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ELECTRONIC NOSE IN EDIBLE INSECTS AREA

Martin Adámek, Anna Adámková, Marie Borkovcová, Jiří Mlček, Martina Bednářová, Lenka Kouřimská, Josef Skácel, Michal Řezníček

ABSTRACT
Edible insect is appraised by many cultures as delicious and nutritionally beneficial food. In western countries this commodity is not fully appreciated, and the worries about edible insect food safety prevail. Electronic noses can become a simple and cheap way of securing the health safety of food, and they can also become a tool for evaluating the quality of certain commodities. This research is a pilot project of using an electronic nose in edible insect culinary treatment, and this manuscript describes the phases of edible insect culinary treatment and methods of distinguishing mealworm (*Tenebrio molitor*) and giant mealworm (*Zophobas morio*) using simple electronic nose. These species were measured in the live stage, after killing with boiling water, after drying and after inserting into the chocolate.The sensing device was based on the Arduino Mega platform with the ability to store the recorded data on the SD memory card, and with the possibility to communicate via internet. Data analysis shows that even a simple, cheap and portable electronic nose can distinguish between the different steps of culinary treatment (native samples, dried samples, samples enriched with chocolate for cooking) and selected species. Another benefit of the electronic nose could be its future introduction into the control mechanisms of food security systems (e.g. HACCP).

Keywords: e-nose; edible insect; mealworm; giant mealworm; Arduino

INTRODUCTION
Edible insect is appraised by many cultures as delicious and nutritionally beneficial food. On the contrary, in the western countries, edible insect is treated with disgust, being associated more with dirt than with food **(Yen, 2009; Looy, Dunkel, and Wood, 2014)**. Despite the many benefits (suitable nutritional values, low ecological burden and low economic cost) this commodity is not yet fully appreciated in western countries **(Tan et al., 2015; Adámková et al., 2016)**. For this reason, insect in these countries is used mainly as menu enrichment, delicacy, sought after mainly because of its sensory properties **(Borkovcová et al., 2009)**. Taste of edible insect is plentiful, depends mainly on the environment and food. Another aspect that influences the edible insect sensory attributes is the culinary processing, during which insect takes the taste of the added ingredients. Majority of insect species is almost without taste due to exoskeleton. Taste and smell of insect are made by the pheromones on the body surface, therefore it is not recommended to wash the insect prior to the eating **(Ramos-Elorduy, 1998)**.

Although the sense of smell is one of the oldest human senses **(Hanboonsong, 2010; Ramos-Elorduy et al., 1997)**, it can be insensitive on this account and smell perception can be strongly subjective. Odour perception is based on a series of chemical reactions that occur upon contact with the gas molecules with smell receptors, which induces the flow of electrical signals through the neurons that are processed by the brain. In humans, the surface of the olfactory mucose membrane creates an area of about 5 cm^2, where 1000 different types of olfactory receptors are scattered. On the contrary, the olfactory mucosal surface of dog occupies an area up to 170 cm^2, which proves their superior sense of smell. Humans can distinguish a maximum of 4000 different compounds. To be able to distinguish differences in intensity of the odour, it is necessary to increase the intensity by at least 30%. The sense of smell have the strongest effect on the mental state of a human of all his senses. In higher concentrations, odors can even cause various health problems (nausea, headache), or conversely, a pleasure feeling **(Carlsson and Kalinová, 2005; The eNose Company, 2016)**.

Humans are able to distinguish many smells and perceive their different intensities. However, the sense of smell is often insufficient, and it is necessary to use other methods for separating and increasing the concentration of odors, which are noticed by human (olfactometry). The other groups are tools that monitor the concentration of each aroma completely independently of the human sense of smell – electronic noses **(The eNose Company, 2016; Gopal, 2015)**. These devices are usually equipped with several semiconductor gas sensors, each one sensitive only to a certain type of gas or group of gases. They also contain a database of reference samples which serves for the evaluation of the measured samples. First, you need to "teach" the electronic nose the different smells. More accurate devices may further combine different methods of measuring the concentration of substances in the gas. Electronic noses may be useful not only as a part of safety systems (detection of flammable, hazardous substances for humans), and environmental protection (determination of air pollution), but also in health care, and especially in the food industry **(Carlsson and Kalinová, 2005; Gopal, 2015)**.

