of identified materials in the FTIR libraries.

Since NIR (near infrared) spectroscopy has been broadly applied in industrial processes with very good results, researchers have sometimes tended to apply NIR to all food analyses while, in some cases, other procedures such as MIR (mid infrared) spectroscopy, using FT-MIR instruments, could provide more useful analytical data. In fact, the MIR spectral region (400-4000 cm^{-1}) is where it can be found most of the fundamental structural information. This is the reason why we decided to apply the FT-MIR technique in our studies related to the evaluation of wine and beer taste attributes.

3.1.1. Instrumental device

There are four basic components in an FT-MIR system: a radiation source, an interferometer, a detector (figure 3.1.) and the compartment where the sample to be measured is placed.

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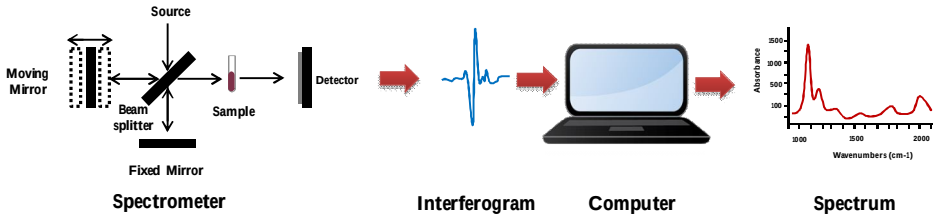

Figure 3.1. Diagram of an FT-MIR instrument.

Radiation source

Infrared energy is emitted from a black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample (and to the detector) [4].

Interferometer

The interferometer is the heart of the FT-MIR instruments. As in our device, the FT-MIR is based on the Michelson interferometer (figure 3.2). This consists basically on a beam splitter and two flat mirrors. One of the mirrors is fixed in one interferometer arm, while the other is movable in a second arm. The beam splitter, as the name suggests, is used to split the incident IR light into two parts of equal intensity. The divided beams are reflected, by the fixed and the movable mirrors, back to the beam splitter, where they recombine and interfere. The displacement of the movable mirror causes differences in distance travelled by the two light beams which is called the optical path difference (OPD). When the two mirrors are at the same distance (zero path difference, ZPD) the reflected beams are in phase and hence they interfere constructively. In this way, the conditions for constructive and destructive interference, as well as all intermediate states between the two, are consecutively met [6].

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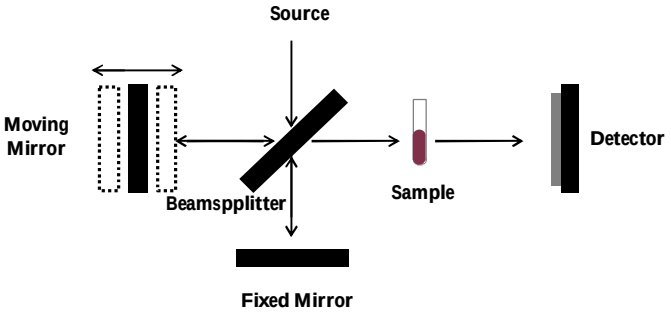

Figure 3.2. Diagram of a Michelson interferometer.

The combined beam containing these interference patterns is called interferogram. An interferogram represents one forward motion of the mirror until the point of ZPD and backward motion to the initial position. So, an interferogram is the plot of the intensity of light (in volts) over the OPD [7]. It contains all of the radiative energy coming from the source and has a wide range of wavelengths [8]. Finally, the recombined IR beam passes through the sample (or the reference) and reaches the detector.

Sample compartment

The beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. The absorption depends of the chemical nature of the sample whose specific frequencies of energy will characterize it [8].

Detector

The beam finally passes to the detector for the final measurement. The detectors used are specially designed to measure the special interferogram signal.

The detector measures the intensity fluctuations produced by the interference effect in real time, resulting in an interferogram that contain all the spectral information related to the sample, as it has been explained. The detector used in the experimental part of this

Doctoral Thesis has been a pyroelectric device incorporating deuterium tryglycine sulfate (DTGS) in a temperature-resistant alkali halide window [9].

Computer

In order to obtain interpretable information, the digital interferogram must be converted to a conventional IR spectrum. The mathematical procedure employed to convert this IR interferogram (intensity versus time) into an IR spectrum (intensity versus frequency) is called Fourier transformation. This operation is carried out by a computer using a fast Fourier Transform (FT) algorithm available in the FT-MIR instruments. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

3.1.2. About this chapter

The purpose of this chapter is, firstly, to present the state of art of the electronic tongues in the analysis and quality control of alcoholic beverages. Nowadays, the usual applications of FTIR technique to this type of beverages include routine wine analyses, such as the simultaneous determination of different oenological parameters such as the alcoholic degree, the pH, the concentration of SO_2 or the sugars' content, among others [10-21]. Because many of these parameters are related to compounds involved in the taste perception, the use of FT-IR could be extended to the description of gustative parameters in the samples when suitable chemometric tools are involved.

However, the specific use of an FT-IR spectrometer as an electronic tongue has not yet been studied. So, in the following pages, we will present a study where FT-MIR is applied to wine samples with the aim of determining if this device could be used as an artificial tongue, emulating the assessments made by a wine panel of tasters. The very good result provided by this study has been published in a prestigious analytical chemistry journal.

3.1.3. **References**

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3.2. Electronic tongue in the quality control of alcoholic beverage: from electrochemical sensors to FTIR

L Vera, L Aceña, J Guasch, R Boqué, M Mestres, O Busto

Submitted to Vibrational Spectroscopy

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Introduction

One of the most important sensory attributes of foods is taste. This attribute is of vital importance when the consumer decides to accept the product tasted. The emerging development of electronic tongues and their increasingly widespread use in food analysis began in the mid-80s. It consisted on applying principles similar to those of the e-noses, but focusing the research in liquid samples, to know their chemical composition related to the non-volatile compounds and, from this information, to classify and discriminate samples in a fast way.

Over time, different types of e-tongues have been developed, starting with the most used potentiometric and voltammetric ones until to arrive to the new generation of sensors, such as the FTIR e-tongue.

The following report reviews the beginning of the e-tongues, the development of the different technologies over the years and their main applications in the alcoholic beverage field.

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Electronic tongue in the quality control of alcoholic beverage: from electrochemical sensors to FTIR

L Vera[1], L Aceña[1], J Guasch[1], R Boqué[2], M Mestres[1], O Busto[1]

[1] Analytical Chemistry of Wine and Food Research Group
[2] Chemometrics, Qualimetrics and Nanosensors Research Group
Department of Analytical Chemistry and Organic Chemistry
Campus Sescelades. Facultat d'Enologia de Tarragona.
Universitat Rovira i Virgili
43007 Tarragona (Spain)

Abstract

The use of electronic tongues has increased in the food analysis area from the first sensors commercially available that appeared in the 90's. The e-tongues have become well-established tools in food quality control and a promising support, when not an alternative, to the conventional tasting panels. The most common types of electronic tongues are those based on electrochemical techniques such as potentiometry, voltammetry and amperometry. To date, the research based on these technologies is ongoing on new food applications being, at the same time, focused in searching new sensors that improve the performance of these devices.

Meanwhile, a new type of e-tongue based on infrared spectroscopy has been successfully applied to the analysis of food samples. The FT-IR e-tongue is capable of delivering chemical information of the liquid samples analyzed, thus allowing disposing of a more specific interpretation, for example, of their organoleptic attributes.

This report presents an overview of electronic tongue applications in the field of the food and, particularly, alcoholic beverages. We describe the e-tongues that have been developed, as well as their main advantages and disadvantages.

Keywords: FT-IR, electronic tongues, quality control, alcoholic beverages, multivariate analysis

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Introduction

The aroma, flavor and color perceptions of a food commodity are highly appreciated by consumers. These properties define not only the quality of the final product, but also the market consumer preferences. Assuming that food standards are strictly complied, the food industry is now aware of driving the customer senses towards their specific products, trying to give a particular value to them. In this way, the first step is usually to control smell and taste sensory attributes, generally conducted by a panel of experts whose work consists in evaluating not only these organoleptic properties, but authenticity and possible defects. The evaluation given by panels affects the economic value of foods and beverages, because they decide, for a brand, whether a product belongs to a specific category or not. So, their work is crucial for the food companies and leads their future trends.

However, these panels require long analytical times and also involve significant training costs. Their results are often limited, mainly due to the subjectivity of their responses, sensory overload and dependence on physical and psychological health of the panel members. That is the main reason why the interest in developing fast analytical techniques, based on artificial sensors, has increased in recent decades. These techniques try to emulate human sensory senses, processing information in very short times and overcoming, as far as possible, the abovementioned problems, since they are able to distinguish sensory parameters from the signals produced by different chemical species in their own detectors. All these signals are collected and processed by data recognition systems, whose main purpose is to decode them in order to give an interpretation based on the organoleptic differences of foods.

In the mid-80's, and after the development of electronic noses, ideas and similar principles were applied to new systems of sensors capable of distinguishing chemical properties of liquid samples using an array of chemical sensors and mathematical signal treatments. These systems closely mimic the working principle of the human taste sense. So they were called at first "taste sensors". It was not until 1995 when the so-called "electronic tongue" was introduced [1]. Since this moment, a rapid development of new types of electronic tongues, whose applications have been extended to various fields of research, started.

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The concept of electronic tongue is based on the model of the biological sensory taste. The taste is strictly defined as the response of the tongue to the non-volatile soluble material [2]: sweet, sour, bitter and salty. A fifth basic taste is *umami*, linked to taste-enhancing chemicals such as glutamate [3]. The human taste sensory system consists on specific sensors called taste receptors. These sensors convert the signals detected on information which is collected and converted in stimuli. These stimuli, after suitable biotransformations, are processed by sensory neurons before arriving to the brain, which, finally, defines the particular taste perceived (Figure 1).

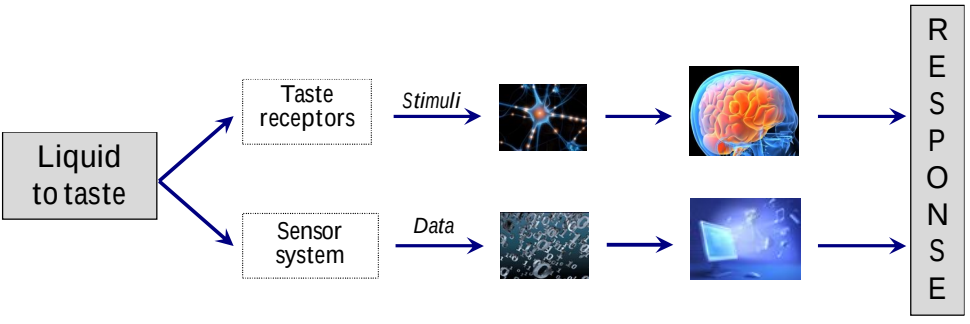

Fig. 1. Analogy between the human and the electronic tongue.

The electronic tongue consists in a system of non-selective chemical sensors (poorly selective) with a complementary program for pre-treatment and processing of signals (data) and which is capable of recognizing and comparing either individual or complex flavors of substances.

A chemical sensor is a small device that responds, ideally in a selective form, to a particular group of chemical substances when it is placed directly on a sample, producing an electrical signal that can be correlated with the concentration of the analytes to be determined. All the chemical sensors are composed of two main parts: the selective receptor whose function is to recognize molecules of the substance analysed, and the transducer, whose function is to transform the changes of the physical properties of the receptors into electrical signals [4].

There are various types of electronic tongues, being the most important those are based on electrochemical techniques such as potentiometry and voltammetry. Both techniques

work with, at least, working modified electrodes that are used as detectors and a reference electrode with a constant potential.

Recently, thanks to its broad utility in food quality control, the Fourier Transform Infrared Spectroscopy (FT-IR) has achieved a good position as an alternative system of the most conventional systems of electronic tongues.

In this report, we present a review of the most important contributions of the e-tongues in food analysis, with especial interest in alcoholic beverages and in its use as an alternative or complementary tool for sensory essays.

Electrochemical sensors

Potentiometric systems

The first studies related to electronic tongues were based on the use of a series of ion-selective electrodes (ISE) which measure the electrical potential generated when a specific ion dissolved in a solution displays activity, according to the Nernst equation. The sensing part of de electrode is usually made as an ion-specific membrane, along with a reference electrode. So, potentiometric sensors make possible both quantitative and selective measurement of ions in liquid samples, since the potential changes can be related to the concentration of the ions dissolved. However, the attempts to use an array of potentiometric ion-selective sensors instead of discrete ISE for quantitative determination of ions were in declining in favor of the use of an array of non-selective chemical sensors with partial specificity for different components in liquid media [5]. If the objective is to measure the amount of chemical substances in liquid food samples using a large number of sensors would be practical. The taste could not be described based on direct measurements of the chemical species present in a sample, as humans cannot distinguish each chemical individually; this led to the conclusion that there was no clear relationship between chemical substances and taste [3].

The first proposal for creating a commercially available sensors that imitate the human taste, was attempted in 1990 by Toko and collaborators, who developed a system of non-selective sensors, also known as sensors with global selectivity, for the analysis of liquids

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[6]. These sensors were composed of several kinds of lipid/polymer membranes to transform information of taste substances into electric signals, which were analysed by a computer. The membranes showed the ability to perceive the taste and transform it into electrical signals, i.e. could imitate the human taste sense by potentiometric measurements [7].

Five years later, A. Legin and coworkers introduced the electronic tongue term and create a system of chalcogenide glass sensors which exhibit cross-sensitivity in solutions of heavy metal ions [1, 8]. The cross sensitivity of these sensors, i.e. the sensitivity to various chemical species, was of vital importance towards obtaining a stable and reproducible response in complex media [9]. Since then, the systems were improved in order to implement the use of this kind of instruments in in-situ food quality control [10] also quantitative measurements of chemical species in solutions complex [11].

