Electronic Nose Based On Metal Oxide Semiconductor Sensors as an Alternative Technique for the Spoilage Classification of Oat Milk

A. Deswal, N. S. Deora, H. N. Mishra

Abstract—The aim of the present study was to develop a rapid method for electronic nose for online quality control of oat milk. Analysis by electronic nose and bacteriological measurements were performed to analyze spoilage kinetics of oat milk samples stored at room temperature and refrigerated conditions for up to 15 days. Principal component analysis (PCA), Discriminant Factorial Analysis (DFA) and Soft Independent Modelling by Class Analogy (SIMCA) classification techniques were used to differentiate the samples of oat milk at different days. The total plate count (bacteriological method) was selected as the reference method to consistently train the electronic nose system. The e-nose was able to differentiate between the oat milk samples of varying microbial load. The results obtained by the bacteria total viable counts showed that the shelf-life of oat milk stored at room temperature and refrigerated conditions were 20hrs and 13 days, respectively. The models built classified oat milk samples based on the total microbial population into "unspoiled" and "spoiled".

Keywords—Electronic-nose, bacteriological, shelf-life, classification.

I. INTRODUCTION

THE evaluation of microbiological quality of foods is very significant for determining the shelf-life of food products. Microbial spoilage also alters the sensory profile of foods and hence plays a very significant role in consumer acceptance as well [1]-[3]. In the past, various instrumental techniques like headspace or dynamic headspace gas chromatography coupled with mass spectrometry (GC–MS) and solid-phase micro-extraction sampling followed by GC–MS have been utilized for objective evaluation of sensory profiling of foods [2], [4].

In the recent years, the e-nose has been successfully used for recording the aroma profiles of foods [5], [6]. The microbial spoilage determination methods consist of plating techniques which are time-consuming. Hence, there is need for methods for microbial detection and identification which are faster, more convenient and more sensitive.

The electronic nose has been defined as an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odors [9]. The electronic nose system parallels the human olfactory system as it assesses the mixture of volatiles released from a sample, while other instrumental methods usually separate the aroma into its individual components. The sensors of electronic noses are mostly based on conductive polymers, metal oxides, or surface acoustic waves utilizing the piezoelectric effect [10].

The aim of this work was to investigate the application of a metal-oxide sensors commercial electronic nose in shelf life of oat milk during storage at different temperatures.

II. MATERIALS AND METHODS

A. Sample Preparation and Sampling

Preparation of oat milk is based on an enzymatic process which involves simultaneous gelatinization & liquefaction of rolled oats at 70-75°C, through an enzymatic reaction [11]. Soluble solids concentration of oat milk was 20°Brix, and pH value was 6.65. The milk thus prepared was filled in sterile bottles under aseptic conditions and stored at room temperature and refrigerated conditions. Measurements were performed every 4th hour for sample stored at room temperature and every third day for samples stored at refrigerated conditions up to 15 days. For every measurement, two replicate samples were withdrawn to undergo microbiological, electronic nose, sensory and chemical analysis.

B. Microbiological Population Enumeration

Total plate count was performed according to the method given in FSSAI Manual of methods of analysis of foods. One gram of oat milk was transferred aseptically to dilution tubes in 0.1% (wt/vol) peptone water and serial dilution was done. Pour plate method was employed for plating and the petriplates were incubated at 35°C for 48±2 hours.

C. pH Measurement

The pH was measured using a Systronics μ-pH System-361 according to the standard method [12].

D. Sensory Analysis

The odor of oat milk samples was evaluated by a 25-member untrained sensory panel consisting of students, from the Agricultural & Food Engineering Department, IIT Kharagpur. A difference-from-control test was performed each time the other analysis was done. The samples were presented to the panelists under daylight conditions.

The samples were kept at room temperature for 30min before the panels. Panelists rated the differences in a 0 to 4

Aastha Deswal and Navneet Singh Deora are with Indian Institute of Technology (IIT), Kharagpur, West Bengal, India (Phone: 09635452961; e-mail: deswalad@gmail.com, navneetsinghdeora@gmail.com).
Hari Niwas Mishra is with the IIT, Kharagpur, West Bengal, India (Phone: +91-9434414288; e-mail: hnm@agfe.iitkgp.ernet.in) .

scale (0 = no difference and 4 = very different) [1].

