An Electronic Nose System Sensitive to the Aroma of Ascomycete Tuber

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Abstract: An “electronic nose” based on a low-cost Metal-Oxide sensor-array is developed and tested for the detection of Volatile Organic Compounds (VOCs) of samples of ascomycete Tuber or truffle. Truffles have highly appreciated gastronomical and nutritive merits and they own a variable characteristic aroma depending on their stage of maturation and species. The response of the gas sensors to a variety of truffle samples is monitored. Principal Component Analysis is applied to the extracted feature vectors. The results show that an intelligent odor-discriminating system based on a gas sensor array is possible and can contribute to the identification and classification of truffles.

Keywords: Electronic nose, Olfactory systems, Gas sensors, Data acquisition, Truffle.

1. Introduction

Odor is commonly used to assess quality in the food, beverage and perfume industry. For such applications electronic nose (e-nose) systems have been developed and tested. E-nose systems are based on gas-sensor arrays that produce a characteristic pattern of each odor under test. First, the system is trained using a set of test samples and then pattern recognition software can identify or classify the product under test [1-3]. Electronic olfactory systems have been applied widely to the recognition and classification of natural products and especially to the assessment or discrimination of various qualities of consumable products [4]. Such systems are finding their way to industrial applications for purposes like food safety by identifying contaminants, biochemical composition by identifying the basic constituents and monitoring the effects of food treatment and processing [5]. Studies based on electronic noses have been directed mainly to the determination of meat and fish freshness [6, 7], the effects of meat processing [8], the detection of fruit and vegetable aroma and ripeness [9-11], the maturity of wine [12] and the assessment of winery processes and the discrimination between various blends of coffee [13].

In the literature, various odor sensors have been reported [14]. All sensors are composed of a chemically sensitive material interfaced to a transducer [15]. Chemical species, which represent the inputs, are measured by allowing the analyzed molecules to interact with the chemically sensitive material of the sensor. In solid-state sensors the sensing element is usually a metal oxide semiconductor (MOS) layer formed on an alumina substrate, together with an integrated heater [16, 17]. They have high sensitivity in a number of volatile organic compounds.

In this work the feasibility to implement an electronic nose system sensitive to the aroma of ascomycete Tuber is explored. This fungus is commonly referred to as truffle. Truffles are a relatively rare species of an under-ground mushroom that grows in the roots of trees or bushes. Truffle is the generic name of all the underground fleshy mushrooms belonging to the family Tuberaceae and...
2. Volatile Compounds in Truffles

The fresh truffles contain a number of organic molecules, like alcohols, aldehydes and ketones. More than 200 VOCs have been described from the fruiting-body of natural truffles in the last twenty years [20-23]. The smell of the truffle is actually due to the molecule dimethyl sulfide or CH₃SCH₃, and to other sulfur-VOCs, like CH₃CH₂CH₂SCH₃ and CH₃CH=CHSCH₃. Other truffle compounds are eight-carbon-containing volatiles (C₈-VOCs), predominantly 1-octen-3-ol [20] and androstenol, which is an animal pheromone. The relative quantity of alcohols to aldehydes and ketones varies, but all genotypes contain dimethyl sulfide molecules. A single truffle fruiting body typically contains 20-50 volatile compounds, and the composition of these volatiles might depend on genotypic variability, geographical location and/or maturation stage [20, 24]. When the truffles are kept over a period of time the volatile sulfur compounds escape faster than the other molecules and their release into the air gives these fungi their strong pungent smell. A lot of research has been focused on the composition of VOCs related to truffle aroma and on their unique bioactive compounds, such as androstenol [25] and dimethyl sulfide [26].