Research in the field of potentiometric sensors to food analyses led to develop new types. Among them, the microsensors based Pb chalcogenide materials which are sensitive to heavy metals [12, 13] or planar-type sensors based on a modified carbon paste [14]. The attempt to improve yields and reduce sample consumption led to the development of miniaturized potentiometric sensors based on silicon technology and membranes of polyvinyl chloride (PVC), which have been used to analyse beverage standards for identification of some characteristic defects [15]. Other electronic tongue, based on potentiometric sensors with PVC solvent polymeric membranes doped with Co and Pt - porphyrinates was developed and applied for the identification of Italian white wine [16]. The use of certain surfaces as a set of un-specific potentiometric sensors allowed the development of an array containing the electrodes; RuO_2, C, Ag, Ni, Cu, Au, Pt, Al, Sn, Pb and C which were used for the qualitative analysis of natural water [17] and fish freshness analysis [18, 19].

As the potentiometric were the first type of e-tongues developed, many food applications can be found in literature [20-34]. The technique is, in fact, considered consolidated, fast, of low cost and a good alternative to the sensory analyses carried out by panelists, spite its known limitations, mainly related to the sensor temperature dependence and the adsorption of solution components that affect the membrane potentials [35].

An important part of the food applications abovementioned are those related to the analysis of alcoholic beverages. In one of the first works published, 29 potentiometric sensors of chalcogenide glass and PVC membranes were applied to differentiate Italian wines and to determine quantitative parameters such as alcohol, pH, acidity, tartaric and shikimic acids [36]. Few years later, an electronic tongue of 23 potentiometric sensors was successfully used to classify wines not only from different geographical areas, but also from vineyards of the same zone. The fusion of the data obtained with those provided by an e-nose based on the gas sensors technology to find correlations between chemical analysis and sensor analyses, leaded to another interesting application to the analysis of red wines [37]. Moreover, the instrument was able to predict the evaluations of a trained tasting panel [38]. Table 1 shows the main application in chronological order of the electronic tongue (potentiometric and voltammetric) in the analysis of alcoholic beverage. Spite most of the works cited in literature are related to wine analysis [36-44], the technique has been also applied to other type of alcoholic beverages, such as beers [10, 14, 24, 45-48], and has also been applied to establish a quantitative method to control the ethanol content in alcoholic beverages [49].

Voltammetric systems

Potentiometric and voltammetric electronic tongues have been developed based on similar ideas, although potentiometric sensors are mainly used to mimic the human taste sense in order to provide an objective scale whereas voltammetric sensors have been used mainly to follow quality of food or to monitor a process. So, their applications are quite different but complementary, as the study given by Ivarsson et al. [50] confirmed.

These devices present high selectivity, high signal-to-noise ratio, low detection limits, various modes of measurement and the possibility to modify conveniently the surface of the electrodes. However, they show disadvantages as temperature dependence, large surface alteration causing drifts in sensor response and their limited applicability to redox-active substances [35].

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Year	Sample	Study	Sensor (number)	Data treatment [1]	Ref.
1997	Beers and beverages	Distinguishing different sorts of beers and beverages	Potentiometric (10)	PCA, ANN	[10]
1999	Wines	Discrimination and quantitative analysis	Potentiometric (29)	PCA, ANN, PLS	[36]
2000	Wines	Correlation between chemical compounds and artificial sense	Potentiometric (6)	PCA, ANN	[37]
2000	Wines, beers and beverages	Discrimination, quantification	Voltammetry	PCA, HCA, PLS, PCR	[62]
2002	Beers and beverages	Discrimination	Potentiometric (6)	PCA, PCR, PLS, ANN	[14]
2003	Wines	Discrimination and quantitative analysis	Potentiometric (23)	PCA, ANN, SIMCA, PLS	[38]
2004	Wines	e-nose/e-tongue (classification and quantification)	Potentiometric (5)	PCA, PLS-DA, PLS	[39]
2004	Wines	Discrimination	Voltammetric (3)	PCA	[73]
2005	Wines	Discrimination	Voltammetric	PCA	[74]
2006	Wines	Classification on the basis of the aging oak	Voltammetric (13)	PCA, SIMCA	[75]
2006	Wines	Discrimination	Voltammetric (12)	PCA, PLS-DA, SIMCA	[76]
2006	Wines	Adulteration	Voltammetric (11)	PCA, PLS2	[77]
2006	Alcoholic beverages	Control of ethanol content	Potentiometric (8)	PCA, SIMCA, PCR, PLS	[49]
2006	Beer, milk, juice	Recognition	Potentiometric (6)	PCA, ANN	[24]
2006	Beers	Qualitative analysis	Potentiometric (10)	PLS, ANN	[45]
2007	Wines	Discrimination between wines aged in barrels or with chips	Voltammetric (6)	PCA, PLS-DA	[78]
2007	Wines Porto	Age prediction	Potentiometric (28)	PCA, PLS	[40]
2007	Wines	Identification	Potentiometric (7)	PCA, PLS-DA, MLR	[16]
2007	Beer and beverages	Recognition	Potentiometric (10)	PCA, PLS-DA	[46]
2008	Wines	Discrimination of wine varieties	Voltammetric (6)	PCA	[79]
2009	Wines	Micro-oxygenation influence and maceration with oak chips	Potentiometric (26)	PCA, ANOVA, ASCA	[41]
2009	Wines	Classification	Voltammetric	PCA, PLS-DA	[80]
2009	Wines	Bisulphite concentration	Voltammetric (4)	PLS	[81]
2009	Beers	Tasting quality	Potentiometric (29)	PCA, CCA, PLS	[47]
2010	Wines	Age prediction and quantitative analysis	Potentiometric (26)	PCA, PLS, ASCA	[42]
2010	Wines	Correlations with sensory perceptions for wines exposed to micro-oxygenation	Potentiometric (26)	PCA, PARAFAC, ANOVA, PLS, PLS2	[43]
2010	Wines	Measurement of bitter taste	Potentiometric (17)	ANOVA, PLS-DA, MLR	[44]
2010	Wines	Oxygenation effect, defects and classification	Voltammetric (5)	PCA, ANN	[83]
2010	Beers	Quality parameters	Potentiometric (18)	PCA, CCA, PLS	[48]

1 PLS: Partial Least Square; PCA: Principal Component Analysis; SIMCA: Soft Independent Modelling of Class Analogy; PCR: Principal Component Regression; PLS-DA: Partial Least Square Discriminant Analysis; ANN: Artificial Neural Network; HCA: Hierarchical Cluster Analysis; MLR: Multiple Linear Regression; ANOVA: Analysis of Variance; ASCA: ANOVA—Simultaneous Component Analysis; CCA: Canonical Correlation Analysis; PARAFAC: Parallel Factor Analysis.

Table 1. Main applications of e-tongues based on voltammetric and potentiometric sensors to the analysis of alcoholic beverages.

In voltammetric measurements, a current is measured between the metal working electrode and the counter-electrode when a voltage pulse is applied over the working electrode and the reference electrode. A set of pulses can be put together to form a pulse train in order to extract as much information as possible from the solution. When the potential is applied, electro-active compounds that react to that potential will be reduced or oxidized and a current, that can be measured, will arise. In e-tongue systems, voltammetric sensors are used to determine redox-active substances, collecting data over the whole pulse to extract as much as possible information of the analytes in the solution [51]. In addition for providing a wide range of information of these analyte redox transformations [52], voltammetric sensors have the advantage of having a very high sensitivity, versatility, simplicity and robustness [11], are less influenced by electrical disturbances which allows high signal to noise ratio [53] and low detection limits and, finally, they permit various modes of measurement [54]. Furthermore, the surface of the electrodes can be modified with various chemosensitive materials obtaining sensors of various sensitivity and selectivity towards a great number of species.

The first attempt to use an e-tongue based on this kind of sensors is attributed to Winquist and colleagues in 1997. They described various voltammetric techniques to generate information when combined with multivariate methods, designing a prototype of e-tongue by combination of pulse voltammetry using two types of working electrodes and PCA [55]. Later, the system was improved by applying the flow injection analysis (FIA) technique to the voltammetric e-tongue [56].

In further studies, developed by the researcher team of Rodríguez-Méndez and De Saja, voltammetric electrodes based of phthalocyanine compounds were used as the sensing units of an e-tongue to evaluate, and discriminate by PCA, the five basic tastes [57].

In addition to these studies, many others have been published whose main objective is to investigate electrode materials appropriate to be used as sensors in voltammetric e-tongues suitable for analyzing complex mixtures [58-61].

Many applications can be found in literature related to the use of voltammetric e-tongues in food analysis. In fact, the usefulness of the first sensors developed was tested against the classification of different beverages [52, 62-70] even combined with e-noses [71].

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Wines are the alcoholic beverages the most analysed by voltammetric e-tongues. Table 1 shows the main works using a voltammetric electronic tongue in wine studies principally. These studies consider, among others, classification, adulteration, quantification works [62, 69-83].

Other types of electrochemical electronic tongues

In 2003, Riul and collaborators developed an electronic tongue based on impedance spectroscopy, which was able to distinguish basic taste substances in solution and also three types of red wines, even detecting the addition of sucrose in one of them [84]. In a further study, this e-tongue allowed to distinguish human tastes and was successfully used to classify red wines [85]. Years later, a prototype of impedimetric e-tongue composed by five different sensors was developed showing selectivity, sensitivity and stability in the analysis of solutions of different taste perceptions [86, 87].

Another type of electronic tongue developed based on amperometric sensors has been used also in food analyses [88]. An interesting study applying this sensor allows the combined use with an electronic nose to classify and characterize wines [89]. This innovative analytical technique of fusion of artificial sensors would allow find substantial and objective differences wines samples analysed. Moreover, applying the amperometric electronic tongue was possible to evaluate the astringency in tea samples through a multi-flow detector design [90].

The electronic noses based on mass sensors are referred on a change of mass, which may be measured as a change in resonance frequency [91]. These types of electronic tongue including surface acoustic wave (SAW) sensors [92] and Quartz Crystal Microbalance (QMB) [93] has been also developed.

Optical Sensors

Other systems of electronic tongues, called optical sensors, are based on several modes of operation such as fluorescence, absorbance, reflectance, etc. Optical electronic tongues involve various kinds of optical sensors. One kind is based on polymeric microspheres

with a chemically modified surface, organized in a sensor array, where the signals are recorded with the use of a color camera. The change of microsphere colors or fluorescence caused by the interaction with the analyte creates characteristic pattern, which can be analyzed with pattern recognition methods [54]. Other kind is the colorimetric electronic tongue is based on the change color when molecular interactions take place [94].

Fourier Transform Infrared Spectroscopy

Another alternative for the most traditional systems of e-tongues, is the Fourier Transform Infrared Spectroscopy (FT-IR), a nondestructive well-established analytical technique, available since the early 1970's [95]. This technique has proved its ability to provide structural information of a large number of compounds and to classify agricultural and food products, as well as detecting subtle compositional differences between and among complex samples.

Infrared spectroscopy detects the vibration characteristics of chemical functional groups in a sample. When an infrared light interacts with the matter, chemical bonds will stretch, contract and bend. The Fourier Transform Infrared (FTIR) spectrometer obtains infrared spectra by collecting a sample interferogram with an interferometer whose beam splitter allows detecting, among others, the mid-infrared region (4000-400 cm^{-1}) wavelengths [96], measuring all the infrared frequencies simultaneously. So, an FTIR spectrometer acquires and digitizes the interferogram, performs the FT function and outputs the spectrum [97].

Despite the advantages of mid-infrared spectroscopy as a potential analytical tool, its use in the food field was low at first and had no interest with respect to the near infrared measures. This was due to the high absorbance of aqueous solutions, which drastically affects the determination sensitivity.

However, considering the good resolution of the absorption bands using mid-infrared region and, most of all, the new instrumental tools, the interest in the applications of the FT-IR to the analysis of foods increased since the mid-80s. Moreover, the improvement of

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the FT-IR software enabled a rapid and effective data processing, what consolidate the technique in fields such as food quality control.

While the FT-IR instruments improved their technology, the ATR-FTIR (Attenuated Total Reflection Fourier Transform Infrared) accessory appeared, offering interesting possibilities for the analysis of solid and liquid samples. The ATR-FTIR technique is perhaps the most versatile and used of those based in infrared spectroscopy.

For the attenuated total reflection infrared (ATR-IR) spectroscopy, the infrared radiation is passed through an infrared transmitting crystal with a high refractive index, allowing the radiation to reflect within the ATR element several times. The sampling surface is pressed into intimate optical contact with the top surface of the crystal. The IR radiation from the spectrometer enters the crystal, and reflects through this crystal by penetrating "into" the sample a finite amount with each reflection along the top surface via the so-called "evanescent" wave. At the output end of the crystal, the beam is directed out of the crystal and back into the normal beam path of the spectrometer [98].

Nowadays, FT-IR instruments have been widely accepted in industries for food quality control. Usually, tests are performed with minimal sample pre-treatment (sometimes beers or wines are only degasified to avoid the CO_2 spectra), or even without. The spectral range obtained with these instruments contain the region of mid infrared (MIR) in the range of 400-4000 cm^{-1}, although this range may vary depending on the study. For example, in wine analysis selection is restricted to the range 960-3600 cm^{-1}, usually avoiding the regions between 1550-1710 and 2970-3630 cm^{-1} due to strong absorption bands of water, and from about 3600 cm^{-1} because to minimal information contained in this zone [99]. In alcoholic beverages is possible to identify typical spectral regions of characteristic compounds. The most abundant absorption is, undoubtedly, the ethanol ranges (between 995-1060 and 2850-2960 cm^{-1}, Table 2) and, it is possible to identify the "fingerprint" region of tannins between 1060-1577 cm^{-1}, with the major peaks in 1445 and 1520 cm^{-1} [100].

Description	Wavelength (cm^{-1})	References
Tannins	1060-1577; 2699-2969	[100]
Ethanol	995-1060; 2850-2960	[152]
Polysaccharides	950-1200	[154]
CO_2	2350	[134]
Esters	1100-1310; 1705-1750	[101]
Aldehydes	1680-1740; 2900-2700	[101]

Table 2. Spectral zones of some relevant compounds in alcoholic beverages.

Due to the strong water absorption throughout the mid-infrared spectrum and taken into account that samples as wines are mainly constituted by compounds with similar chemical characteristics (so, with similar absorption spectra), the IR instruments were at first applied specifically to the analysis of milk [102], meat [103, 104], fats and oils [105-114], coffee [115, 116], butter [97], mashed fruit [117, 118], apples [119], cheese [120], vinegar [121, 122], sweetened condensed milk and juices [94, 96].

