Detection of different densities of *Ephestia kuehniella* pest on white flour at different larvae instar by an electronic nose system

Behzad Nouri a, Kobra Fotouhi b, Seyed Saeid Mohtasebi a,*, Amin Nasiri a, Seyed Hosein Goldansaz a

a Department of Agricultural Machinery Engineering, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran
b Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

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**A B S T R A C T**

Warehouse pests reduce the quantity, quality, and health of storage products. These parameters are protected by detecting and controlling related pests. *Ephestia kuehniella* (*E. kuehniella*) causes intense damage to the storage products, such as flour, almond, date and cereals. Thus, diagnosing pest densities for preventing and monitoring them by online alarming systems is important. The present study was designed to detect pest densities in white flour. For this purpose, an electronic nose (E-nose) system was applied by MOS sensors for pest density detection. PCA/LDA multivariate statistical models were built and relevant performances were compared for different instars of larvae. LDA provided the highest prediction abilities on the fifth instar with an accuracy of 90%. According to results, this system had the capability to differentiate between the pests densities, and thus the detection accuracy reflected the ability of the E-nose system for such purposes.

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1. Introduction

Every year million tons of nuts and cereals in warehouses are damaged due to warehouse pests attack. Warehouse pests impose many disadvantages in terms of the quantity, quality and health of stored products, through the presence of alive pests and chemical disposals of their bodies. (Moraglio et al., 2018). On the other hand, for being in the world markets, agricultural products should have obligatory standards that mean products have not been polluted by insects, eggs or larvae (Kader et al., 2001). Flour moth, *E. kuehniella* is one of the pests which causes intense damage to storage products every year. This pest is a cosmopolite and causes damages to warehouse products such as cereals, beans, nuts and etc. Moreover, each adult female pest lays 300–350 eggs during its life, so controlling *E. kuehniella* is one of the most important processes after harvesting such a kind product. According to the economic importance of fighting against pests and troubles due to using chemical pesticides and fumigants, it is required to replace them with friendly environment ways (Salehi et al., 2014). Nowadays, contact insecticides and fumigants are being used to control damages from flour moth. Irrational use of pesticides in controlling insects in warehouses has harmful side-effects like remaining pesticides on saved products and increased resistance to pesticides (Grieshop et al., 2006). Thus, knowing pest density is an important factor to reduce the use of pesticides and chemical materials, especially in flour products. Berlese funnel method (Jian et al., 2016), *Uric acid method* (Leelaja et al., 2007) and *Hidden infestation detector* (Jamshidi et al., 2019) are the methods to detect insect density in food grains. However, these methods are time consuming and destructive. Therefore, it is recommended to apply alternative and healthy methods to control the related pest. An electronic nose (E-nose) is a modern technique with a solid-state sensor-based system, consisting of the data collection unit and the computerized statistical data processing tool. Metal oxide semiconductor (MOS) E-nose is a rapid, objective and intelligent instrument for flavor analysis, which allows volatile organic compounds (VOCs) are assessed directly in their original matrix (Chen et al., 2013; López et al., 2016). The metal oxide sensors of the E-nose can collect the VOCs data and give outputs as the so-called ‘fingerprints’ to represent the characteristic flavor/aroma (Liu et al., 2018).
technology has been applied successfully in various food analysis such as quality control, adulteration and spoilage (Feng et al., 2018; Sung et al., 2014; Zhang and Wang, 2007). Furthermore, the chemical parameter spectrometry has been used to employ a quality classification of grain with some successes (Laopongsit et al., 2014; Stetter et al., 1993). Solid phase micro-extraction (SPME) and E-nose are olfactory-based method techniques for insect detection in stored food grains (Banga et al., 2018). In a similar study (Zhang and Wang, 2007), the E-nose system was applied to detect instars and insect damages incurred by wheat. They successfully classified samples into the five groups of different storage-age wheat and the 15 groups of different degrees of insect-damaged wheat. The use of E-nose reflects its ability to assess and distinguish between crops and food industries such as peach (Huang et al., 2017), peanuts (Raigar et al., 2017; Shen et al., 2016), rice (Abdullah et al., 2016; Timsorn et al., 2017; Zhou and Wang, 2011), milk (Yang et al., 2015), honey (Huang et al., 2015), coffee (Sberveglieri et al., 2011; Severini et al., 2015), saffron (Heidarbeigi et al., 2015), Chocolate (Tan and Kerr, 2018; Valdez and Gutiérrez, 2016) and etc. Some studies have suggested that insect interactions, can produce distinguishable VOCs (Henderson et al., 2010; Lan et al., 2008; Rains et al., 2004). The results of previous studies showed the ability and applicability of the E-nose as a reliable, fast, low cost and non-destructive method. Despite these encouraging examples, E-nose application is still scarce to detect insect population on flour products. Moreover, nowadays storage applications are going to prevent and monitor warehouse pests by online alarming systems. In storage products, the amount of VOCs changes due to the physiological activity of pests. The objective of this study is to develop a reliable method based on E-nose system with the purpose of detecting E. kuehniella densities in storage white flour.