Parameter	Measurement
Quantity of sample in the vial	1ml
Headspace generation time	300s
Headspace generation temperature	50°C
Syringe volume	2.5ml
Injected volume	1ml
Injected speed	1ml/s
Acquisition time	120s
Runtime	1200s
Headspace generation time	300s

E. Electronic-Nose

The electronic nose used for this study was a Fox 4000 (ALPHA MOS, Toulouse FR) with three Metal Oxide Sensors chambers equipped with 18 sensors. The carrier gas was pure air at a pressure of 5 psi. The samples were injected to the Fox system with an autosampler HS100 (Alpha MOS, Toulouse FR) from 10ml sealed vials with flowrate of 150 ml/min. The other parameters of e-nose set for experiment are shown in Table I.

The concept of the Electronic Nose can be described like the sensory perception of human being; the Electronic Nose needs to be trained with correctly selected sample set to ensure good recognition and reproducibility. The E-nose consists of the following major components: Autosampler, injection system, temperature controlled sensor chamber with sensor array, mass flow controller and a computer. The autosampler injects a sample into the instrument through the injection port. Gaseous compounds (samples) pass through the chambers that contain 6 sensors each. The carrier gas pushes the headspace injected and every sensor reacts with the chemical compounds in the air flux. Chemicals will be adsorbed on each sensor that will modify the resistivity, continuously measured by the instrument. Sensors resistivity is monitored for 120 seconds. At the end of the analysis time, the system requires a period to allow a return to the baseline for each sensor.

III. RESULT AND DISCUSSION

A. Microbial Flora Evolution

The bacteria populations have been counted during the oat milk analysis [7], [8]. As expected, growth is a lot faster at ambient temperature. It was found that as the bacterial population increases, pH decreases in both the cases. This can be attributed to the uncontrolled fermentation resulting from action of bacteria on nutrients present in food, converting carbohydrates to acid.

B. Electronic Nose Response

1. Sensor Response Analysis

In Fig 1, the response of all the 18 sensors to volatiles generated is shown. The X coordinate corresponds to the acquisition time and Y coordinate corresponds to the response intensity. The response intensity on the ordinate axis

corresponds to the rate of change of the relative resistance of the different sensors [(R0-R)/R0], where R0 is the resistance value at t=0, and R is the resistance value with the changes in time [13].
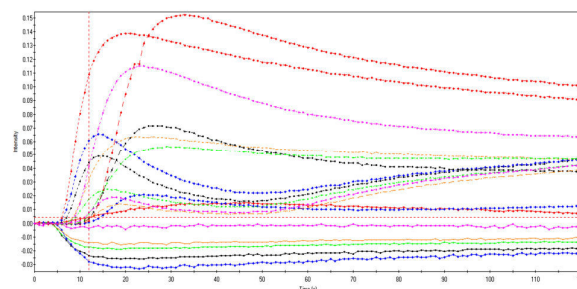

Fig. 1 Variation of response of 18 sensors with time of analysis

2. Multivariate Statistical Techniques

Comparison of the data produced by an e-nose can be made visually by comparing graphs of raw data or sensor response for various samples.

There are several multivariate statistical techniques which can be applied to the sensors data. Some of the techniques used in this study are as follows:

- Principal component analysis (PCA) is used to evaluate discrimination performance (that is, the ability to determine which references are significant and to what extent).
- Discriminant factorial analysis (DFA) is used to identify unknown samples in one of the training groups.
- Soft independent modeling by class analogy (SIMCA) is used to compare an unknown sample with a reference.

PCA

By using PCA data may be expressed and presented in such a way as to highlight the similarities and differences in analyzed samples. PCA achieves this by computing the eigenvectors and eigenvalues of the covariance matrix of the dataset. Keeping only a few eigenvectors corresponding to the largest eigenvalues, PCA can be also used as a tool to reduce the dimensions of the dataset while retaining the major variation of the data [14]. PCA has been used by many researchers for monitoring the changes in volatiles as recorded by e-nose [6], [15], [16].

As a first step, in order to evaluate the ability of the electronic nose to discriminate among different oat milk samples, the sensor responses were elaborated by principal component analysis (PCA) and it was found that PCA was able to differentiate between the samples, both for room and refrigerated storage.