As the new FT-IR tools and software pretreatment of spectrum data improved, the problem of water was resolved by subtracting its spectrum from the sample spectrum or by selecting the most relevant parts of the spectrum [99]. So, the analysis of alcoholic beverages was much more possible and many applications can be found in literature since then.

Analysis of alcoholic beverages

The application of the FT-IR to the analysis of alcoholic beverages is varied and with increasing interest in quality control areas. For example, the ethanol content in beers is a parameter that determines their flavor and also determines their economic value as the fees of alcoholic beverages are based on their ethanol content [123]. For this reason, strategies to optimize the methods in the determination of ethanol in alcoholic beverages by FT-IR have been frequent [123-127]. Besides the many works related to quality control in beer [128-130], the FTIR has been used in fruit liquors [131] and in discrimination studies related to brandy and other distilled spirits [132], tequilas [133] and cider [134]. The main applications of FT-IR in alcoholic beverages are summarized in Table 3.

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A large number of applications of FT-IR in wine analysis can be found, most of them related to the determination of alcohol, glycerol, volatile acidity, pH, SO_2, anthocyanins, glucose, fructose, mannose, and organic acids, among others [135-147].

Because of the great quantity of spectral information obtained by the FT-IR technique, many studies of classification, differentiation and characterization of wines have been developed [148-154]. Its applicability in the detection of fermentation problems has also been proved [155].

It is important to remark those studies related to wine phenolics [148, 156], because of their contribution to the flavor, color and bitterness and therefore the quality of the product. They are also closely related to the sensation of astringency, which in some countries is recognized as a basic taste sensation [2]. Some of these works involved tannin determinations, including characterization of their molecular structures [157], quantification by using suitable calibration models [158] and wine discrimination based on the tannin type and concentration [159].

FT-IR as electronic tongue

Edelmann and Lendl explained the singular sensory perceptions of astringency by evaluating the chemical composition of the hydrolysable and condensed tannins from their differences in their respective FT-IR spectra [161]. Some years later, some spectral areas were identified as the absorption zones of tannins, thus allowing their quantitative determination in wines using multivariate PLS calibration models and variable selection techniques [100].

Since the FT-IR seemed to be suitable to explain sensory properties from the different spectra of the samples, as well as to identify a large number of parameters in liquid samples -many of them related to taste- the FT-IR was pointed as an alternative electronic tongue. So, by means of ATR-FTIR, different tomato varieties were classified according to their sweetness in 2005 [162]. A year later, the same researchers concluded that the ATR-FTIR was able to offer a better rating than the potentiometric electronic tongue on measurements of important flavor-related compounds such as acid and sugar content [163, 164].

Year	Sample	Study	Detection	Spectrum zone (cm^{-1})	Data treatment [1]	Ref.
2001	Wine	Variety discrimination	ATR-FTIR	940-1760 950-1640	HCA, SIMCA	[148]
2002	Wine	Polysaccharide characterization	ATR-FTIR	1200-800	PCA, CCA, PLS	[137]
2002	Wines, Brandy, Distillates	Differentiation, classification and characterization	FTIR	926-2971 ~3670-5012	PCA, LDA, PLS	[149]
2004	Beers	Spectra-composition relation	ATR-FTIR	800-1200	PCA	[128]
2005	Wines	Organic acid analysis	FTIR	929-1582 1698-2971	PLS	[145]
2005	Tequila	Authenticity	FTIR	930-1447 1887-2971 3696-4996	PCA	[133]
2005	Wines	Mannose quantification	ATR-FTIR	800-1200	PLS	[143]
2006	Beers	Quality parameters of beers	ATR-FTIR	840-2314 2382-3050	HCA, PLS	[129]
2006	Brandy & distillates	Discrimination	ATR-FTIR	400-1430 1480-2849 2918-4000	PCA, PLS-DA	[132]
2007	Wines	Tannin amount classification	FTIR	400-700 740-1430 1480-2849 2918-4000	DA, SIMCA	[159]
2007	Wines	Characterization and polysaccharide quantification	ATR-FTIR	950-1850	PLS	[154]
2007	Wines	Tannin quantification	FTIR	650-4000	iPLS, mw-PLS	[158]
2008	Wines	Variety differentiation	FTIR	926-5012	PCA, LDA	[150]
2008	Wines	Fermentation problems	FTIR	698-3564	PLS	[155]
2008	Wines	Tannin spectral region identification	FTIR	933-1577 1716-1812 2699-2969	Bi-PLS, si-PLS, ga-PLS, IBECSI-PLS	[100]
2009	Wines	Differentiation	FTIR	929-1542 1717-2971	PCA, LDA	[151]
2009	Wines	Organic and non organic wine classification	FTIR	400-4000	PCA, PLS-DA, LDA	[152]
2010	Wines	Antioxidant capacity prediction	ATR-FTIR	965-1543 1717-2280 2435-2971	PLS	[160]
2010	Wines	emulation the gustative mouthfeel “tannin amount”	FTIR	1002-1542 1720-1808 2703-2966	PLS, IPW-PLS, mi-PLS, ti-PLS, mw-PLS, UVE, Select	[165]

1 PLS: Partial Least Square; PCA: Principal Component Analysis; SIMCA: Soft Independent Modelling of Class Analogy; PLS-DA: Partial Least Square Discriminant Analysis; HCA: Hierarchical Cluster Analysis; CCA: Canonical Correlation Analysis; DA: Discriminant Analysis, LDA: Linear Discriminant Analysis; iPLS: interval PLS; mw-PLS: moving window-PLS; bi-PLS: backward interval-PLS; si-PLS: synergy interval PLS; GA-PLS: Genetic Algorithm-PLS; IBECSI-PLS: Iterative Backward Elimination of Changeable Size Intervals-PLS; IPW-PLS: Iterative Predictor Weighting-PLS; mi-PLS: main intervals-PLS; ti-PLS: tree interval-PLS; UVE: Uninformative Variable Elimination.

Table 3. Principal FT-IR applications to the analysis of alcoholic beverages.

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Nowadays, the work has been focused on the study of the relationship between the FT-IR spectra and the sensory response of panels of experts when tasting wines, among other beverages. In this way, a very good correlation between the FTIR and sensory panel responses has been obtained in the evaluation of the red wine "tannin amount" attribute, so pointing the FT-IR as a good alternative electronic tongue [165].

Multivariate analysis

The great amount of data collected from the instrumental measurements makes it necessary the use of chemometric techniques for multivariate analysis. The raw instrumental signals are arranged as a matrix X of n samples by m variables, which correspond to the values recorded by the electrochemical or spectroscopic sensors. These raw signals are rarely adequate to be directly used for multivariate analysis, mainly because of their noise levels, and they need to be preprocessed. The most common preprocessing technique is probably standardization, which consists on giving equal weight to the variables. For spectroscopic signals, other common preprocessing techniques are multiplicative scattering correction (MSC), standard normal variate (SNV), smoothing and derivatives. When the analytical objective is regression (calibration), the orthogonal signal correction (OSC) is a useful method since it removes the part of X not correlated (orthogonal), to Y. OSC has mainly been applied to spectroscopic signals, although it has also been applied to potentiometric e-tongue signals [39]. Sometimes it may be appropriate to use more than one preprocessing technique. For example, in FT-IR, SNV is first applied to correct for changes in the spectral baseline. After that, smoothing and derivatives can be used to improve the signal. However, if not used properly, the combined use of different preprocessing techniques can cause a loss of useful information; therefore, the criterion for using a given preprocessing technique must take into account the type of instrument and the objective of the study.

Pattern recognition techniques

Pattern recognition techniques are divided into non supervised and supervised. The first do not use information about the class of the samples and its objective is to identify trends or to find groups. On the other hand, supervised techniques are used to build classification rules for a series of samples whose class has been previously specified. So, new unknown samples can be assigned to a certain class previously modelled [166].

Unsupervised

Usually, principal component analysis (PCA) is used as a preliminary method for data visualization. PCA reduces the dimension of the original X matrix and represents the maximal possible information in a new system of variables, linear combination of the original ones, called principal components, which are orthogonal [167]. The first PC (PC1) spans the direction of maximal variance (information) and is orthogonal to PC2. PC2 spans the direction of maximal residual variance (not explained by PC1) and is orthogonal to PC3, and so on. In PCA, data are usually shown in two-dimensional plots (PC1 vs PC2), where samples and variables are projected, giving rise to score and loading plots, respectively.

An alternative, and also commonly used, method is hierarchical cluster analysis (HCA), which allows visualising the relationship between samples (objects) and find natural groups (clusters) of samples. HCA groups similar objects based on the distances between them. Data are shown in a plot called dendogram, where objects are hierarchically plotted as a function of their similarity.

Supervised

A common method used in FT-IR for classification and discrimination of alcoholic beverages is linear discriminant analysis (LDA). This method is based on the hypothesis that data belonging to different classes follow a normal distribution and are linearly separated. The spread of each class is described by the same variance-covariance matrix, and classes are only differentiated by their position (centroid). A variant of LDA is canonical correlation analysis (CCA), which studies the relationship between two systems of variables. CCA has been used in analyses with potentiometric e-tongues to

correlate the signals of potentiometric sensors with the sensory attributes evaluated by a tasting panel [39] o with physicochemical parameters of beers [40].

SIMCA is probably the class modelling technique most used. With SIMCA a PCA model is applied independently to each class [168]. PLS-DA (partial least-squares discriminant analysis) is a discrimination technique based on PLS multivariate regression. In PLS-DA the matrix X contains the original data, and the matrix Y is a codified matrix, containing 1's and 0's, where 1 represents the class of interest and 0 the rest of classes [169]. PLS-DA has been widely used in alcoholic beverages studies performed with potentiometric and voltametric e-tongues [16, 39, 44, 46, 76, 78, 80] and FT-IR [132, 152]. Finally, artificial neural networks (ANN) are a powerful tool for multivariate analysis, especially when data are not linear, as it is the case in potentiometric sensors [170].

Multivariate regression

Many studies have been performed for the quantification of different components in alcoholic beverages or for sensory analysis. This is done by correlating the multivariate instrumental signal with the reference values. As an example, alcohol, glycerol, volatile acidity, pH or tartaric acid, among others, and sensory properties, have been quantified using the results obtained by standard analytical techniques or tasting panels, as reference values [136-147, 165]. The most commonly used multivariate regression method is partial least-squares (PLS) regression.

However, in multivariate regression one of the most common problems is the presence of variables showing little or no information. These variables may decrease the predictive ability of the calibration models. As a consequence, variable selection techniques are required to build more accurate models. They have been used to select useful spectral regions for quantifying tannins in red wines [100, 158], or to find spectral regions correlated with sensory attributes related to tannins [165]. Among the different variable selection techniques found in the literature, we mention backward interval PLS (biPLS), synergy interval PLS (siPLS), genetic algorithms (GA-PLS), iterative backward elimination of changeable size intervals (IBECSI-PLS) [100], interval PLS (iPLS), changeable size moving window (CSMWPLS), iterative predictor weighting (IPW-PLS)

[158, 165], main interval (mi-PLS), moving windows (mw-PLS), tree interval (ti-PLS), uninformative variable elimination (UVE-PLS) and SELECT [165]. The use of any of these techniques must be justified, since its indiscriminate use may lead to model overfitting.

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3.3. Application of an electronic tongue based on FT-MIR to emulate the gustative mouthfeel "tannin amount" in red wines

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Introduction

In the following paper a newfangled study where the FT-MIR was used as an e-tongue to predict a common attribute in wine tasting is presented. A set of red wines, properly evaluated by a panel of experts with respect to the tasting attribute "tannin amount", was used to calibrate the instrument in such way that it was able to mimic the sensory analysis done by the human panel. With this sensory attribute, the characteristic mouthfeel intensity due to the presence of tannins in red wines is evaluated.

The study attempted initially the elaboration of calibration models, using Partial Least Squares (PLS), capable of reproducing the panel response. However, the calibrations instrument-panel did not show at first important correlations for the different attributes evaluated. For this reason, the following step was to apply variable selection techniques which would allow working with those variables most correlated with the dependent variable (panel response) and, therefore, avoiding the spectral noise or absorption spectral regions of chemical compounds which are not related with tannins. Six techniques of variable selection were applied. Three of them select discrete wavenumbers: Iterative Predictor Weighting (IPW), SELECT, and Uninformative Variable Elimination (UVE) and the other three select continuous wavelength regions: Moving Window-PLS (mw-PLS), Tree Interval PLS (ti-PLS), and Main Intervals-PLS (mi-PLS). The results showed that the selection of discrete variables in complex spectra seems to be the best alternative. Then, with the selected variables, the FT-MIR spectra were modeled against the sensory responses evaluated in 37 red wines by means of partial least squares (PLS) regression.

On the other hand, it has to be also noted that the advantages offered by the FT-MIR instrument, besides the ones already mentioned, are related to the possibility of having chemical information of the samples. So, it was possible to find the relationship between those spectral regions selected and the spectral absorption provided by the tannins, which allows getting chemical interpretations to explain the differences between samples.

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Application of an electronic tongue based on FT-MIR to emulate the gustative mouthfeel "tannin amount" in red wines.

L Vera[1], L Aceña[1], J Guasch[1], R Boqué[2], M Mestres[1], O Busto[1]

[1] Analytical Chemistry of Wine and Food Research Group
[2] Chemometrics, Qualimetrics and Nanosensors Research Group
Department of Analytical Chemistry and Organic Chemistry
Campus Sescelades. Facultat d'Enologia de Tarragona.
Universitat Rovira i Virgili
43007 Tarragona (Spain)

Abstract

In this work, the ability of an electronic tongue based on Fourier-Transform Mid Infrared (FT-MIR) spectroscopy as a gustative sensor is assessed by emulating the responses of a tasting panel for the gustative mouthfeel "tannin amount". The FT-MIR spectra were modeled against the sensory responses evaluated in 37 red wines by means of partial least squares (PLS) regression models. In order to find the wavenumbers more correlated with the sensorial attribute and thus providing the best predictive models, six different variable selection techniques were tested. The iterative predictor weighting IPW-PLS technique showed the best results with the smallest RMSEC and RMSECV values (0.07 and 0.13, respectively) using 20 selected wavenumbers. The coincident wavenumbers selected by the six variable selection techniques were interpreted based on the absorption bands of tannin and then a calibration model using these wavenumbers was built to validate the interpretation made.

