Application of electrochemical sensors as an alternative tool for perfume evaluation

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ABSTRACT

The capability to discriminate perfumes based on their specific aroma profiles is of utmost relevance for the perfume industry considering that, the identification of more than three aromas is a very difficult task even for a trained human nose. Currently, no analytical tool can completely substitute the human nose for aroma evaluation. Also, no analytical system can fully mimic the human perception, being the recognition of perfume aroma patterns usually carried out by gas chromatography coupled with olfactometry or sniffing techniques or even by applying electronic noses, although it still is a difficult analytical task. In this work, the possibility of applying a potentiometric electronic tongue as an analytical sensors tool for perfume analysis, was evaluated for the first time. In fact, the perfume aroma pattern will depend on the composition of the liquid perfume phase and on the diffusion properties of the volatile components, making the proposed strategy feasible from a theoretical point of view. A multi-sensor potentiometric device, comprising a set of 40 lipid sensor membranes with cross-sensitivity, was applied together with chemometric techniques to identify and establish unique chemical perfume fragrances' fingerprints for discriminating perfumes according to the target consumer (men – women perfumes), the perfume olfactory family (Citric-Aromatic, Floral, Floral-Fruity, Floral-Oriental, Floral-Woody, Woody-Oriental and Woody-Spicy) or the perfume storage time-period ($\leq$ 9 months; 9-24 months; and, $\geq$ 24 months). Linear discriminant multivariate models were established, based on potentiometric profiles gathered by sub-sets of sensors selected using the simulated annealing algorithm, and allowed correct classification rates of 93-100% (for leave-one-out cross-validation procedure). The satisfactory analytical performance of the electronic tongue demonstrates the versatility of the proposed approach, as a practical device for preliminary perfume classification, which industrial application may be foreseen in a near future, contributing to a green-sustained economic growth of the perfume industry.

Keywords: Perfume olfactory family; Perfume storage time-period; Potentiometric electronic tongue; Linear discriminant analysis; Simulated annealing algorithm

RESUMO

A capacidade de discriminar perfumes com base em seus perfis de aromas específicos é de extrema relevância para a indústria de perfumes, considerando que a identificação de mais de três aromas é uma tarefa muito difícil, mesmo para um nariz humano treinado. Atualmente, nenhuma ferramenta analítica pode substituir completamente o nariz humano na avaliação do aroma. Além disso, nenhum sistema analítico pode imitar completamente a percepção humana, sendo o reconhecimento de padrões de aroma de perfume geralmente realizados por cromatografia em fase gasosa acoplada a técnicas de olfatometria ou cheirar ou mesmo pela aplicação de narizes eletrónicos, embora ainda seja uma tarefa analítica difícil. Neste trabalho, a possibilidade de aplicar uma língua eletrónica potenciométrica como uma ferramenta analítica de sensores para a análise de perfumes foi avaliada pela primeira vez. De fato, o perfil de aromas do perfume dependerá da composição da fase líquida do perfume e das propriedades de difusão dos componentes voláteis, viabilizando a estratégia proposta do ponto de vista teórico. Um dispositivo potenciométrico de multisensores, com um conjunto de 40 membranas lipídicas com sensibilidade cruzada foi aplicado, em conjunto com técnicas quimiométricas para identificar e estabelecer perfis típicos de fragrâncias químicas para discriminação de perfumes de acordo com o consumidor-alvo (perfumes masculinos - femininos), a família olfativa do perfume (cítrico-aromático, floral, floral-frutado, floral-oriental, floral-amadeirado, amadeirado-oriental e amadeirado-especiado) ou o período de armazenamento do perfume (≤ 9 meses; 9-24 meses e ≥ 24 meses). Modelos multivariados discriminantes lineares foram estabelecidos, com base em perfis potenciométricos de subconjuntos de sensores selecionados usando o algoritmo de recozimento simulado, e permitiram obter taxas de classificação corretas de 93 a 100% (para a validação cruzada “leave-one-out”). O desempenho analítico satisfatório da língua eletrónica demonstra a versatilidade da abordagem proposta, como um dispositivo prático para a classificação preliminar de perfumes, cuja aplicação industrial pode ser prevista em um futuro próximo, contribuindo para um crescimento económico sustentado da indústria de perfume.

Palavras chave: Família olfativa de perfumes; período de armazenamento de perfume; língua eletrónica potenciométrica; análise discriminante linear; algoritmo de recozimento simulado

Index

List of Figures

List of tables

Objectives

The present work aims, for the first time, to evaluate the possibility of using a potentiometric electronic tongue (E-tongue) together with linear discriminant analysis (LDA) coupled with the simulated annealing (SA) variable selection algorithm, as a practical perfume classifier device, minimizing or even avoiding the need of applying complex hybrid statistical techniques. Contrary to other research areas (e.g., food science [1]), in the perfume field, the use of E-tongues is not common. Only one work reported the use of a voltammetric E-tongue for perfume evaluation [2]. The study evaluated the performance of the voltammetric E-tongue to detect the type and concentration of different perfume's fragrances. On the other hand, E-tongues have been widely used to assess positive and negative sensory attributes of foods [3-4]. Moreover, sensor lipid membranes can interact with different polar compounds (e.g., phenolic compounds, esters, alcohols and aldehydes) via the establishment of electrostatic or hydrophobic interactions [5] and, since some of these chemical families are present in perfumes (as fragrances and scent ingredients), the possible application of this type of E-tongues may be foreseen. In fact, it has been reported that lipid bilayer membranes could be effectively applied within a synthetic sensing system to discriminate odorants and successfully differentiate perfumes by brand [6]. On the other hand, it was shown that a simple technique like ultraviolet-visible (UV-Vis) spectrophotometry, coupled with multivariate statistical tools, allowed obtaining a preliminary chemical fingerprint of perfume samples, enabling perfume classification [7]. Therefore, and although the advantages of using an E-tongue could not be obvious, considering that perfume analysis is usually associated to the olfactory perception of aroma fragrances, its use can be foreseen. Actually, the analysis of the perfume' liquid phase, which contains the chemical compounds responsible for the aroma profile, may be extremely relevant, allowing gathering complementary but relevant chemical information of the perfumes' main fragrances notes as well as their age, i.e., the storage time-period, during which a chemical profile change is expected.

I. Introduction

It is expected that the global market of Fragrances and Perfumes exceeds US$40 billion by 2020 [4]. A perfume may comprise from 10 to 100 individual ingredients [5], which are usually complex mixtures of synthetic or natural (e.g. essential oils) organic compounds (e.g., aldehydes, alcohols, lactones, esters and terpene derivatives). So, assessing the perfume composition, identifying the main aroma family as well as assessing perfume-stability and shelf life is not a straightforward task [4,5]. As most of perfume ingredients are volatile or semi-volatile, gas chromatography (GC), in combination with mass spectrometry (MS) is, by far, the most used analytical technique [6]. However, GC-MS does not provide qualitative information about sensor perception of the aroma molecules [4]. Thus, GC-Olfactometry (GCO) or GC-sniffing techniques coupled with condensed Phase Fourier-transform infrared (FTIR) spectroscopy or Time of Flight-MS (ToFMS) may be required [5,7]. These techniques are time-consuming, expensive and require skilled technicians, which may be beyond the economic possibilities of low-medium local perfume companies. Thus, the development of fast, low-cost and green sensor-based techniques, which may be applied on-line, to monitor in-situ perfume aroma-fragrance profiles is highly envisaged by the industry. Electronic noses (E-noses) have been proposed for perfume analysis namely for discriminating original brand perfumes or recognizing fake counterparts [8-9]; for identifying simple aromas [10-11]; for recognizing unknown fragrance mixtures [16]; to classify different perfume classes [17]; as quality control method of musk samples [14]; for generating analyte-specific fingerprints of odorants [12]; to differentiate perfumes by brand [6,15]; or, for highlight the differences of perfumes according to the producers, using odorant maps [16]. An E-nose was also applied to detect counterfeit perfumed cleaner products as well as to quantify the perfume added amount [13]. Despite the satisfactory results reported so far, the identification of more than three perfumes remains difficult for the human nose and for E-nose devices with multiple sensors [13]. To overcome this problem, complex hybrid multiple statistical classifier methodologies have been proposed [16].

The present work aims, for the first time, to evaluate the possibility of using a potentiometric electronic tongue (E-tongue) together with linear discriminant analysis (LDA) coupled with the simulated annealing (SA) variable selection algorithm, as a practical perfume classifier device, minimizing or even avoiding the need of applying complex hybrid statistical techniques. Contrary to other research areas (e.g., food science [1]), in the perfume field, the use of E-tongues is not common. Only one work reported the use of a voltammetric E-tongue for perfume evaluation [2]. The study evaluated the performance of the voltammetric E-tongue to detect the type and concentration of different perfume's fragrances. The versatility of applying E-tongues to assess both positive and negative flavor sensations of food matrices have been extensively evaluated [3-4]. Moreover, sensor lipid membranes can interact with different polar compounds (e.g., phenolic compounds, esters, alcohols and aldehydes) via the establishment of electrostatic or hydrophobic interactions [5] and, since some of these chemical families are present in perfumes (as fragrances and scent ingredients), the possible application of this type of E-tongues may be foreseen. In fact, it has been reported that lipid bilayer membranes could be effectively applied within a synthetic sensing system to discriminate odorants and successfully differentiate perfumes by brand [6]. It was also shown that a simple technique like ultraviolet-visible (UV-Vis) spectrophotometry, coupled with multivariate statistical tools, allowed obtaining a preliminary chemical fingerprint of perfume samples, enabling perfume classification [7]. Therefore, and although the advantages of using an E-tongue for perfume analysis is not obvious, considering that this type of analysis is usually associated to the olfactory perception of aroma fragrances, its use can be foreseen. Actually, the analysis of the perfume' liquid phase, which contains the chemical compounds responsible for the aroma profile, may be extremely relevant, allowing gathering complementary but relevant chemical information of the perfumes' main fragrances notes as well as their age, i.e., the storage time-period, during which a chemical profile change is expected.

I.1. Perfumes: an overview

A perfume is a complex matrix, being alcohol, water and natural and/or synthetic fragrances the main components. In more detail, usually a perfume comprises a denatured alcohol, an undisclosed mixture of several scent chemicals and ingredients used as fragrances, and water. Other ingredients such as fragrance additives, masking ingredients and scents are also present. According to the International Fragrance Association (IFRA) [18] "*scent is one of the most powerful of senses*", being present in the usual daily life, and sometimes may alter the person's mood, diminishing or increasing stress, or being used to reduce pain sensation[19]**.** Perfumes are widely used, being incorporated in several cosmetic products, like shampoos, deodorants, soaps, and fine fragrances; in household products such as laundry detergents, cleaners, and bleaches **(Figure1)**.

Figure 1: Examples of products containing perfumes [24]

Perfumes can be classified according to their nature, in natural or synthetic. Natural perfumes are obtained from plants (e.g., lavender, geranium) or from some of their parts, like flowers (e.g., jasmine, rose, gardenia), fruits (e.g., lemon, orange, vanilla), roots (e.g., vetiver, cistus, angelica), leaves (e.g., violet, patchouli, peppermint), wood (e.g., vetiver, sandalwood, cedarwood), bark (e.g., cinnamon, nutmeg), resin (e.g., benjui, tolu, galbanum), and seeds (e.g., angelica, celery, anis). [20] Also, some perfumes may be obtained from animal glands and organs, like musk, civet, ambergris and castoreum. These natural perfumes, or essential oils, are obtained using different extraction methods (e.g., steam distillation, hydro distillation, solvent extraction, supercritical fluid extraction or manual/mechanical pressing extraction), which selection depends on the raw material and the chemical fragrances to be extracted [20]. Contrary, synthetic perfumes are obtained by mixing synthetic fragrance related chemicals, which are synthesized in the laboratory, aiming to mimic the aroma of a natural fragrance or to obtain a new and original scent. This latter type of fragrances has many practical advantages, namely their low cost compared to natural perfumes. Also, since the amount and quality of the natural source are often unpredictable, due to their dependence on crop quality or weather, synthetic perfumes may be an interesting alternative. Nevertheless, synthetic perfumes also comprise some problems. For example, a natural essential oil is made up by hundreds or thousands of different compounds, which makes difficult to reproduce the desired perfume exactly by just mixing a limited number of different synthetic fragrances. Moreover, the final scent of a natural perfume depends not only of the characteristic odors of the main components but also the minor or trace components, which may affect considerably the final scent of the perfume, turning out the development of a synthetic perfume able to mimic the natural one a challenging and sometimes impossible task. Additionally, if a natural perfume contains isomeric forms of a chemical compound, which may possess different aromas, the development of a similar synthetic perfume would require the use of chiral synthesis.

Perfumes and the pure fragrance chemicals within the perfumes, can be classified according to the olfactory note they provide, that is, according to the fragrance type. For example,

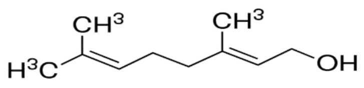
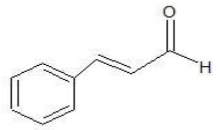
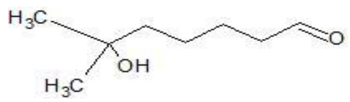
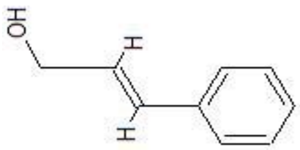
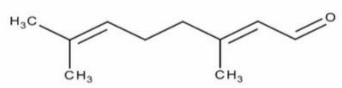
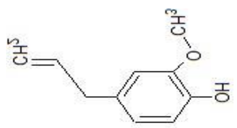
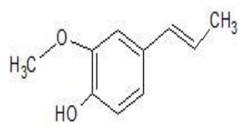
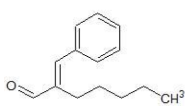
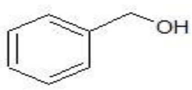
- floral, which reminds one of scents like jasmine, rose, heliotrope;
- citrus, which are aromas reminiscent of lemon, orange, lime, grapefruit;
- fruity, based on non-citrus fruity odors like peach, apple, banana;
- green, which creates the sensation of smelling recently cut grass and leaves;
- woody, which reminds one of dry wood and trees;
- oriental, referring to sweet strong fragrances reminiscent of vanilla, ambergris;
- spicy, giving off a redolence coming from clove, cinnamon, thyme, pepper;
- animal, comprising scents provided by musk, civet, and castoreum; and,
- leather, which tries to reproduce the characteristic smell of leather, tobacco and smoke.

The most typical fragrance compounds include a variety of chemical compounds namely, octadienes, hexyloxyacetonitriles, cyclopentanederivatives, α-oxo (oxo) sulfides, aliphatic dibasic acid diesters, 3-(10-undecenyloxy) propionitrile, tricyclodecane-methylol derivatives,2-methyl-2-alkyl-alkanoic esters, trimethylcyclonexylethylethers, cyanoethylidene-bicyclo-heptenes, crotonyl-trimethylcyclohexanes, nonanols, nonenols, α-oxo (oxo) mercaptanes, safranic acid esters and maltyl-2-methyl alkenoates [21].Examples of some of these compounds, which are commonly used in the industry, are shown in **Table 1**.

Commercial fragrances usually contain 22% (not always) of fragrance oils with alcohol, dye solutions, water and propylene glycol with extenders, fixers and stabilizers [22]**.** Several base fragrance oils are formulated to generate specific olfactory effects in the final fragrance. As mentioned, fragrances or perfumes consist of a combination of numerous ingredients including basic odor botanical or animal derived compounds.

The olfactory families allow individual perfumes to be classified according to their key olfactory characteristics. They are created either by grouping together raw materials such as flowers, woods, aromatics or citrus fruits, or by taking inspiration from traditional accords (e.g., oriental or chypre).

Table 1: Examples of different natural and synthetic fragrances of perfume commonly used in the industry [21]

Fragances compounds	**Formula**	**Chemical composition**
Geraniol	$C_{10}H_{18}O$	
Cinnamaldehyde	C_9H_8O	
Hydroxycitronellal	$C_{10}H_{20}O_2$	
Cinnamylalcohol	$C_9H_{10}O$	
Citral	$C_{10}H_{16}O$	
Eugenol	$C_{10}H_{12}O_2$	
Isoeugenol	$C_{10}H_{12}O_2$	
α-Amylcinnamaldehyde	$C_{14}H_{18}O$	
Benzyl alcohol	C_7H_8O	

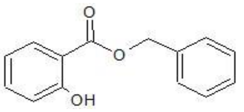
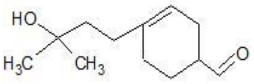
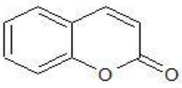
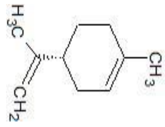
Benzyl salicylate	$C_{14}H_{12}O_3$	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	$C_{13}H_{22}O_2$	
Coumarin	$C_9H_6O_2$	
Limonene	$C_{10}H_{16}$	

Table 1 continued.

The families can be classified as feminine, masculine or unisex, which may be further grouped according to the olfactory family as citrus, floral, aromatic, woody, oriental, or chypre.

Chypre: this family includes a blend of bergamot, rose, jasmine, oak moss, patchouli and *Cistus* Labdanum.

Floral: this category comprises two main groups, the sweet florals (e.g., jasmine, ylang-ylang, tuberose) and fresh florals (e.g., lily of the valley, lilac, freesia).

Citrus: this olfactory family includes scents from citrus.

Oriental: this category includes notes associated with sensual and warm sensations. It includes sweet base notes like vanilla, patchouli, ambery and powdery notes as well as spicy, animal ones.

Aromatic: this family includes blends of lavender, geranium, oak moss, vetiver, coumarin, being usually associated with very masculine scents with fresh, sweet and aromatic notes.

Woody: woody notes can be split into six subcategories: dry, humid, mossy, ambery, smoky, resinous, milky. These notes are related to elegance, warmth and character, particularly represented by cedar, vetiver, sandalwood and birch [22].

Leather: leathery notes came from the master glove and fragrance makers in Grasse. These smoky notes were created by infusing scraps of tanned leather with burnt birch bark. Animal notes (ambergris, civet, castoreum and musk) were included more recently.

