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Discrimination of selected fungi species based on their odour profile using prototypes of electronic nose instruments

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ABSTRACT

The paper presents practical application of an electronic nose technique to fast and efficient discrimination of the samples of different fungi species such as: *Penicillium chrysogenum, Cladosporium herbarum, Rhizopus oryzae, Alternaria alternata.* Two prototypes of electronic nose instrument were utilized for investigation of discrimination capability with respect to odour profile of these fungi: the first prototype was based on MOS-type chemical sensors, the second one was based on gas chromatography technique. The fungi samples were prepared as the aqueous suspensions, the headspace of which was subjected to analysis. The data were analysed using the multidimensional methods: PCA, LDA and k-NN. The obtained results confirmed legitimacy of application of the electronic nose technique to identification and discrimination of fungi species. In case of the gas chromatography-based electronic nose prototype correct classification of the fungi species was at the level of 80–100% depending on the classification method employed, in case of the electronic nose prototype utilizing the MOS-type sensors correct classification was at the level of 55–100%.

1. Introduction

Recent years have witnessed a significant increase in the interest in application of artificial senses to chemical analytics. A special place is occupied by an instrument intended to mimic the sense of smell – an electronic nose. It is the instrument enabling holistic composition analysis of gas mixture without its separation and identification of its particular components [7,19,23,3]. The main task for these devices is automatic discrimination of the samples based on differences in a composition of their volatile fraction (odour profile). Intrinsically, each sensor of the electronic nose exhibits different selectivity and sensitivity with respect to particular components of the sample, however, as a whole they generate characteristic chemical image of the gas mixture, so called 'fingerprint'. This task determines analytical usefulness of the aforementioned instruments; they are used mainly for fast, qualitative analysis [13,15,1,21]. The electronic nose instruments often take the place of traditional olfactometers, the devices utilizing human sense of smell for odour measurement, due to significantly shorter time of analysis and possibility of automation [9,10]. Main disadvantages of the e-nose instruments include: multidimensional character of obtained results and necessity of utilization of complicated mathematical-statistical approach for results analysis. Significant interest in this analytical technique resulted in construction of the devices dedicated to particular analytical applications, which are employed in many fields of science and industry (for instance medicine, safety, food industry, pharmaceutical and chemical industry as well as environmental protection) [5,4,2,14,17,6,22]. Currently classic approach to utilization of the chemical sensors in the electronic nose instruments is frequently substituted with a hybrid approach, which takes advantage of the pros of two known and long-used techniques of odour analysis: gas chromatography and electronic nose techniques. Combination of these two made it possible to elaborate the electronic nose instruments, which identify volatile substances using detectors and properly selected chromatographic columns instead of standard chemical sensors matrix. In case of such devices investigated volatile fraction is directed to a set (typically two) of parallel chromatographic columns differing in polarity of stationary phase. The electronic nose technique employs short columns for ultrafast gas chromatography yielding the time of analysis of ca. 1–2 min [24,18]. The obtained chromatograms are analysed with chemometric methods of comparison with the reference, derived from classic version of the electronic nose; however in this case the chromatographic peaks play the role of sensors.

Application of gas chromatography technique allows utilization of the standard chromatographic detectors such as FID, TCD, PID or ECD. An advantage is also a possibility of application of the analyte enrichment techniques, for example HS-SPME – headspace solid-phase microextraction. Gas chromatography technique provides sensitivity at the concentration level of a few ppb (depending on the detector used).

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Indisputable drawback of this type of devices is high cost, as compared to traditional e-nose instruments, and necessity of provision of high-purity gases utilized in chromatographic analysis (carrier gas, hydrogen, air, etc.). High energy consumption of these analytical instruments also constitutes a disadvantage.

Among various aforementioned applications of the electronic nose an important place is occupied by biological and microbiological investigations as well as medical diagnostics [11,8,16,12]. These branches of science are directly connected with human activity; hence introduction of fast methodologies employing the e-nose technique and allowing fast identification of infections or diseases can substantially contribute to an increase in life quality. This paper is an attempt of discrimination of four types of fungi based on the differences in their odour profile. The volatile chemical compounds (alcohols, aldehydes, ethers, esters, etc. produced by microorganisms as a result of their metabolism can be a kind of 'fingerprint' allowing unequivocal identification of the fungi type. The literature scarcely reports on application of the e-nose devices to identification and discrimination of fungi, especially those, which are directly connected with human health and life aspects [20]. That is why the investigation described in this paper was focused on four types of microorganisms, which are utilized by human or exhibit pathogenic character (especially with respect to people with impaired immunological response):

Penicillium chrysogenum (widely utilized in biotechnological processes for production of penicillin),
Cladosporium herbarum (strong allergen),
Rhizopus oryzae (fungi causing mucormycosis),
Alternaria alternata (strong allergen).