Keywords: FT-MIR e-tongue, variable selection, sensory analysis, tannins, calibration model

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Introduction

There are several chemicals known as polyphenolic compounds that affect the taste, color and mouthfeel of wines. Among them we find tannins, which are polymeric flavonoid compounds mainly present in red wine and that arise from the grape seeds, skins, and stems, but also from the oak barrels in which wine is aged.

From a sensory point of view, tannins play an important role in the perception of astringency, an important attribute for determining the quality of wines [1]. This major taste property in red wines is often the last principal sapid sensation to be detected and slowly declines over a period of several minutes [2]. This puckering rough or drying mouthfeel is due to the precipitation of salivary proteins when these interact with tannins present in wine. On the other hand, natural tannins are considered a sign of potential longevity and ageability of a wine because, although they impart a mouth-puckering astringency when the wine is young, they become into part of "bottle bouquet" when the wine is cellared under appropriate conditions [2, 3]. Given the importance of the mouthfeel note that these compounds can bring to wine, one of the attributes that the tasting panel considers when conducting a wine evaluation is called "tannin amount".

In recent decades, techniques such as electronic nose (e-nose)—based both on metal oxide semiconductors [4, 5] and on mass spectra (MS e-nose) [6]—near infrared (NIR) spectroscopy [7], electronic tongue (e-tongue) [8] or combined techniques such as e-nose/Visible-NIR [9] or e-nose/e-tongue [10, 11] have been used to establish a relationship between sensory attributes and chemical composition of wine with the purpose of better understanding what components influence the sensory properties and the final quality of the product.

The most common instrumental technique for the study of gustative attributes in wine is the electronic tongue based on an array of nonspecific, poorly selective, chemical sensors with cross-sensitivity to different compounds and an appropriate chemometric tool for data processing [12]. The responses of every sensor to different compounds of the different types of wines are represented as pattern signals, which are subsequently processed with chemometric techniques for discrimination, classification or characterization purposes.

An alternative for the evaluation of gustative attributes is the electronic nose based on Fourier-Transform Mid Infrared (FT-MIR) spectroscopy. This technique provides structural information about molecular features of a large range of compounds, most of them related with the sensorial characteristics. Its main advantage is its speed and automation [13]. In wine analysis, this technique has successfully been applied for classification or characterization purposes [14], but also for quality control [15], antocyanins determination [16], and polysaccharides studies [17]. Related to tannins, there are also some recent studies where FT-MIR has been used for determining the tannin content [18-21].

Taking into account the great amount of information provided by each of the FT-MIR spectra, the use of multivariate regression methods is imperative. The most common is partial least squares (PLS) regression. However, in multivariate regression, one of the most common problems to be tackled is the presence of non-informative or noisy variables in the spectra, which may worsen the predictive ability of the regression model. To select the most informative variables, that is, the spectral variables more correlated with the property to be predicted, several variable selection techniques have been developed [22-26].

The aim of this study is to assess the FT-MIR ability to reproduce—by using different variable selection techniques— the response that an expert tasting panel provided on the evaluation of the attribute "tannin amount" in red wine samples.

Material and methods

Instrumental

All analyses were performed with an FT-MIR Nexus (Thermo Nicolet, USA) spectrometer, with a DTGS (deuterate triglycine sulfate) detector. The software package OMNIC version 6.2 from Thermo Nicolet was used for spectra acquisition. The spectra were recorded at 4 cm^{-1} resolution, from 400 to 4,000 cm^{-1}. Prior to the analysis of the samples, a blank with distilled water was made. Although the whole spectral range was stored for each sample, only 234 data points were selected for the analysis, restricted to

the ranges 1002.8-1542.8, 1720.2-1808.9, and 2703.7-2966.0 cm^{-1} (Figure 1). The region between 1543 and 1716 cm^{-1} contains strong water absorption bands and the region between 1812 and 2699 is a non-informative absorption region [18]. Prior to the analysis by FT-MIR, the samples were placed into an ultrasound bath for 15 min to eliminate CO_2. All the samples were analyzed in duplicates. The software used for data analysis, calibration, and variable selection was the package PARVUS [24].

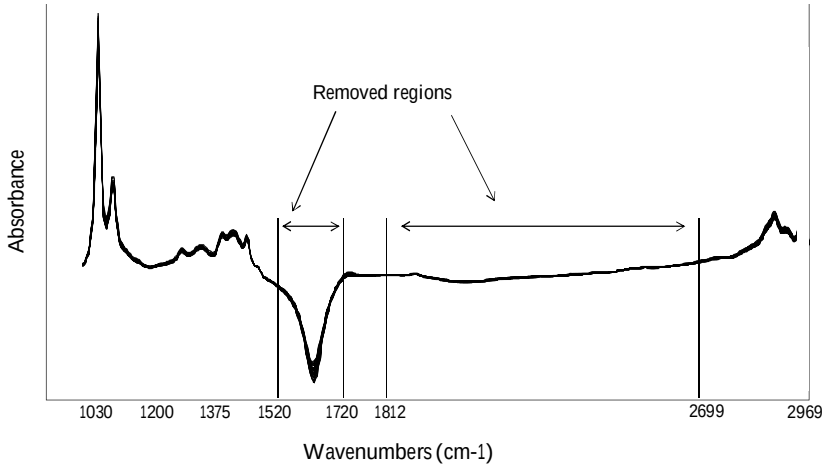

Figure 1. Full spectra of the wine samples and removed spectral regions. The absorbance region of water appears modified because the spectrum obtained when analyzing the blank (distilled water) was subtracted.

Samples

Thirty-seven samples supplied by different wineries of the AOC Priorat, one of the most important wine growing zones located on the North-East of Spain, were analyzed. All the samples were red wines of the same vintage (2006). Their varietal compositions were coupages of different proportions of Garnacha, Cariñena, Cabernet Sauvignon, Merlot, and Syrah.

The sensorial attribute studied was “tannin amount” and was evaluated by 33 expert wine tasting panelists in a sensory panel room. Panelists were always asked to score this

mouthfeel intensity in each sample from 1 to 6, where 1 means low amount of tannins and 6 means high amount. The registered evaluations by the panelists were averaged for each one of the 37 wines to obtain a representative sensorial evaluation. The mean and standard deviation of the 37 resulting scores were 3.90 and 0.31 respectively.

Multivariate analysis and variable selection

The PLS regression method was used to build the multivariate calibration models between the FT-MIR spectra and the Tannin Amount attribute. The quality of the models was checked by means of the root mean square error of calibration (RMSEC) and the root mean square error of cross validation (RMSECV), calculated as:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{i=n}\left(y_i - \hat{y}_i^C\right)^2}{n - F - 1}} \qquad \text{Eq. 1a}$$

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

were y_i is the value of the attribute measured by the tasting panel, $\hat{y}_i^C$ and $\hat{y}_i^{CV}$ are the values computed by the model in calibration and predicted by cross validation, respectively, n is the number of samples in the calibration set and F is the number of factors of the model, which is chosen by selecting the smallest number of factors for which the predictive residual error sum of squares (PRESS) value (Eq. 2) is below 5% (calculated as a percentage between the maximal and minimal observed PRESS values).

$$PRESS = \sum_{i=1}^{i=n}\left(y_i - \hat{y}_i^{CV}\right)^2 \qquad \text{Eq. 2}$$

k-fold cross validation (CV) was used to determine the prediction ability of the models. This technique partitions the original set of n samples into k sets ("folds" or "cancellation groups") of size n/k. A given fold is left out and the whole process of variable selection

and calibration model building is performed with the remaining folds. The model is then tested on the left-out fold. The process is repeated until all k-folds have been left out once. Additionally, for each model the residual predictive deviation (RPD) was calculated as the ratio between the standard deviation (SD) of the tannin amount values and the RMSECV. An RPD value around 3 is indicative of a suitable calibration model. The relative standard deviation, RSD, was used to evaluate the percentage of variation of the RMSECV with respect to the average ($\overline{x}$) of the *tannin amount* values for all the wines analyzed:

$$RSD(\%) = \left(\frac{RMSECV}{\overline{x}} \right) \times 100 \qquad \text{Eq.3}$$

In order to find the wavenumbers more correlated with the sensorial attribute evaluated and thus providing the best predictive models, different variable selection techniques were studied.

Iterative predictor weighting (IPW-PLS) is a technique that builds the calibration model based on the cyclic repetition of the PLS algorithm, multiplying each spectral variable by their importance computed in the previous cycle [23]. The importance Z of each variable j in the first cycle is one and in subsequent cycles is computed as:

$$Z_j = \frac{|b_j| s_j}{\sum_{j=1}^{J} |b_j| s_j} \qquad \text{Eq. 4}$$

where s_j and b_j are the standard deviation and the regression coefficient of the variable j respectively. The regression coefficient b is obtained in the regression model, which is developed starting with all the variables:

$$y = b_0 + b_1 x_1 + \ldots + b_j x_j + \ldots + b_J x_J \qquad \text{Eq. 5}$$

When a spectral variable has an importance less than a given cut-off value, it is deleted.

Main intervals PLS (mi-PLS) [24] is based on the interval PLS (iPLS) method developed by Nørgaard et al. [22] and splits the spectral variables in M (between 2 and 5) main

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intervals. Within each main interval, the spectral variables are divided into regular intervals of maximum 12/M+1 size (i.e., 7 with M=2, 3 with M=5,...) and the best combination of them is found by means of systematic search. The selected intervals (max. 16) within the main intervals are joined for the final systematic search. The selected final combination of intervals is the one providing the minimal RMSECV.

Tree interval PLS (ti-PLS) [24] is also based on iPLS. In this case, the spectral variables are divided in M main intervals. Other M-1 main overlapping intervals are obtained with the variables of the right half of the main interval m with the left half of the main interval m+1. For each of the 2M-1 selected intervals, the usual iPLS method is applied and the selected variables are then joined and the whole procedure is repeated again. In this second step, the variables selected are splitted in M-1 main intervals and M-2 main overlapping intervals. The procedure continues until there is only one main interval, and the final systematic search is done. At each step, the best combination of variables is the one providing the minimal RMSECV.

Moving window PLS (mw-PLS) [25] is a technique that builds a series of PLS models in a window that moves along the spectral direction, taking advantage of the continuity of the spectral responses. For each variable, a PLS model is calculated with the given window size. Then, useful spectral intervals are found by deleting those predictors with a RMSECV lower than a specific cut-off value.

Uninformative Variable Elimination (UVE) [26], adds to the original spectral variables an equal number of random variables with very small values (~10^{-10}) and it is based on an analysis of the *b* coefficients of the PLS model. The reliability, C_j, of each variable is:

$$C_j = \frac{b_j}{S_{b_j}} \qquad \text{Eq. 6}$$

where b_j is the regression coefficient of each variable *j* and S_{b_j} is its standard deviation, obtained from the variation of the *b* coefficients by leave-one-out jack-knifing. The maximum absolute value of C_j of the added artificial variables is the cut-off value for the elimination of non-informative original variables [24].

SELECT [24], searches the spectral variable with the maximum correlation coefficient with the variable to be predicted. This spectral variable is selected and decorrelated from other spectral variables. Then, another variable with the maximum correlation coefficient is selected and decorrelated from the other remaining variables, and so on, until a specified number of variables are selected.

Results and discussion

For building the different PLS models we used a data matrix of 37 rows (wine samples) and 234 columns (FT-IR wavenumbers) and a vector of the average values for the tannin amount attribute of each wine evaluated by the panel. The standard deviation (SD) of the tannin amount values was 0.31. The data matrix was pre-treated with standard normal variate to correct the baseline shifts. In the application of the variable selection, each algorithm was validated by 5-fold cross validation (CV) as explained above, by dividing the whole data set in 5 cancelation groups. In SELECT and mw-PLS the number of retained wavenumbers was selected by the user: it was about 10% of the total (around 25 predictors). In the other cases, the wavenumbers were selected directly by the algorithms according to minimal RMSECV. For building the final multivariate models, the PRESS criterion (threshold 5%) was used.

All the wavenumbers selected by the six variable selection algorithms are shown in Fig. 2. Although the retained wavenumbers by each technique were not exactly the same, certain regions of the full spectra were retained mostly (Fig. 3). Three are the principal selected regions. They cover the 1022-1072, 1361-1450, and 1520-1750 cm^{-1} regions, the latter in discontinuous range. The three regions will be named regions 1, 2, and 3, respectively.

In region 1 (1022-1072 cm^{-1}), we find the absorption of grape tannins (933-1060 cm^{-1}) [18]. This region also belongs to the more extended range 1000-1300 cm^{-1} where the C-O stretching in phenols appears [27]. Furthermore, it has been reported in the literature [18] that the region between 995 and 1060 cm^{-1} was selected by other variable selection techniques in the study of the quantification of red wine tannins by FT-MIR. Wavenumbers related to the selected regions are shown in Table 1.

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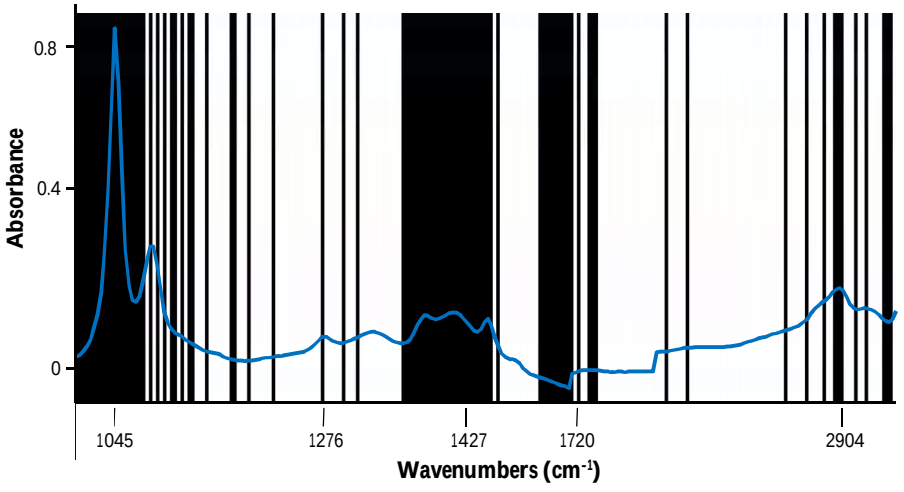

Figure 2. Wine FT-MIR spectra and all the wavenumbers selected by each variable selection techniques.