I.2. Analytical techniques and their application for perfume analysis

As already pointed out, perfume is a very complex chemical matrix, which may comprise several chemical compounds. Depending on the final use a contemporary perfume may comprise between 10 to over 100 individual perfumery raw materials (e.g., home-care products, personal-care perfumes or fine fragrances) [23]. Nevertheless, perfumes are high economic revenue products (approximately 95% profit) and so very prone to counterfeit, which may lead to the introduction in the market of low-quality products that may even pose serious health safety problems to unaware or less informed consumers. Indeed, due to the nature of perfumes use (i.e., leave-on cosmetics) there is a high potential of human exposure, which requires the correctness of the ingredient labelling to avoid skin reaction or other adverse effects [23]. For example, the European legislation requires monitoring 27 volatile compounds (VOCs) used in perfumery as they might elicit skin sensitization, i.e., the so-called potentially allergenic fragrance-related substances (PAS) or fragrance allergens, musks (despite their pleasant aroma they are considered persistent pollutants) and phthalates (used as denaturants, fixatives or solvents for some fragrances and as film formers, have been proven to be harmful to living organisms), which use is legally restricted or forbidden for cosmetic purposes [24,10,25] .

To accomplish these key tasks, among others, gas chromatography (GC) in combination with mass spectrometry (GC-MS) together with suitable extraction or thermal desorption methodologies are used [8]. Since GC-MS does not provide qualitative information about sensory perception of the aroma molecules [8].

usually a GC-Olfactometry (GCO) or GC-sniffing technique are needed, allowing improving the performance of GC-MS systems in terms of odor analysis [26]. Since, odors are detectable by the human nose at very low concentrations (low ppt), to minimize the risk of cases of odour detected by the nose without a spectral signal, the use of Time of Flight-Mass Spectrometer (ToFMS) to detect molecular traces may be envisaged. Linking the molecular information provided by GC-ToFMS with the perceived intensity and odour description by the sniffing technique, may allow detailed understanding of the key odour impact molecules present in the perfume.

Perfumes were also analyzed by the HPLC, For the determination of the concentrations of 25 fragrance allergens in perfumes products containing fragrances are widely used and are in direct contact with human skin, because Certain ingredients present in perfumes may contain compounds that are responsible for cosmetic-related allergic contact dermatitis(allergens),and legal restrictions imposed by the European Commission Scientific Committee on Cosmetics and Other Non-food Products (SCCNFP) limit the use of 24 fragrance agents suspected of producing cutaneous contact Allergy [27]. The analyzes were made to assess the risk for dermal exposure based on a "worst-case scenario" related to 107 perfumes, to examine the human health risk of skin exposure to a fragrance present in consumer products on the Korean market [28, 29; 30]. In conclusion, according to the results obtained from HPLC perfume analysis, the ingredients of the perfumes evaluated were shown to pose no apparent significant health risk at the maximum concentration used, except for lilial, HICC, citral, isoeugenol, and methyl2-octynoate. This risk assessment approach is recommended to be used to establish improved guidelines for specific ingredients in consumer products, and for setting limits for newly developed raw ingredients that might pose potential dermal hazards [31, 32, and 33].

Other analytical techniques like E-noses, voltammetry, E-tongues and UV/Vis spectrophotometry, which are recognized as more cost-effective, fast and use-friendly, have also been applied, although the latter two in a less extent, for perfume analysis.

I.2.1. UV/Vis Spectrophotometry

The UV/Vis spectrophotometer used in this work was a dual-beam apparatus, which is with the double-beam UV-Vis spectrophotometer, you can measure the both groups simultaneously. So that the double beam UV-Vis spectrophotometer is more accurate, because you don't need to recalibrate the instrument, before you measure the second sample [34]**.**

Double Beam: There are two light sources, two cuvettes and two detectors. It has not only the excellent performance, but also the price is economic, which is a cost-effective instrument for most of the industries and research organizations [35].

The following studies are examples of perfume analysis using UV/Vis spectrophotometry technic :The UV spectrophotometry is a promising analytical tool to be used as alternative to other instrumental methods already available in research laboratories, since this technique has been used with efficiency to classify, identify and distinguish original products from fake copies

[36,37]. The traditional treatment of data did not lead to a conclusive evaluation, due to the complexity of qualitative interpretation of UV spectra, but with the statistical chemometric techniques it was possible to draw interesting conclusions [35,38].

This study focuses on perfumes classification, by highlighting the use of UV spectrophotometry as a rapid and low-cost technique, using the applicability of statistical chemometric techniques such as PCA, SIMCA and LDA.

I.2.2. GC Olfactometry

A breakthrough in aroma research was achieved with by combining olfactometry and gas chromatography (GC-O), a new technique that associates the resolution power of capillary GC with the selectivity and sensitivity of the human nose [39]. As alternatives to the GC-O technique, two types of equipment based on electronic sensors are increasingly being employed. The first performs an aroma analysis (volatile compounds) of the gas phase, without separating the aroma into individual components. The second allows is to determine components with low and medium volatility, which are dissolved in a liquid phase. Both types of equipment consist of arrays of non-selective gas or liquid sensors and, coupled with appropriate pattern recognition tools, can identify simple or complex aroma or taste profiles [40]. These devices are usually quick-acting, easy to operate and, in some cases do not require any complex sample pre-treatment [41]. This combination (instrumental and olfaction) allows the method to be treated as artificial olfaction. Otherwise, there is many factors which can influence the correct detection and assessment of odor compounds when GC-O is used, namely the extraction procedure, the method of data collection and separation capability of the GC column [42].The GC-O technique uses the human nose as a detection device, generally, thus permits rapid identification of so-called odorant zones in a chromatogram. An analysis using the human sense of smell is carried out by trained technicians or a group of evaluating persons (panelists) who in the course of the assay, sniff the eluate from the column and relate the aromatic impressions to the retention times. A critical comparison between GC-O methodologies may be found in the literature [43]. Despite the commonly use of GC-O, further researches still being conducted in order to improve its capability, to achieve a higher sensitivity and better repeatability of the results. Simultaneous detection is achieved by splitting the eluent stream at an appropriate ratio, so, that it reaches both detectors. The most favorable is the simultaneous use of an olfactometric detector and mass spectrometer. Such an approach allows for both the description of odors and an identification of the compounds responsible for them, followed by a determination of which of them is characterized by the most intense aroma.

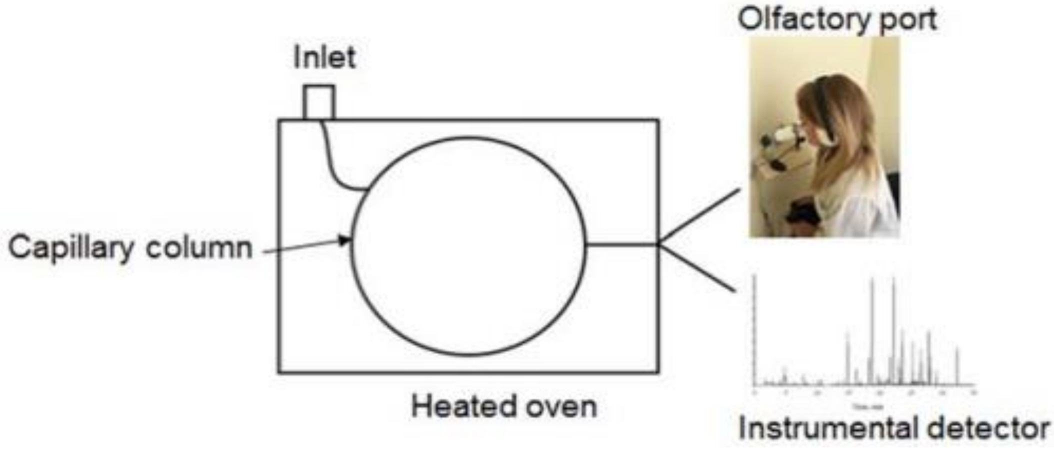


Figure 2: Schematic of GC-O analysis [44]

I.2.3. E-nose

The e-nose has many synonyms: artificial nose, mechanical nose, odour sensor, flavour sensor, aroma sensor, odor-sensing system, multi-sensor array technology and electronic olfactometry, and Depending on the type of analytes, electronic nose (e-nose) and electronic tongue (e-tongue) instruments are usually used [45]. Additionally, the e-nose is an instrument that aims mimicking the sense of smell. Traditionally, the human nose is used to evaluate quality parameters of different food matrices as well as of perfumes. However, this suffers from several drawbacks. For example, discrepancies can occur due to human fatigue or stress and clearly cannot be used for online measurements [46]. Thus the development of alternative methods is highly desirable the Electronic noses (E-noses) have been proposed for perfume analysis namely for discriminating original brand perfumes or recognizing fake counterparts [47, 48]; for identifying simple odors [49, 1]; for recognition of unknown fragrance mixtures [12, 13]; as quality control method of musk samples [14]; or for generating analyte-specific fingerprints of odorants [15]. Despite the satisfactory results reported so far, the identification of more than three perfumes remains difficult for the human nose and for E-nose devices with multiple sensors [13]. To overcome this problem, complex hybrid multiple statistical classifier methodologies have been proposed [13].

I.2.4. *E-tongue*

The electronic tongue presents a novel, smart sensing system developed for the analysis of liquids and it is controlled by a computer which is an instrument that measures and may compare different tastes. there is a Chemical compound responsible for different tastes which are detected by human taste receptors, and the chemical sensors comprised on the e-tongue may also detect the same dissolved organic and inorganic compounds. Like human receptors, each sensor has a spectrum of reactions different from the other. The information acquired by each sensor is complementary and the combination of all sensors' profiles generates a unique fingerprint of a liquid matrix. Most of the detection thresholds of sensors are similar to or better than those of human receptors. In the biological mechanism, taste signals are transduced by nerves in the brain into electric signals and the E-tongue present a similar sensors process: they generate electric signals as potentiometric variations. Taste quality perception and recognition is based on building or recognition of activated sensory nerve patterns by the brain and on the taste fingerprint of the product. Despite the great advances of the (bio)electrochemical technologies, sensor arrays/E-tongue and aptasensor devices that are recognized as promising tools for medical and pharmaceutical applications, there are still relevant challenges in the design and applications of electrochemical sensors in order to meet the demands of modern health care. Indeed, there is an urgent need to take advantage of the unique capabilities of these sensors, such as low-cost, miniaturization, portability, and short response times minimum, or no sample pretreatment, and wide applicability, already demonstrated at research level, in real-world applications.

Multisensory chemical arrays and aptasensors, are generally electrochemical-based sensor devices, such as; potentiometric, voltammetry, and impedance spectroscopy.

Electronic tongues based on voltammetry (VET) have been intensively investigated in recent years due to their high sensitivity and high signal-to-noise ratio.

A setoff potential pulses is applied to different metallic electrodes and the resulting current is sampled; the current data are then processed using multivariate analysis tools. Analyses of food stuff [16], water quality [49], wines [18] and urine for disease detection [6] and sensing of explosive material [66] are some of the applications of this type of electronic tongue.

E-tongue based on potentiometric sensors they generate electric signals as potentiometric variations, Taste quality perception and recognition is based on building or recognition of activated sensory nerve patterns by the brain and on the taste fingerprint of the product. This step is achieved by the e-tongue's statistical software which interprets the sensor data into taste patterns. in this case, Liquid samples are directly analyzed without any preparation.in fact, in this work the analysis of perfume was done with this type of electronic tongue.

In this work, it will be analyzed, for the first time, perfumes with a potentiometric electronic tongue [50], which has already been successfully used to analyze foods, like honey, milk, olive oil mineral waters and soft drinks. The use of the Electronic tongue can help to classify perfume according the aroma. The aroma pattern will depend on the composition of the liquid phase and on the diffusion properties of their volatile components, a novel smart electronic tongue classifier will be developed for recognizing the type of perfume and follow the maturation process, aiming establishing perfumes' olfactory-gustatory unique fingerprints through chemo metric tools

II. Materials and methods

II.1. Samples

Perfume samples were supplied by NORTEMPRESA Perfume Lab in (Braga, Portugal). In total, 33 independent samples were collected, being 18 women perfumes and the other 15 men perfumes, which main details are given in **Table 2**. According to the label information and based on the olfactory pyramid data perfumes were grouped into 7 different main aroma/olfactory families. Women perfumes were classified as Floral (5 perfumes), Floral-Fruity (5 perfumes), Floral-Oriental (5 perfumes) and Floral-Woody (3 perfumes), Men perfumes were grouped into 4 aroma families, being one of them common to the women perfumes, namely Citric-Aromatic (3 perfumes), Floral-Woody (4 perfumes), Woody-Oriental (4 perfumes) and Woody-Spicy (4 perfumes). The perfumes were from different production lots and had different storage time-periods (ranging from 6 to more than 24 months), being grouped into 3 main classes: 6 to 9 months, 9 to 24 months and more than 24 months. According to the label information and data from the perfume company, all perfume samples contained denatured alcohol (a mixture of ethanol with a denaturing agent) that has antimicrobial, masking and viscosity controlling functions; parfum, meaning an undisclosed mixture of several scent chemicals and ingredients used as fragrances); aqua (i.e., water); and, propylene glycol, an organic alcohol used as a skin conditioning agent, fragrance and humectant, allowing controlling the final viscosity of the perfume. Besides, the samples could contain a mixture of other ingredients, in different proportions, which could include fragrance additive and masking ingredients (e.g., hydroxyisohexyl 3-cyclohexene carboxaldehyde that has a delicate sweet, light, floral aroma; Evernia prunastri that is an extract of the oakmoss; benzyl salicylate that is a salicylic acid benzyl ester; among others) and scents (e.g., limonene that has a fresh and sweet citrus aroma; coumarin that is an aromatic organic chemical compound, used as a sweet, vanilla, nutty scent; geraniol, a monoterpenoid and alcohol, which is a natural scent ingredient; butylphenyl methylpropional, an aromatic aldehyde, which is a synthetic fragrance with a strong floral scent; among others).

Table 2. Perfume samples details (label information: sample code, type, olfactory pyramid notes, aroma family classes; and, storage time-period classes)

Sample code	Type	Aroma family class	Storage time-period class
100001	Woman	Floral-Fruity	6-9 months
100005	Woman	Floral	> 24 months
100006	Woman	Floral-Fruity	9-24 months
100012	Woman	Floral-Woody	> 24 months
100014	Woman	Floral-Fruity	9-24 months
100015	Woman	Floral-Oriental	> 24 months
100016	Woman	Floral-Oriental	> 24 months
100017	Woman	Floral-Woody	9-24 months
100018	Woman	Floral	6-9 months
100019	Woman	Floral-Oriental	9-24 months
100020	Woman	Floral-Woody	> 24 months
100023	Woman	Floral	> 24 months
100029	Woman	Floral-Fruity	6-9 months
100031	Woman	Floral-Oriental	6-9 months
100032	Woman	Floral-Fruity	6-9 months
100033	Woman	Floral	6-9 months
100034	Woman	Floral	24 months
100040	Woman	Floral-Oriental	6-9 months
200201	Man	Woody-Spicy	6-9 months
200204	Man	Citric-Aromatic	6-9 months
200206	Man	Woody-Oriental	6-9 months
200208	Man	Floral-Woody	> 24 months
200209	Man	Woody-Oriental	9-24 months
200210	Man	Woody-Spicy	9-24 months
200216	Man	Woody-Oriental	> 24 months
200217	Man	Woody-Oriental	> 24 months
200218	Man	Floral-Woody	> 24 months
200219	Man	Citric-Aromatic	> 24 months
200221	Man	Woody-Spicy	> 24 months
200222	Man	Woody-Spicy	9-24 months

200223	Man	Floral-Woody	> 24 months
200226	Man	Floral-Woody	9-24 months
200227	Man	Citric-Aromatic	> 24 months

Table 2 continued.

II.2. UV-Vis analysis

UV-Vis spectrophotometry was applied to acquire a preliminary insight of each perfume composition, following the experimental methodology described by Gomes and co-authors [51], with some adaptations. Perfume samples were firstly diluted in the proportion of 1:4000, withdrawing 2.5 μL of perfume, measured using a Gilson micropipette (0.4-10 μL), to a 10 mL glass volumetric flask, which was filled with absolute ethanol (+99%, Extra Pure, SLR, Fisher Chemical®). Each perfume-ethanol mixture was agitated, placed into a quartz cuvette (with 1 cm of path length) and then, the UV spectra (200-1100 nm, at intervals of 5 nm) was recorded, using a SPECORD®200 spectrophotometer (Analytik Jena®) (Figure 3) and treated using the WinASPECT® software. Absorption was detected in a near UV wavelength interval (200–350 nm).

Figure 3. SPECORD®200 spectrophotometer used in this work

II.3. Potentiometric E-tongue

II.3.1. E-tongue device and set-up

A lab-made E-tongue like that previously used by the research team for food analysis [51], was designed and built specially for the perfume analysis considering the need to minimize the perfume volume needed for each electrochemical assay. The new device included two potentiometric arrays built in an acrylic cylinder body with height of 6.5 cm, diameter of 1.5

cm; wells of 0.5 cm of width and 1 mm of depth with support of Araldite epoxy resin and graphite in the proportion of 50% **(Figure 4).** Each array one had the same 20 sensors (lipid polymeric membranes) obtained from the combination of 4 lipid additives (octadecylamine, oleyl alcohol, methyltrioctylammonium chloride and oleic acid; ≈3%); 5 plasticizers (2-nitrophenyl-octylether, tris(2-ethylhexyl) phosphate, bis (1-butylpentyl) adipate, dibutylsebacate, and Bis(2-ethylhexyl)phthalate; ≈65%) and high molecular weight polyvinyl chloride (PVC; ≈32%) [52].

