Advances in Natural Science
Vol. **3**, No. **2**, **2010**, pp. 185-191
www.cscanada.net

ISSN 1715-7862 [PRINT]
ISSN 1715-7870 [ONLINE]
www.cscanada.org

The 3rd International Conference of Bionic Engineering

Rapid Detection of *E. coli* on Goat Meat by Electronic Nose

DING Ning-ye[1]
LAN Yu-bin[2]
ZHENG Xian-zhe[3]

ABSTRACT: Much attention has been paid on the foodborne illness of food, which is easily contaminated with bacteria or pathogens. *Escherichia coli* (*E.coli*) is one of these bacteria that commonly live in the contaminated animal meat. There is a growing need in the food industry for pathogen detection systems that are sensitive to low levels of bacteria, specific to the target organisms, capable of yielding results at or near real time. Both contaminated and non-contaminated goat meat were tested using an electronic nose (Cyranose-320) which consists of 32 polymer sensors. We developed an electronic nose method for the rapid detection of *E. coli* O157:H7 in goat meat. Principal Component Analysis (PCA) method was applied to analyze the experimental data, and the results indicated that they either overlap or are very close making it very difficult for the device to correctly identify. E-nose has a potential for being used as a tool for rapid detection of contamination, although it is not able to detect very low concentration of the contaminant.

Keywords: Goat meat; bacteria (*E.coli*); electronic nose; quality detection

INTRODUCTION

Foodborne illness results from eating food contaminated with bacteria (or their toxins) or other pathogens such as parasites or viruses. Most of the time it is the harmful bacteria which are found responsible. Some bacteria may be present at the source, included from the environment, or from the animal, or during the human handling at the processing plants. As for the meat, the main source of contamination is the feces in the slaughter plants. Although most foodborne infections are undiagnosed and unreported, the Center for Disease Control and Prevention estimates that every year about 76 million people in the United States become ill from pathogens in food, 5000 of whom die. Nine pathogenic bacteria cause most foodborne illness associated with meats. *Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella sp. Staphylococcus aureus,*

[1] College of Engineering, Northeast Agricultural University, Harbin 150030, P.R. China.
[2] Corresponding author, USDA-ARS, 2771 F&B Road, College Station, TX 77845, USA. Email: Yubin.lan@ars.usda.gov.
[3] College of Engineering, Northeast Agricultural University, Harbin 150030, P.R. China.
*Received 3 May 2010; accepted 28 July 2010

and Yersinia enterolitica can all be found in meat products either by nature, contamination, or product abuse[4]. This contamination is of great loss to humans both on health and economic grounds. This calls for the need of detecting the contaminants, inspecting the microbial load, and their elimination to ensure the food safety. Hazard Analysis Critical Control Point (HACCP) helps to prevent the foodborne illnesses and when detected it has the steps to correct the deviations. This control system is preventive instead of reactive and is acknowledged worldwide as one of the most effective approaches to food safety[5].

There is a growing need in the food industry for pathogen detection systems that are sensitive to low levels of bacteria, specific to the target organisms, capable of yielding results at or near real time. Traditional testing methods such as conventional plating, molecular and immunological methods for microorganisms are relatively costly and time consuming. They have their own limitations. Moreover they are expensive and time consuming. On the other hand the analytical instruments like electronic nose have less preparation time, much efficiency in detection of volatiles, greater safety and low costs.

Gases are intrinsically linked to everyday life and their odors significantly influence the "image" of our environment. The human nose serves as a highly advanced sensing instrument that is able to differentiate between thousands of smells but fails if absolute gas concentrations or odorless gases need to be detected. The demand for gas sensing devices that support the human nose is therefore large. Support is desired in safety critical applications where combustible or toxic gases are present and in comfort applications, such as climate controls of buildings and vehicles where good air quality is required. Additionally, gas monitoring is needed in process control and laboratory analysis. Whilst traditional gas chromatography and mass spectrometry techniques separate, quantify and identify individual volatile chemicals, they cannot tell us if a compound has an odor. Only large scale process control and laboratory analysis can justify the high cost and space of these gas analyzers. For all other purposes, the space and cost cannot be justified. Therefore, the electronic nose, a versatile, reliable, and user-friendly gas sensing device, was developed to augment these systems and human sensory panels in situations where knowledge of a sample's odor is deemed important.