2. Materials and methods

2.1. E. kuehniella growth

An artificial food for flour moth growth was provided by mixing 750 g white flour, 250 g wheat bran, and 30 g yeast. During the study period, temperature and relative humidity with 16/8 lightening diet were 25 ± 1 °C and 65 ± 5%, as an environmental condition, respectively. At the first day, eggs were collected from adult insects situated in funnel and stored in a refrigerator. After collecting more than 2500 eggs, (the least eggs needed for study beginning), they were transferred to sample chambers.

2.2. Sample preparation

The sample chambers were selected with dimensions of 60*50*35 mm (millimeters) and sealed lids. On the lid, a slice of 30*20 mm was cut-off, and a fine mesh was installed so that the larvae could receive oxygen and continue to live without escaping the colony. White flour with an approximate weight of 50 ± 2 g (gram) was poured into 110 chambers. Ten different densities (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50) of the eggs with ten densities were placed in each chamber. Furthermore, ten chambers were considered as control samples without any infection. The study was performed from 0 to 5 larvae instars of E. kuehniella. During the whole process, the samples were kept under identical conditions with temperature, humidity and light period as flour moth growth.

2.3. Developed electronic nose system

The E-nose system consists of three units: (1) the sampling unit, (2) the gas detection system, and (3) the pattern recognition software (Liu et al., 2018). The overall scheme of used E-nose system is shown in Fig. 1. The prepared system includes a power supply, filtration unit, sample chamber, control valve, Diaphragm pump with the rate of 1.2 l/min (liter per minute), sensor chamber and Arduino- Mega2560 board.

For transferring all gas compounds of the sample chamber to the sensory one, only by one test cycle, chamber volumes were matched together. As a result, the sensors could react faster and better to the gas transferred to the sensor chamber. Thus, a cylinder with 6 cm diameter and 8 cm in height, was prepared and six sensors were installed on a regular basis Fig. 2. The sensors sensitivity and their related range are shown in Table 1.

A 4 mm diameter needle and tube were used as sample VOCs transfer probe. Thus, a small hole (size of needle diameter) was created on each lid. After each test, the hole was filled with a tape strip to prevent inside and outside VOCs exchange.

Before the test, the sensors were preheated for 24 h as recommended by sensors datasheet. The procedure of the system Fig. 3 can be expressed as follows: at first, the pump starts and causes to flow air through both carbon-active filter and control-valve to the sensory chamber (step 1). After 40 s (the response of the sensor leading the baseline) when the sensor chamber been purging from unwanted VOCs, flow path of the control-valve changes and direct the sample VOCs to the sensor chamber (step 2). Due to low VOC contents on the headspace, after 10 s pumping, it was cut off for about 30 s, so that sensors were able to do a reliable reaction to the contents (step 3). Finally, in order to discharge the sensor chamber from contents, the pump restarted (for about 40 s) along flow path changing of the control-valve to its original state to direct the filtered air into the sensor chamber (step 4). The tests were taken continuously, thus, step 4 would be step 1 of the next test. During one cycle (80 s), sensory voltages were transferred and saved to the portable computer by the Arduino Mega2560 board with a rate of 10 Hz (hertz).

2.4. Pre-processing data

To perform the analysis of the obtained data from the experiment, at first, data pre-processing should be done. For this purpose, different features such as MAX, MIN, MAX-MIN, and (MAX-MIN)/MIN were examined and the best feature extraction was reported as follows:

\[ X = \frac{\max(x) - \min(x)}{\min(x)} \]

Collected data were processed using the Unscrambler X10.4 software. In order to investigate the data separation, the Principal Component Analysis method was used. Then, linear discriminant analysis (LDA) was applied for evaluating and analyzing the accuracy of the system by sample classification.

2.5. Principal component analysis (PCA)

PCA has been utilized widely as pattern recognition method for analyzing and reducing the dimensionality of numerical data sets in a multivariate data which these data have been collected from sample VOCs by E-nose application (Heidarbeigi et al., 2015; Huang et al., 2015; Xu et al., 2016). PCA summarizes the data based on their similarities and differences by dimension reduction of data without losing much information (Janshidi et al., 2019; Moon et al., 2016). In this study, PCA was applied to obtain similarities among different flour moth densities so the dimension was reduced from six components as the number of variables to two principal components, while the most of the original information in the data set was kept.
2.6. Linear discriminant analysis (LDA)

LDA is known to be an appropriate method to obtain the capability of the sensory arrays in detection (Zhang and Wang, 2007). Compared with the PCA method, LDA can take into the distribution of points in same category and distances among them (Xu et al., 2016), by increasing the inter-class variance in relation to intra-class variance (Ghasemi-Varnamkhasti et al., 2018). Therefore, the specific value of the LDA is analyzed to obtain the relationship between the data factors in the model. In this study, the Unscrambler X 10.4 (CAMO ASA, Oslo, Norway) software was utilized to carry out PCA and LDA analyses.

3. Results

3.1. PCA analysis

Fig. 4, shows the results of the PCA of different densities and instars of E. kuehniella in white flour.