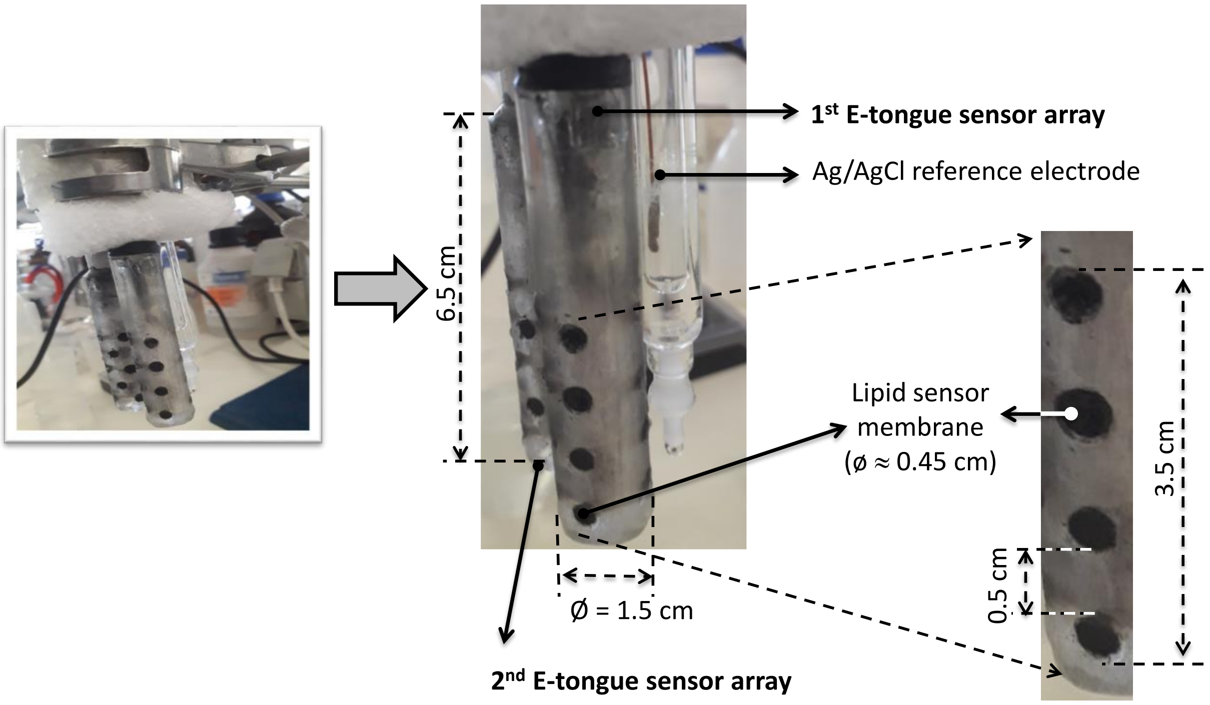


Figure 4.Potentiometric E-tongue device used in this work: geometry and basic dimensions of the array and lipid membranes.

Each sensor was identified with a letter S (for sensor) followed by a code for the number of the sensor array (1: or 2:) and the number of the membrane (1–20, corresponding to different combinations of plasticizer and additive used) [52]. For example, the first 4 sensors follow the order of the 4 additives as a function of the first plasticizer presented (2-nitrophenyl-octylether) and the order of the four additives being subsequently maintained for the presented sequence of the remaining plasticizers. The sensor membranes were linked to a multiplexer Agilent Data Acquisition Switch Unit (model 34970A), which was controlled by an Agilent BenchLink Data Logger software (Figure 5). Each perfume analysis (each experimental assay) took 5 min, which allowed signals' stabilization, being recorded the potentiometric signals of the 40 sensor membranes, generated by the establishment of electrostatic and/or hydrophobic interactions [53]. An Ag/AgCl double-junction glass electrode (Crison, 5241) was used as the reference electrode.

The E-tongue was stored in a HCl solution (0.01 M) that was also used to evaluate the signals intra-day stability or the occurrence of signal drifts.

Figure 5.The equipment of the Electronic Tongue used in this work

II.3.2. E-tongue perfume analysis: sample preparation and potentiometric assays

The E-tongue comprised lipid polymeric membranes used as sensor units and since solutions with high alcoholic levels may degraded them, the perfume samples were diluted with deionized water in order to obtain an 80:20 (v/v) water-perfume solution. This proportion was selected based on the previous experience of the research team, which observed a satisfactory E-tongue performance when used to analyze water-ethanol solutions (80:20, v/v) [53]. So, from each perfume, 8 mL were withdrawn and diluted in 32 mL of deionized water, allowing to obtain a total sample volume of 40 mL, enough to completely immerse the two cylindrical E-tongue arrays, allowing the contact of the sensor membranes with the aqueous perfume solution. The solution system was then agitated during 2 min, after which the potentiometric assays were performed in duplicate for each sample, with a third assay carried out if the recorded signals of any of the 40 sensors showed a coefficient of variation for the inter-assays greater than 20%. Besides, for evaluating the sensors' intra-day signal stability (i.e., signal stability over-time, for a typical daily analysis time-period), E-tongue potentiometric profiles of solutions of HCl (0.1

M) were recorded ten times in the same day, being the assays carried out over an 8-h time-period, within the usual perfume samples set of assays. The intra- and inter-day signal repeatability was further checked using selected perfume samples (sample codes: 100001, 100019, 100020, 100023, for woman perfumes and 200204, 200206, 200210 and 200226 for man perfumes; Table 2) one from each different olfactory family studied (men's perfumes: citric aromatic, woody oriental, floral woody, woody spicy; women's perfumes: floral, floral fruity, floral woody, floral oriental). So, each sample was analyzed five times in each day (8h period) during three consecutive days. A satisfactory overtime signal stability (i.e., negligible signal drift) would correspond to a coefficient of variation (%CV) lower than 5% for a 5 min period and an intra- and inter-day repeatability lower than 10 and 15%, respectively [54].

II.4. Statistical analysis

The potentiometric E-tongue data collected was statistically analyzed (at a 5% significance level) using the statistics program R version 3.2.0 (the R Foundation for Statistical Computing, Vienna, Austria), a free software environment for statistical computing and graphics [54]. The R statistical packages Sub select [7,55], ggplot2 [5] and MASS [56] were used.

The work aimed to establish models for perfume samples discrimination using the potentiometric signals, by using linear discriminant analysis (LDA) coupled with the meta-heuristic simulated annealing (SA) variable selection algorithm. This approach was used to evaluate the capability of the potentiometric E-tongue to:

- differentiate men from women perfumes
- classify perfumes according to the main olfactory family;
- semi-quantitatively determine the storage time-period.

The potentiometric signals were centered and scaled (autoscaling) to minimize the possible effects of magnitude differences in signal strength (by subtracting the mean and dividing by the standard deviation of the variable, resulting in a variable with mean of 0 and a standard deviation of 1). The E-tongue-LDA-SA best models were established based on the best sub-set of sensors selected between the 40 potentiometric auto scaled signals, by the SA algorithm, which allowed minimizing noise effects due to the inclusion of redundant variables (sensors' signals). The model's predictive performance was verified using the leave-one-out cross-validation (LOO-CV) technique. In this cross-validation variant the number of models established equals the number of samples in the dataset, being in each try-out run one sample used as the test group (for model performance assessment) and remaining samples included in the training group and used to establish the multivariate model [57]. The overall performance of each LDA model established was assessed based on the sensitivity values (percentage of correct classifications) and visualized using 2D plots of the main discriminant functions, being the class membership boundary ellipses determined based on the posterior probabilities computed using the Bayes' theorem (which enables controlling over-fitting issues) [57].

Linear discriminant analysis (LDA) this technique is based on recognizing supervised patterns. Its approach to classification to maximize the variance between categories and to minimize the variance in the categories, generating a series of orthogonal linear discriminants in functions equal to the number of categories minus one.

III. Results and discussion

III.1. UV-Vis spectra of perfume samples

The possibility of using UV spectrophotometry in combination with chemometric techniques for perfume classification was described [58]. In the present work, it was observed that the diluted perfume-ethanol samples showed a significant absorption in the range of 200-350 nm, corresponding to the near-UV region. **Figure 6** shows examples of the absorption spectra recorded for each olfactory family of men or women perfumes studied (**Figure 6A-B**, respectively) as well as the UV spectra trend with the storage time-period for Woody-Spicy men perfumes and Floral-Oriental women perfumes (**Figure 6C-D**, respectively).

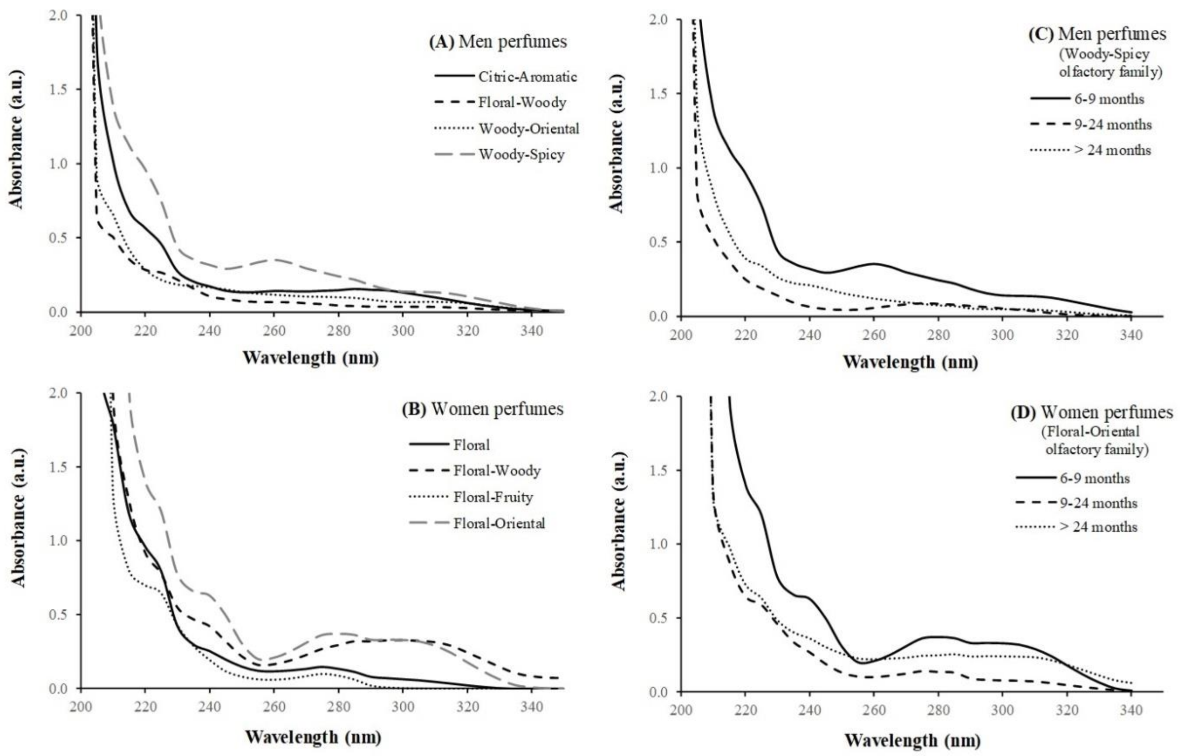


Figure 6.UV spectra of diluted perfume samples with ethanol (1:4000 v/v) in the absorption region from 200-350 nm.

(A) Olfactory families of men perfumes: Citric-Aromatic (sample #200204), Floral-Woody (sample #200226), Woody-Oriental (sample #200206) and Woody-Spicy (sample #200201); (B) Olfactory families of women perfumes: Floral (sample #100018), Floral-Woody (sample #100017), Floral-Fruity (sample #100001) and Floral-Oriental (sample #100031); (C) Storage time-periods of Woody-Spicy men perfumes: 6-9 months (sample #200201), 9-24 months (sample #200210) and > 24 months (sample #200221); (D) Storage time-periods of Floral-Oriental women perfumes: 6-9 months (sample #100015), 9-24 months (sample #100031) and > 24 months (sample # 100019).

In the recorded spectra several peaks (major and minor bands) can be found in the region of 210-340 nm that, as pointed out by Gomes et al· [59], may be due to the chemical diversity of chemical of the perfume fragrances, which include into terpenoids, musk's, aliphatic derivatives and aromatic derivatives, characterized by the presence of unsaturated conjugated or unconjugated carbon-carbon and/or the presence of carbonyl groups [59,60]. It should also be remarked that, globally, the perfume bands observed agree with those found by Gomes et al. [59] for perfumes as well as for individual ethanolic standard solution of scents (e.g., limonene, linalool, citral, eugenol, coumarin, eugenol, isoeugenol and cinnamic derivatives). This similarity could be attributed to the fact the perfumes evaluated in both studies several equal scents in their composition, namely, limonene, linalool, citral, coumarin, eugenol, isoeugenol, cinnamyl alcohol and cinnamal. It should also be noticed that the observed spectra confirmed the presence of polar compound families with which electrostatic and hydrophobic interactions could be established by the polymeric lipid sensor membranes comprised on the lab-made potentiometric E-tongue, as also pointed out for lipid bilayer membranes of synthetic sensing systems previously used to discriminate odorants [61]. Finally, the UV absorption spectra recorded changed with the perfume's olfactory family and, even for the same olfactory family (e.g., Floral-Woody men and women perfumes) different absorption spectra were obtained (**Figure 6A-B**). Indeed, it should be kept in mind that, perfumes may be classified as belonging to the same olfactory family, although having different top, heart and base olfactory notes due to the different composition in fragrances and scents. In fact, as previously stated, a perfume is a complex matrix that may comprise from 10 to 100 individual ingredients [62]. Finally, different UV absorption spectra could be observed for different storage time-periods of perfumes belonging to the olfactory family (**Figure 6 C-D)**, being the main differences found between perfumes with less than 9 months of storage compared to those with more than 9 months of storage, showing that the perfumes, although kept in adequate storage conditions, their composition slightly change with time.

III.2. E-tongue signal stability over time and perfume samples' signal profiles

Potentiometric sensor devices may exhibit signal drifts, which can be minimized or overcome when daily calibrations are carried out or if signal standardization statistical treatments are applied. In which concerns potentiometric E-tongues, comprising lipid polymeric membranes (both print-screen and cylindrical arrays geometries) it was previously observed that intra-day signals were quite stable showing negligible drifts (with coefficients of variation lower than 5%) [6,9,22, 63,64,65]. To further checked the reported stability of this kind of E-tongue, comprising similar sensors, HCl (0.1 M) solutions were randomly analysed (10 times), during the perfumes' assays, within the usual 8-h time-period of analysis. The results pointed out **(Figure 7)** that with the new device, the intra-day signal coefficients of variation (%CV) varied, in general, from 1.3 to 5.7%, showing the overall satisfactory signal stability over-time.

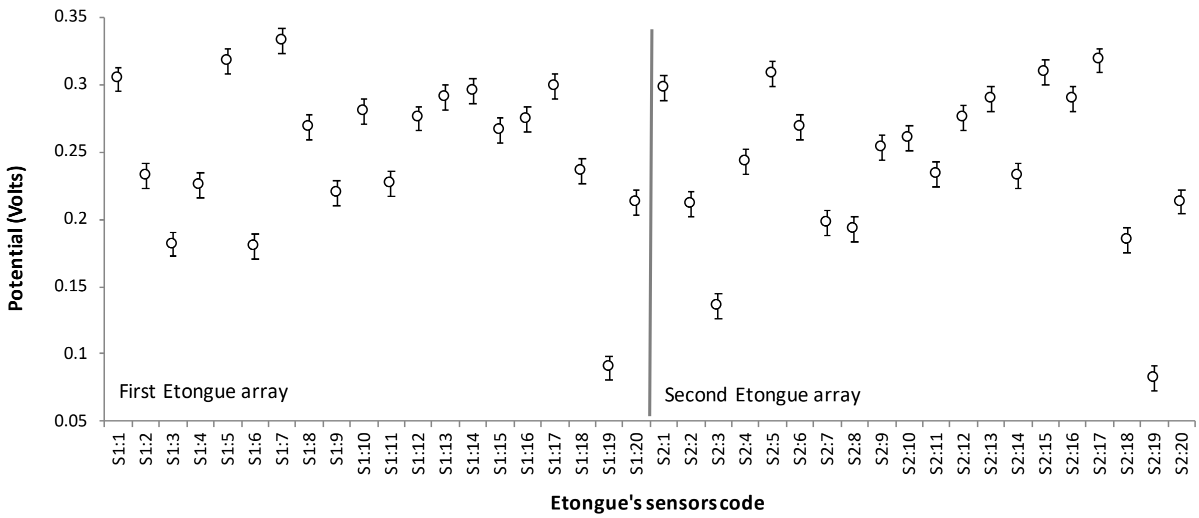


Figure 7.E-tongue potentiometric signals intra-day repeatability (10 assays in the same day) during the analysis of a standard aqueous solution of HCl (0.1 M).

Regarding the inter-day signals, it was expected an higher variability in the sensors' response due to the usual signal drifts between the 3 days analysis. This variability was confirmed by the potential range, which varied from +12 to +340 mV, and the %CV, which varied between 17 and 78%. Due to these results, the experimental work in perfume analysis was carried out in intra-day analysis, avoiding inter-day analysis. However, this not implies that it is not possible to do experimental work in inter-days assays, since it would require to carry out daily E-tongue calibrations in order to reduce or eliminate significant inter-day signal drifts. As can be seen in Figure 8, the E-tongue potentiometric profiles recorded by the 40 lipid sensor membranes (1st sensor array: S1:1 to S1:20; 2nd sensor array: S2:1 to S2:20), showed also slightly differences (regarding signal intensity/signal dynamic range). These differences obtained in the intra- and inter-day assays are not relevant considering that the E-tongue application is related to the use of samples signal profile instead of a singular sensor response.

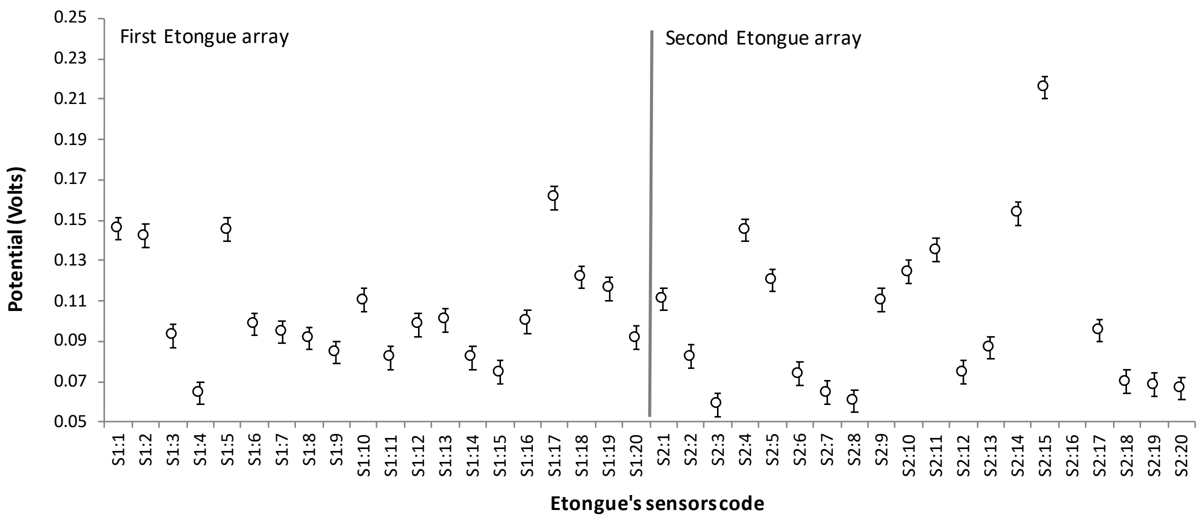


Figure 8. E-tongue potentiometric signals inter-day repeatability (assays in the 3 days) during the analysis of a standard aqueous solution of HCl (0.1 M).