Sensory panels, which could be either human sensory evaluation or advanced analytical instruments like electronic nose, identify these odors and flavors. Moreover, the human sensory evaluation has its own limitations and which are expensive and time consuming. On the other hand the analytical instruments (like electronic nose) have less preparation time, much efficiency in detection of volatiles, greater safety and low costs. This calls for the increase in demand for the pathogen detecting systems which are sensitive even to the lowest level detection of microorganisms in the meat industry.

Fecal contamination is the major source of contamination with bacteria like *Salmonella and Escherichia coli* O157:H7 and generic *Escherichia coli* is a good microbial indicator of fecal contamination (Pierce & Knight, 1996). The bacteria are transmitted to food products through the fecal-oral route or by cross-contamination. Fecal contamination is the major concern for *E. coli* O157:H7(Banwart, 1979). This occurs when the organism is deposited in the food from direct or cross-contamination during slaughter, processing and preparation. Kudva et al. conducted a study in which cattle and sheep manure was inoculated with *E. coli* O157:H7 to determine survivability. The study found that the bacterium survived in sheep manure for 21 months and positive culture was found in bovine manure at 47 days. The bacterium in bovine manure frozen at -20°C survived for at least 100 days, whereas it survived for 100 days in bovine manure incubated at 4°C or 10°C (Kudva & Blanch, 1998). Ruminant animals are naturally infected with the pathogen *E. coli* O157:H7, annually responsible for numerous meat recalls, foodborne illnesses, and deaths.

HACCP rulings are more sensitive than the historical way of inspection by sight and smell. But total elimination of harmful bacteria is not possible. Only the risk may be reduced substantially (Pierce & Knight, 1996). This has called for new rapid methods of detection of microbial load in the meat. Time is the major limiting factor in the detection and elimination of pathogenic microorganisms in the slaughter plant. Traditional testing methods are tedious and time consuming and may not give the desired results (Bruce,

[4] Food Safety and Inspection Service (FSIS): Overview of biological, chemical and physical properties, Section 1 in the USDA Meat and Poultry Products Hazards and Control Guide, USDA, 1997.
[5] Food Safety and Inspection Service (FSIS): Pathogen reduction and HACCP systems, Final rule, USDA, 1998.

1996). Thus an electronic nose or a bio-sensor could be a more user friendly method for rapid detection of the foodborne pathogens.

An electronic nose works similar to a human olfactory system. There are several types of e-nose instruments currently implemented in the food industry (Bartlett et al., 1997). Among them, the most popular sensors used in the industry are tin oxide sensors, quartz microbalance sensors, and conducting polymer sensors. Conducting polymer sensors are more popular because of the rapid response and reversibility of the polymer (Hobbs, 2003). Polymer sensors show quick results under odor influences and are easily reverted to a baseline after each test. Cyranose 320 (CYRANOSE Sciences, Inc., CA) is such a polymer composite sensor-based, handheld electronic nose (e-nose). The term electronic nose (e-nose) describes an electronic system that is able to mimic the human sense of smell. It contains an array of 32 sensors, each of which is made of a composite material consisting of conductive carbon black homogeneously blended throughout a non-conducting polymer. Each sensor contains its own type of polymer, allowing the entire array to distinguish among many different types of vapors. The device can be trained to distinguish complex vapor mixtures as easily as simple vapors. It is designed to follow the simple process of training, sampling and reporting so that decisions can be made immediately without leaving the sample site (Hobbs, 2003).

This can be possible with the use of electronic nose technology. The development in electronic nose technology has brought about new possibilities for its use within medical environmental and food industries (Winquist et al., 1993). There are reports of the use of e-nose (Cyranose 320) for the classification of bacteria (Dutta et al., 2002) and distinguishing bacterial species (Rossi et al., 1995; Holmberg et al., 1998; Gardner et al., 1998). It has been shown that Cyranose-320 could differentiate between samples with and without *E.coli* between the concentration of 5.3 and 6.3 log10 cfu/ml (Powell et al., 2002). E-nose has been used to discriminate between ground lamb stored either in carbon dioxide or frozen in vacuum packages (Braggin & Frost, 1997). Odor change in tallow that had been oxygen abused has been recorded using e-nose (Braggin et al., 1999). Similarly, its capability to discriminate between ground pork and ground beefs as well as storage estimation of meat samples have been studied (Winquist et al., 1993). In similar work, a relationship between e-nose and sensory odor evaluation for frozen ground beef have been studied (Spanier & Braggin, 1999). It has been shown that this technology can be used to detect changes in the volatile compounds associated with chicken meat based on the storage time and temperature (Boothe & Amold, 2002).