These densities start from 0 (control) up to 50 eggs with a sequence of 5 densities, which were characterized by a unique color and sign for each one. In Fig. 4a. It is clear that the control samples placed far away from the other merged samples.

In addition, according to the first and second components of PCA results and their sum (Table 2) The range of data discrimination percentage varied from 89 to 98 percent. While changes in the second instar reached the lowest level.

3.2. LDA analysis

Fig. 5, shows the results of the LDA analysis for the density of 5 sequences from egg infection up to the fifth instar period.

According to PCA analysis results, the highest and lowest detection accuracy were obtained at the fifth and second instars, respectively. While the lowest accuracy of 50% was obtained by the LDA method for the second instar. Next, the detection accuracies of 69%, 77%, and 90% were reached for the third, fourth and fifth instars, respectively.

4. Discussion

According to Fig. 4, the presence of egg samples and effect of the sensors sensitivity to constant VOCs (Table 1.), caused to merge egg samples. Because the similar VOCs of flour existed due to the physiological activities of the larvae with different densities. Except for the second instar, the first two components of PCA varied from 94% to 98%. Therefore, further results can be obtained with high certainty by LDA or other detection methods. In addition to increasing the discrimination percentage of the PCA (out of second instar), the distance between the different observed densities changed with the passage of time and larvae instar. Increasing physiological activities caused more VOC changes between larvae and surrounding areas. Thus, each density was classified easily from others. Moreover, the results of LDA method showed that as the VOCs emitted from the E. kuehniella larvae has caused more differentiation between the density of the second, third and the other instars. Therefore, according to the sensors sensitivity Table 1., some VOCs indicated hydrogen, alcohols, methane (decomposes and decays from organic materials) and natural gas were probably

![Fig. 1. Overall schematic view of the electronic nose (E-nose) system.](image)

![Fig. 2. Cylindrical sensor chamber with 6 metal oxide semi-conductor sensors.](image)

Table 1

<table>
<thead>
<tr>
<th>Name</th>
<th>General description</th>
<th>Reference parts-per-million (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQ-2</td>
<td>LPG, Propane and Hydrogen</td>
<td>300-10,000</td>
</tr>
<tr>
<td>MQ-3</td>
<td>Alcohol vapor</td>
<td>10–300</td>
</tr>
<tr>
<td>MQ-4</td>
<td>CH₄, Natural gas</td>
<td>200-10,000</td>
</tr>
<tr>
<td>MQ-5</td>
<td>LPG, methane, coal gas</td>
<td>300-10,000</td>
</tr>
<tr>
<td>MQ-6</td>
<td>LPG, ISO-butane, propane</td>
<td>200-10,000</td>
</tr>
<tr>
<td>MQ-8</td>
<td>Hydrogen (H₂)</td>
<td>100-10,000</td>
</tr>
</tbody>
</table>
being found in white flour containing *E. kuehniella* pest. In summary, an electronic nose (E-nose) system was applied to detect different densities of *Ephesia kuehniella* (*E. kuehniella*) pest in white flour. Principal component analysis (PCA) and Linear discriminant analysis (LDA) methods detected the densities with data separation of 98% and an accuracy of 90%, respectively. According to results, the E-nose system detected different densities of pests with control samples by using PCA and LDA methods. Volatile organic compounds (VOCs) changes between larvae and surrounding areas had a direct relation with physiological activities.

### Table 2

First two component and their sum obtained from PCA results related to various instars.

<table>
<thead>
<tr>
<th>Age</th>
<th>PC1%</th>
<th>PC2%</th>
<th>SUM%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>85</td>
<td>9</td>
<td>94</td>
</tr>
<tr>
<td>First</td>
<td>89</td>
<td>8</td>
<td>97</td>
</tr>
<tr>
<td>Second</td>
<td>72</td>
<td>17</td>
<td>89</td>
</tr>
<tr>
<td>Third</td>
<td>61</td>
<td>33</td>
<td>94</td>
</tr>
<tr>
<td>Forth</td>
<td>70</td>
<td>28</td>
<td>98</td>
</tr>
<tr>
<td>Fifth</td>
<td>87</td>
<td>9</td>
<td>96</td>
</tr>
</tbody>
</table>

![Fig. 3. System procedure diagram with pump flow work.](image)

![Fig. 4. PCA results for different instars: (a) egg, (b) first instar, (c) second instar, (d) third instar, (e) fourth instar and (f) fifth instar.](image)
The results showed the ability of the E-nose application with the aim of detecting different *E. kuehniella* densities with high accuracy in comparison with other assessment methods. This study provided a new perspective on the use of the E-nose system in the qualitative and quantitative assessment of flour products especially at storage time as a non-destructive, reliable and fast method. A comparative study can be done to analyze the effects of lesser densities of pests on the released VOCs of stored products like white flour.

**Appendix A. Supplementary data**

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jspr.2019.101522.

**References**


Leelaja, B.C., Rajashekar, V., Rajendran, S., 2007. Detection of eggs of stored-product