III.3. E-tongue classification performance

The performance of the proposed potentiometric E-tongue, comprising non-specific and cross-sensitive sensors, for simultaneously classifying, based on a single-run assay, the perfume type (men or women), perfume main aroma/olfactory family and perfume storage time-period was evaluated for the first time. This type of sensor device has been reported as a powerful taste sensor device for assessing different positive and negative sensory attributes of foods [66 ,7. Moreover, the use of a multisensory arrays, with the above-mentioned characteristics may allow gathering the unique fingerprint of a perfume and so, overcoming the known limitation of applying a single sensor, which results in and unspecific response towards the complex perfume composition (10 to 100 individual ingredients [67]) that can deliver exactly the same potentiometric signal for different chemical compounds in solution, which are related to the specific aroma/olfactory perfume notes [3,68].

III.3.1. Discrimination of men and women perfumes

Although men and women perfumes may be differentiated according to the olfactory notes. For the perfume industry it is important to have an analytical technique that could be implemented (on-line and *in-situ*) for monitoring the production line, allowing a fast and easy discrimination of men from women perfumes. So, the E-tongue performance was evaluated keeping in mind this objective. An E-tongue-LDA-SA model was established based on the potentiometric data of 12 sensors (1st arrayS1:3, S1:4; S1:7, S1:14 and S1:20; 2nd array: S2:2, S2:4, S2:6, S2:9, S2:12, S2:13 and S2:17), enabling to correctly differentiate men from women perfumes, with sensitivities of 100% for both original grouped data (**Figure 9**) and LOO-CV internal-validation procedure [4].

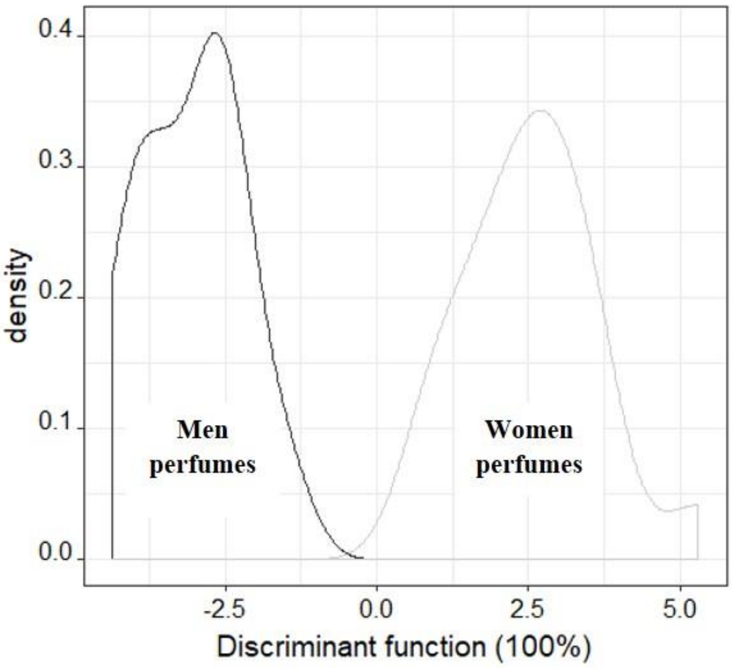


Figure 9. Density distribution (one-dimension plot) for the discriminant function of the E-tongue-LDA-SA classification model based on 12 selected sensors' signals (1st array: S1:3, S1:4; S1:7, S1:14 and S1:20; 2nd array: S2:2, S2:4, S2:6, S2:9, S2:12, S2:13 and S2:17) established for discriminating men and women perfumes, regardless the perfume's olfactory family or storage time-period.

All samples were correctly classified, pointing out the versatility and powerful of the classifier potentiometric device for discriminating men and women perfumes, comprising a total of 7 different olfactory families and have being stored during 6 to more than 24 months. The satisfactory results also strengthen the initial idea that E-tongues could be a practical tool for perfume analysis even if, at a first view it was expected to correlate a sensor-based device with the olfactory profile of a perfume sample.

III.3.2. Classification of perfumes according to the main aroma family

Men and women perfumes possess a complex composition, being a mixture of a multitude of ingredients, which include a basis of alcohol denatured, perfume, aqua and propylene glycol combined with a several other chemical compounds (e.g., fragrances and scents). Depending of the different top, heart and base olfactory notes (olfactory pyramid), each perfume may be commercially classified according to the main aroma/olfactory family (**Table 2**). Thus, in this study it was evaluated the E-tongue performance for classifying perfumes taking into account the main olfactory family, independently of the perfume type (men or women) or the perfume's storage time-period (*i.e.*, perfume's age), using a LDA-SA chemometric approach. The 33 perfumes were grouped into 7 different olfactory families (**Table 2**) including, Citric-Aromatic (3 men perfumes with 6-9 months or more than 24 months of storage), Floral (5 women perfumes with 6-9 months or more than 24 months of storage), Floral-Fruity (5 women perfumes with 6-9 months or 9-24 months of storage), Floral-Oriental (5 women perfumes with 6-9 months, 9-24 months or more than 24 months of storage), Floral-Woody (4 men and 3 women perfumes with 9-24 months or more than 24 months of storage), Woody-Oriental (4 men perfumes with 6-9 months, 9-24 months or more than 24 months of storage) and Woody-Spicy (4 men perfumes with 6-9 months, 9-24 months or more than 24 months of storage). For this purpose, a classification E-tongue-LDA-SA model, which 2 first discriminant functions accounted for 99.97% of the total variance, was developed based on the potentiometric signals gathered by 18 selected sensors (1st array: S1:1, S1:2, S1:4, S2:6, S1:7, S1:8, S1:11, S1:12 and S1:16; 2nd array: S2:1, S2:2, S2:11 and S2:13 to S2:18). The model allowed obtaining sensitivities (i.e., percentage of correct classifications) of 100% and 94% for the original grouped data (**Figure 9**) and for the LOO-CV internal-validation procedure, respectively. An overall satisfactory predictive performance was achieved, being the olfactory family of only 2 of the 33 perfumes incorrectly assessed.

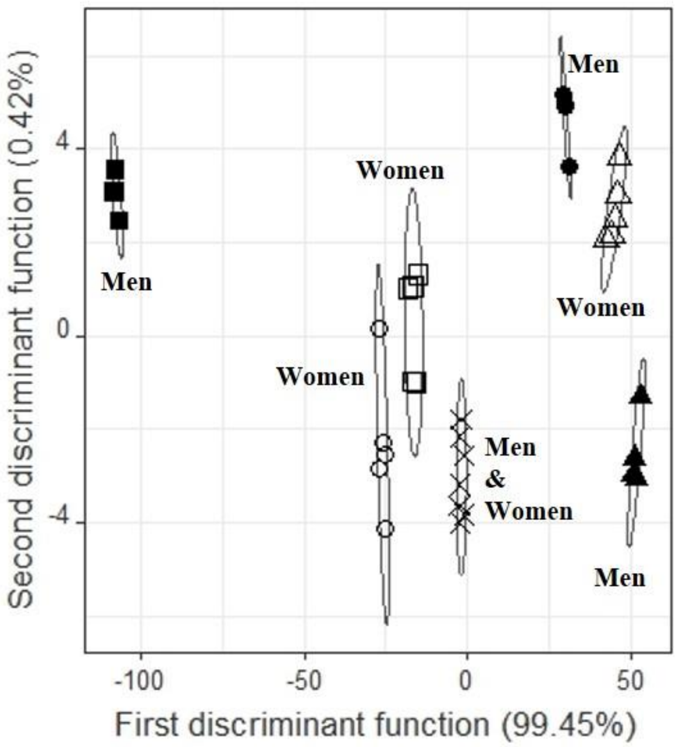


Figure 10.Perfumes' discrimination (2D plot of the first 2 discriminant functions and respective class membership boundary ellipses) according to the main aroma/olfactory family (■ Citric-Aromatic, ○ Floral, ∆ Floral-Fruity, □ Floral-Oriental, × Floral-Woody, ∆ Woody-Oriental and ▲ Woody-Spicy; being fill symbols used for men fragrances, open symbols for women fragrances and other symbols for men & women fragrances), regardless the perfume type (men or women) and the storage time-period.

It should be noticed that, if the perfumes were split by men or women type, 100% of correct predictive classifications could be obtained (LOO-CV procedure) using LDA models based on the signal profiles of 8 and 9 E-tongue sensors, respectively, selected by the SA algorithm **(Figure 10).**

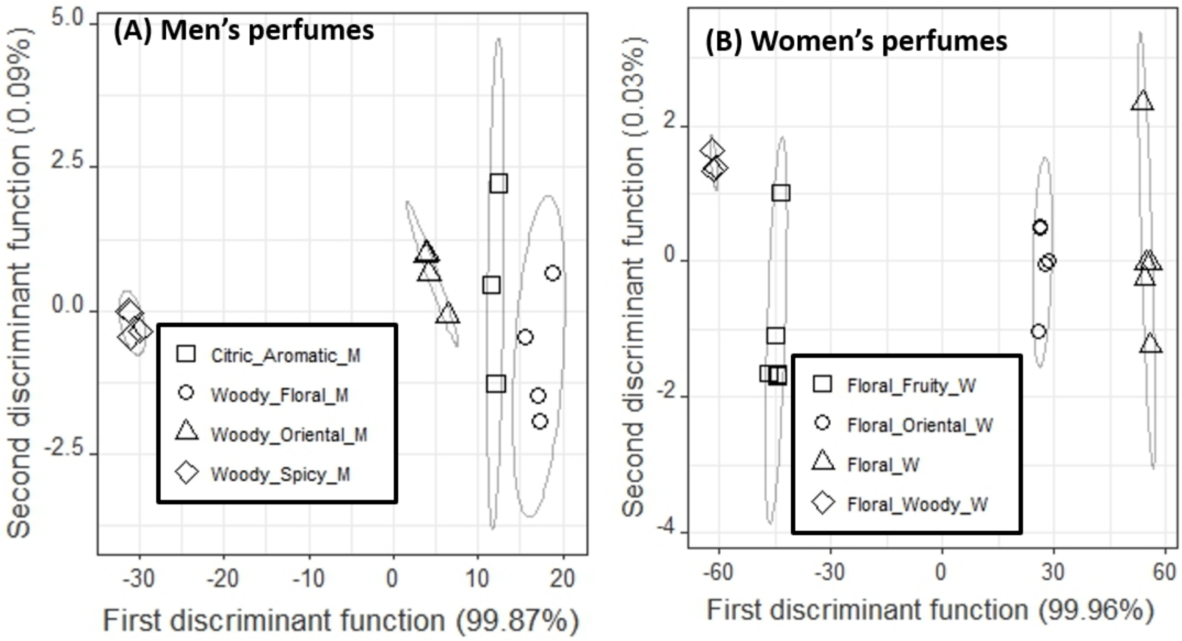


Figure 11.Perfumes' discrimination (2D plot of the first 2 discriminant functions and respective class membership boundary ellipses) according to the main aroma/olfactory family for men's' (A) or women's perfumes (B).

The overall correct classification rates achieved with the lab-made potentiometric E-tongue are of the same order of magnitude as those reported in the literature using E-nose devices coupled with different chemometric techniques (which predictive sensitivities ranged from 71-98% when classifying different perfume classes or discriminating them by brand) [69,70] or ev0en with a voltammetric E-tongue [71,72]. Furthermore, compared to the reported performances achieved with E-nose These results showed, for the first time, that a potentiometric E-tongue could be used as a classifier sensor device for perfume analysis, namely for identifying the main olfactory family. This is of utmost practical and economical relevance since this evaluation and classification requires the availability of trained sensory panellists, leading to an expensive and time-consuming task that may be beyond the economic possibilities of local small-medium perfume companies.

III.3.3 Assessment of the storage time-period of the perfume samples

For the perfume industry it is relevant to have a fast and user-friendly analytical tool for classifying perfumes considering the storage time-period (*i.e.*, the time after production until commercialization). This possibility is even of greater practical application if it could be used regardless the type of perfume (men or women) and the perfume's aroma/olfactory family. So, the E-tongue performance to assess the storage-time period (6 to 9 months; 9 to 24 months; and more than 24 months) was further evaluated [10,73]. An E-tongue-LDA-SA model, with two discriminant functions (accounting 98.36% and 1.64% of the total variability, respectively), was established based on the potentiometric signals recorded by a sub-set of 20 sensors selected by the SA algorithm (1st array: S1:3, S1:4, S2:6, S1:7, S1:9, S1:12, S1:14, S1:15, S1:19 and S1:20; 2nd array: S2:2 to S2:6, S2:9, S2:12, S2:14, S2:18 and S2:20). The multivariate linear classification model allowed the correct classification of the storage time-period of 100% of the original data samples (**Figure 12**) and of 97% of the samples for the LOO-CV internal validation procedure (being only one sample of the 9-24 months erroneously classified as being stored for more than 24 months).

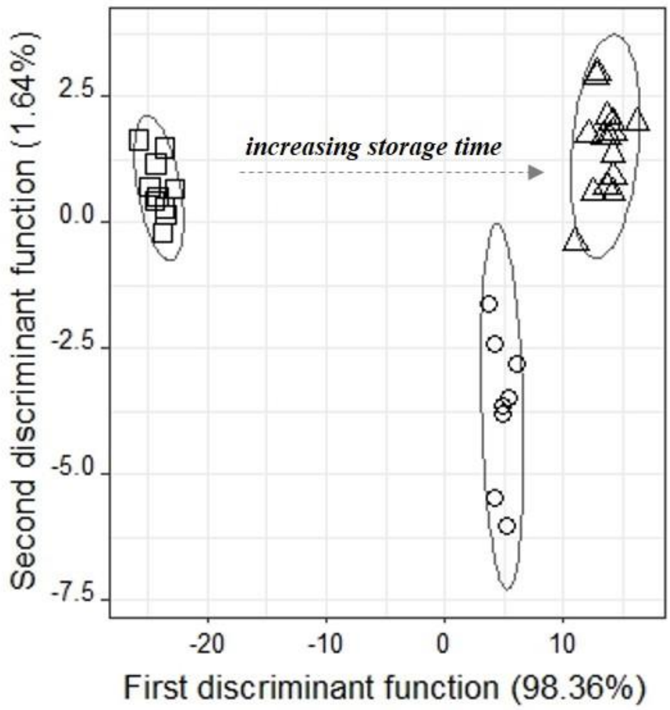


Figure 12. Perfumes' storage time-period (□ 6-9 months; ○ 9-24 months; ∆ >24 months) assessment (2D plot and respective class membership boundary ellipses) using an E-tongue-LDA-SA classification model based on the potentiometric signals of 20 selected lipid sensor membranes (1st array: S1:3, S1:4, S2:6, S1:7, S1:9, S1:12, S1:14, S1:15, S1:19 and S1:20; 2nd array: S2:2 to S2:6, S2:9, S2:12, S2:14, S2:18 and S2:20), regardless the type of perfume (men or women) and the aroma/olfactory family.

The predictive performance achieved was very satisfactory considering the variability of the perfumes included in each storage time-period (6-9 months: 3 men and 7 women perfumes from Citric-Aromatic, Floral, Floral-Fruity, Floral-Oriental, Woody-Oriental and Woody Spicy olfactory families; 9-24 months: 4 men and 4 women perfumes from Floral-Fruity, Floral-Oriental, Floral-Woody, Woody-Oriental and Woody-Spicy olfactory families; and, > 24 months: 8 men and 7 women perfumes from Citric-Aromatic, Floral, Floral-Oriental, Floral-

Woody, Woody-Oriental and Woody Spicy olfactory families). This fact clearly pointed out the versatility of the E-tongue-LDA-SA proposed approach, which has proven to be a powerful semi-quantitative classifier tool of perfume's age assessment. Furthermore, if the perfumes were split by men and women type, the correct predictive classification percentages (sensitivity values) would reach 100% (E-tongue-LDA-SA models based on the signal profiles of 7 selected sensors, (**Figure 13**), strengthen the above-mentioned powerful of the classifier potentiometric device.

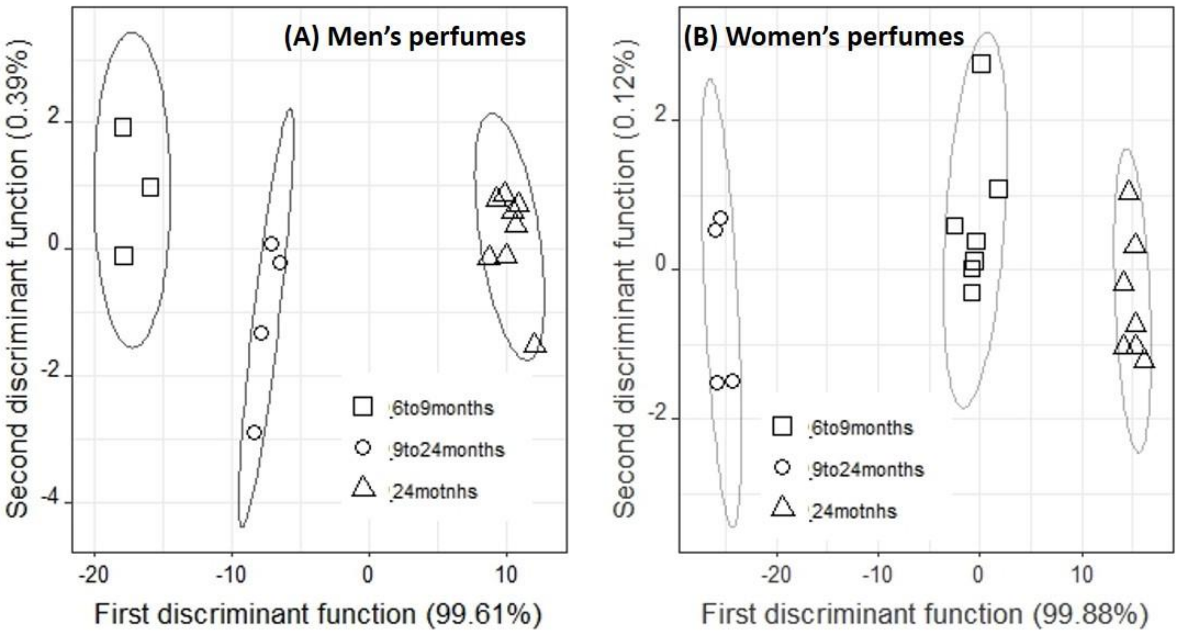


Figure 13.Perfumes' storage time-period (□ 6-9 months; ○ 9-24 months; ∆ >24 months) assessment (2D plot and respective class membership boundary ellipses) using an E-tongue-LDA-SA classification model based on the potentiometric signals for men's' (A) or women's perfumes (B).