3. Measurement System

An array of different gas sensors, each one able to capture different volatile compounds is used in the preliminary stage of our research. A gas sensor array permits to improve the selectivity of a single gas sensor, and shows the ability to classify different odors and quantify component concentrations [27]. A trial system has been designed and implemented based on low-cost solid state MOS sensors. Their sensing element is metal oxide, most typically tin dioxide (SnO₂). In the presence of detectable volatile compounds the sensor’s conductivity increases, depending on the gas concentration in the air. The sensors use a heater in order to increase oxygen adsorption at grain boundaries. Oxygen forms potential barriers which control the sensor’s resistance. In the presence of deoxidizing gases, like organic volatiles, potential barriers fall and the sensor conductivity is increased [6].

Each sensor resistance is measured using a simple measurement circuit formed with a load resistor RL and a buffer. For calibration purposes RL is partly formed by a potentiometer. The load resistance can also be varied to adjust the sensor sensitivity to a particular volatile compound. Fig. 2 presents a typical sensor diagram, where the heater, the sensor electrodes and the load resistance are shown.

In the present implementation, six solid-state gas sensors are used. Five sensors are from the MQ series manufactured by Hanwei Electronics and two from the TGS series manufactured by Figaro Engineering Inc. They are presented in Table 1, along with their corresponding main target gases. However, all sensors are sensitive to a variety of gases, according to their data sheets. A diagram of the sensor array is presented in Fig. 3. The sensor array is sealed in the measurement compartment, where volatiles are injected through air-tubes. A diagram of the system is shown in Fig. 4. The samples to be measured are monitored. A feature vector is extracted based on the response to a variety of truffle specimens is tested against the truffle volatiles. The reported in [19]. An array of six different metal-oxide sensors is tested against the truffle volatiles. The developed sensor array can discriminate truffle odors and quantify component concentrations [27]. A trial system has been designed and implemented based on low-cost solid state MOS sensors. Their sensing element is metal oxide, most typically tin dioxide (SnO₂). In the presence of detectable volatile compounds the sensor’s conductivity increases, depending on the gas concentration in the air. The sensors use a heater in order to increase oxygen adsorption at grain boundaries. Oxygen forms potential barriers which control the sensor’s resistance. In the presence of deoxidizing gases, like organic volatiles, potential barriers fall and the sensor conductivity is increased [6].

In the present study, a low-cost, microcontroller-based electronic nose, able to respond to the presence of truffle specimens is presented, following preliminary results in [18] and reviewing results reported in [19]. An array of six different metal-oxide gas sensors is tested against the truffle volatiles. The response to a variety of truffle specimens is monitored. A feature vector is extracted based on the transient and steady-state response of the MOS sensors. The system can basically support truffle detection and identification in laboratory conditions. Principal component analysis (PCA) indicates that the developed sensor array can discriminate truffle aroma from other fruity odors such as mature apple, as well as alcohol, to which most of the sensors typically respond. It also tends to discriminate between different truffle species, like Tuber borchii, Tuber brumale, Tuber magnatum and Tuber macrosporum. Artificially scented oil and conserved truffles have been tested as well.

As truffles do not have above-ground organs, it is very difficult to find them in nature and in most cases this happens randomly. In order to determine the locations where they grow, various indications are used, as for example the specific trees of the region, the total lack of grass, swarms of yellow flies flying in low altitude over the truffle areas, the light elevation of the soil and the clefts of the ground under which this mushroom grows. But the most remarkable indication of all is that when truffles mature, they emanate an intense smell that can be detected from a long distance by some animals like pigs, squirrels, deers, dogs and bears. During the maturity period, each genus of truffle emits its own smell.

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Fig. 1. Tuber aestivum (left) and Tuber borchii.
placed in the sample compartment, where gas fumes are accumulated. At the beginning of each measurement the air pump is turned on and air is pumped out of the sensor compartment. Then valve 1 is turned to allow gas from the sample compartment to fill the sensor chamber and the air pump is turned off. The measurements reach a steady state in about one minute and a half and the sample is measured for a total of about 3 minutes. Following the sample measurement the air pump is turned on again and a “cleaning” step takes place by injecting dry air in the measurement compartment. A period of 10 minutes is allowed for the sensors to settle to their initial values.

