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Discrimination and characterization of breath from smokers and non-smokers via electronic nose and GC/MS analysis

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***Abstract*—The objective of this study was to prove the general applicability of an electronic nose for analyzing exhaled breath considering the dependency on smoking. At first, odor compounds from spices (n=6) were detected via the electronic nose and further characterized and classified with gas chromatography/ mass spectrometry to demonstrate the principle ability of the electronic nose. Then, the exhaled breath from smokers and non-smokers were analyzed to prove the influence of smoking on breath analyses with the electronic nose. The exhaled breath was sampled from 11 smokers and 11 non-smokers in a special sampling bag with the mounted sensor chip of the electronic nose. Additionally, solid phase micro-extraction (SPME) technique was established for detection of the specific chemical compounds with gas chromatography and mass spectrometry (GC/MS). For analyses of the sensor signals the principle component analysis (PCA) was applied and the groups were differentiated by linear discriminant function analysis. In accordance to the discrimination between the different spices and between smokers and non-smokers the PCA analysis leads to an optimum accuracy of 100%. The results of this study show that an electronic nose has the ability to detect different changes of odor components and provides separation of smoking side effects in smelling different diseases.**

I. Introduction

ELECTRONIC noses (eNose) were described as an "instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognizing simple or complex odors" [1]. For this reason the application of such devices is or will be useful in food industry, environmental technology and medical fields [2-4] to qualify and observe characteristic odors. In clinical studies electronic noses are applied for medical diagnosis and monitoring due to the fact that effects to metabolism caused by illness lead to distinctive human odors. Therefore, recent investigations observe whether noninvasive biomarkers of different diseases can be determined with eNose. For the detection of volatile organic compounds (VOC) the measurements can be performed on breath, sweat or other excreta from humans.

Concerning the investigation of breath samples, an eNose was used to differentiate patients with lung cancer from healthy controls with an accuracy of 90% [5, 6], A further study showed that breath from patients with asthma includes other VOC than breath from healthy controls [7].

We could show the ability of an eNose to detect changes in the human body odor in consequence of renal dysfunction. All healthy subjects (n=11) could completely be distinguished from patients with renal failure (n=62) applying principle component analysis (PCA) and quadratic discriminant analysis, whereas a correct classification of 95.2% of patients between end stage renal failure (n=42) and chronic renal failure was found. [8].

To enhance identification of odorous samples analytical methods such as gas chromatography and mass spectrometry (GC/MS) are additionally applied [8, 9]. In that way it is possible to separate and specify VOC that electronic sensors cannot provide. Previous studies showed that spices could be differentiated applying eNose with neural networks for data analysis and GC/MS to interpret [10, 11] the findings. Results from chemical examinations revealed that VOC from spices mainly differ in the presence and fraction of alcohols and sulfides.

For the distinction of different human odors analyses of breath samples from smokers and non-smokers were already performed [12, 13]. By means of principal component analysis applied for data evaluation 95% of known smokers were correctly identified [13]. A further GC/MS analyses revealed VOC such as furan, acetonitrile and benzene amongst others to be specific analytes in exhaled breath from smokers [12].

The objective of this study is divided in two parts. In the first part the general applicability of our eNose system should be proved by analyzing different aromatic spices and characterized (validated) via GC/MS. In the second part our eNose system is applied together with GC/MS analysis to investigate the exhaled breath from smokers and non-smokers and to characterize its components.

II. Methods

A. Electronic nose

The applied eNose system is based on a metal oxide gas sensor chip that consists of three thin oxide layers. Each thin oxide layer has different dopings, leading to different sensitivities and selectivities for various gas components. In addition these

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sensitivities and selectivities for various gas components might be changed actively by changing the heating temperature. The interactions between gas molecules and sensor layers include reactions (oxidizing and reducing procedures by volatile gases such as oxygen resp. carbon monoxide) on the sensor surfaces by changing their conductivity. These reactions lead to a change of free charge carrier concentration in the conducting metal oxide.
The sensor resistance (conductivity) is measured within a temperature range of the heating system between 200°C and 400°C.The changes in conductivity are continuously recorded, amplified, processed by a microcontroller and finally stored on a computer. The configuration of the applied eNose system is shown in Fig. 1.

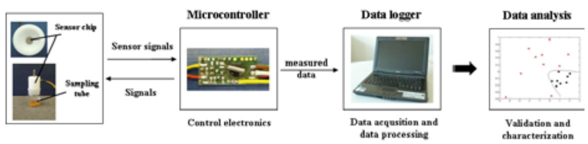

Fig. 1. Scheme of the applied eNose system.