DFA

To classify the samples into different groups, the data obtained by e-nose was analyzed using DFA as done by other researchers [17]-[19]. Microbial counts was used as grouping variable, and 18 e-nose sensor outputs as independent variables. DFA was used to develop predictive models for

classification of samples based on grouping variables. The electronic nose was able to classify the odor changes in oat milk samples based on microbial counts with accuracies of 100% for storage temperatures of 4°C and ambient temperature. The DFA model built classified oat milk samples based on the total microbial population into "unspoiled" and "spoiled". Fig. 2 shows results from the DFA using the discriminant functions for oat milk samples at different storage conditions.
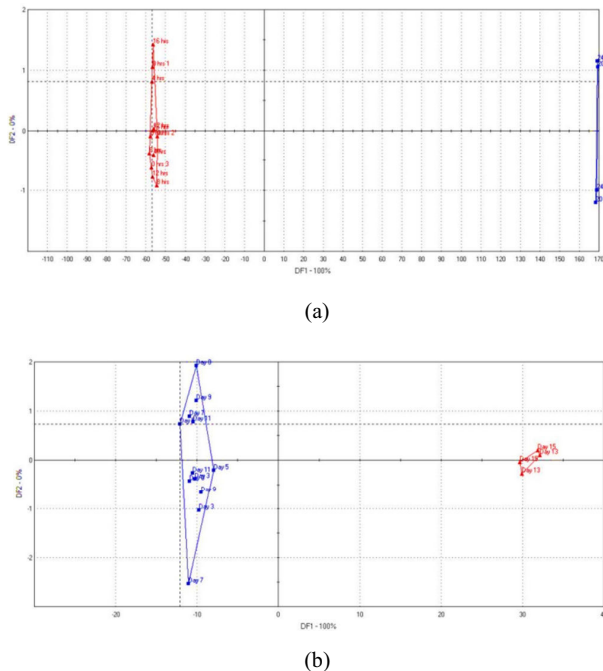

(a)

(b)

Fig. 2 DFA of odors of oat milk samples based on microbial counts and electronic nose readings (a) Refrigerated temperature (b) Room temperature

Soft Independent Modeling of Class Analogy (SIMCA)

Soft independent modeling by class analogy (SIMCA) is used to compare an unknown sample with a reference. Soft independent modeling of class analogy is interesting because it allows building a model with a single group considered as the "reference". The model is used to identify unknown samples as belonging or not to the only group defined. SIMCA has been used by many researchers for classification of samples on the basis of responses obtained by e-nose in various classes [20]-[22]. SIMCA was used to develop models for classifying unknown samples into acceptable and unacceptable category based on microbial count. Fresh samples with known microbial load were used as reference to train e-nose. The validity of the model is verified by determining the recognition rate obtained by cross-validation. The models developed had a validation score of 100% which means that e-nose was able to correctly identify samples if they belong to the reference category or not. The SIMCA plots for both the storage conditions are shown in Fig. 3.
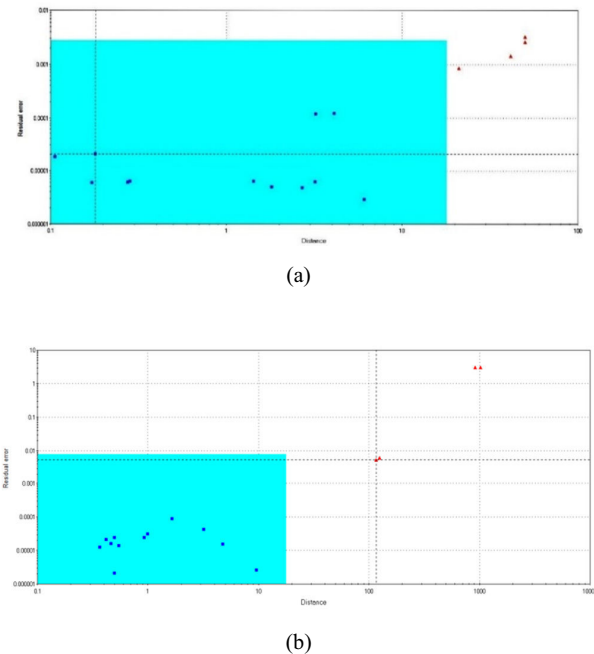

(a)

(b)

Fig. 3 SIMCA models for oat milk samples based on microbial counts and electronic nose readings (a) Refrigerated temperature (b) Room temperature

IV. CONCLUSION

This study suggests that the e-nose can correlate the odor change of oat milk samples with different degree of microbial spoilage, by using DFA as the pattern recognition technique. Predictive models for sensory evaluation and microbial levels of milk were developed. SIMCA model has been successfully developed to compare an unknown sample with a reference. The e-nose has potential to be used in a rapid and easy method for determining the shelf life assessment of oat milk, with no sample preparation.

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