In the food industry, a scent that satisfies consumers is one of the most important sensory properties **(Haščík et al., 2013)**. To assess it, knowledgeable people in the field of sensory perception are used **(Kinclová, Jarošová and Tremlová, 2004)**. However, layman evaluates the smell only subjectively, based on his experience and preferences. People commonly use the sense of smell to assess the quality of the food (consumer connects fresh food with a pleasant smell and a deteriorating food with unpleasant smells - odour). However, this form of assessment is not exact, so it is preferable to use a more precise method based on electronic sensors and computer processing – an electronic nose. Using the electronic nose can prevent economic losses and health complications due to spoiled food poisoning.

The aim of this work was to test edible insect samples using simple and cheap prototype of electronic nose, in order to evaluate its ability to identify insect species and stage of processing through simple culinary treatment.

MATERIAL AND METHODOLOGY

Insect

Larvae of mealworm *(Tenebrio molitor)* and giant mealworm (*Zophobas morio*) were used for the analysis. Samples were bought in pet store Krmiva Hostivice.

Insect processing

The insect was raised in optimal condition for development of each species. Both species were fed with brans and a mixture of chopped vegetables and fruits. Prior to the analysis larvae in the last and penultimate instar development (the full length of the body just before pupation) were collected from breeding for subsequent culinary processing and measurement.

Measurement of gas concentrations

Measurement of gas concentrations was done using the experimental prototype of electronic nose (Figure 1), constructed as a simple, cheap and mobile device. The tool itself was based on the Arduino Mega platform controlled by ATmega1280 microcontroller with the ability to store the recorded data on the SD memory card, and with the possibility to communicate with the web server. The measuring cell was equipped with sensors based on the chemo-resistive principle. It uses MQ-6 sensor (Zhengzhou Winsen Electronics Technology Co., Ltd, Zhengzhou, China), which is sensitive especially to the propane or isobutane (300 – 10000 ppm) and less sensitive to alcohol. Other sensors are MQ-3 (Zhengzhou Winsen Electronics Technology Co., Ltd, Zhengzhou, China), which is very sensitive to alcohol (25 – 500 ppm) and MQ-8 sensor (Zhengzhou Winsen Electronics Technology Co., Ltd, Zhengzhou, China), used to detect hydrogen (100 – 1000 ppm). This device should only compare its measurements among themselves for final data evaluation, and precise measurement of absolute concentration of each gas molecules within the smell was not taken into account. Therefore, we used the plugging recommended by the manufacturer, without subsequent calibration for evaluating the absolute gas concentration. US voltage [V] from the individual sensors was converted to a digital value d [-] (voltage of 0V and 5V corresponds to a digital level 0 and 1023) using the internal 10-bit A/D microcontroller converters. These values were then mathematically processed.

Insect culinary processing and smell evaluation

Measurements were done in several stages. After starving for 24 hours the first measurement was done. Subsequently, the insect was killed with boiling water (100 °C) and the second measurement was carried out.