IV. Conclusions and future work

The present study outlined, for the first time, the application of a E-tongue for perfume analysis, which allowed, in a single-run assay, to establish a unique perfume potentiometric fingerprint capable of discriminating men and women perfumes, for differentiating perfumes according to the main olfactory family and for semi-quantitatively assessing the storage time-period of perfumes. The work also highlighted the predictive satisfactory performance of a multisensory device, comprising non-specific lipid polymeric membranes, coupled with classification chemometric techniques and variable selection algorithm, showing that the proposed approach could be used by the perfume industrials as a practical, cost-effective and fast perfume classifier analytical technique as well as a complementary sensory preliminary tool, minimizing the need to recourse to trained/official perfume panelists. Thus, the study carried out may also contribute to enlarge the E-tongue field of application, mainly focused on the food and environmental analysis, to the perfume emerging and promising area. Perfume is a product of great economic importance in the cosmetics industry Because economic value of this sector, counterfeit products has emerged damaging to the economy of this product, by reducing tax revenues and affect the cosmetics industry sales [6,2]**.** Such products could also represent a risk to public health, due to low-quality raw materials and inappropriate concentrations [74,9], which can cause allergic reactions, especially on the skin, as dermatitis. The quality of the final product is very important because it may exhibit a short life of the expected smell [10]**.** Perfume development demands high specialized technicians for flavor and fragrance creation the so-called perfume-formulation process, which confers a high commercial value. So, it is important to have new analytical methodologies to asses commercial perfume quality, perfume-stability, longevity on the skin, detection of potentially allergenic fragrance-related substances, musks etc.In this context, it is pretended to verify the performance of a potentiometric electronic tongue to discriminate perfumes by typical aroma classes or to detect the presence of legally restricted or forbidden fragrances-related substances [11].

V. References

1. M.Cole, J.A.Covington, J.W. Gardner, Combined electronic nose and tongue for a flavour sensing system, Sens. Actuat. B: Chem. 156; (2011); 832-839.
2. P.Rattanawarinchai, P.Krongkrachang, T.Chodjarusawad, D.Phromyothin, Electrochemical sensor: Preparation technique based on electronic tongue in fragrance, Mater. Today: Proceed. 4; (2017); 6410-6414.
3. A.C.A. Veloso, L.G. Dias, N. Rodrigues, J.A. Pereira, A.M. Peres, Sensory intensity assessment of olive oils using an electronic tongue, Talanta 146; (2016); 585–593.
4. U. Harzalli, N. Rodrigues, A.C.A. Veloso, L.G. Dias, J.A. Pereira, S. Oueslati, A.M. Peres, A taste sensor device for unmasking admixing of rancid or winey-vinegary olive oil to extra virgin olive oil, Comput. Electron. Agr. 144; (2018); 222–231.
5. Y. Kobayashi, M. Habara, H. Ikezazki, R. Chen, Y. Naito, K. Toko, Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores, Sensors 10; (2010); 3411–3443.
6. T. Takeuchi, J. Montenegro, A. Hennig, M. Stefan, Pattern generation with synthetic sensing systems in lipid bilayer membranes, Chem. Sci. 2; (2011); 303-307.
7. C.L. Gomes, A.C.A. Lima, M.C.L. Cândido, A.B.R. Silva, A.R. Loiola, R.F. Nascimento, Multivariate analysis of perfumes by ultraviolet spectrophotometry, J. Braz. Chem Soc. 26; (2015); 1730-1736.
8. C. Villatoroa, L. Vera, H. Gygax, Comparative study of odours present in twin fragrances by GC-sniffing-To FMS, Chem. Eng. Trans. 54; (2016); 133-138.
9. A. van Asten, The importance of GC and GC-MS in perfume analysis, Trends Analyt. Chem. 21; (2002); 698-708.
10. A. Cisvert, M. López-Nogueroles, and A. Salvador, Essential Oils: Analytical Methods to Control the Quality of Perfumes, In K. Ramawat, J.M. Mérillon (Eds.), Natural Products. Springer, Berlin, Heidelberg; (2013); 3287-3310.
11. N. Nibbe, H. Gygax, B. Maxeiner, Odour Measurement for Improved Scent Performance in Consumer Goods, SOFW-J. 141; (2015); 42-46.
12. J. Gebicki, B. Szulczynski, M. Kaminski, Determination of authenticity of brand perfume using electronic nose prototypes, Meas. Sci. Technol. 26; (2015); 125103 (11pp).

13. M. Cano, V. Borrego, J. Roales, J. Idígoras, T. Lopes-Costa, P. Mendoza, J.M. Pedrosa, Rapid discrimination and counterfeit detection of perfumes by an electronic olfactory system, Sens. Actuat. B: Chem. 156; (2011); 319-324.
14. R. Singh, An intelligent system for odour discrimination, Proceedings - 1st IEEE International Workshop on Electronic Design, Test and Applications DELTA; (2002); 994681; 489-491.
15. T. Temel, B. Karlik, an Improved Odor Recognition System Using Learning Vector Quantization with a New Linear Discriminant Analysis, Neural Netw. World 17; (2007); 287-294.
16. W. Jatmiko, T. Fukuda, F. Arai, B. Kusumoputro, Artificial odor discrimination system using multiple qartz resonator sensors and various neural networks for recognizing fragrance mixtures, IEEE Sens. J. 6; (2006); 223-233.
17. E. Esme, B. Karlikb, Fuzzy c-means based support vector machines classifier for perfume recognition, Appl. Soft. Comput. J. 46; (2016); 452-458.
18. T. Ye, C. Jin, J. Zhou, X. Li, H. Wang, P. Deng, Y. Yang, Y. Wu, X. Xiao, Can odors of TCM be captured by electronic nose. The novel quality control method for musk by electronic nose coupled with chemo metrics, J. Pharm. Biomed. Anal. 55; (2011); 1239-1244.
19. M. Penza, G. Cassano, F. Tortorella, G. Zaccaria, Classification of food, beverages and perfumes by WO3 thin-film sensors array and pattern recognition techniques, Sens. Actuat. B: Chem. 73; (2001); 76-87.
20. W. Guo, F. Gan, H. Kong, J. Wu, Signal model of electronic noses with metal oxide semiconductor, Chemometr. Intell. Lab. Syst. 143; (2015); 130-135.
21. J. Poprawski, P. Boilot, F. Tetelin, Counterfeiting and quantification using an electronic nose in the perfumed cleaner industry, Sens. Actuat. B: Chem. 116; (2006); 156-160.
22. https://ifrafragrance.org/self-regulation/understanding-the-standards(accessed on 10 April 2019).
23. D. Fernandes, S. Gomes, A. Fontes, M. Costa, B. Almeida, E. Araújo, U. Galvão, H. Véras, J. Braz. Chem. Soc. 25; (2014); 169.
24. A. Chisvert, A. Salvador Perfumes in cosmetics. Regulatory aspects and analytical methods for fragrance ingredients and other related chemicals in cosmetics. In: Salvador A, Chisvert A (eds) Analysis of cosmetic products. Elsevier, Amsterdam, 2007.

25. http://www.osmoz.com/encyclopedia/olfactory groups?fbclid=IwAR1HMsjpVetKNtSRG5z3vllyEGV4AW0X8mklcbdKxoG3x-BHFzYnlwGNwTw (accessed on17 may 2019).
26. https://www.scentys.com/blog/en/the-seven-olfactory-families/?fbclid=IwAR3ScqvAwyicD1gI_ETMSl8laL_ZL2N05U5uORMUNuRo6Ogny8Hw87X_u-A (accessed on17 may 2019).
27. A. van Asten, Trends Analyt. Chem the importance of GC and GC-MS in perfume analysis 21; (2002); 698
28. Regueiro J, Garcia-Jares C, Llompart M, Lamas JP, Cela R.J Chromatogr A. Landfills and waste water treatment plants as sources of polyfluorinated compounds, polybrominated diphenyl ethers and synthetic musk fragrances to ambient air 1216 (14); (2009); 2805-15.
29. Regulation (EC) no 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products; Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending council directive 76/768/EEC on the approximation of the laws of the member states relating to cosmetic products. Official Journal of the European Union (accessed on 10 February 2019).
30. L. Sanchez-Prado, M. Llompart, J.P. Lamas, C. Garcia-Jares, M. Lores, Talanta. Multicomponent analytical methodology to control phthalates, synthetic musks, fragrance allergens and preservatives in perfumes 85; (2011); 370.
31. P. Marriott, R. Shellie, J. Fergeus, P. Morrison, Flavour, Fragr. J. 15; (2000); 225.
32. Pybus, D. H., and C. S. Shell. The Chemistry of Fragrances. The Royal Society of Chemistry, Cambridge, UK, 1999.
33. J. Cheng, K. A. Zug. Fragrance allergic contact dermatitis. Dermatitis. 25; (2014); 232–45.
34. EC (European Commission). Opinion Concerning Fragrance Allergy in Consumers. SCCNFP. (1999); 8. Available (accessed on 21 February 2019)
35. http://europa.eu.int/comm/health/ph_risk/committeessccp/documents/out98_en.pdf. (accessed on 11 April 2019)
36. A. Nardelli, A. Carbonez, W. Ottoy, J. Drieghe, and A. Goossens. Frequency of and trends in fragrance allergy over a 15-year period (2008).
37. S. Choi, M. T. H. Roh, D. S. Lim, S. Kacew, H. S. Kim, and B. M. Lee. Risk assessment of benzalkonium chloride in cosmetic products. J. Toxicol. Environ. Health B 21; (2018); 8–23.

38. J. Kim, H.T. Kim, H. Yoon, A. Jo, D. Lee, P. Kim, and J. Seo. Health risk assessment of dermal and inhalation exposure to deodorants in Korea. Sci. Total Environ. 625; (2018); 1369–79.
39. D. S. Lim, T. H. Roh, M. K. Kim, Y. C. Kwon, S. M. Choi, S. J. Kwack, K. B. Kim, S. Yoon, H. S. Kim, and B. M. Lee. 1184 D. SOO LIM ET AL. Risk assessment of N-nitrosodiethylamine (NDEA) and N-nitrosodiethanolamine (NDELA) in cosmetics. J. Toxicol. Environ. Health Part A. 81; (2018); 465–80.
40. Hartz Way, NJ. Caucus, (070944) V.1- 1973- 14, 111, 1985 (AECTCV) Australian; (1994): Australian-. January 18, 1994, page 4. Archives of Env. Contam. and Tox., Archives of Environmental Contamination and Toxicology, Springer Verlag New York, Inc., Service center, 4, 1985.
41. http://www.acttr.com/en/en-faq/en-faq-uv-vis/128-en-faq-what-is-uv-vis-spectrophotometer.html?fbclid=IwAR1ktoMiK55qqU1-ab7OfWfgFw8S-Y802fml-xb1Y-4yOZASvRM_7LSy7cA (accessed on 25 May 2019)
42. G. Véras ; A. L. B. Brito ; A. C. Silva ; P. Silva ; G. B. Costa, L. C. N. Félix ; D. D. S. Fernandes ; M. M. Fontes ; Quim. Nova UV-Vis spectrometric detection of biodiesel/diesel blend adulterations with soybean oil35; (2012); 315.
43. D. D. S. Fernandes ; A. A .Gomes ; M. M. Fontes ; G. B. Costa ; V. E. Almeida ; M. C. U. Araujo ; R. K. H. Galvão; G. Véras ; J. Braz. Chem. Soc. Dental Therapy: Evolving in Minnesota's Safety Net. 25; (2014); 169.
44. U.Contreras; O. Barbosa-García; J. L. M. Pichardo; G. Ramos-Ortíz,; J. L.Maldonad,; M. A. Meneses-Nava; N. E. Ornelas Soto; P. L. Lopes-Alba,; Food Res. Int. 43; (2012); 2356.
45. B.Plutowska and W.Wardencki, Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review, Food Chem 107(1); (2008); 449.
46. C. Apetrei et al. Combination of an e-nose, an e-tongue and an e-eye for the characterization of olive oils with different degree of bitterness, Anal Chim Acta 663(1); (2010); 91.
47. J.E. Haugen et al, Rapid control of smoked Atlantic salmon (Salmo salar) quality by electronic nose: correlation with classical evaluation methods, Sens Actuators B. 116(1–2); (2006); 72.

48. E. Falque Lopez and E. Fernandez Gomez, Comparison of solvents for determination of monoterpenes in wine using liquid-liquid extraction, Chromatography 52; (2000); 798–802.
49. E.A. Nonato, F .Carazza, F.C. Silva, C. Carvalho Rand, Z. Cardeal, A headspace solid-phase microextraction method for the determination of some secondary compounds of Brazilian sugar cane spirits by gas chromatography, J Agric Food Chem. 49; (2001), 3533–3539.
50. B. Zellner, P. Dugo, G .Dugo and L .Mondello, Gas chromatography–olfactometry in food flavour analysis, J Chromatogr A 1186; (2008), 123–143.
51. C. M. Delahunty, G. Eyres and J.P. Dufour, Gas chromatography-olfactometry, J Sep Sci. 29; (2006); 2107–2125.
52. L. Gagliardi, D. De Orsi, L. Manna, D. Tonelli, J. Liq, Chromatogr. R. T. 20; (1997); 1797–1808.
53. C. Apetrei, Combination of an e-nose, an e-tongue and an e-eye for the characterisation of olive oils with different degree of bitterness, Anal Chim Acta 663(1); (2010); 91.
54. R. Singh, Proceedings of the1st IEEE International Workshop on Electronic Design, Test and Applications 1453; (2002); 489.
55. Z. Wei, Y. Yang, J. Wang, W. Zhang, Q. Ren, the measurement principles, working Parameters and configurations of voltammetric electronic tongues and its applications for foodstuff analysis, J. Food Eng. 217; (2018); 75–92.
56. M.C. Martínez-Bisbal, E. Loeff, E. Olivas, N. Carbó, F.J. García-Castillo, J. López Carrero, et al., A voltammetric electronic tongue for the quantitative analysis of quality parameters in wastewater, Electroanalysis 29; (2017); 1147–1153.
57. X. Cetó, J.M. Gutiérrez, M. Gutiérrez, F. Céspedes, J. Capdevila, S. Mínguez, et al., Determination of total polyphenol index in wines employing a voltammetric electronic tongue, Anal. Chim. Acta. 732 ;(2012); 172–179.
58. L. Pascual, I. Campos, J.-L. Vivancos, G. Quintás, A. Loras, M.C. Martínez-Bisbal, et al. Detection of prostate cancer using a voltammetric electronic tongue, Analyst 141; (2016); 4562–4567.
59. E. García Breijo, C. Olguin Pinatti, R. Masot Peris, M. Alcañiz Fillol, R. Martínez-Máñez, J. Soto Camino, TNT detection using a voltammetric electronic tongue based on neural networks, Sens. Actuators A Phys. 192; (2013); 1–8.
60. S. M. Van Ruth. Methods for gas chromatography-olfactometry: A review, Biomol Eng; (2001);17, p.121.

61. L. G. Dias, A.Fernandes, A. C. A Veloso, A. A. S. C .Machado, J. A. Pereira, A. M. Peres, Single-cultivar extra virgin olive oil classification using a potentiometric electronic tongue. Food Chemistry 160; (2014); 321–329.
62. L.A. Dias, A. M. Peres, A. C. A. Veloso, F. S. Reis, M. Vilas-Boas, A. A. S. C.Machado An electronic tongue taste evaluation: Identification of goat milk adulteration with bovine milk. Sensors and Actuators B: Chemical 136; (2009); 209–217.
63. R.Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, (2013).
64. URL http://www.R-project.org/.(accessed on 09 June 2019)
65. J.O. Cadima, M Cerdeira. Minhoto, Computational aspects of algorithms for variable selection in the 410 contexts of principal components. Computational Statistics & Data Analysis 47; (2004); 225-236.
66. J.O. Cadima, P.D. Cerdeira, M.Silva, Minhoto, The subselect R package, (2012).
67. http://cran.rproject.org/web/packages/subselect/vignettes/subselect.pdf. (accessed on 15 June 2019)
68. H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York, (2009). (accessed on 20 June 2019)
69. W.N. Venables and B. D. Ripley, Modern Applied Statistics with S, Springer, fourth Edition, New York, C.M. Bishop, Pattern recognition and machine learning, ;(2006); first edn. Springer, New York, (2002).
70. N. Rodrigues, Í.M.G. Marx, S. Casal, L.G. Dias, A.C.A. Veloso, J.A. Pereira, A.M. Peres, Application of an electronic tongue as a single-run tool for olive oils' physicochemical and sensory simultaneous assessment, 197; (2019); 363-373.
71. S. Slim, N. Rodrigues L.G. Dias, A.C.A. Veloso, J.A. Pereira, S. Oueslati, A.M. Peres, Application of an electronic tongue for Tunisian olive oils' classification according to olive cultivar or physicochemical parameters, Eur. Food Res. Technol. 243; (2017); 1459-1470.
72. R. Prata, J.A. Pereira, N. Rodrigues, L.G. Dias, A.C.A. Veloso, S. Casal, A.M. Peres Olive oil total phenolic contents and sensory sensations trends during oven and microwave heating processes and their discrimination using an electronic tongue. J. Food Qual. 22; (2018); 10 pages.
73. http://cran.rproject.org/web/packages/subselect/vignettes/subselect.pdf. (accessed on 18 June 2019)

74. L. A. Marques, R. R. Catharino, R. E. Bruns; M. N. Eberlin, Rapid Commun. Mass Spectrom. 20; (2006); 3654.

Appendix

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Journal Pre-proof

An electronic tongue as a classifier tool for assessing perfume olfactory family and storage time-period

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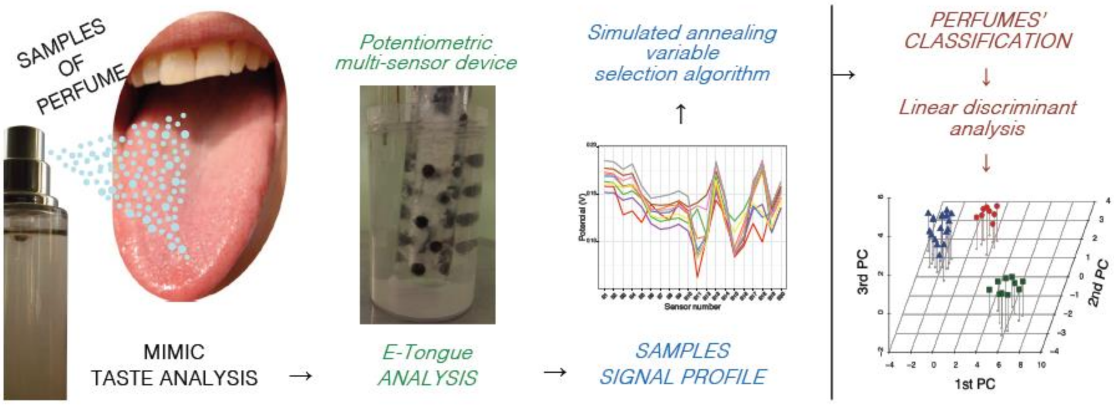
Graphical abstract:



Highlights:

Highlights:

- A lab-made potentiometric E-tongue was designed for perfume analysis
- Lipid sensor membranes allowed establishing interactions with odorant compounds
- E-tongue-LDA-SA showed to be a powerful perfume classifier tool
- Men and women perfumes successful discrimination
- Perfume olfactory family and storage time-periods correctly assessed

An electronic tongue as a classifier tool for assessing perfume olfactory family and storage time-period

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Abstract

The identification of more than three perfumes is difficult and no analytical tool can completely replace the human olfactory system for fragrance classification. Indeed, no analytical system can mimic the human fragrance perception, being the recognition of perfume aroma patterns by conventional or sensor-based analytical tools a challenging task. For the perfume sector, the possibility of applying fast, cost-effective and green analytical devices for perfume analysis would represent a huge economic revenue. Since the perfume aroma pattern will depend on the composition of the liquid phase and on the diffusion properties of their volatile components, this work aimed to apply a potentiometric electronic tongue, comprising non-specific cross-sensitive lipid polymeric membranes, combined with chemometric techniques, as a novel perfume classifier. The multisensors device allowed establishing perfumes' unique fingerprints, which were successfully used to discriminate men from women perfumes, to identify the perfume aroma family (Citric-Aromatic, Floral, Floral-Fruity, Floral-Oriental, Floral-Woody, Woody-Oriental and Woody-Spicy) and, assessing the perfume storage time-period (≤ 9 months; 9-24 months; and, ≥ 24 months). The established linear discriminant models were based on the a single-run potentiometric profiles gathered by sub-sets of sensors selected using the simulated annealing algorithm, which enabled achieving correct classification rates of 93-100% (for leave-one-out cross-validation procedure). The satisfactory performance of the electronic tongue demonstrates the versatility of the proposed approach as a practical perfume preliminary classifier sensor device, which industrial application may be foreseen in a near future, contributing to a green-sustained economic growth of the perfume industry.