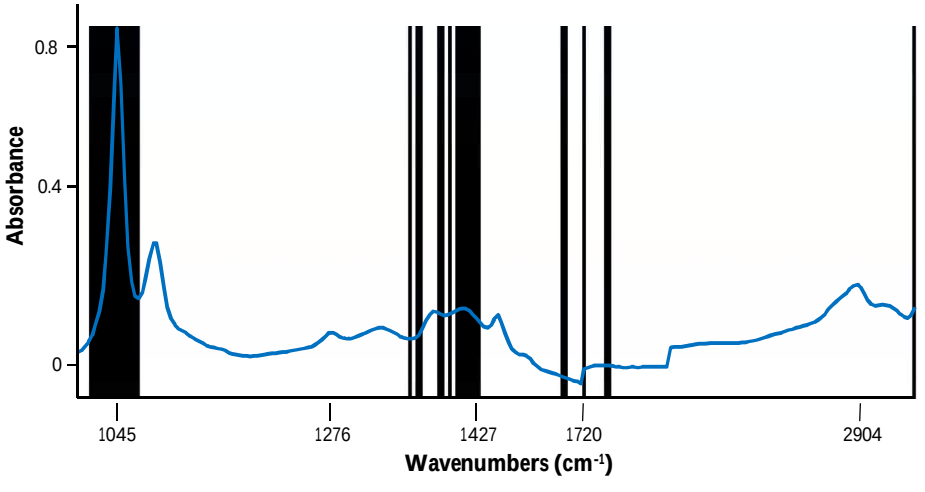

Figure 3. Coincident wavenumbers selected by the six different variable selection techniques.

Description	Wavenumbers	Reference
Grape tannins	933-1060	[18]
Grape tannins	1716-1812	[18]
Selected by algorithms	1425-1485	[18]
C-O stretching in phenol	1000-1300	[27]
Selected by algorithms	995-1060	[18]
OH deformation	1340-1420	[28]
OH-absorption	1260-1410	[29]
Grape tannins peak	1445, 1520	[18]
C=O stretch of esters	1740-1750	[18]

Table 1. Important spectral regions related to tannin compounds.

The discontinuous region 3 (1520-1750 cm^{-1}) contains the grape tannin peak at 1520 cm^{-1} in the typical region of aromatic ring stretches [18]. Moreover, there are peaks in the range of 1720-1750 cm^{-1} that belong to the region of grape tannin absorption (1716-1812 cm^{-1}) [18]. The 1740-1750 cm^{-1} range is assigned to the C=O stretches of the ester group, which matches the spectral absorption of hydrolysable tannin [18].

	Variable selection techniques (a)						**Variables** (b)	
	IPW	mi-PLS	SELECT	TREE	mw-PLS	UVE-PLS	ORIGINAL	RETAINED
Predictors	20	24	25	40	27	7	234	33
Factors	10	5	11	8	10	6	10	12
RMSEC	0.07	0.2	0.09	0.13	0.13	0.21	0.16	0.07
RMSE CV	0.13	0.24	0.18	0.23	0.24	0.22	0.30	0.18
RSD % (CV)	3.33	6.15	4.61	5.89	6.15	5.64	7.69	4.61
RPD (CV)	2.41	1.30	1.74	1.36	1.30	1.42	1.04	1.74
Slope	0.98	0.86	0.80	0.82	0.78	0.95	0.57	0.89
Corr. Coef.	0.92	0.65	0.83	0.64	0.67	0.70	0.42	0.81

Table 2. Analytical results for the models built with each variable selection algorithm (a) and the models built with the original predictors and predictors retained in region 1, 2 and 3 (b).

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The results of each model built using the wavenumbers selected by the six variable selection techniques are shown in Table 2(a). Because of the low standard deviation of the tannin amount values, we consider important to include also in Table 2 the values of the slope and the correlation coefficient of the regression lines "reference vs predicted value" for the cross-validation data.

The best results were provided by IPW-PLS, which retained 20 wavenumbers. Figure 4 shows the importance of the selected variables, computed using Eq. 4. The final PLS regression model with this algorithm was built with ten factors. This model shows the smallest RMSEC and RMSECV values. The values of slope (0.98) and correlation coefficient (0.92) indicate a fairly good fit (Fig. 5) between spectra and tannin amount for cross-validation data. The model also shows an acceptable RPD value considering that the reference attribute values are a subjective evaluation of a tasting panel. Table 3 shows the predicted and reference values obtained with the model built with the IPW-PLS technique. It can be observed that the error associated to each prediction, calculated as the difference between predicted and reference values, is quite low (less than 5%).

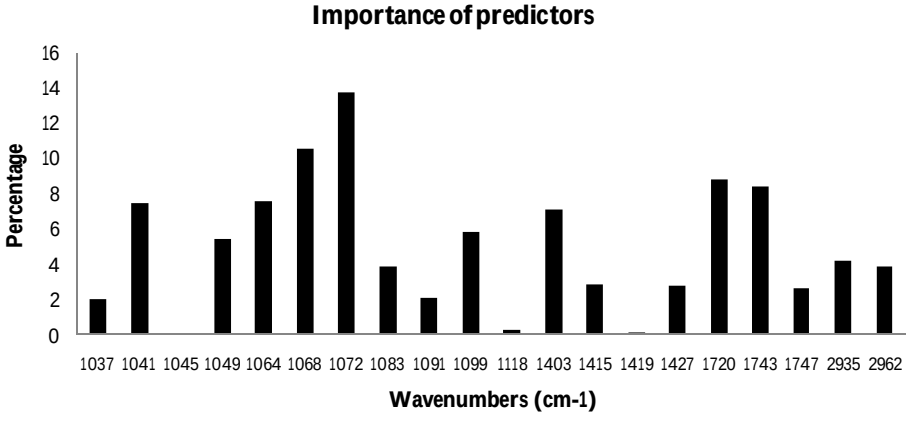

Figure 4. Importance of the 20 selected predictors by the IPW method expressed in percentage.

The other models do not show as good fit as IPW-PLS. When working with sensory data, the difficulty of finding an optimal regression model lies in the low dispersion of the sensorial evaluations of the tasting panel. The standard deviation (SD) of the tannin amount values is 0.31, which represents only the 8.02% respect to the average, in a range of 3.3 to 4.4 units of tannin amount.

IPW results							
Wine	Pred. CV	Reference	Error	Wine	Pred. CV	Reference	Error
1	3.33	3.47	0.14	20	3.95	4.03	0.08
2	4.34	4.16	0.18	21	3.40	3.27	0.13
3	3.35	3.30	0.05	22	3.73	3.72	0.01
4	4.25	4.19	0.06	23	4.35	4.03	0.32
5	3.78	3.79	0.01	24	4.21	4.12	0.09
6	3.75	3.72	0.03	25	3.60	3.42	0.18
7	4.07	4.06	0.01	26	3.93	3.82	0.12
8	3.53	3.64	0.11	27	3.88	3.97	0.09
9	3.52	3.48	0.04	28	3.94	3.82	0.12
10	4.04	4.24	0.20	29	3.71	3.75	0.04
11	3.42	3.28	0.14	30	4.01	3.91	0.10
12	3.94	3.94	0.00	31	4.30	4.30	0.00
13	4.11	4.09	0.01	32	4.10	4.03	0.07
14	4.02	4.18	0.16	33	4.08	4.06	0.02
15	3.85	3.48	0.37	34	4.19	4.09	0.10
16	4.18	4.26	0.08	35	4.15	4.15	0.00
17	4.14	4.28	0.15	36	4.17	4.29	0.12
18	3.88	3.87	0.01	37	4.28	4.35	0.08
19	3.72	3.81	0.09				

Table 3. Predicted values with cross-validation (pred. CV), reference and error values obtained with the IPW-PLS mode.

To confirm the validity of the interpretation made in regions 1, 2 and 3 (Fig. 3) with respect to the tannin amount, a PLS model, named “retained”, elaborated with these regions was built (Table 2(b) and Fig. 5). This model shows a good fit unlike the model built with all the original variables, which uses noisy variables and variables not correlated with the attribute values.

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Based on the results obtained by the variable selection algorithms we observe that those discrete variable selection techniques (IPW, SELECT, and UVE) give the best results, unlike mw-PLS, TREE, and mi-PLS that select continuous ranges of variables. Although in the FT-IR spectrum the signals are correlated it is possible that in mw-PLS, ti-PLS, and mi-PLS not all the wavenumbers in a selected range belong to absorptions of chemical compounds related to the tannin amount. However, IPW, SELECT, and UVE are more selective because they are based on analysis of correlation of each independent variable with the vector of attribute values.

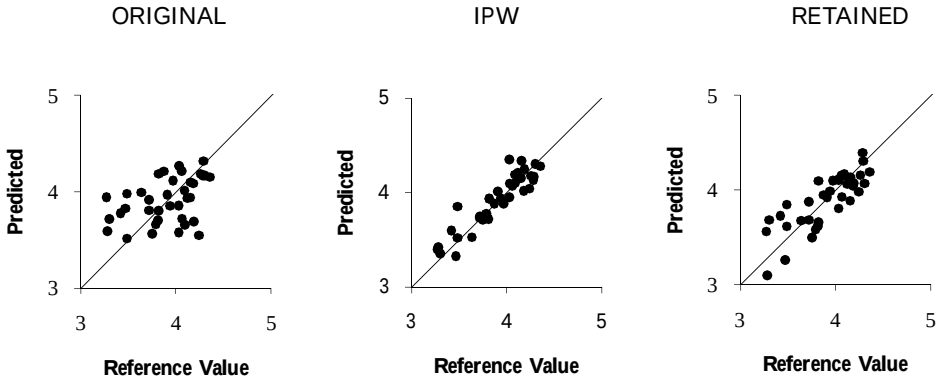

Figure 5. Predicted vs. reference plots (cross-validation data) for the models elaborated by original, IPW, and retained PLS.

Conclusions

From the results obtained in this study we can consider FT-MIR as an alternative tool for its use as a gustative sensor in sensory analysis. Considering that the reference values provided by a sensory panel are subjective values, the most notable aspect of the use of different variable selection techniques in the FT-MIR spectrum is the interpretation of the wavenumbers retained. Based on the results of this study, the retained wavenumbers

have a direct relationship with the absorption of the functional groups associated to tannin compounds, mainly responsible of the astringency attribute in wine.

Concerning the models built with the six variable selection techniques, the IPW-PLS was the best, despite the low dispersion of the sensorial evaluation. The selection of discrete variables in complex spectra seems to be an advantage in this algorithm.

The combined use of variable selection techniques with the FT-MIR technique has been able to reproduce, in this study, the sensory evaluation of tannin amount attribute of a tasting panel.

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Chapter 4

Future Trends: Instrumental Data Fusion In Sensory Analysis

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One of the main challenges related to food sensory analysis through instrumental chemical sensors is how to develop a multifaceted instrumental system capable of performing a complete sensory analysis to obtain as much information as possible related to smell, taste and colour. If this system is used together with the appropriate data processing techniques and multivariate analysis to interpret the collected information, then, the system could emulate the work done by the human sensory system. Nowadays, it is not possible to carry out this type of analysis by using a single instrument. However, it is possible to merge information from several individual chemical sensors by processing the data obtained as a single information system. In that way, the data collected by the different sensors would provide a system of complementary and comprehensive information of the product analysed. The strategies used to combine these data lead to the concept of *data fusion*.

Data fusion provides an approach to improve the performance obtained when using several single sensors. The different sensory instruments used to perform the analysis of a same sample provide many data that can be jointly processed with data fusion techniques to obtain a great amount of information, which overcomes the performance of each sensor when working individually.

Due to the interesting philosophy of the data fusion and the promising results that this technique could provide, we decided to check its feasibility when fusing the data obtained with the equipments used in the present Doctoral Thesis.

Indeed, despite the good results obtained when we used the instruments individually, it was possible to study the quality of the information that could be obtained by combining the olfactive and gustative data obtained through e-nose and e-tongue, respectively, and, even the visual data obtained through an Ultra Violet (UV)-visible spectrometer. The use of the UV-visible was chosen because it could provide colour information by analysing

the samples in the visible spectral range (chapter 1). So, we had additional sensory information.

4.1. About this chapter

This chapter presents an application of data fusion to the data obtained with an MS e-nose, an FT-IR e-tongue and a UV-Visible e-eye when analysing beers from different factories of the same brand and, therefore, commercialized as the same product. The aim of the study was to improve the results obtained when using the different techniques individually and provide a strategy to extract more sensory information through the use of chemometric tools.

The results and conclusions of this work will be a starting point for future studies aimed to the achievement of a future electronic taster.

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4.2. Study of the feasibility of two levels of data fusion in signals obtained from an MS e-nose, an FTIR e-tongue and a UV-visible e-eye for a comprehensive sensory description of beers

L Vera, L Aceña, J Guasch, R Boqué, M Mestres, O Busto

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Study of the feasibility of two levels of data fusion in signals obtained from an MS e-nose, an FTIR e-tongue and a UV-visible e-eye for a comprehensive sensory description of beers

L Vera[1], L Aceña[1], J Guasch[1], R Boqué[2], M Mestres[1], O Busto[1]

[1] Analytical Chemistry of Wine and Food Research Group
[2] Chemometrics, Qualimetrics and Nanosensors Research Group
Department of Analytical Chemistry and Organic Chemistry
Campus Sescelades. Facultat d'Enologia de Tarragona
Universitat Rovira i Virgili
43007 Tarragona (Spain)

Abstract

The signals of three electronic sensors, an MS e-nose, an FTIR e-tongue and a UV-visible e-eye have been fused to study if the ability of classification improves with respect to the individual use of the techniques in the sensory analysis of beer samples. Two levels of data fusion have been studied: low and mid level fusion, and the classification was performed by Linear Discriminant Analysis (LDA). The beer samples analysed belonged to four different factories but were commercialized as a same product. Mid-level fusion provided better classification results (above 95% correct classification) than those of low-level fusion and also than those obtained when using the individual techniques. Moreover, by means of the score and loading plots obtained by Fisher-LDA, it was possible to interpret the chemical information provided by the three sensors, and we were able to relate the variables associated to each sensor to the main compounds responsible of the sensory perception.