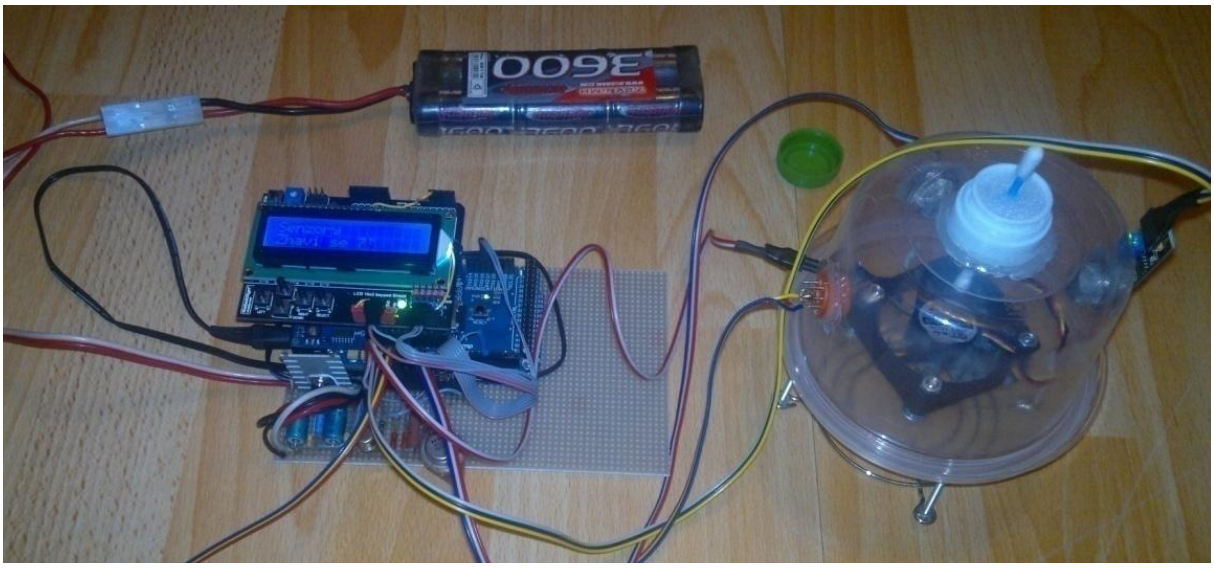

Figure 1 Experimental prototype of electronic nose.

a) TM after killing with boiling water
b) ZM after killing with boiling water
c) TM after drying
d) ZM after drying
e) Chocolate with TM samples
f) Chocolate with ZM samples

Figure 2 Insect samples after each processing phase.

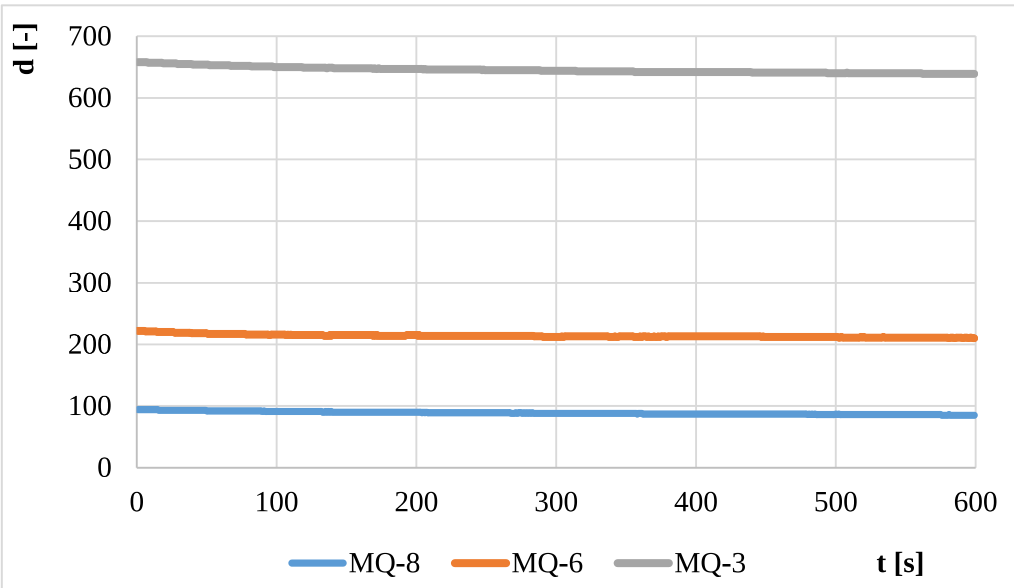

Figure 3 Example of time dependence of data for each of the sensors MQ-8, MQ-6 and MQ-3 in samples of dried larvae of the giant mealworm (*Zophobas morio*).

Table 1 Mean values for each sensor and phase of culinary processing.

mealworm (*Tenebrio molitor*)				giant mealworm (*Zophobas morio*)			
Sample	MQ-8	MQ-6	MQ-3	Sample	MQ-8	MQ-6	MQ-3
live larvae				live larvae			
1	74.4	186.2	590.3	1	72.1	188.3	598.2
2	77.8	200.5	628.6	2	71.9	186.1	593.4
3	74.2	195.1	617.2	3	73.0	184.4	589.5
M	75.5	193.9	612.0	M	72.4	186.3	593.7
±SD	2.0	7.2	19.7	±SD	0.6	2.0	4.3
larvae killed with boiling water				larvae killed with boiling water			
1	100.2	281.5	679.3	1	98.3	287.7	661.3
2	84.0	219.6	640.5	2	91.6	220.5	628.9
3	72.5	199.3	617.5	3	75.5	191.9	599.1
M	85.6	233.5	645.7	M	88.5	233.4	629.7
±SD	13.9	42.8	31.3	±SD	11.7	49.2	31.1
dried larvae				dried larvae			
1	66.9	172.6	579.6	1	84.1	210.8	635.4
2	66.0	172.7	574.8	2	82.7	217.2	639.0
3	66.7	175.2	565.0	3	88.7	213.8	645.2
M	66.5	173.5	573.2	M	85.2	213.9	639.9
±SD	0.5	1.4	7.4	±SD	3.1	3.2	5.0
larvae in chocolate				larvae in chocolate			
1	96.4	267.0	806.1	1	97.2	263.2	800.9
2	93.6	257.6	789.4	2	103.0	263.9	799.5
3	97.9	261.2	799.4	3	97.2	257.0	785.9
M	95.9	261.9	798.3	M	99.1	261.4	795.4
±SD	2.2	4.8	8.4	±SD	3.4	3.8	8.3