Keywords: Perfume olfactory family; Perfume storage time-period; Potentiometric electronic tongue; Linear discriminant analysis; Simulated annealing algorithm

1. Introduction

It is expected that the global market for Fragrances and Perfume exceeds US$40 billion by 2020 [1]. A perfume may comprise from 10 to 100 individual ingredients [2], which are usually complex mixtures of synthetic or natural (e.g. essential oils) organic compounds (e.g., aldehydes, alcohols, lactones, esters and terpene derivatives). So, assessing the perfume composition, identifying the main aroma family as well as assessing perfume-stability and longevity is not a straightforward task [1,2]. As most of perfume ingredients are volatile or semi-volatile, gas chromatography (GC), in combination with mass spectrometry (MS) is, by far, the most used analytical technique [3]. However, GC-MS does not provide a direct qualitative information about the sensory perception of the aroma molecules, being needed to establish calibration models for correlating this qualitative information [1]. Thus, GC-Olfactometry (GCO) or GC-sniffing techniques coupled with condensed Phase Fourier-transform infrared (FTIR) spectroscopy or Time of Flight-MS (ToFMS) may be required [2,4]. These techniques are time-consuming, expensive and require skilled technicians, which may be beyond the economic possibilities of low-medium local perfume companies. Thus, the development of fast, low-cost and green sensor-based techniques, which may be applied on-line, to monitor *in-situ* perfume aroma-fragrance profiles is highly envisaged by the industry. Electronic noses (E-noses) have been proposed for perfume analysis namely for discriminating original brand perfumes or recognizing fake counterparts [5-7]; for identifying simple aromas [8-12]; for recognizing unknown fragrance mixtures [13]; to classify different perfume classes [14]; as quality control method of musk samples [15]; for generating analyte-specific fingerprints of odorants [16]; to differentiate perfumes by brand [16,17]; or, for highlight the differences of perfumes according to the producers, using odorant maps [18]. An E-nose was also applied to detect counterfeit perfumed cleaner products as well as to quantify the perfume added amount [19]. Despite the satisfactory results reported so far, the identification of more than three perfumes remains difficult for the human nose and for E-nose devices with multiple sensors [14]. To overcome this problem, complex hybrid multiple statistical classifier methodologies have been proposed [14].

As an alternative/complementary approach, the present work aims, for the first time, to evaluate the possibility of using a potentiometric electronic tongue (E-tongue) together with linear discriminant analysis (LDA) coupled with the simulated annealing (SA) variable selection algorithm, as a practical perfume classifier device, minimizing or even avoiding the need of applying complex hybrid statistical techniques. Contrary to other research areas (e.g., food science [20]), in the perfume field, the use of E-tongues is not common. Only one work reported the use of a voltammetric E-tongue for perfume evaluation [21]. The study evaluated the performance of a voltammetric E-tongue to detect the type and concentration of different perfume's fragrances. On the other hand, E-tongues have been widely used to assess positive and negative sensory attributes of foods [22-28]. Moreover, sensor lipid membranes can interact with different polar compounds (e.g., phenolic compounds, esters, alcohols and aldehydes) via the establishment of electrostatic or hydrophobic interactions [29] and, since some of these chemical families are present in perfumes (as fragrances and scent ingredients), the possible application of this type of E-tongues may be foreseen. In fact, it has been reported that lipid bilayer membranes could be effectively applied within a synthetic sensing system to discriminate odorants and successfully differentiate perfumes by brand [16]. It was also shown that a simple technique like ultraviolet-visible (UV-Vis) spectrophotometry, coupled with multivariate statistical tools, allowed obtaining a preliminary chemical fingerprint of perfume samples, enabling perfume classification [30]. Therefore, and although the advantages of using an E-tongue could not be obvious, considering that perfume analysis is usually associated to the olfactory perception of aroma fragrances, its use can be foreseen. Actually, the analysis of the perfume' liquid phase, which contains the chemical compounds responsible for the aroma profile, may be extremely relevant, allowing gathering complementary but relevant chemical information of the perfumes' main fragrances notes as well as their age, i.e., the storage time-period, during which a chemical profile change is expected.

2. Materials and methods

2.1. Perfume samples

Perfume samples were supplied by NORTEMPRESA Perfume Lab (Braga, Portugal). In total, 33 independent samples were collected, being 18 women perfumes and the other 15 men perfumes, which main details are given in **Table 1**. According to the label information and based on the olfactory pyramid data perfumes were grouped into 7 different main aroma/olfactory families. Women perfumes were classified as Floral (5 perfumes), Floral-Fruity (5 perfumes), Floral-Oriental (5 perfumes) and Floral-Woody (3 perfumes). Men perfumes were grouped into 4 aroma families, being one of them common to the women perfumes, namely Citric-Aromatic (3 perfumes), Floral-Woody (4 perfumes), Woody-Oriental (4 perfumes) and Woody-Spicy (4 perfumes). The perfumes were from different production lots and had different storage time-periods (ranging from 6 to more than 24 months), being grouped into 3 main classes: 6 to 9 months, 9 to 24 months and more than 24 months. According to the label information and data from the perfume company, all perfume samples contained denatured alcohol (a mixture of ethanol with a denaturing agent) that has antimicrobial, masking and viscosity controlling functions; parfum, meaning an undisclosed mixture of several scent chemicals and ingredients used as fragrances); aqua (*i.e.*, water); and, propylene glycol, an organic alcohol used as a skin conditioning agent, fragrance and humectant, allowing controlling the final viscosity of the perfume. Besides, the samples could contain a mixture of other ingredients, in different proportions, which could include fragrance additive and masking ingredients (e.g., hydroxyisohexyl 3-cyclohexene carboxaldehyde that has a delicate sweet, light, floral aroma; *evernia prunastri* that is an extract of the oakmoss; benzyl salicylate that is a salicylic acid benzyl ester; among others) and scents (e.g., limonene that has a fresh and sweet citrus aroma; coumarin that is an aromatic organic chemical compound, used as a sweet, vanilla, nutty scent; geraniol, a monoterpenoid and alcohol, which is a natural scent ingredient; butylphenyl methylpropional, an aromatic aldehyde, which is a synthetic fragrance with a strong floral scent; among others).

2.2. UV-Vis perfume evaluation

UV-Vis spectrophotometry was applied to acquire a preliminary insight of each perfume composition, following the experimental methodology described by Gomes et al., [30], with some adaptations. Perfume samples were firstly diluted in the proportion of 1:4000, withdrawing 2.5 µL of perfume, measured using a Gilson micropipette (0.4-10 µL), to a 10 mL glass volumetric flask, which was filled with absolute ethanol (+99%, Extra Pure, SLR, Fisher Chemical®). Each perfume-ethanol mixture was agitated, placed into a quartz cuvette (with 1 cm of path length) and then, the UV spectra (200-1100 nm, at intervals of 5 nm) was recorded, using a SPECORD®200 spectrophotometer (Analytik Jena®) and treated using the WinASPECT® software. Absorption was detected in a near UV wavelength interval (200–350 nm).

2.3. E-tongue

2.3.1. E-tongue device and set-up

A new lab-made potentiometric E-tongue multisensor device, comprising two cylindrical arrays, similar to that previous described [31], was re-designed and built (**Figure 1**) specifically for the perfume analysis, aiming to minimize the total perfume volume required for each assay. Indeed, for high-value samples the required volume for each assay may be an economic concern and so, the new system was miniaturized aiming to reduce the amount of perfume used in each experimental assay. The arrays comprised the same 40 lipid polymeric cross-sensitive sensor membranes (20 sensors for each array), with the composition (lipid additive, 3%; plasticizer, 32%; and, polyvinyl chloride, 65%) [31]. The sensor membranes were linked to a multiplexer Agilent Data Acquisition Switch Unit (model 34970A), which was controlled by an Agilent BenchLink Data Logger software. Each perfume analysis took 5 min, being recorded the potentiometric signals of the 40 sensor membranes, generated by the establishment of electrostatic and/or hydrophobic interactions [29]. An Ag/AgCl double-junction glass electrode (Crison, 5241) was used as the reference electrode. The E-tongue was stored in a HCl solution (0.01 M) that was also used to evaluate the signals intra- and inter-day stability or the occurrence of signal drifts. Similarly, intra- and inter-day repeatability of the E-tongue potentiometric signals were also evaluated for the perfume samples of each olfactory family studied. The same sensor coding used in previous works was adopted: each sensor was identified with a letter S (for sensor) followed by the number of the array (1 or 2) and the number of the membrane (1 to 20, corresponding to different combinations of plasticizers and additives).

2.3.2. E-tongue perfume analysis: sample preparation and potentiometric assays

Since lipid polymeric membranes were used and taking into account their possible degradation when high alcoholic solutions are being analysed, each perfume sample (that had a high level of alcohol) was previously diluted with deionized water in order to obtain an 80:20 (v/v) water-perfume solution. This proportion was selected based on the previous experience of the research team, which observed a satisfactory E-tongue performance when used to analyse water-ethanol solutions (80:20, v/v) [31]. So, from each perfume, 8 mL were withdrawn and diluted in 32 mL of deionized water, allowing to obtain a total sample volume of 40 mL, sufficient to completely immerse the two cylindrical E-tongue arrays, allowing the contact of the sensor membranes with the aqueous perfume solution. The solution system was then agitated during 2 min, after which the potentiometric assays were performed in duplicate for each sample, with a third assay carried out if the recorded signals of any of the 40 sensors showed a coefficient of variation for the inter-assays greater than 20%. Besides, for evaluating the sensors' intra-day signal stability (i.e., signal stability over-time, for a typical daily analysis time-period), E-tongue potentiometric profiles of solutions of HCl (0.01 M) were recorded 10×, in the same day, being the assays carried out over an 8-h time-period, within the usual perfume samples set of assays.

2.4. Statistical analysis

Linear discriminant analysis (LDA) coupled with the meta-heuristic simulated annealing (SA) variable selection algorithm was used to evaluate the capability of the potentiometric E-tongue to differentiate men from women perfumes, to classify perfumes according to the main olfactory family and to semi-quantitatively determine the storage time-period. E-tongue-LDA-SA models were established based on the best sub-sets of the 40 normalized signal profiles generated during the potentiometric analysis, which were selected by the SA algorithm, aiming to minimize noise effects due to the inclusion of redundant signals. The LDA predictive performance was assessed using the leave-one-out cross-validation (LOO-CV) technique taking into account the dimension of the independent dataset. The classification performance of each LDA model was also graphically evaluated using 2D plots of the main discriminant functions, being the class membership boundary ellipses determined based on the posterior probabilities computed using the Bayes' theorem (which enables controlling over-fitting issues) [32]. Finally, for each LDA model established the overall performance was also assessed based on the sensitivity values, i.e., based on the percentage of correct classifications. All statistical analyses were performed using the Subselect [33,34] and MASS [35] packages of the open source statistical program R (version 2.15.1), at a 5% significance level.

3. Results and discussion

3.1. UV-Vis spectra of perfume samples

Recently, the possibility of using UV spectrophotometry in combination with chemometric techniques for perfume classification was described [30]. In the present work, it was observed that the diluted perfume-ethanol samples showed a significant absorption in the range of 200-350 nm, corresponding to the near-UV region. **Figure 2** shows examples of the absorption spectra recorded for each olfactory family of men or women perfumes studied (**Figure 2A-B**, respectively) as well as the UV spectra trend with the storage time-period for Woody-Spicy men perfumes and Floral-Oriental women perfumes (**Figure 2C-D**, respectively). It can be inferred that several peaks (major and minor bands) can be found in the region of 210-340 nm that, as pointed out by Gomes et al. [30], may be due to the chemical diversity of chemical of the perfume fragrances, which include into terpenoids, musks, aliphatic derivatives and aromatic derivatives, characterized by the presence of unsaturated conjugated or unconjugated carbon-carbon and/or the presence of carbonyl groups [19,30]. It should also be remarked that, globally, the perfume bands observed are in agreement with those found by Gomes et al. [30] for perfumes as well as for individual ethanolic standard solution of scents (e.g., limonene, linalool, citral, eugenol, coumarin, eugenol, isoeugenol and cinnamic derivatives). This similarity could be attributed to the fact that the perfumes evaluated in both studies contained several equal scents in their composition, namely, limonene, linalool, citral, coumarin, eugenol, isoeugenol, cinnamyl alcohol and cinnamal. It should also be noticed that the observed spectra confirmed the presence of polar compound families with which electrostatic and hydrophobic interactions could be established by the polymeric lipid sensor membranes comprised on the lab-made potentiometric E-tongue, as also pointed out for lipid bilayer membranes of synthetic sensing systems previously used to discriminate odorants [16]. Finally, it should be pointed out that, the UV absorption spectra recorded changed with the perfume's olfactory family and, even for the same olfactory family (e.g., Floral-Woody men and women perfumes) different absorption spectra were obtained (**Figure 2A-B**). Indeed, it should be kept in mind that, perfumes may be classified as belonging to the same olfactory family, although having different top, heart and base olfactory notes due to the different composition in fragrances and scents. In fact, as previously stated, a perfume is a complex matrix that may comprise from 10 to 100 individual ingredients [2]. Finally, different UV absorption spectra could be observed for different storage time-periods of perfumes belonging to the olfactory family (**Figure 2C-D**), being the main differences found between perfumes with less than 9 months of storage compared to those with more than 9 months of storage, showing that the perfumes, although kept in adequate storage conditions, their composition slightly change with time.

3.2. E-tongue signal stability over time and perfume samples' signal profiles

Potentiometric sensor devices may exhibit signal drifts, which can be minimized or overcome when daily calibrations are carried out or if signal standardization statistical treatments are applied. In which concerns potentiometric E-tongues, comprising lipid polymeric membranes (both print-screen or cylindrical arrays geometries) it was previously observed that intra-day signals were quite stable showing negligible drifts (with coefficients of variation lower than 5%) [22,26,28,31,36,37]. To further checked the literature reported stability of this kind of E-tongue, comprising similar sensors, HCl (0.01 M) solutions were randomly analysed (10×), during the perfumes' assays, within the usual 8-h time-period of analysis, in one day and in three consecutive days. The results pointed out that, with the new device, the intra -and inter-day signal coefficients of variation varied, in general, in the ranges of 1.3-5.7% and 2.5-

8

13.9%, showing the overall satisfactory signal stability over-time (**Figure 3**). Regarding the analysis of the diluted perfume samples (perfume-water solutions, 20:80 v/v), typical potentiometric signal profiles were acquired, varying the recorded potentials from +12 to +340 mV, showing satisfactory intra- and inter-day signal repeatabilities (coefficients of variation varying from 1.8-11.4% and 4.7-16.2%) and similar profiles to those shown in Figure 3, although with slightly higher signal variations. The E-tongue potentiometric profiles recorded by the 40 lipid sensor membranes (1^{st} sensor array: S1:1 to S1:20; 2^{nd} sensor array: S2:1 to S2:20), showed slightly differences (regarding signal intensity/signal dynamic range) according to the perfume olfactory family (7 different olfactory families; men perfumes: Citric-Aromatic, Floral-Woody, Woody-Oriental and Woody-Spicy; women perfumes: Floral, Floral-Fruity, Floral-Oriental and Floral-Woody).

3.3. E-tongue classification performance

The performance of the proposed potentiometric E-tongue, comprising non-specific and cross-sensitive sensors, for simultaneously classifying, based on a single-run assay, the perfume type (men or women), perfume main aroma/olfactory family and perfume storage time-period was evaluated for the first time. This type of sensor device has been reported as a powerful taste sensor device for assessing different positive and negative sensory attributes of foods [22-28]. Moreover, the use of a multisensors arrays, with the above-mentioned characteristics may allow gathering the unique fingerprint of a perfume and so, overcoming the known limitation of applying a single sensor, which results in and unspecific response towards the complex perfume composition (10 to 100 individual ingredients [2]) that can deliver exactly the same potentiometric signal for different chemical compounds in solution, which are related to the specific aroma/olfactory perfume notes [3].