This eNose system was developed in cooperation with our partners UST Umweltsensortechnik GmbH, Jencontrol GmbH, enverdis GmbH and the Department of Internal Medicine I, Friedrich-Schiller-University of Jena.

B. Data Analysis

The three sensor signals were analyzed with principle component analysis (PCA) and linear discriminant analysis (LDA). PCA is a mathematical procedure which reduces the multidimensional data space to a set of main components. The main components are linear combinations of the sensor values which contain the maximum variance and can be obtained in mutually orthogonal dimensions [14]. Hence, the first component has the largest variance and the second component is orthogonal to the first with the next largest variance. To assess dissimilarities in the odor we reduced the complete sensor signals information to its first and second principal component that composed the score plot. For each unique substance or subject a definite color is allocated. In conclusion, the LDA was applied as a statistical analysis technique for separating the odor components.

C. GC/MS analysis

Analyzing the sensor signals from the eNose provides only changes of the sensor property (conductivity) but not any characteristics of the existing odor components. Therefore, GC/MS was established as a reference method for such gas component analyses. For sampling the gas probes the solid phase micro extraction (SPME) method was used. Analyzing the SPME fibers we used a GC/MS from Agilent Technologies.

D. Analysis of spices

In this study six different spices were investigated: curry powder, caraway, nutmeg, garlic, pepper and cinnamon. Each spice was analyzed in a separate vial, on whose top the sensor head was placed. After each spice measurement the sensor was decontaminated to guarantee comparable measuring conditions. The measurement time of each spice took 35min.

For characterization of the spice's odors profile in parallel a SPME-fiber was placed together with the spice in a second sampling vessel.

All measurements were performed within 1 day in the same air-conditioned room to avoid environmental influences.

E. Analysis of exhaled breath in smokers and non-smokers

The study population was recruited from the University of Applied Sciences Jena. For the analysis of the exhaled breath 11 smokers who smoked regularly and 11 non-smokers who smoked occasionally or never were enrolled (Tab. I). At least two hours before starting the measurement teeth brushing, beverage consumption (alcohol, coffee) and cigarette smoking were strictly forbidden. A single exhaled breath was collected in a non-gassing sampling bag (500 ml) which remained closed and is assembled with the sensor chip of the eNose and the SPME fiber. The analysis of this breath sample takes about 35min.
All measurements were performed within 2 weeks in the same air-conditioned room to avoid environmental influences.

TABLE I
CHARACTERISTICS OF THE STUDY POPULATION

Groups	N (male/female)	Age [year]
Smokers	11 (7/4)	31.67 ± 8.72
Non-smokers	11 (8/3)	27.91 ± 3.62

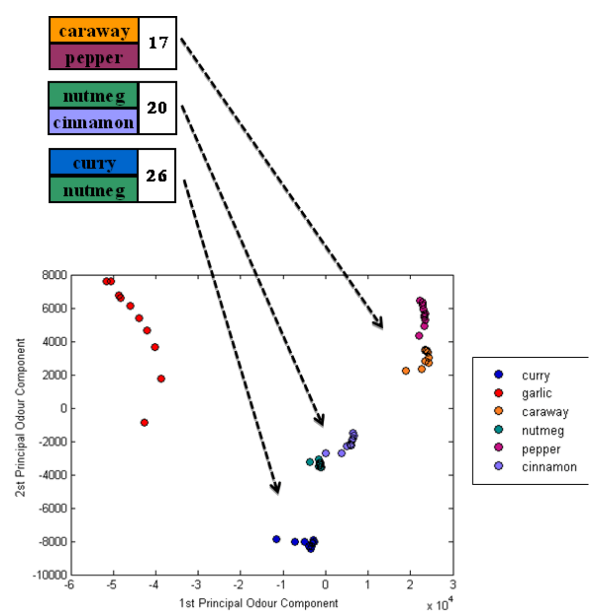

Fig. 2. PCA plot of the 1st and 2nd principal odor components of six different spices.

III. RESULTS

A. Analysis of spices

The GC/MS analysis was implemented to identify the characteristically gas components (flavors) of the spices. For the qualitative analysis of the chromatograms, only peaks with a quality higher than 80% were considered. We could detect components that are unique in one spice and components that were found in more than one spice. As examples, 26 common compounds were found in curry and nutmeg, 17 in caraway and pepper and 20 in nutmeg and cinnamon. The spices' typical odors allow a complete discrimination from all other spices by applying the eNose system. Some spices exhibit clusters which are partly close together with a cluster from another spice (in the cases of various common compounds) but can be separated completely. Fig. 2 shows the PCA result and the distribution of the principal components.