Fig. 2. The measuring circuit used with MOS sensors. A heater voltage and a measurement bias voltage are required.

Table 1. Elements of the sensor array.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Type/Manufacturer</th>
<th>Detectable gases</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGS 822 MOS/ Figaro Eng. Inc.</td>
<td>Organic solvent vapors, acetone, ethanol</td>
<td></td>
</tr>
<tr>
<td>TGS 2602 MOS/ Figaro Eng. Inc</td>
<td>VOCs, odorous gases</td>
<td></td>
</tr>
<tr>
<td>MQ2    MOS/ Hanwei Electronics</td>
<td>General combustible gases-LPG</td>
<td></td>
</tr>
<tr>
<td>MQ3    MOS/ Hanwei Electronics</td>
<td>Alcohol</td>
<td></td>
</tr>
<tr>
<td>MQ4    MOS/ Hanwei Electronics</td>
<td>CH₄, natural gas</td>
<td></td>
</tr>
<tr>
<td>MQ5    MOS/ Hanwei Electronics</td>
<td>LPG, natural gas, town gas</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Diagram of the six MOS sensors array. Potentiometers are used for calibration.

Fig. 4. A diagram of the e-nose system.

Data acquisition is implemented with a PIC16F877 microcontroller by Microchip Corporation, using a six-channel on-chip analog to digital converter with 10-bit analysis. Depending on the noise present in the measurements, the analysis can be reduced to 8-bits. The measurement dynamic range can be adjusted by the potentiometers and in our experiments it is 2.0-5.0 Volts, the lower value corresponding to dry air. Sampling rate is 50 Hz and total measurement cycle is approximately 15 minutes. An RS232 serial interface is used for communication with a host computer. Since sensor response is not very fast, serial communication is adequate for data transmission. A LabVIEW™ host application performs data analysis and displays the results. Signal conditioning, consisting mainly of low-pass filters, is implemented in software and is used for noise suppression. A screenshot of the front panel is shown in Fig. 5.

Fig. 5. Front panel of the host application used for data monitoring and analysis.

4. Experimental Results

Samples of several Tuber species were measured in the present study. All samples were collected from forests in eastern Macedonia, Greece, by a specialized cultivator and truffle hunter. Most truffle specimens were gathered during maturity; however the measured samples of Tuber magnatum are
probably in their early stage of maturation and are relatively small. This truffle was discovered in Greece only recently [28] and is considered of great value since it is generally difficult to find. The bodies of the truffles of different kinds have different colors and textures and their aroma has some characteristic differences. The fruiting body of the truffle was put into the sample compartment for a few minutes, according to the procedure described in Section 3. Typical results are shown in Figs. 6 to 8. The rising part of the curves corresponds to the injection of the truffle fumes into the measurement compartment, while the falling part is monitored at the cleaning step. The most intense responses are given by sensors TGS2602 and MQ3 which are designed to detect general VOCs and alcohol, respectively. TGS822 which is a general-purpose gas sensor and MQ5, which is sensitive to natural gas, also give strong responses in most of the experiments. MQ4 and MQ2 also respond in all experiments, usually giving lower steady state values. The measured quantity is the resistivity of the metal-oxide sensor, translated to quantum states in the y-axis of Figs. 6, 7 and 8. The patterns generated by the VOCs sensors in most other measurements are similar to those described above and differ only in details.

Beside the wild truffle specimens, the sensor-array response to a conserved truffle product was monitored. The odor of small truffle slices pickled in artificially scented olive oil was measured. The sensors respond in a way similar to that of Fig. 8, however the measured values are lower. Again TGS2602 and MQ3 give the higher response, with TGS822, MQ5 and MQ2 following, with lower responses. Finally, sensor MQ4 is rather insensitive to the volatile compounds emitted by the artificially scented truffle samples.