3.3.1. Discrimination of men and women perfumes

Although men and women perfumes may be differentiated according to the olfactory notes. For the perfume industry it is important to have an analytical technique that could be implemented (on-line and *in-situ*) for monitoring the production line, allowing a fast and easy discrimination of men from women perfumes. So, the E-tongue performance was evaluated keeping in mind this objective. An E-tongue-LDA-SA model was established based on the potentiometric data of 12 sensors (1^{st} array: S1:3, S1:4; S1:7, S1:14 and S1:20; 2^{nd} array: S2:2, S2:4, S2:6, S2:9, S2:12, S2:13 and S2:17), enabling to correctly differentiate men from women perfumes, with sensitivities and specificities (overall and for each group) of 100% for both original grouped data (**Figure 4**) and LOO-CV internal-validation procedure. All samples were correctly classified, pointing out the versatility and powerful of the classifier potentiometric device for discriminating men and women perfumes, comprising a total of 7 different olfactory families and have being stored during 6 to more than 24 months. The satisfactory results also strengthen the initial idea that E-tongues could be a practical tool for perfume analysis even if, at a first view it was expected to correlate a sensor-based device with the olfactory profile of a perfume sample.

3.3.2. Classification of perfumes according to the main aroma family

Men and women perfumes possess a complex composition, being a mixture of a multitude of ingredients, which include a basis of alcohol denatured, parfum, aqua and propylene glycol combined with a several other chemical compounds (e.g., fragrances and scents). Depending of the different top, heart and base olfactory notes (olfactory pyramid), each perfume may be commercially classified according to the main aroma/olfactory family (**Table 1**). Thus, in this study it was evaluated the E-tongue performance for classifying perfumes taking into account the main olfactory family, independently of the perfume type (men or women) or the perfume's storage time-period (*i.e.*, perfume's age), using a LDA-SA chemometric approach. The 33 perfumes were grouped into 7 different olfactory families (**Table 1**) including, Citric-Aromatic (3 men perfumes with 6-9 months or more than 24 months of storage), Floral (5 women perfumes with 6-9 months or more than 24 months of storage), Floral-Fruity (5 women perfumes with 6-9 months or 9-24 months of storage), Floral-Oriental (5 women perfumes with 6-9 months, 9-24 months or more than 24 months of storage), Floral-Woody (4 men and 3 women perfumes with 9-24 months or more than 24 months of storage), Woody-Oriental (4 men perfumes with 6-9 months, 9-24 months or more than 24 months of storage) and Woody-Spicy (4 men perfumes with 6-9 months, 9-24 months or more than 24 months of storage). For this purpose, a classification E-tongue-LDA-SA model, which 2 first discriminant functions accounted for 99.97% of the total variance, was developed based on the potentiometric signals gathered by 18 selected sensors (1^{st} array: S1:1, S1:2, S1:4, S2:6, S1:7, S1:8, S1:11, S1:12 and S1:16; 2^{nd} array: S2:1, S2:2, S2:11 and S2:13 to S2:18). The model allowed obtaining overall sensitivities (i.e., percentage of correct classifications) of 100% and 94% and global specificities (i.e., the proportion of true negatives that are correctly classified) of 100% and 95% for the original grouped data (**Figure 5**) and for the LOO-CV internal-validation procedure, respectively.

An overall satisfactory predictive performance was achieved, being the olfactory family of only 2 of the 33 perfumes incorrectly assessed (**Table 2**), being both predictive sensitivity and specificity per group (LOO-CV procedure) within the range of 80-100%. It should be noticed that, if the perfumes were split by men or women type, 100% of correct predictive classifications could be obtained (LOO-CV procedure) using LDA models based on the signal profiles of 8 and 9 E-tongue sensors, respectively, selected by the SA algorithm (*data not shown*). The overall correct classification rates achieved with the lab-made potentiometric E-tongue are of the same order of magnitude as those reported in the literature using E-nose devices coupled with different chemometric techniques (which predictive sensitivities ranged from 71-98% when classifying different perfume classes or discriminating them by brand) [14,16] or even with a voltammetric E-tongue [21]. Furthermore, compared to the reported performances achieved with E-nose These results showed, for the first time, that a potentiometric E-tongue could be used as a classifier sensor device for perfume analysis, namely for identifying the main olfactory family. This is of utmost practical and economical relevance since this evaluation and classification requires the availability of trained sensory panelists, leading to an expensive and time-consuming task that may be beyond the economic possibilities of local small-medium perfume companies.

3.3.3. Assessment of the storage time-period of the perfume samples

For the perfume industry it is relevant to have a fast and user-friendly analytical tool for classifying perfumes according to the storage time-period (*i.e.*, the time after production until commercialization). This possibility is even of greater practical application if it could be used regardless the type of perfume (men or women) and the perfume's aroma/olfactory family. So, the E-tongue performance to assess the storage time-period (6 to 9 months; 9 to 24 months; and more than 24 months) was further evaluated. An E-tongue-LDA-SA model, with two discriminant functions (accounting 98.36% and 1.64% of the total variability, respectively), was established based on the potentiometric signals recorded by a sub-set of 20 sensors selected by the SA algorithm (1^{st} array: S1:3, S1:4, S2:6, S1:7, S1:9, S1:12, S1:14, S1:15, S1:19 and S1:20; 2^{nd} array: S2:2 to S2:6, S2:9, S2:12, S2:14, S2:18 and S2:20). The multivariate linear classification model allowed an overall correct classification of the storage time-period of 100% of the original data samples (**Figure 6**) and of 97% of the samples for the LOO-CV internal validation procedure (being only one sample of the 9-24 months erroneously classified as being stored for more than 24 months). The model overall specificities were of 100% and 98% for the original grouped data and for the LOO-CV procedure, respectively. The sensitivity and specificity per group, for LOO-CV procedure, ranged from 88-100% and 93-100%, respectively, as shown in **Table 3**. The predictive performance achieved was very satisfactory considering the variability of the perfumes included in each storage time-period (6-9 months: 3 men and 7 women perfumes from Citric-Aromatic, Floral, Floral-Fruity, Floral-Oriental, Woody-Oriental and Woody Spicy olfactory families; 9-24 months: 4 men and 4 women perfumes from Floral-Fruity, Floral-Oriental, Floral-Woody, Woody-Oriental and Woody-Spicy olfactory families; and, > 24 months: 8 men and 7 women perfumes from Citric-Aromatic, Floral, Floral-Oriental, Floral-Woody, Woody-Oriental and Woody Spicy olfactory families). This fact, clearly pointed out the versatility of the E-tongue-LDA-SA proposed approach, which has proven to be a powerful semi-quantitative classifier tool of perfume's age assessment. Furthermore, if the perfumes were split by men and women type, the correct predictive classification percentages (sensitivity values) would reach 100% (E-tongue-LDA-SA models based on the signal profiles of 7 selected sensors; *data not shown*), strengthen the above-mentioned powerful of the classifier potentiometric device.

4. Conclusions

The present study outlined, for the first time, the application of an E-tongue for perfume analysis, which allowed, in a single-run assay, to establish a unique perfume potentiometric fingerprint capable of discriminating men and women perfumes, for differentiating perfumes according to the main olfactory family and for semi-quantitatively assessing the storage time-period of perfumes. The work also highlighted the predictive satisfactory performance of a multisensor device, comprising non-specific lipid polymeric membranes, coupled with classification chemometric techniques and variable selection algorithm, showing that the proposed approach could be used by the perfume industrials as a practical, cost-effective and fast perfume classifier analytical technique as well as a complementary sensory preliminary tool, minimizing the need to recourse to trained/official perfume panelists. Thus, the study carried out may also contribute to enlarge the E-tongue field of application, mainly focused on the food and environmental analysis, to the perfume emerging and promising area. Several challenging applications may be foreseen in the future for electrochemical based sensor devices, namely to monitor the maceration and maturation critical phases of a perfume design, to detect the presence of legally restricted or forbidden fragrance-related substances or even to recognize the perfume brand allowing discriminating original and copied perfumes.

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Compliance with Ethics Requirements

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

[1] C. Villatoroa, L. Vera, H. Gygax, Comparative study of odours present in twin fragrances by GC-sniffing-ToFMS, Chem. Eng. Trans. 54 (2016) 133-138. https://doi.org/10.3303/CET1654023.

[2] A. van Asten, The importance o GC and GC-MS in perfume analysis, Trends Analyt. Chem. 21 (2002) 698-708. https://doi.org/10.1016/S0165-9936(02)00807-5.

[3] A. Cisvert, M. López-Nogueroles, A. Salvador, Essential Oils: Analytical Methods to Control the Quality of Perfumes, in: K. Ramawat, J.M. Mérillon (Eds.), Natural Products. Springer, Berlin, Heidelberg, 2013, pp. 3287-3310.

[4] N. Nibbe, H. Gygax, B. Maxeiner, Odour Measurement for Improved Scent Performance in Consumer Goods, SOFW-J. 141 (2015) 42-46.

[5] J. Gebicki, B. Szulczynski, M. Kaminski, Determination of authenticity of brand perfume using electronic nose prototypes, Meas. Sci. Technol. 26 (2015) art. no. 125103. https://doi.org/10.1088/0957-0233/26/12/125103.

[6] T. Nakamoto, A. Fukuda, T. Moriizumi, Perfume and flavor identification by odor sensing system using quartz-resonator sensor array and neural-network pattern recognition, Transducers '91 (1991) 355-358.

[7] M. Cano, V. Borrego, J. Roales, J. Idígoras, T. Lopes-Costa, P. Mendoza, J.M. Pedrosa, Rapid discrimination and counterfeit detection of perfumes by an electronic olfactory system, Sens. Actuat. B: Chem. 156 (2011) 319-324. https://doi.org/10.1016/j.snb.2011.04.039.

[8] R. Singh, An intelligent system for odour discrimination, Proceedings - 1st IEEE International Workshop on Electronic Design, Test and Applications DELTA (2002) art. no. 994681, 489-491. https://doi.org/10.1109/DELTA.2002.994681.

[9] B. Bahraminejad, S. Basri, M. Isa, Z. Hambali, Application of a sensor array based on capillary-attached conductive gas sensors for odor identification, Meas. Sci. Technol. 21 (2010) art. No. 085204. https://doi.org/10.1088/0957-0233/21/8/085204.

[10] A. Branca, P. Simonian, M. Ferrante, E. Novas, R.M. Negri, Electronic nose based discrimination of a perfumery compound in a fragrance, Sens. Actuat. B: Chem. 92 (2003) 222-227. https://doi.org/10.1016/S0925-4005(03)00270-3

[11] B. Karlik, K. Yuksek, Fuzzy clustering neural networks for real-time odor recognition system, J. Autom. Methods Manage. Chem. 2007 (2007) art. no. 38405. https://doi.org/10.1155/2007/38405.

[12] T. Temel, B. Karlik, An Improved Odor Recognition System Using Learning Vector Quantization with a New Linear Discriminant Analysis, Neural Netw. World 17 (2007) 287-294.

[13] W. Jatmiko, T. Fukuda, F. Arai, B. Kusumoputro, Artificial odor discrimination system using multiple qartz resonator sensors and various neural networks for recognizing fragrance mixtures, IEEE Sens. J. 6 (2006) 223-233. https://doi.org/10.1109/JSEN.2005.858435.

[14] E. Esme, B. Karlikb, Fuzzy c-means based support vector machines classifier for perfume recognition, Appl. Soft. Comput. J. 46 (2016) 452-458. https://doi.org/10.1016/j.asoc.2016.05.030

[15] T. Ye, C. Jin, J. Zhou, X. Li, H. Wang, P. Deng, Y. Yang, Y. Wu, X. Xiao, Can odors of TCM be captured by electronic nose? The novel quality control method for musk by electronic nose coupled with chemometrics, J. Pharm. Biomed. Anal. 55 (2011) 1239-1244. https://doi.org/10.1016/j.jpba.2011.03.018.

[16] T. Takeuchi, J. Montenegro, A. Hennig, M. Stefan, Pattern generation with synthetic sensing systems in lipid bilayer membranes, Chem. Sci. 2 (2011) 303-307. https://doi.org/10.1039/c0sc00386g.

[17] M. Penza, G. Cassano, F. Tortorella, G. Zaccaria, Classification of food, beverages and perfumes by WO3 thin-film sensors array and pattern recognition techniques, Sens. Actuat. B: Chem. 73 (2001) 76-87. https://doi.org/10.1016/S0925-4005(00)00687-0.

[18] W. Guo, F. Gan, H. Kong, J. Wu, Signal model of electronic noses with metal oxide semiconductor, Chemometr. Intell. Lab. Syst. 143 (2015) 130-135. https://doi.org/10.1016/j.chemolab.2015.02.021.

[19] J. Poprawski, P. Boilot, F. Tetelin, Counterfeiting and quantification using an electronic nose in the perfumed cleaner industry, Sens. Actuat. B: Chem. 116 (2006) 156-160. https://doi.org/10.1016/j.snb.2005.11.074.

[20] M. Cole, J.A. Covington, J.W. Gardner, Combined electronic nose and tongue for a flavour sensing system, Sens. Actuat. B: Chem. 156 (2011) 832-839. https://doi.org/10.1016/j.snb.2011.02.049.

[21] P. Rattanawarinchai, P. Krongkrachang, T. Chodjarusawad, D. Phromyothin, Electrochemical sensor: Preparation technique based on electronic tongue in fragrance, Mater. Today: Proceed. 4 (2017) 6410-6414. https://doi.org/10.1016/j.matpr.2017.06.146.

[22] A.C.A. Veloso, L.G. Dias, N. Rodrigues, J.A. Pereira, A.M. Peres, Sensory intensity assessment of olive oils using an electronic tongue, Talanta 146 (2016) 585–593. https://doi.org/10.1016/j.talanta.2015.08.071.

[23] L.G. Dias, N. Rodrigues, A.C.A. Veloso, J.A. Pereira, A.M. Peres, Monovarietal extra-virgin olive oil classification: a fusion of human sensory attributes and an electronic tongue, Eur. Food Res. Technol. 242 (2016) 259-270. https://doi.org/10.1007/s00217-015-2537-4.

[24] Í. Marx, N. Rodrigues, L.G. Dias, A.C.A. Veloso, J.A. Pereira, D.A. Drunkler, A.M. Peres, Sensory classification of table olives using an electronic tongue: Analysis of aqueous pastes and brines, Talanta 162 (2017) 98-106. https://doi.org/10.1016/j.talanta.2016.10.028

[25] Í.M.G. Marx, N. Rodrigues, L.G. Dias, A.C.A. Veloso, J.A. Pereira, D.A. Drunkler, A.M. Peres, Quantification of table olives' acid, bitter and salty tastes using potentiometric electronic tongue fingerprints, LWT-Food Sci. Technol. 79 (2017) 394–401. https://doi.org/10.1016/j.lwt.2017.01.060.

[26] S. Slim, N. Rodrigues L.G. Dias, A.C.A. Veloso, J.A. Pereira, S. Oueslati, A.M. Peres, Application of an electronic tongue for Tunisian olive oils' classification according to olive cultivar or physicochemical parameters, Eur. Food Res. Technol. 243 (2017) 1459-1470. https://doi.org/10.1007/s00217-017-2856-8

[27] A.C.A. Veloso, L.M. Silva, N. Rodrigues, L.P.G. Rebello, L.G. Dias, J.A. Pereira, A.M. Peres, Perception of olive oils sensory defects using a potentiometric taste device, Talanta 176 (2018) 610-618. https://doi.org/10.1016/j.talanta.2017.08.066

[28] U. Harzalli, N. Rodrigues, A.C.A. Veloso, L.G. Dias, J.A. Pereira, S. Oueslati, A.M. Peres, A taste sensor device for unmasking admixing of rancid or winey-vinegary olive oil to extra virgin olive oil, Comput. Elelctron. Agr. 144 (2018) 222–231. https://doi.org/10.1016/j.compag.2017.12.016

[29] Y. Kobayashi, M. Habara, H. Ikezazki, R. Chen, Y. Naito, K. Toko, Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores, Sensors 10 (2010) 3411–3443. https://doi.org/10.3390/s100403411.

[30] C.L. Gomes, A.C.A. Lima, M.C.L. Cândido, A.B.R. Silva, A.R. Loiola, R.F. Nascimento, Multivariate analysis of perfumes by ultraviolet spectrophotometry, J. Braz. Chem Soc. 26 (2015) 1730-1736. https://doi.org/10.5935/0103-5053.20150135

[31] N. Rodrigues, Í.M.G. Marx, S. Casal, L.G. Dias, A.C.A. Veloso, J.A. Pereira, A.M. Peres, Application of an electronic tongue as a single-run tool for olive oils' physicochemical and sensory simultaneous assessment, 197 (2019) 363-373. https://doi.org/10.1016/j.talanta.2019.01.055.

[32] C.M. Bishop, Pattern recognition and machine learning, (2006) 1st edn. Springer, New York

[33] J. Cadima, J.O. Cerdeira, M. Minhoto, Computational aspects of algorithms for variable selection in the context of principal components, Comput. Stat. Data An. 47 (2004) 225–236. https://doi.org/10.1016/j.csda.2003.11.001.

[34] J. Cadima, J.O. Cerdeira, P.D. Silva, M. Minhoto (2012), The subselect R package. http://cran.rproject.org/web/packages/subselect/vignettes/subselect.pdf/, Accessed date: 15 February 2016.

[35] W.N. Venables, B.D. Ripley (2002). Modern applied statistics with S (statistics and computing) (4th ed.). New York: Springer

[36] R. Prata, J.A. Pereira, N. Rodrigues, L.G. Dias, A.C.A. Veloso, S. Casal, A.M. Peres (2018). Olive oil total phenolic contents and sensory sensations trends during oven and microwave heating processes and their discrimination using an electronic tongue. J. Food Qual. 2018, Article ID 7826428. https://doi.org/10.1155/2018/7826428.

[37] L.G. Dias, A.M. Peres, A.C.A. Veloso, F.S. Reis, M. Vilas Boas, A.A.S.C. Machado, An electronic tongue taste evaluation: identification goat milk adulterations with bovine milk, Sens. Actuators B Chem. 136 (2009) 209-217. https://doi.org/10.1016/j.snb.2008.09.025.