B. Analysis of exhaled breath in smokers and non-smokers

The GC/MS analysis of the exhaled breath led to a total of 291 relevant VOC. Considering the quality level (higher than 80%) the peak geometry and contribution to the group separation the number of compounds was reduced to 40 compounds that were examined by NIST mass spectral databases. In comparing exhaled breath of smokers and non-smoker 8 compounds were identified to contribute to differentiate between the groups. These were ketones, alcohols, aldehydes, ester and aromatic compounds. Table II presents all 8 compounds and their significances.

TABLE II
COMPOUNDS MEASURED IN EXHALED BREATH OF SMOKERS AND NON-SMOKERS

Compound	p-Value
Furan, 2,5-dimethyl-	***
2-Butanone, 3-hydroxy-	*
Toluene	**
1-Octene	*
Phenol	***
Octanal	*
Cyclohexanol, 5-methyl-2-(1-methyl	*
Benzophenone	*

P – Value represents the significance of Mann–Whitney U test:
* : p-Value 0.01 – 0.05
** : p-Value 0.01 – 0.001
***: p-Value 0.001 – 0

The PCA analysis of the sensor signals confirms the results of the GC/MS showing clear differences in exhaled breath between the two groups. The results from the PCA are shown in Fig. 3. This PCA plot shows that the exhaled breath of smokers can be separated completely (100%) from the non-smokers applying linear discriminant analysis.

IV. DISCUSSION

This study demonstrated that an electronic nose system might distinguish olfactory compounds of different spices and VOC within exhaled breath of smokers and non-smoker. Some of these results confirm findings from other studies [10-13]. However, we used spices directly from the supermarket in contrast to [10] who analyzed originally berries and leaves which produce (if prepared) a considerable higher aroma density.

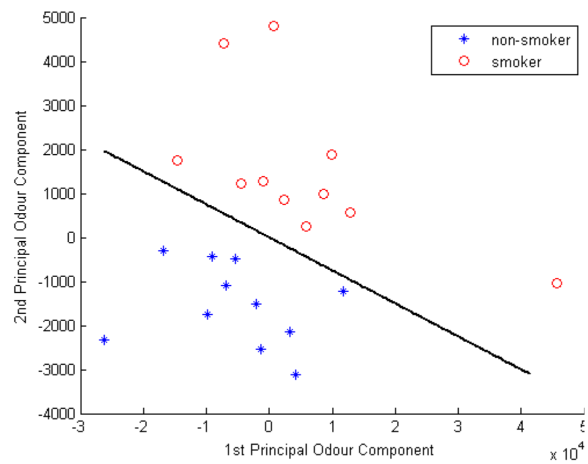

Fig. 3. PCA plot of the 1st and 2nd principal odour components of smokers (circles) and non-smokers (stars).

GC-MS analysis of breath samples from smokers revealed the specific substance Furan, 2,5-dimethyl as one biomarker in the exhaled breath of smokers. This compound has been found already in several studies [12, 15, 16]. In these studies additionally compounds (1,3-butadiene, benzene) were detected which are able to discriminate between smokers and non-smokers. However, these additional substances could not be detected in our study. Some reasons for this can be the selection of another type of column in the used GC, another temperature profile and the use of different fiber coatings. Other effects which might influence the exhaled breath could be e.g. the consumption of different kinds of tobacco, food and beverages.

The analysis of the sensor signals was performed with PCA analysis which is a potential tool for differentiation of great amounts of data. A further study is intended to investigate the separation power between different degrees of tobacco consumption and different kinds of tobacco.

The GC/MS analyses using SPME sampling techniques was proven to be an efficient method for the chemical characterization of the breath compounds. In this way the sensor layers might be optimized to be more sensitive to the desired specific gas components (to exclude the tobacco influence when analyzing diseases).

V. Conclusion

This study investigated the ability of an electronic nose to differentiate spices and to classify the exhaled breath of smokers and non-smokers. For the statistical analysis the principle component analysis in combination with linear discriminant function analysis was performed. An accuracy of separation of 100% was achieved between spices and between breath samples from smokers and non-smokers. Consequently, the smoking habits of patients and controls have to be considered in prospective studies analyzing the breath in diseased patients. Thus, the analyses of exhaled breath via electronic nose could contribute to medical applications such as diagnosing and monitoring of especially pulmonary diseases under the consideration of potentially smoking side effects.

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