Keywords: data fusion, MS e-nose, FT-IR e-tongue, UV-Visible, LDA, classification, beer characterization

Introduction

The great advance of the so-called chemical sensors and their growing applications in food analysis has attracted a considerable research interest in developing alternative techniques or new applications in the field of food sensory analysis. So, mass spectrometry (MS) and spectroscopic techniques, also known as optical sensors, such as near and mid infrared (NIR, MIR), Raman and Ultra Violet-Visible spectroscopy, have been adapted to be used as sensory instruments in food analysis.

The analysis carried out by each instrument provides important sensory information from the samples, which is related to the attributes that allow their characterization. However, the complete characterization of the samples requires the simultaneous use of several techniques that can describe the gustative, olfactive and visual aspects. The compilation of data coming from different sources provides complementary interpretations and facilitates a full product description. The combination of information provided by several analytical instruments is what is called *data fusion*. Data fusion involves treating a large number of multivariate signals of a different nature, and implies the use of chemometric strategies.

One of the first works related to data fusion in food analysis involved the combination of auditive, tactile and olfactive data from a microphone, a force sensor and an electronic nose, respectively, to mimic the human appreciation of potato chips [1]. The authors of this work also proposed to include vision and taste sensors in further studies. Thereafter, some studies have been carried out fusing the data obtained from electronic tongues and noses [2-7]. The first attempt to develop a sensory system to mimic a taste panel was carried out by Rodríguez-Méndez *et al.* [8]. In this study, the combination of three sensory modalities: an array of gas sensors (e-nose), an array of electrochemical liquid sensors (e-tongue), and an optical system to measure colour by means of CIElab coordinates was successfully used for the discrimination of wines. Similar studies in wines [9, 11] and olive oils [12, 13] can be found in the literature.

There is a distinction between the levels at which the sensor data can be fused [14]. The most basic, the so-called low-level fusion, consists on building a single data vector with the fused signals from different sensors to form a sort of supra-sensor system [3]. The

medium-level fusion consists of extracting features from the signal of each sensor and then fusing them [15]. A basic application is to fuse the first principal components (the ones explaining the maximum variance of the data) of the signals collected from each sensor. Finally, in the high-level fusion [16], multivariate models (e.g. classification models) are built separately for each instrumental technique and, then, the individual classification results are combined to produce the final classification.

In this study we present the use of low- and mid-level data fusion with the data obtained by an MS e-nose, an FT-MIR e-tongue and a UV-visible e-eye in the analysis of beers. The beer samples were brewed in different factories of the same brand and are commercialized as a same product. The purpose of this study was to try to improve the classification results performed with each individual technique and provide an alternative strategy to represent and interpret the sensory information. For data processing, Bayesian-LDA was used to classify the beers and Fisher-LDA to obtain the canonical variables and loadings and to find relationships between samples and variables. Additionally, the Wilks lambda criterion was used to select those most discriminant variables and so to provide a chemical interpretation for that discrimination.

Materials and methods

Samples

67 lager beer samples of a same trademark were obtained from four different factories: 11 from factory A, 15 from factory B, 21 from factory C and 20 from factory D. To ensure the representativeness of the samples, the sampling was conducted over six months and, for each sample, we took, randomly, 3 bottles of freshly bottled beer.

Prior the analyses of volatiles, the beer samples were degassed by ultrasonication for 15 min. Then, aliquots of 5.0 ml of beer were placed in 10 mL vials with NaCl (2 M). The vials were hermetically capped with PFTE/silicone septum under N_2 atmosphere. All the samples were prepared and analyzed in triplicate. For the FT-IR and UV-visible analyses, the beer samples were also degassed by ultrasonication for 15 min to avoid the

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interference of the high contents of CO_2 of the beers in the spectra. For FT-IR, aliquots of 10.0 ml of beer were analyzed in triplicate.

Chemicals and Reagents

The standards of the different sugars and acids studied were of analytical grade. D(-)-fructose, D(+)-glucose anhydrous, sucrose and maltose were supplied by Panreac (Barcelona, Spain). 2-methyl butanoic and butanoic acid were supplied by Aldrich (Madrid, Spain); propanoic, 2-methyl propanoic and 3-methyl butanoic by Fluka (Madrid, Spain) and acetic acid by Sharlab (Barcelona, Spain). Finally the iso-α-acids was supplied by Barth Haas Group (Nuremberg, Germany).

Instruments

The volatile composition of beers was analysed with an HS-MS from Hewlett-Packard (Waldbronn, Germany), composed of an HP 7694 static headspace sampler, an HP 6890 gas chromatograph and an HP 5973 quadrupole mass spectrometer with a diffusion pump. To transfer the volatiles from the headspace sampler to the MS detector, an apolar analytical column (HP-5MS) on the gas chromatograph was kept at the suitable temperature to guarantee the transference of the volatiles in less than 5 min to the MS and to avoid the chromatographic separation.

The analyses of the liquid composition were performed with an FT-IR Nexus (Thermo Nicolet, USA) spectrometer, with a DTGS (deuterate triglycine sulfate) detector. The software package OMNIC version 6.2 from Thermo Nicolet was used for spectra acquisition. The spectra were recorded at 4 cm^{-1} resolution, from 400 to 4000 cm^{-1}. Prior to the analysis of the samples, a blank with distilled water was made.

Finally, the analysis of the colour was carried out with an UV-Vis spectrophotometer Thermo Spectronic, Helios γ model (Thermo Electron Corporation, Cambridge, UK). The spectra were recorded at 0.5 nm intervals from 320 to 800 nm. For all measurements,

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rectangular quartz cells of 1 mm path length were used. The radiation source was a tungsten lamp (visible wavelength range).

All the data analysis were made using the chemometric package PARVUS [17].

Multivariate Analysis

Principal Component Analysis (PCA) was used for a preliminary visualization of the 67 beers after low and mid-level fusion was applied. PCA allows visualizing most of the information contained in a data matrix in a few dimensions (called principal components, PCs), which are orthogonal to each other, thus describing complementary information.

Bayesian Linear Discriminant Analysis (Bayesian-LDA) was used to discriminate the beer samples according to their factories. It is based on the hypothesis that the data in the classes follow a normal distribution, being their dispersion described by the same covariance matrix and differing only in the position of their centroid. The classification rule is based on linear discriminant scores, which are directly derived from plugging the density of the multivariate normal distribution into the equation for the *a posteriori* probabilities [17]. A sample (object) is classified in the class for which it has the highest probability. LDA cannot be applied if the number of variables is greater than the number of samples. In such cases, an alternative is to perform a preliminary PCA to the data matrix and then run LDA on the scores of the selected PCs.

Fisher Linear Discriminant Analysis (Fisher-LDA) was used to discriminate the beer samples according to their factories and to obtain a visual representation of the data by overlapping the scores and loading plots (bi-plot). Fisher-LDA calculates the canonical variables, that is, the directions with the maximum discriminant power between classes, which are obtained by maximizing the ratio between the between-class variance and the within-class variance, w/p:

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$$w = \frac{C}{C-1}\frac{\sum_{c=1}^{C} I_c(\overline{d_c} - \overline{d})^2}{I} \qquad \text{Eq. 1A}$$

$$p = \frac{\sum_{c=1}^{C}\sum_{i=1}^{I_c}\left(d_{ic} - \overline{d_c}\right)^2}{\left(I - C\right)} \qquad \text{Eq. 1B}$$

with:

$$\overline{d}_c = \sum_{i=1}^{I_c} d_{ic} \Big/ I_c \qquad\qquad \overline{d} = \sum_{c=1}^{C}\sum_{i=1}^{I_c} d_{ic} \Big/ I$$

where *C* is the number of classes, I is the total number of objects, I_c is the number of objects of class *c* and d_{ic} is a coordinate where the points are projected.

This means that the distances among centroides of each class must be the longest possible compared to the within-class spread. So, the first canonical variable will be the direction with the maximal w/p ratio.

Additionally, the *Stepwise-LDA* (SLDA) [18] algorithm was applied to find the variables that best discriminate the factories and to study their potential contribution to the sensory perception of beers. SLDA was applied using the Wilks lambda criterion, which computes the ratio between the determinants of the within-class variance matrix and of the variance matrix for the whole set of samples. The selected variable is the one that produces the largest decrease of this ratio.

Due to the limited number of samples, all models were cross-validated, with five cancellation groups. In this process the original set is split in five subsets (or cancellation groups), where a given subset is left out to be used as an evaluation set while the remaining four subsets are used to compute the classification or variable selection rule.

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The process is executed until each cancellation group is used as an evaluation set (5 times).

Data pre-treatment

MS e-nose. For the analysis of volatiles the range of m/z used was restricted from 50 to 150 to avoid the ethanol influence (m/z=45 and 46) and because the main compounds that contribute to the beer aroma are fragmented in m/z less than 150. This information was collected in a data matrix of 67 samples (rows) by 101 m/z ratios (columns). The initial matrix was normalized with row profile (RPR) to avoid possible signal shifts between injections. This algorithm divides, for a given sample, each m/z abundance by the sum of all m/z abundances for that sample.

FT-MIR e-tongue. The data matrix was initially pre-treated with standard normal variate (SNV) and first-derivative to correct for baseline shifts [19]. The first-derivative of the spectra was taken using the Savitzky and Golay method [20] with third-order smoothing polynomials through seven points. The MIR spectra were collected in a matrix of 67 samples by 234 wavenumbers, in the ranges 1002-1542, 1720-1808 and 2800-2966 cm^{-1}. The region between 1546 and 1716 cm^{-1} contains strong water absorption bands and the one between 1812 and 2796 cm^{-1} is a non-informative absorption region.

UV-Visible e-eye. The spectral region considered for the colour analysis was 380 to 550 nm. The matrix obtained had dimensions 67 samples by 341 and wavelengths. This data matrix was initially pre-treated with standard normal variate (SNV) and, as in FT-MIR, the first derivative was performed to the spectra.

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Results and discussion

Low-level fusion

In the low-level fusion approach, the individual spectra obtained from the three techniques were fused in a single matrix of 67 samples by 652 variables (see Figure 1). After that, data were autoscaled to compensate for the scale differences and a PCA was carried out on the whole dataset.

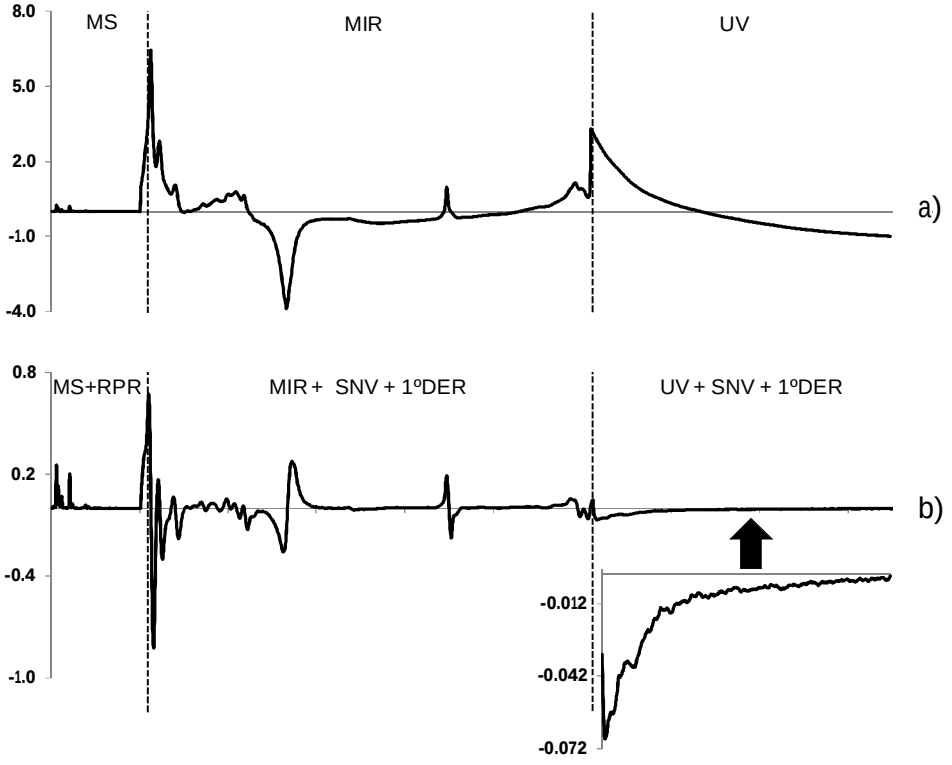

Figure 1. Representative spectrum of a beer sample, showing the process used to fuse the individual matrices: a) whole original spectra of the three techniques and b) final spectra obtained with the pre-processed and transformed signals.

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The PCA score plot (Figure 2) shows a different location of the samples of factory A along the first PC compared to the others factories. The second PC shows a difference between the samples of factories B and D and the ones of factory C. As it can be seen, the samples of each factory, although not totally separated, are located in different regions of the plot.