Table 1. Perfume samples details (label information: sample code, type, olfactory pyramid notes, aroma family classes; and, storage time-period classes)

Sample code	Type	Aroma family class	Storage time-period class
100001	Woman	Floral-Fruity	6-9 months
100005	Woman	Floral	> 24 months
100006	Woman	Floral-Fruity	9-24 months
100012	Woman	Floral-Woody	> 24 months
100014	Woman	Floral-Fruity	9-24 months
100015	Woman	Floral-Oriental	> 24 months
100016	Woman	Floral-Oriental	> 24 months
100017	Woman	Floral-Woody	9-24 months
100018	Woman	Floral	6-9 months
100019	Woman	Floral-Oriental	9-24 months
100020	Woman	Floral-Woody	> 24 months
100023	Woman	Floral	> 24 months
100029	Woman	Floral-Fruity	6-9 months
100031	Woman	Floral-Oriental	6-9 months
100032	Woman	Floral-Fruity	6-9 months
100033	Woman	Floral	6-9 months
100034	Woman	Floral	24 months
100040	Woman	Floral-Oriental	6-9 months
200201	Man	Woody-Spicy	6-9 months
200204	Man	Citric-Aromatic	6-9 months
200206	Man	Woody-Oriental	6-9 months
200208	Man	Floral-Woody	> 24 months
200209	Man	Woody-Oriental	9-24 months
200210	Man	Woody-Spicy	9-24 months
200216	Man	Woody-Oriental	> 24 months
200217	Man	Woody-Oriental	> 24 months
200218	Man	Floral-Woody	> 24 months
200219	Man	Citric-Aromatic	> 24 months
200221	Man	Woody-Spicy	> 24 months
200222	Man	Woody-Spicy	9-24 months
200223	Man	Floral-Woody	> 24 months
200226	Man	Floral-Woody	9-24 months
200227	Man	Citric-Aromatic	> 24 months

Table 2. Discriminant analysis (sensitivity and specificity data) for perfumes classification according to the main olfactory family, based on an E-tongue-LDA-SA model based on the potentiometric profiles gathered by 18 selected sensors (1st array: S1:1, S1:2, S1:4, S2:6, S1:7, S1:8, S1:11, S1:12 and S1:16; 2nd array: S2:1, S2:2, S2:11 and S2:13 to S2:18).

Actual perfume olfactory family	Predicted perfume olfactory family (LOO-CV internal validation procedure)							Total	Sensitivity (%)
	Citric-Aromatic	Floral	Floral-Fruity	Floral-Oriental	Floral-Woody	Woody-Oriental	Woody-Spicy		
Citric-Aromatic	3	0	0	0	0	0	0	3	100
Floral	0	5	0	0	0	0	0	5	100
Floral-Fruity	0	0	4	0	0	0	1	5	80
Floral-Oriental	0	0	0	5	0	0	0	5	100
Floral-Woody	0	0	0	1	6	0	0	7	86
Woody-Oriental	0	0	0	0	0	4	0	4	100
Woody-Spicy	0	0	0	0	0	0	4	4	100
Total	3	5	5	6	6	4	5	33	94
Specificity(%)	100	100	100	83	100	100	80	95	

Table 3. Discriminant analysis (sensitivity and specificity data) for perfumes classification according to the storage time-period, based on an E-tongue-LDA-SA model based on the potentiometric profiles gathered by 20 selected sensors (1st array: S1:3, S1:4, S2:6, S1:7, S1:9, S1:12, S1:14, S1:15, S1:19 and S1:20; 2nd array: S2:2 to S2:6, S2:9, S2:12, S2:14, S2:18 and S2:20).

Actual perfume storage time-period	**Predicted perfume storage time-period (LOO-CV internal validation procedure)**			**Total**	**Sensitivity (%)**
	6-9 months	**9-24 months**	**> 24 months**		
6-9 months	10	0	0	10	100
9-24 months	0	7	1	8	88
> 24 months	0	0	15	15	100
Total	10	7	16	33	97
Specificity(%)	100	100	94	98	

Figure Captions (No color for any Figure in printed version)

Figure 1. E-tongue device: geometry and basic dimensions of the array and lipid membranes.

Figure 2. UV spectra of diluted perfume samples with ethanol (1:4000 v/v) in the absorption region from 200-350 nm. (A) Olfactory families of men perfumes: Citric-Aromatic (sample #200204), Floral-Woody (sample #200226), Woody-Oriental (sample #200206) and Woody-Spicy (sample #200201); (B) Olfactory families of women perfumes: Floral (sample #100018), Floral-Woody (sample #100017), Floral-Fruity (sample #100001) and Floral-Oriental (sample #100031); (C) Storage time-periods of Woody-Spicy men perfumes: 6-9 months (sample #200201), 9-24 months (sample #200210) and > 24 months (sample #200221); (D) Storage time-periods of Floral-Oriental women perfumes: 6-9 months (sample #100015), 9-24 months (sample #100031) and > 24 months (sample # 100019).

Figure 3. E-tongue potentiometric signal profiles recorded during the analysis of a standard HCl solution (0.01 M): **(A)** intra-day repeatability assays (10 assays performed in the same day, within a 8-h time-period); **(B)** inter-day repeatability assays (12 assays performed in three consecutive days, being 4 assays carried out per day within a 8-h time-period).

Figure 4. Density distribution (one-dimension plot) for the discriminant function of the E-tongue-LDA-SA classification model based on 12 selected sensors' signals (1st array: S1:3, S1:4; S1:7, S1:14 and S1:20; 2nd array: S2:2, S2:4, S2:6, S2:9, S2:12, S2:13 and S2:17) established for discriminating men and women perfumes, regardless the perfume's olfactory family or storage time-period.

Figure 5. Perfumes' discrimination (2D plot of the first 2 discriminant functions and respective class membership boundary ellipses) according to the main aroma/olfactory family (■ Citric-Aromatic, ○ Floral, Δ Floral-Fruity, □ Floral-Oriental, × Floral-Wo●dy, Woody-Oriental and ▲ Woody-Spicy; being fill symbols used for men fragrances, open symbols for women fragrances and other symbols for men & women fragrances), regardless the perfume type (men or women) and the storage time-period.

Figure 6. Perfumes' storage time-period (□ 6-9 months; ○ 9-24 months; Δ >24 months) assessment (2D plot and respective class membership boundary ellipses) using an E-tongue-LDA-SA classification model based on the potentiometric signals of 20 selected lipid sensor membranes (1st array: S1:3, S1:4, S2:6, S1:7, S1:9, S1:12, S1:14, S1:15, S1:19 and S1:20; 2nd array: S2:2 to S2:6, S2:9, S2:12, S2:14, S2:18 and S2:20), regardless the type of perfume (men or women) and the aroma/olfactory family.

514

515 **Figure 1**

516

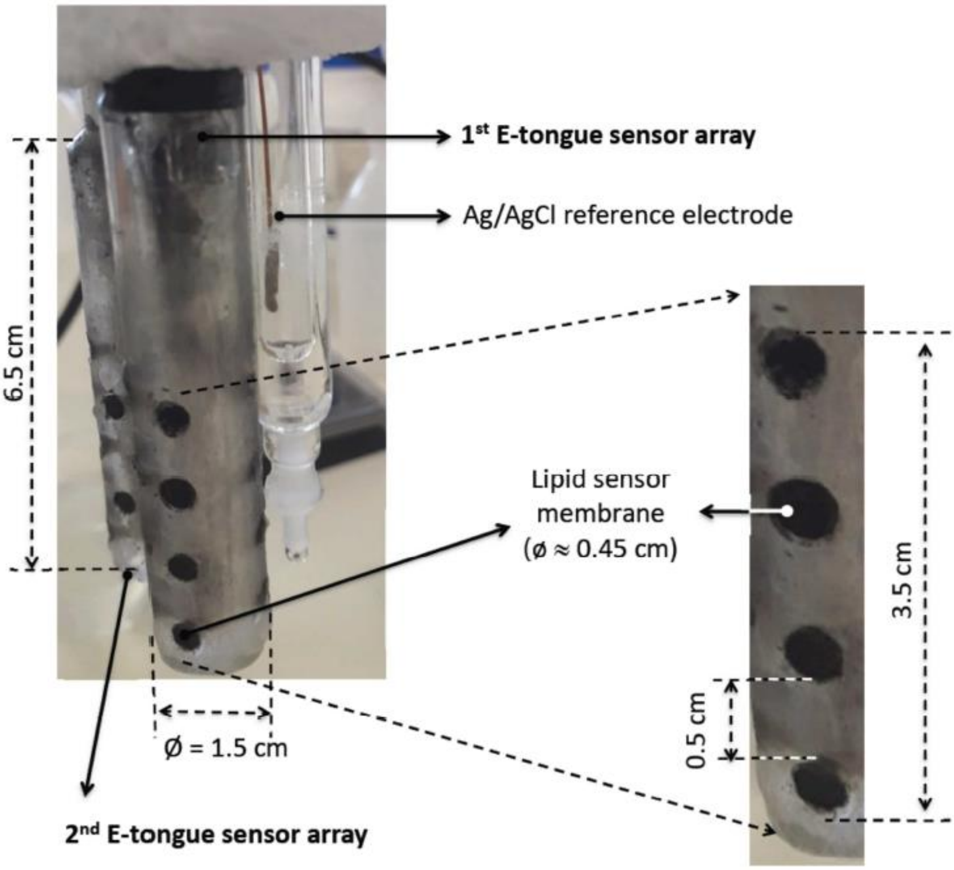

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Figure 3

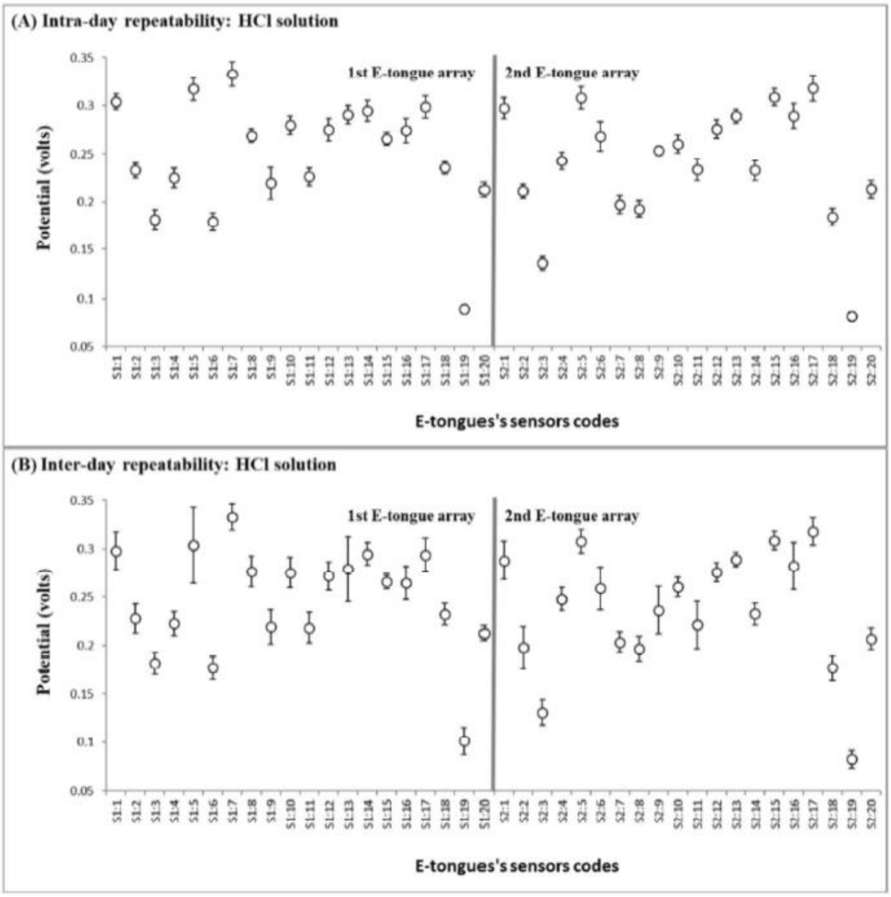

(A) Intra-day repeatability: HCl solution
Potential (volts)
1st E-tongue array
2nd E-tongue array
E-tongues's sensors codes
(B) Inter-day repeatability: HCl solution
Potential (volts)
1st E-tongue array
2nd E-tongue array
E-tongues's sensors codes

Figure 4

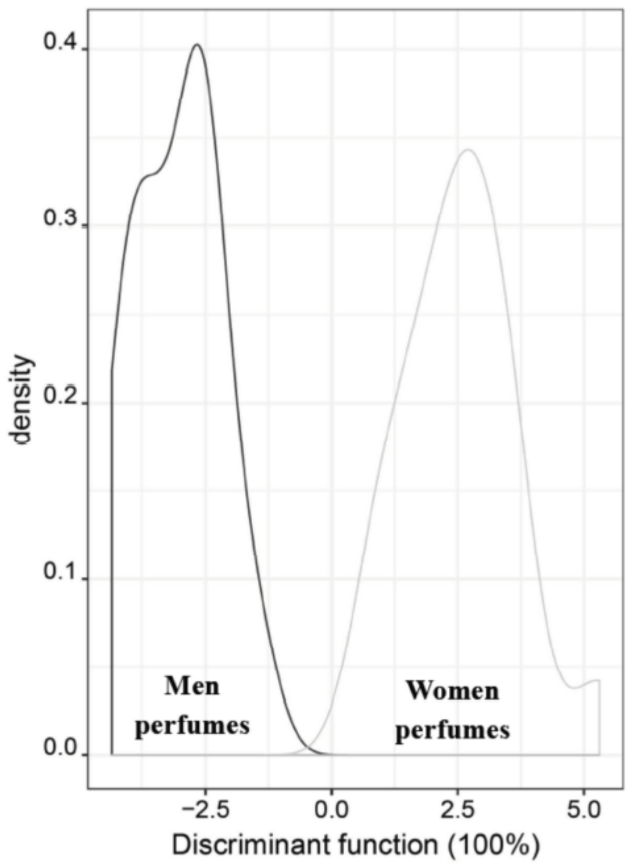

0.4
0.3
0.2
0.1
0.0
density
Men
perfumes
Women
perfumes
−2.5
0.0
2.5
5.0
Discriminant function (100%)

Figure 5

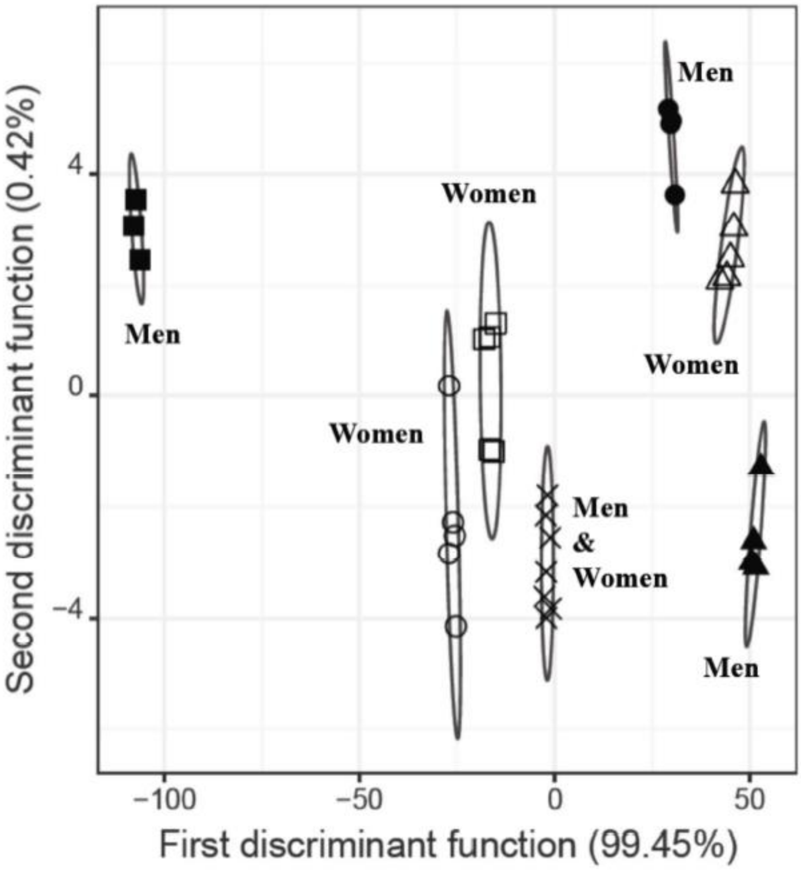
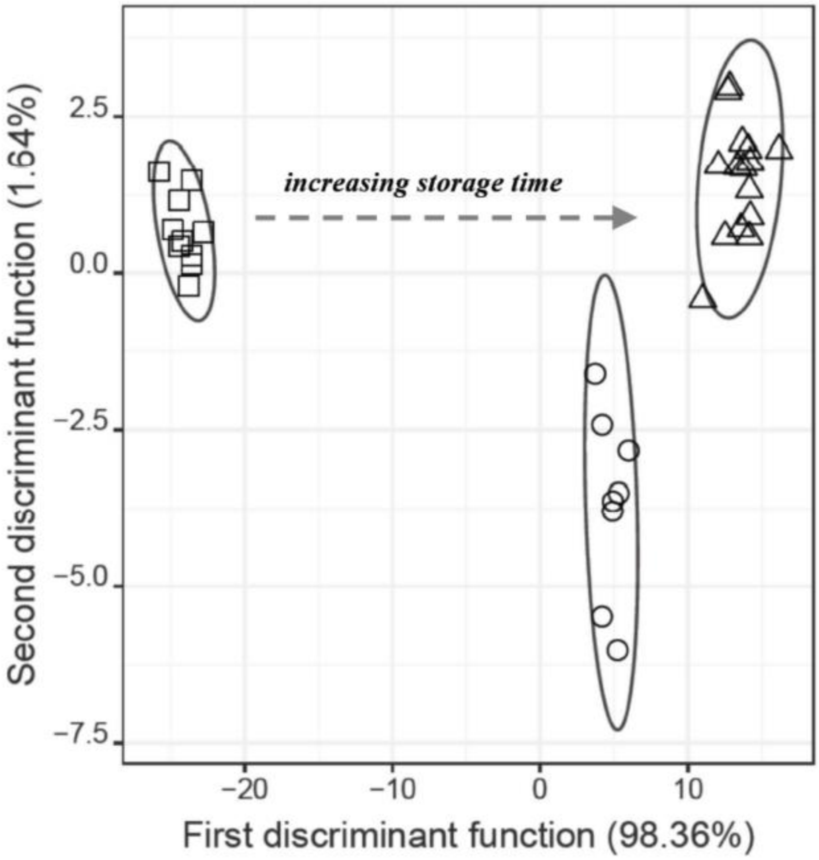

Men
Women
Men
Women
Women
Men
&
Women
Men
Second discriminant function (0.42%)
First discriminant function (99.45%)
4
0
−4
−100
−50
0
50

Figure 6


increasing storage time
Second discriminant function (1.64%)
First discriminant function (98.36%)
2.5
0.0
−2.5
−5.0
−7.5
−20
−10
0
10