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Prediction of *Sitophilus granarius* infestation in stored wheat grain using multivariate chemometrics & fuzzy logic-based electronic nose analysis

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ABSTRACT

Insect infestation is an alarming concern in stored wheat grain, accounting for losses in quality as well as quantity. Infested wheat with *Sitophilus granarius* for four different storage periods with various degrees of infestation were evaluated through E-nose. A fuzzy logic based approach was undertaken to screen the relatively more sensitive sensors towards infestation. Multiple linear regression (MLR) models were further used to predict the uric acid and protein content as infestation indices. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) efficiently classified the most infested wheat grain samples among the stored samples. The developed MLR models were found best fit with higher regression co-efficient (R^2) value and lower root mean square error (RMSE). The E-nose sensor responses closely predicted the uric acid ($R^2 = 0.958$; RMSE = 1.401) and protein content ($R^2 = 0.978$; RMSE = 0.275). The findings of this study will open up a convenient, rapid yet nondestructive approach for quality determination of insect infested wheat grains at various stages during the storage.

1. Introduction

Wheat is one of the most significant food crop consumed globally as staple food. It is a spring harvest crop and can be stored over a year at 12–13% (wb) moisture content, however, it requires proper storage conditions and environment to restrict insect infestation, rodent and fungal infestation, for retaining acceptable quality. Storage is the obligatory step in the wheat supply chain. Losses due to insect infestation during storage shares a major part (10–30%) of the post production losses of wheat grain (Paliwal et al., 2004), worth about US $1 trillion per year globally (Kumar and Kalita, 2017). Along with the quantitative loss, the metabolites produced by the insects such as excreta and by-products of protein metabolism, evolves nuisance odor. Also, during storage, wheat develops different volatiles, which with changing time, is called as storage odor. The volatiles generated during storage are generally aliphatic alcohols, amine compounds, ketones and other carbonyl compounds in traces (Zhang and Wang, 2007). The principal off odor producing volatile in *Rhyzopertha dominica*, *Tribolium castaneum, and Cryptolestes pusillus* infested wheat are esters, aldehydes or hydrocarbons (Laopongsit et al., 2014). With increase in storage duration, the medium-polarity volatiles also increase with a simultaneous decrease in the low-polarity volatiles (Olsson et al., 2000). Due to the production of these off odor in the stored grain, there is a reduction in the consumer's acceptance and export value.

The routine methods of chemical analysis such as Berlese-funnels test (Neethirajan et al., 2007), uric acid content analysis (FSSAI, 2012) are time-consuming, labor intensive, costly and sometimes hazardous also. The perception of volatile compounds emerging from the food material by a human nose for evaluation of quality can be risky as it may contain allergic compounds. Many researchers have developed methods for quality control of wheat grain using SPME-GC or headspace coupled with GC (Borjesson et al., 1992; Olsson et al., 2000; Laopongsit et al., 2014; Niu et al., 2015), but, due to the involvement of complexity, high operational cost and tough sample preparation methods, GCMS lacks wide acceptance in regular practice. Nowadays, rapid methods for quality analysis is paid more attention (Verdú et al., 2016; Mishra et al., 2017; Srivastava et al., 2018a; 2018b). Electronic nose (E-nose) is reported as a specific biochemical marker for early spoilage detection with adequate reproducibility (Zhang et al., 2012; Lippolis et al., 2014), which reduces sample preparation complexity and analysis time (Ridgway et al., 1999; Magan and Evans, 2000).

E-nose consists of various types of electrochemical gas sensors, which enables recognition of complex odors. Different types of sensors are used for commercial E-noses viz. metal oxide semiconductors (MOS), MOS field effect transistors (MOSFET), piezoelectric crystal sensors, etc. (Schaller et al., 1998), which has partial specificity to

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volatile organic compounds. E-nose has been widely used for quality assessment of various food products such as fruits & vegetables (Brezmes & Llobet, 2016), meat (Kodogiannis, 2017), fish (Güney & Atasoy, 2015), garlic (Trirongjitmoah et al., 2015), medicinal and aromatic plant products (Kiani et al., 2016) in the last decade. Stored wheat grain of five storage periods and 15 levels of *Rhyzopertha dominica* infestation were evaluated by Zhang and Wang (2007) using an E-nose and classified into five groups starting from severely damaged to unremarkable damaged with control. However, they didn't calculate the infestation indices. They created the level of infestation by mixing insect infested grain with fresh grain, which should not be the ideal case. Wu et al. (2013) studied the feasibility of E-nose in determining infestation in wheat due to various insects and reported that it could only detect the presence of red flour beetle with high infestation level and failed to detect the presence of rusty grain and herbst insect (*Tribolium castaneum*). Recent researches are evidence that E-nose can be used as a tool for detection of insect infestation in stored cereal grains by analyzing the volatiles emerged by the metabolic activity of insects; however, the work area is still limited. The presence of multiple MOS sensors during analysis results in poor discrimination efficiency due to highly precise nature of the MOS sensors (Upadhyay et al., 2017a). Therefore, it is essential to screen the most responsible sensors before analysis, which can be accomplished by fuzzy logic analysis of the MOS sensor responses. Fuzzy logic is a computational approach based on 'degrees of truth' was found to be applied for sensory analysis of coffee products (Lazim and Suriani, 2009), dahi powder (Routray and Mishra, 2012), high pressure processed mango pulp (Kaushik et al., 2015) and also for sensor screening (Upadhyay et al., 2017b) in scientific literatures. Therefore, the aim of this study was to develop an efficient and reliable classification method using fuzzy screened E-nose sensors for determination of granary weevil infestation in stored wheat grain. The study will be helpful for development of the software that would automatically receive the signal from selected sensors connected with the afore-mentioned infestation. This approach will be helpful for exporters, graders and grain processing & storage industries for rapid routine quality control of stored wheat to maintain the consumer's acceptability.