5. Truffle Specimens Classification

The basic characteristics of a typical sensor response to the volatiles of truffle specimens are presented in Fig. 9. In order to extract features, several attributes of the curves are used. The feature extraction procedure is as follows:

1) The baseline \( x(0) \) is subtracted from the sensor response \( x(t) \): \( y(t) = x(t) - x(0) \). In this way, noise or drift is compensated.

2) The upper part of the curve is used in order to extract the steady state value \( st \).

3) The gradient \( \text{grad}_1 \) of the rising edge of the measurement ("on" derivative) is obtained. For this purpose the ratio \( \Delta y_1/\Delta t_1 \) is used, where \( \Delta y_1 \) stands for the 70% of the steady state value \( st \) and \( \Delta t_1 \) is the corresponding time interval.

4) The gradient \( \text{grad}_2 \) of the falling edge ("off" derivative) is obtained, by calculating \( \Delta y_2/\Delta t_2 \), where \( \Delta y_2 \) is \((1-0.7)st\) and \( \Delta t_2 \) is the time interval of the corresponding fall of the response. The above parameters \( st \), \( \text{grad}_1 \) and \( \text{grad}_2 \) are extracted from each sensor measurement. A feature vector with eighteen dimensions is extracted from the six sensors. The features are rescaled using appropriate scale factors. Alternatively, only the steady state \( st \) of the sensor response can be chosen, in order to form a six-dimensional vector. Although both methods achieve some level of classification and recognition of the truffle samples, the gradient-enriched vectors generally produce better results.
It is well known that PCA uses the most expressive features by computing the $m$ eigenvectors with the largest eigenvalues of the $d$-dimensional feature vectors [29]. PCA computes the most meaningful basis to re-express the data-set and in this way it reveals hidden data dynamics, while it suppresses noise and reduces redundancy in the measurement data. In the presented feature space eighteen dimensions were defined, as described above. Three or two principal components are extracted and plotted, while the possible clustering of data according to their corresponding species is examined. Principal eigenvectors are extracted using Singular Value Decomposition (SVD).

In a first experiment, the variance between truffle samples of various species and other fruity samples, like mature apple was studied. Ethanol is also added to the measurement set, because most of the sensors used in our sensor array are sensitive to alcohol. Fig. 10 presents the resulting PCA plot. It is shown that truffles can be basically discriminated from other samples, especially ethanol. It seems that truffle specimens present a variance themselves. This variance is presented in Fig. 11. In this figure, PCA is applied to measurements of three different Tuber species, namely *Tuber magnatum*, gathered at an early stage of maturation, *Tuber uncinatum* and *Tuber macrosporum*. All these specimens are measured fresh as gathered, within three days from the day they were collected. The measurements of the mature apple specimens are also included for comparison. The relative contribution of the three principal components is 60 %, 30 % and 10 % respectively.

Finally, a k-Nearest Neighbors (k-NN) classifier was trained and tested in the detection of truffle specimens under laboratory conditions. It is found that k-NN has a success rate of about 85 % in identifying truffle samples against other odors to which the sensors respond, like mature apple, ethanol, oranges and ethereal oils. Artificially scented truffle products can also be discriminated from fresh specimens. Such tests have not yet been implemented in the field, where truffles are cultivated or grow spontaneously.

6. Conclusions

An electronic olfactory system based on a Metal-Oxide sensor array is designed and tested against truffle volatiles. All the tested sensors respond to the aroma of truffles, with the best response given by Figaro TGS2602 and Hanwei MQ3, MQ5, which are VOCs, alcohol and natural gas sensors, respectively. The system can basically support truffle detection and identification in laboratory conditions. It also tends to discriminate between different truffle species, like *Tuber borchii*, *Tuber brumale*, *Tuber magnatum* and *Tuber macrosporum*. The presented results encourage that a low-cost electronic nose system can be developed and can contribute to the investigation and identification of truffles, especially in the manufacturing industry. Such a device can be used for truffle maturity testing and quality control.
References


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