After drying at 105 °C for 120 minutes, the third measurement was done. The last measurement was performed after inserting the dried larvae into a bowl and pouring with chocolate for cooking (Nestlé Czech Ltd., Praha). Samples after each processing phase are shown in Figure 2.

Statistical analysis

Each measurement was carried out three times. Data was processed and evaluated using the applications Excel 2013 (Microsoft Corporation, USA) and Gnuplot 5.0: an interactive plotting program **(Williams and Kelley, 2016)**.

RESULTS AND DISCUSSION

The results presented are data from first available measurements of insect properties in different stages of culinary processing gained using the simple electronic nose. Available literature did not present any comparable data.

The data obtained for each of the sensors MQ-8, MQ-6 and MQ-3 was used to create curves to show the dependency on measured time. Time of measurement was set to 600 s. Example of time dependence is shown in Figure 3.

Each curve was used to obtain basic statistical quantities: mean, standard deviation, minimum and maximum. Mean values were inserted into Table 1 and into a 3D graph

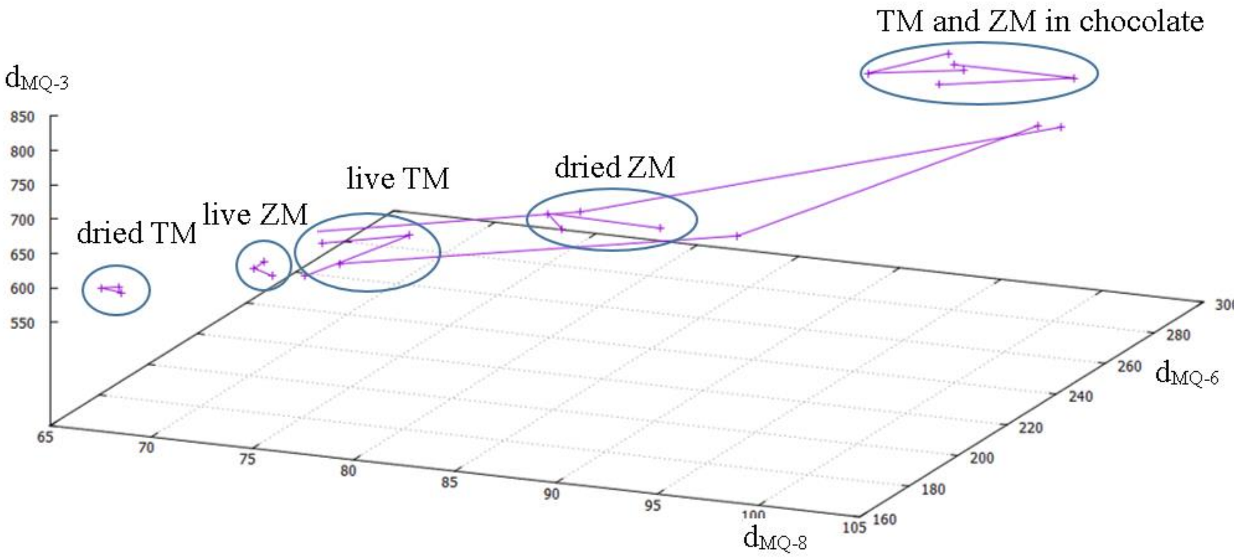

Figure 4 Measured spots graph.

using the Gnuplot application.