The investigations were carried out with two prototypes of electronic nose: the first one possessed a matrix of gas chemical sensors, the second one utilized gas chromatography technique. Obtained data were analysed with three chemometric methods: principal component analysis (PCA), linear discriminant analysis (LDA) and nearest neighbours algorithm (k-NN).

2. Materials and methods

2.1. Materials

The fungi: *Penicillium chrysogenum* (Fig. 1B), *Cladosporium herbarum* (Fig. 1C), *Rhizopus oryzae* (Fig. 1D) and *Alternaria alternata* (Fig. 1A) were cultured on the Sabourauda substrate. The culture of each species was carried out in five heats, in parallel. A sample was collected from each heat. The sample was a 1 cm^2 fragment of the substrate together with mycelium, which then was placed in 10 ml of deionized water and transferred to the test-tubes intended for headspace analysis. The test-tubes were closed with the stoppers equipped with a membrane enabling further analysis. 5 samples of each species were prepared, which gives the total of 20 samples.

2.2. Instruments

A scheme of the test station is presented in Fig. 2. The set-up of two e-nose prototypes connected in parallel consisted of the following elements:

- containers with compressed gases: air and nitrogen (Linde Gas Poland Ltd.),
- hydrogen generator (Packard Model 7525),
- cut-off valves (Z1, Z2),
- rotameter Dwyer,
- three-way valve (Z3),
- six-way valve (Z4) with dosing loop of 500 µl volume,
- electronic nose prototype equipped with a matrix of six sensors of MOS type (TGS 2104, TGS 2106, TGS 2180, TGS 2600, TGS 2602, TGS 2201),
- gas chromatograph Philips PU-4500 equipped with two chromatographic columns (DB-WAX of 30 m length, 0,53 mm internal diameter, 1 µm film thickness and HP-1 of 30 m length, 0,53 mm internal diameter, 1,5 µm film thickness) and two FID detectors,
- electrometer INCO EL-1AN,
- converter Perkin Elmer 888-PE CHROM,

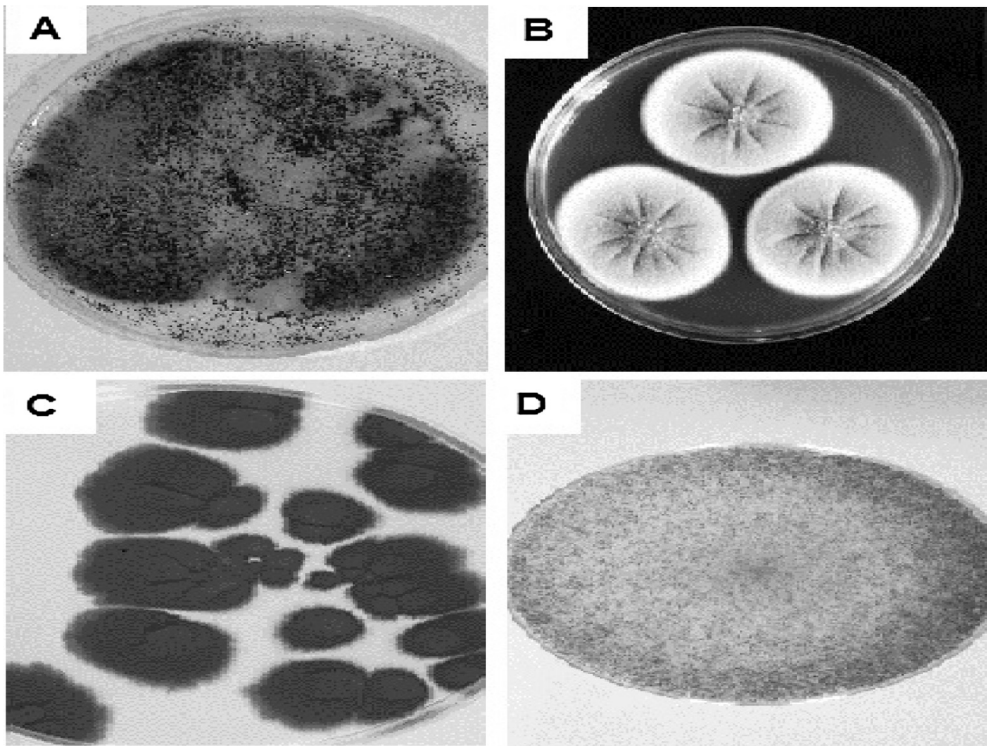

Fig. 1. Species of the fungi. A- Alternaria alternata, B- Penicillum chrysogenum, C- Cladosporium herbarum, D- Rhizopus oryzae.