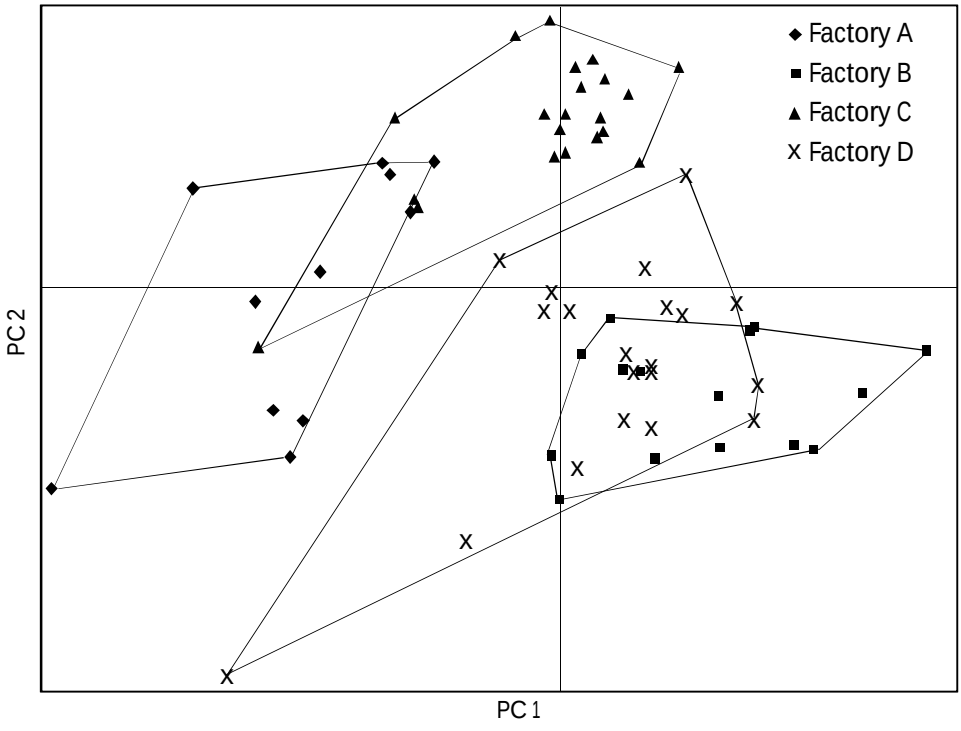

Figure 2. PCA performed with the fused matrix using the low-level fusion strategy (PC1: 18%, PC2: 14%).

To check if the beers of the four factories could be discriminated and to analyse the contribution of each technique to this discrimination, Fisher-LDA was applied. As Fisher-LDA cannot work with a large number of variables, PCA was applied to the data and Fisher-LDA was computed with the first seven PCs (60% of explained variance). Seven PCs were enough in this particular case to discriminate between factories according to the Bayesian LDA. Others LDA models were made with 10, 15 and 20 PCs

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(68, 77 and 83% of explained variance respectively); however, the classification results were not better than with 7 PCs.

Figure 3 shows the projections of the sample beers (cross-validated with 5 cancellation groups) on the two first canonical variables (CVs), computed by Fisher-LDA and using the seven PCs of the fused matrix. All factories are well discriminated: factories A and B by the first CV and factories C and D by the second CV.

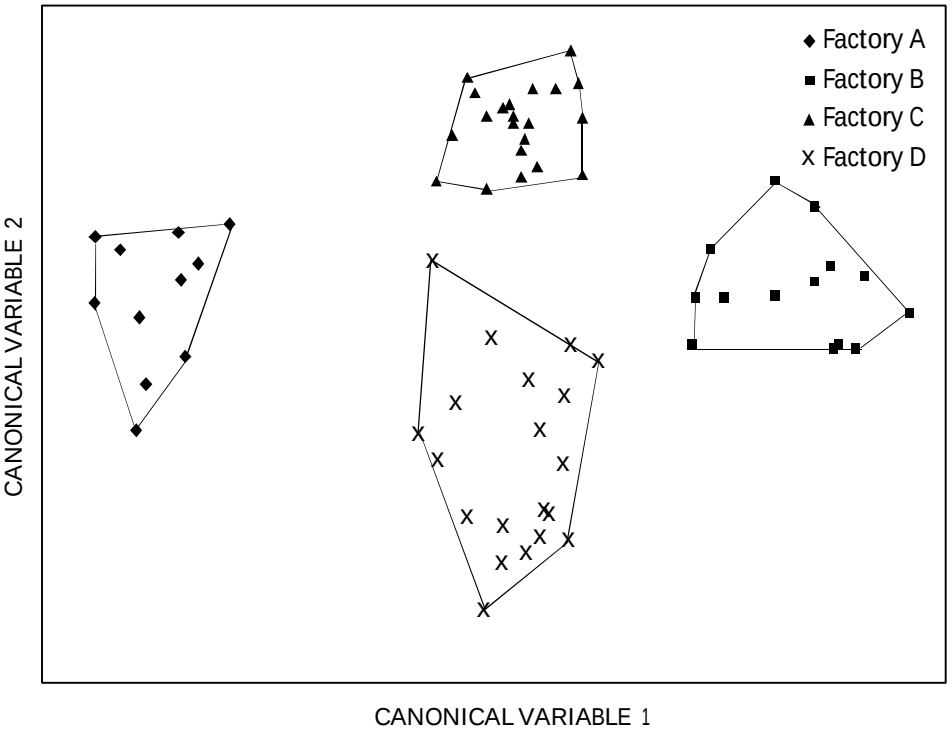

Figure 3. Score plot of the Fisher-LDA, where the discrimination between the samples of the different factories can be observed.

Figure 3 does not show what variables are responsible for the discrimination, what would facilitate a sensory interpretation. In order to get this information, SLDA using the Wilks lambda criterion [18] was applied for variable selection. The number of selected variables

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was initially ten, but only 6 of them are the most discriminative according to the Wilks lambda decrease order (figure 4).

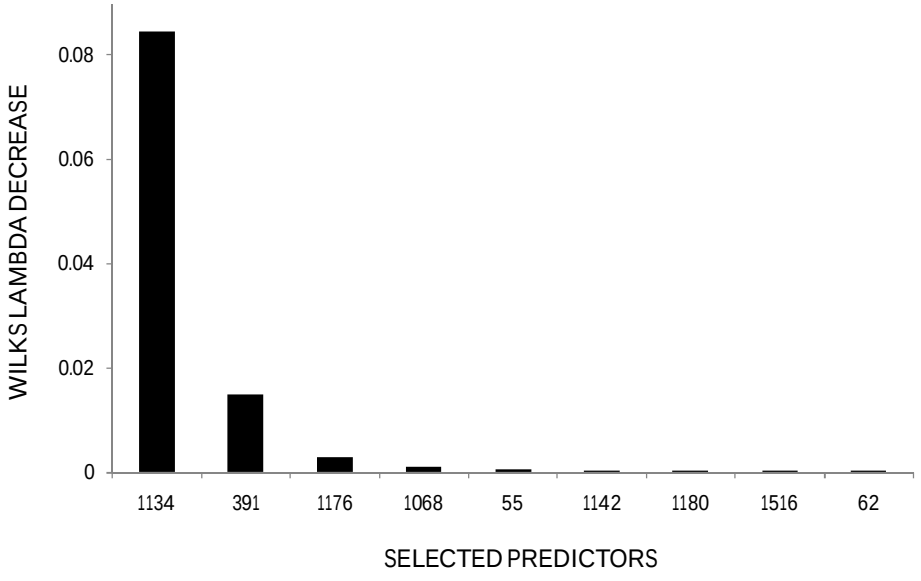

Figure 4. Selected variables sorted according to the Wilks lambda decrease value.

This means that six variables are enough to discriminate the factories. The variables considered to perform the Fisher-LDA were: m/z 55 (e-nose); 1068, 1134, 1176 and 1538 cm^{-1} (e-tongue); and 391 nm (e-eye). The first selected variable, the FT-IR 1538 cm^{-1} wavenumber (not represented in figure 4), was obtained by maximizing the ratio between the total sum of squares and the within-class sum of squares.

Figure 5 shows the biplot (samples and variables) of the Fisher-LDA using the selected variables. It can be seen that the four factories are also discriminated, although with a slight overlap between beers of factories C and D.

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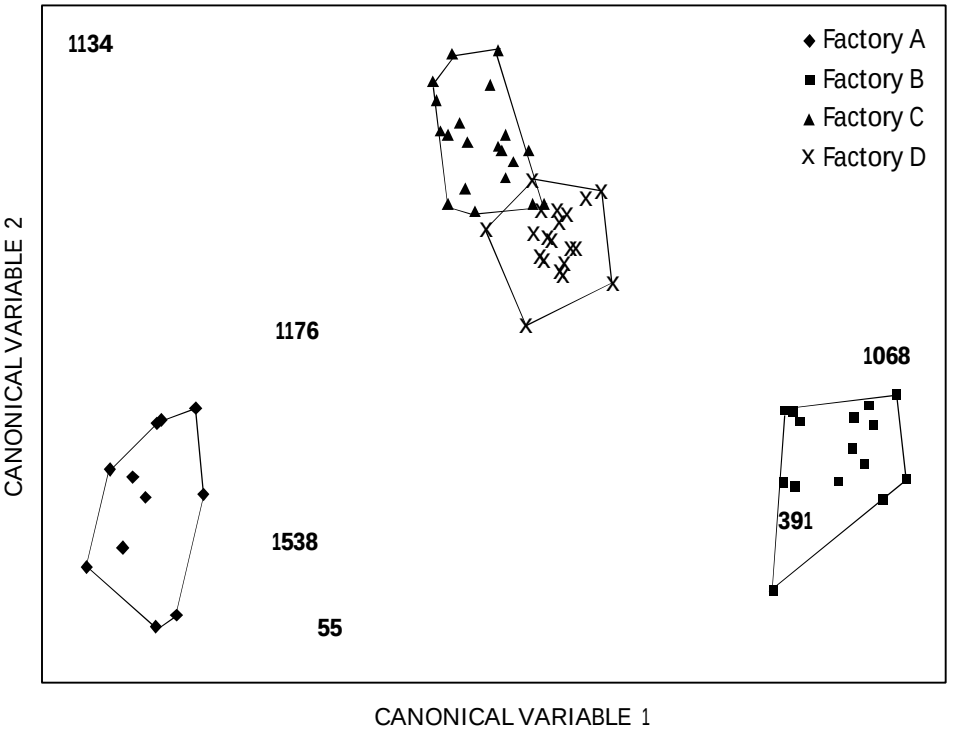

Figure 5. Fisher-LDA biplot showing the 6 selected variables and the distribution of the beers.

Once we obtained the selected variables, the next step was trying to relate them to the absorptions and/or fragmentations of important components in beers in order to extract chemical information related to the differences between samples. The important m/z ions considered by the individual use of MS e-nose related to the main olfactive volatile compounds were presented in a previous study [21]. This study clearly showed a relationship between the samples from factories A and D with alcohols (m/z 55 and 56) and esters (m/z 71, 74, 87, 99, 102 and 104) respectively.

To determine and associate the absorption wavenumbers in FT-MIR to the taste perception, we prepared 1 g L^{-1} of four sugars (glucose, maltose, fructose and sucrose), 0.1 g L^{-1} of six acids (propanoic, 2-methyl butanoic, acetic, butanoic, 2-methyl propanoic and

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3-methyl butanoic) and 0.1 g L^{-1} of a mixture of six iso-α-acids (related to bitterness) in aqueous solutions with 5 % of ethanol. Then, these solutions were analyzed by FT-MIR under the same conditions than the beer samples. The absorption wavelength ranges of each compound, together with the wavelength corresponding to the main peaks, are shown in table 1.

Main compounds responsible of the taste in beers				
Taste	Compound	mgL^{-1}	Main absorption range	Main peaks
Sweetness	Glucose	40-1100	1026-1041; 1068-1110	1033; 1080; 1106
	Maltose	700-3000	1026-1068; 1137-1157	1033; 1056; 1110
	Fructose	0-190	1056-1072	1064; 1149
	Sucrose	0-3300	995-1010; 1049-1068; 1130-1145	1002; 1056;1137
Sourness	Propanoic	1-5	1211-1242; 1704-1735	1230; 1724
	2-Methyl butanoic	0.1-0.5	1211-1242; 1693-1728	1230; 1708
	Acetic	30-200	1249-1311; 1689-1731	1280; 1708
	Butanoic	0.5-1.5	1199-1222; 1701-1728	1211; 1712
	2-Methyl Propanoic	0.1-2	1203-1242; 1697-1728	1222; 1708
	3-Methyl Butanoic	0.1-2	1207-1230; 1697-1724	1218; 1708
	Octanoic	2-12	1200-1310; 1675-1750	1711
	Lactic	20-80	1000-1500; 1650-1770	1128; 1220;1732
	Pyruvic	15-150	1340-1360; 1710-1800	1348; 1726; 1790
	Succinic	16-140	1180-1220; 1650-1750	1204;1696
Bitterness	Iso- α -acids	10-100	1400-1473; 1510-1608	1431; 1542

Table 1. Main responsible compounds of taste in beers and their absorption peaks in the infrared region. The absorbances were obtained from FT-MIR measurements. The absorbance of octanoic, lactic, pyruvic and succinic acids were obtained from reference [23].

The first selected variable (1538 cm^{-1} in the MIR spectrum), lies in the absorption region of the iso-α-acids, which are directly related to the bitterness sensation. This absorption seems to be related to factory A (figure 5), as it also happens with the 55 m/z ion of the MS technique. This ion is characteristic of volatile alcohols such as 3-methyl 1-butanol and 1-pentanol, among others [21]. Close to factory A, the variable 1176 cm^{-1} corresponds to the absorption of fructose [22], which provides sweetness to beers. The MIR variable

1134 cm^{-1} has a high value of Wilks lambda decrease (figure 4), which means that it is a key variable to discriminate the factories. This is important in factory A in the first CV and factory C in the second CV. This absorption is typical of sucrose, an important carbohydrate related to the sweetness of beers [23].

Finally, as it can also be seen in figure 5, there are two variables very related to factory B: the MIR wavenumber 1068 cm^{-1} and the UV-Vis wavelength 391 nm. The first corresponds to an important absorption of fructose, but also of sucrose, glucose and maltose, so it could be related to the sweetness of the samples. The 390 nm wavelength is an absorption of the violet colour whose sensory perception is the yellow-green colour [24] and this belongs to the typical range of absorbance of flavonols [25], a common type of phenols in beers [26], which are yellow pigments [27].

From all these elucidations, it can be said that the main contribution of the low-level fusion strategy is the direct chemical information that can be obtained. Moreover, taking into account that the information contained in the fused spectra comes from analytical tools that register absorptions or abundances of specific compounds related to sensory attributes, the variables selected could be preliminarily interpreted as those attributes that highlight the differences between the samples. However, to corroborate these interpretations and execute a complete validation, a further study, involving a sensory panel, is needed.

Mid-level fusion

This second fusion strategy consists on fusing the principal components obtained individually for each data matrix (instrumental technique). Different pre-treatment were performed to the matrices prior to PCA. The MS matrix was centred, and SNV and first-derivative were applied to the MIR and UV-visble spectra. The number of PCs was selected in each case based on the explained variance. Two PCs (97% explained variance) were selected in the MS matrix, three PCs (96% explained variance) in the MIR matrix and six PCs (91% explained variance) in the UV-visible matrix, to obtain a final matrix of 67 samples by 11 variables.