The results of our analyses suggest that even the simple electronic nose can recognize the species of edible insects and phase of its culinary processing. Figure 4 shows a graph of measured values (smell) monitored by the each gas sensor for mealworm (*Tenebrio molitor)* and giant mealworm (*Zophobas morio*) after starving – in the native state, after killing (boiling water 100 °C), after drying (105 °C) and after pouring with chocolate for cooking. In the graph we can distinguish different parts (smells) for larvae in the native state, dried samples and larvae in chocolate for cooking. Values for samples in chocolate are very similar in each species. The difference between species is clear in native and dried samples. Values for samples after killing with boiling water are scattered through the graph. This may be due to elevated temperature and humidity, to which the sensors are sensitive and which were not measured and corrected.

It is supposed that by detecting the scent it will be possible not only to evaluate the danger of spoiled food that can cause foodborne illness, but also the various stages of technological preparation and species. This could be used to document the screening of foods to avoid their potential spoofing. The information obtained may serve as a basis for exclusion of food from the food chain. In the case of breeding of edible insects, this may also serve as a primary screening, not only of the species, but also to evaluate the amount of dead individuals in the rearing box.

Using the electronic nose for monitoring the scents of insects is examined in other areas, e.g. the control of volatile substances released from cotton bolls in response to feeding by stink bugs **(Degenhardt, Greene and Khalilian, 2012; Henderson et al., 2010)**. The other area is the control of the degree of insect infestation in wheat, when **Zhang and Wang (2007)** proved it is possible to evaluate the food aging and insect infestation in wheat using the commercial E-nose (PEN2) comprising 10 metal-oxide semiconductor (MOS) sensors. Similarly **Wu et al. (2013)** proved the possibility to detect the presence of red flour beetle (*Tribolium castaneum*) using the electronic nose in wheat with certain humidity, however, their electronic nose did not detect the presence of rusty grain beetle (*Cryptolestes ferrugineus*). Also evaluation of rice infestation by *Nilaparvata lugens* was carried out using electronic nose **(Zhou and Wang, 2011)**. Electronic nose can be used to precisely set up the bait used in boll weevil eradication programs **(Suh, Ding and Lan, 2011)**. Available literature also mentions and demonstrates the possibility of using the electronic nose as an effective tool to monitor the red palm weevil pest (*Rhynchophorus ferrugineus* Olivier) **(Rizzolo et al., 2015)**. Electronic nose also enables the identification of species and sex of stink bugs **(Lan et al., 2008)**.

CONCLUSION

This research dealt with the comparison of the smells of mealworm *(Tenebrio molitor)* and super worm (*Zophobas morio*) larvae; using simple, cheap and mobile device based on the Arduino Mega platform. Although the device is simple, it enables measuring and detection of different stages of edible insect culinary processing. Considering the food safety, electronic nose can be used to monitor the processing of edible insect and to distinguish each species of edible insect in certain phases of culinary processing. Electronic noses can be the source of data used as input for HACCP, applied from agricultural primary production, through culinary processing and selling to the final consumer (protection of the consumer's health).

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Contact address:

Martin Adámek, Brno University of Technology, Faculty of Electrical Engineering and Communication, Department of Microelectronics, Technická 3058/10, 616 00 Brno, Czech Republic, E-mail: adamek@feec.vutbr.cz

Anna Adámková, Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Quality of Agricultural Products, Kamýcká 129, 165 21 Prague 6 – Suchdol, Czech Republic, E-mail: adamkovaa@af.czu.cz

Marie Borkovcová, Mendel University in Brno, Faculty of Agronomy, Department of Zoology, Fisheries, Hydrobiology and Agriculture, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: marie.borkovcova@mendelu.cz

Jiří Mlček, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, Vavreckova 275, 760 01 Zlin, Czech Republic, E-mail: mlcek@ft.utb.cz

Martina Bednářová, Mendel University in Brno, Department of Information Technology, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: bednarova@mendelu.cz

Lenka Kouřimská, Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Quality of Agricultural Products, Department of Microbiology, Nutrition and Dietetics, Kamýcká 129, 165 21 Prague 6 – Suchdol, Czech Republic, E-mail: kourimska@af.czu.cz

Josef Skácel, Brno University of Technology, Faculty of Electrical Engineering and Communication, Department of Microelectronics, Technická 3058/10, 616 00 Brno, Czech Republic, E-mail: xskace09@stud.feec.vutbr.cz

Michal Řezníček, Faculty of Electrical Engineering and Communication, Department of Microelectronics, Technická 3058/10, 616 00 Brno, Czech Republic, E-mail: reznicek@feec.vutbr.cz