2. Materials and methods

2.1. Sample preparation

Freshly harvested wheat grains (var. Sharbati), procured from the local market of Kharagpur, West Bengal, India, were cleaned and any foreign matter in it were removed by a pneumatic separator before the experiment. The wheat grains were exposed to −20 °C for one week to eliminate unwanted proliferated insects. For experiment, 5 kg of wheat kept in a container were infested with *Sitophilus granarius* (SG) insects, collected from entomology department of GB Pant University of Agriculture and Technology, Pantnagar, India. To produce bulk culture of insects, 5 kg clean grain were taken and insects were added @ 10 adults/kg and stored in an incubator, set thermostatically at 27 ± 1 °C and 65 ± 5% relative humidity (RH). The insects required for the study were taken from the bulk culture grown previously. The clean wheat grains were conditioned up to desired moisture level viz. 10, 12, 14 and 16% wb by adding calculated amount of water and conditioned in moisture lock compartment of a refrigerator. The conditioned grain samples were taken for artificial infestation of the insects for production of different levels of infested samples. The detail experimental design is provided in Table 1.

2.2. Grouping of infested samples

Wheat grains were distributed into two sample sets viz. training set (T) and storage set (S). The E-Nose was trained using duplicate set of highly infested wheat grains to acquaint with the volatile fingerprints of the stored wheat grains. The training set samples were named as T_1 to T_{15} indicating different levels of infestation. Moisture conditioned samples were infested with 5, 10 and 15 number of SGs per 100 g, and zero insect infestation was taken as control. All the infested samples were stored in small polyethylene terephthalate (PET) containers closed with lids. Holes having 3 cm diameter were created over the lids of the container and cover with stretched 100 μ mesh cloth for ventilation. The containers were stored in an incubator set at 27 ± 1 °C and 65 ± 5% RH for different storage periods, viz. 45, 90, 135 and 180 days. Fungal infested samples were discarded as the volatile organic compound produced due to fungi will interfere with the final odor of the stored wheat samples. A total of 53 numbers of stored samples of different infestation level were taken for volatile analysis in E-nose. About 2 g of the wheat samples were placed in screw cap vials for headspace analysis and rest were packed in LDPE pouches and stored at −20 °C deep freezer to stop the growth. For validation of the model, unknown samples were collected by imitating the infestation using a known number of insects stored for certain duration.

Table 1
Experimental design for production of different degrees of infestation.

Fixed parameters	Independent variables	Levels	Dependent variables
Insect: *Sitophilus granarius*	Moisture content (%wb)	10, 12, 14, 16	MOS sensor references
Wheat variety: Sharbati	Number insects per 100 g	0, 5, 10, 15	
Storage condition: 27 °C and 65% relative humidity	Storage duration (days)	45, 90, 135, 180	

2.3. E-Nose instrumentation

Fox 4000 E-nose system (Alpha MOS, Toulouse, France) was used which detects the quality changes in the stored wheat grain samples by measuring the volatiles generated. The screw cap vials were placed in the sample tray from where the automated HS 100 auto-sampler picked the samples to the heater for volatile organic compounds (VOCs) generation. The syringe was heated to 60 °C before injection. The VOCs were injected in the Fox 4000 system by the auto-sampler at a flow rate of 150 mL/min. The E-nose system consists of an array of 18 MOS sensors and an electronic data acquisition system. The MOS sensors are of three types depending on the class of volatiles that are L-type (LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTI, LY2/gCT), sensitive to aldehydes; P-type (P10/1, P10/2, P40/1, P30/1, P30/2, P40/2, PA2), susceptible to non-polar hydrocarbons, methane, propane, ammonia and ethanol and T-type (T30/1, T70/2, T40/2, T40/1, TA/2), sensitive to alcoholic and amine compounds (Chatterjee et al., 2014). Post injection, a carrier gas was blown to the sensor chambers to return to the baseline of the sensor response. The relative change in the sensor resistance (ΔR/R) value indicated the reaction of a sensor to the stored wheat grain and the value was monitored for 120 s followed by the dormancy period to allow the sensor to return to its baseline. Odor profiles for stored wheat samples of different duration and infestation levels were generated using the E-nose system.

2.4. Sensor screening using fuzzy logic analysis

Based on the innumerable literature reports by various researchers on fuzzy logic analysis of sensory data, The MOS sensor response (ΔR/R) of E-nose are considered as the sensory score for fuzzy interpretation (Upadhyay et al., 2017a,b; Mukhopadhyay et al., 2013; Singh et al., 2012). The absolute value of the maximum ΔR/R values of the 18 MOS sensors were subjected to fuzzy logic analysis using MATLAB 2013a (The MathWorks, Natick, MA, USA) and then the most sensitive sensors