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Figure 6 shows the PCA score plot of the data. As it can be seen, the samples from factories C and D overlap in both first and second PC (this overlaping is not observed in figure 2) and the samples from factories A and B are well separated by the first and second PC.

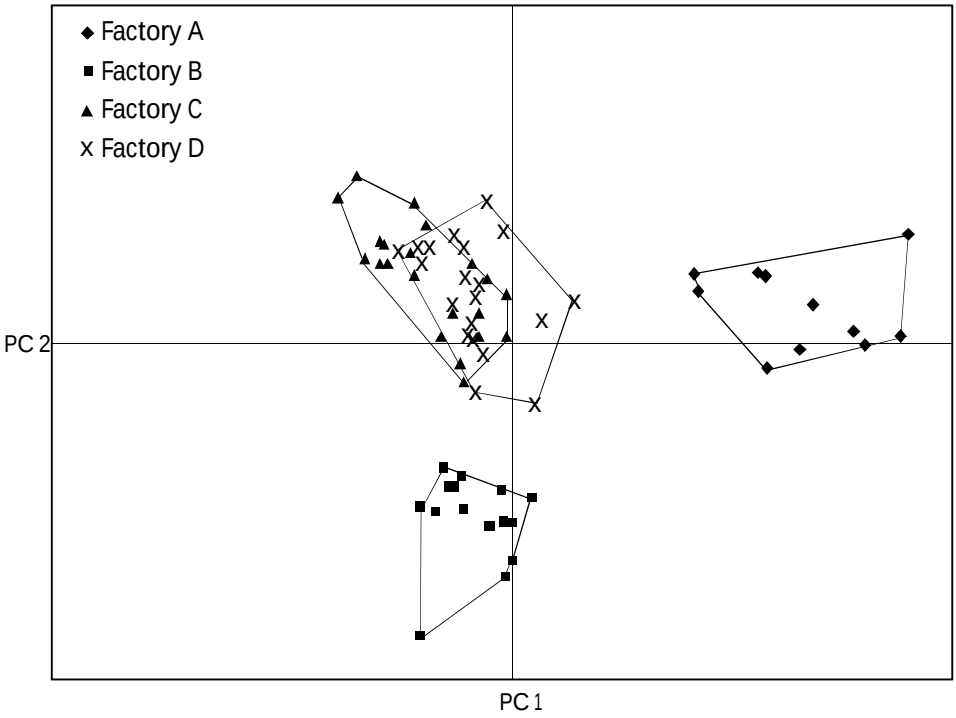

Figure 6. Score plot of the PCA obtained from the fused matrix using the mid-level fusion strategy. (PC1: 21% and PC2: 20%).

Fisher-LDA was applied to the data matrix (67x11) and the biplot is shown in figure 7. This figure is similar to figure 3, especially in the first canonical variable, where factories A and B are discriminated. Factories C and D are also discriminated in the second CV. The first PC of the MS matrix, which explains a 91% of variance, is highly related in the first canonical variable to factory A, and seems to indicate the important influence of the aroma (volatile compounds) in the differentiation of this factory from the rest. The first PC of the MIR matrix (81% explained variance) is in an intermediate region between

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factories A, C and D in both CVs and it seems to be unrelated to factory A in the first CV. Finally, the information of the colour, contained in UVPC1, is more related to factory D and it is an indication of the importance of the colour in the characterization of these samples.

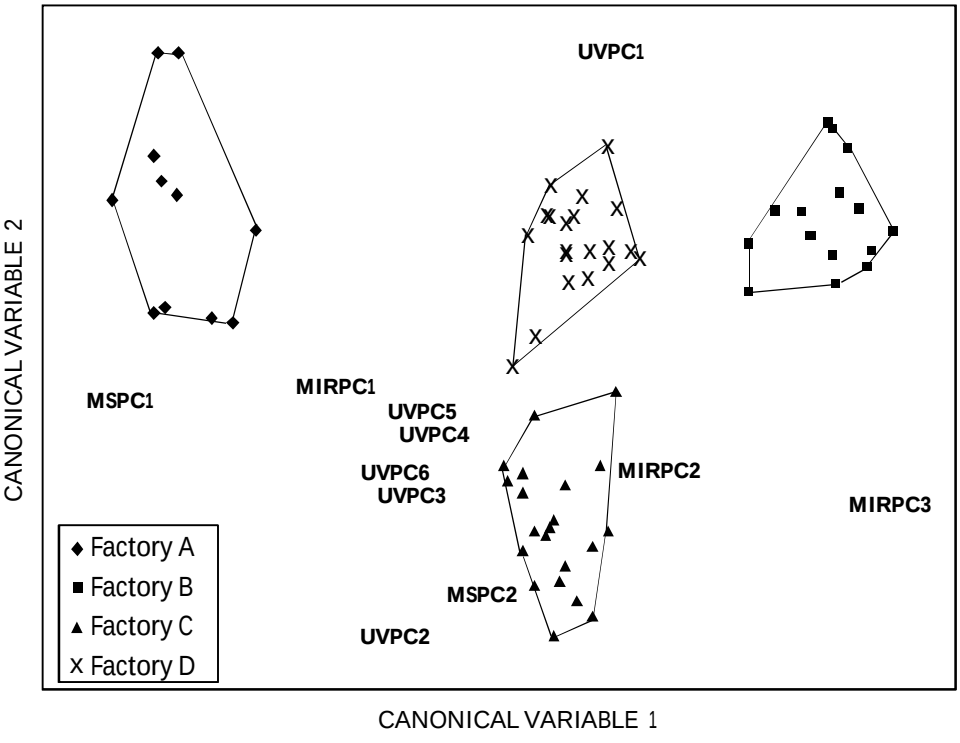

Figure 7. Fisher-LDA biplot of the first two canonical variables. The legend refers to the spectral nature of the variable (MS, MIR and UV) and the PC number.

The discussion above, based on figure 7, refers only to the importance of the information provided by each technique in the discrimination of the beers analyzed, and not to a quantitative interpretation of the olfactive, gustative and visual information. This would mean knowing, *a priori*, what sensory properties (olfactory, gustatory, or visual) are the ones that allow a better description and characterization of the beers grouped according to its factory of origin.

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Data fusion vs individual sensors

In the last part of this study, we tried to determine what strategy (individual sensors, low-level fusion or mid-level fusion) provides the highest ability of classification of the beer samples into their brewery of origin. For this purpose, we applied Bayesian-LDA. Table 2 shows the results, in percentage of classification, after using the MS e-nose, the FTIR e-tongue or the UV-Vis e-eye individually, and also after applying the two levels of data fusion.

SINGLE INSTRUMENTS

MS e-Nose

	Assigned to class				% class
Class	A	B	C	D	
A	10	0	0	1	90.9
B	0	14	0	1	93.3
C	0	0	20	1	95.2
D	1	0	3	16	80.0

FT-IR e-Tongue

	Assigned to class				% class
Class	A	B	C	D	
A	11	0	0	0	100
B	0	15	0	0	100
C	0	0	20	1	95.2
D	0	0	2	18	90.0

UV-Vis e-eye

	Assigned to class				% class
Class	A	B	C	D	
A	10	0	0	1	54.6
B	0	14	0	1	86.7
C	0	0	20	1	90.5
D	1	0	3	16	65.0

DATA FUSION

Low -level Fusion

	Assigned to class				% class
Class	A	B	C	D	
A	10	0	0	1	90.9
B	0	15	0	0	100
C	0	0	21	0	100
D	1	0	1	18	95.0

Mid -level Fusion

	Assigned to class				% class
Class	A	B	C	D	
A	11	0	0	0	100
B	0	15	0	0	100
C	0	0	21	0	100
D	0	0	1	19	95.0

Table 2. Results of Bayesian-LDA applied to the data obtained by the single instruments and also to the fused data. The percentage values of correct classification (% class) correspond to the values of the cross-validation sets.

Although the results from each single instrument, mainly from the MS e-nose or from the FT-IR e-tongue, show a good classification of the samples, data fusion clearly improves them. Low-level fusion gave the same classification results for factory A than the MS e-nose and, in both cases, the sample misclassified was the same. This behaviour means that factory A was strongly influenced by the information provided by the e-nose, as it can be seen in figure 7, where the first PC of the e-nose data (MSPC1) is closely related to the samples of factory A.

The additional information that can be extracted by fusing the data allows, in many cases, to improve the classification results. This is what happens with the percentage of classification of beers from factory C. When using the e-nose or the e-tongue individually, this percentage was 95.2%, and when using the UV-Vis e-eye, this value decreased to 90.5%. However, after applying low-level or mid-level fusion to the data, the correct classification of the samples was 100%.

Finally, it can be observed that mid-level fusion shows better classification results (table 2) than low-level fusion. This better behaviour can be due to the fact that, whereas mid-level fusion considers the first PCs of each individual matrix -which implies that the information of the three techniques is well represented- the low-level fusion only considers the most relevant information from the fused matrix so, possibly, some information is not considered. The percentage of explained variance of the three sensors, MS e-nose, FT-IR e-tongue and UV-Visible e-eye, obtained with mid-level fusion was of 97, 96 and 91%, respectively, unlike low-level where the seven firsts PCs with only 60% of explained variance were considered. So, the application of mid-level fusion represents a gain of information, which can be useful to represent the samples. In turn, low-level fusion allowed sensory characterizations from the direct interpretations of variables (spectrum) and samples.

Conclusions

Two levels of data fusion have been applied to spectral measurements obtained when analysing beers of the same brand and type but produced in different factories, by using an MS e-nose, an FT-IR e-tongue and a UV-visible spectrophotometer. Bayesian and

Fisher-LDA were used to classify the samples according to the factory of origin and to provide chemical information about those more discriminant variables in the classification of the factories. With the low-level fusion strategy, it was possible to obtain direct chemical interpretation of the differences between factories by comparing the selected variables with the responses given by important chemical compound related to the sensory perception. With the mid-level fusion, the importance of the information provided by each technique in the discrimination of the samples could be established.

The influence of using individual or fused matrices in the classification was discussed. Data fusion (low and mid level fusion) provided a better classification, with mid-level fusion showing the best classification results.

This work intends to be a preliminary study of the potential of the data fusion methodology and represents a starting point for future research that involves studies aimed at developing an electronic taster. However, to perform a complete study and validate the sensory interpretations, is necessary the information provided by a tasting panel or by consumers.

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Chapter 5

Conclusions

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The appearance of the electronic nose and the electronic tongue techniques in the 80's and 90's and its rapid development have enabled many applications in food analysis and sensory analysis. Indeed, these have become well-established and consolidated techniques in quality control analysis of foods, mainly due to its speed, sensitivity and portability. The traditional electronic nose and electronic tongue devices consist on a detection system based in an array of gas or liquid sensors which collect the information from electrochemical signals produced when the volatile or liquid substances reach the sensors.

More recently, other types of techniques, widely used in chemical analysis, have been adapted as electronic nose and tongue: the MS e-nose and FT-IR e-tongue. These techniques, based on Mass Spectrometry (MS) and Fourier Transform Infrared (FT-IR) Spectroscopy, collect spectral information of the volatile phase and the liquid phase of the samples, respectively. The principal characteristic of these techniques is the great amount of information collected which comes from the spectra of m/z ions (produced by the fragmentation of volatile compounds) in the case of MS e-nose or from the spectra of absorption at different wavenumbers registered (produced by the absorption of the chemical compounds present in the liquid phase of the sample) in the case of FT-IR e-tongue.

Related to the MS e-nose, there are many studies where this technique has been applied both to food analysis and to sensory analysis. However, since the use of FT-IR in sensory analysis is recent, its application to this field is still reduced but the results found show this technique as a potential alternative to conventional electronic tongues.

This Doctoral Thesis is focused on the applications of MS e-nose and FT-IR e-tongue in the sensory analysis of alcoholic beverages. These studies were made possible through the use of chemometric techniques which allowed interpreting the vast amount of information collected.

5.1. Conclusions

According to the results reached in the different studies performed during the development of this Doctoral Thesis, some interesting conclusions have been obtained.

1) The MS e-nose can be considered as a reliable tool to be used in the sensory analysis of alcoholic beverage. This assertion is based on the following conclusions:

1.1. It has been possible to correct the problem of signal instability in the MS e-nose through the model transfer strategy. In such way we could build durable calibration models over time that is important for studies of sensory analysis such as calibration instrument-panel. The elaboration of synthetic wine to be used as transfer set is a solution to the problem of getting a set of samples stable over time with a similar composition to that of wine. Although this strategy has been applied to the analysis of wine aroma, it could be also used in the analysis of other alcoholic beverage by using a suitable synthetic matrix.

1.2. It has been possible to assume that the MS e-nose represent a complete aroma sensor based on its capacity to discriminate and characterize beer samples of a same type but elaborated in different factories when using chemometric strategies. Indeed, the use of these strategies, and more precisely LDA and variable selection algorithms, provides interesting interpretations of the information collected by this instrument.

2) The ability shown by the FT-IR e-tongue to reproduce the values of a taste panel for the gustative attribute “tannin amount” in red wines, allows considering the FT-IR technique as a potential and alternative gustative sensor in sensory analysis. A suitable interpretation of the wavenumbers was made by using variable selection techniques and it was possible to find a direct relationship with the absorption of the functional groups associated to tannin compounds, mainly responsible of the astringency attribute in wine. So, the combined use of the FT-IR and variable selection technique is a powerful tool to reproduce sensory evaluations of a tasting panel.

3) The combined use of the electronic sensors and chemometric tools (PLS, LDA and the selection variables algorithm) allow to obtain important sensory information related to the sensory attributes of wine and beers and even to interpret sensory differences.

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4) The development of strategies for data fusion (low and mid-level fusion) can improve the performance and quality of the information provided by the individual use of sensory instruments, considering the large amount of information obtained from them. By applying Fisher-LDA in beers of different origins, but of a same type, it has been possible to obtain, with low-level fusion, direct chemical interpretation of the differences between samples and, with mid-level fusion, the importance of the information provided by each technique in the discrimination of the samples. So, the results obtained mean an important advance toward the development of an electronic taster through the fusion of three artificial sensors: electronic nose, electronic tongue and electronic eye.

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