In this paper, the mainly objective is on the detection of *E. coli* O157:H7 in the goat meat using the Electronic nose (Cyranose-320), which is available in our laboratory. The objective of this project is to develop an electronic nose method for the rapid detection of *E. coli* O157:H7 in goat meat and see if it can differentiate contaminated and non-contaminated samples. Apart from this, the method will be used to detect different concentration of bacteria and find the least concentration of bacteria the e-nose can detect.

2 MATERIALS AND METHODS

2.1 Cyranose-320

This is a polymer composite sensor-based electronic nose, which has 32 sensors arranged in an array. The sensors are made of a composite material consisting of conducive carbon black and are homogenously blended completely by a non-conducting polymer. When the sensors come in contact with a vapor, the polymer absorbs the vapor and swells like a sponge. During swelling, the distance between the conductive carbon particles increases, increasing the resistance of the composite. This change in the resistance is transmitted to a processor, and the pattern of change in the sensor array is used to identify the vapor.

The various parts include:

1) PC nose software program run on a personal computer
2) The Cyranose 320

The functions of PC nose are to set up the methods, view data collected on the Cyranose 320, to save data and to download the saved methods to the Cyranose 320. In addition, training sets and instrument

settings generated on the Cyranose 320 can be archived on the PC. The Cyranose 320 controls and performs the execution of all the samplings and data analysis functions.

The Cyranose 320 operation needs training for the purpose of identifying the unknown samples. A minimum of five exposures is required to get acclimatized with the instrument. *Escherichia coli* (k-12): The bacteria were brought from the Department of Microbiology at Fort Valley State University, US.

2) Goat Meat: This was obtained from the Goat Meat Research Center at Fort Valley State University, US.

2.2 Methods

E.coli (K-12) was grown overnight at 37°C with continuous shaking (150 rpm) in a 125 ml flask. The optical density (O.D) of this culture was adjusted to giving a concentration of about 108 cfu/ml (colony forming units per ml). This culture was used to inoculate the meat pieces.

Meat pieces of about 2x3 cm was prepared and placed in jars of 50 ml volume. These were then inoculated with 300 µl of the bacterial culture, covered with parafilm to make it air tight and left at room temperature for 2-4 hours to collect the gas produced by the bacteria. The head space thus generated was then sniffed using the trained e-nose (Cyranose-320) to detect the presence of bacteria.

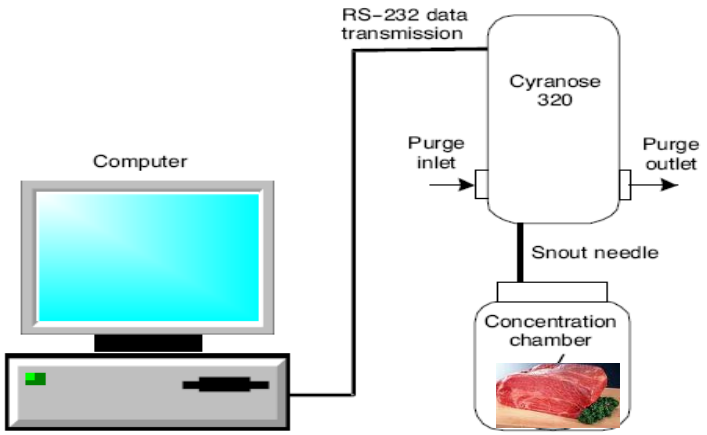

Fig. 1: Experimental schematic for the Cyranose 320 data collection

The e-nose was first trained before using it for the identification of unknown samples as shown in Fig.1. The operating parameters of e-nose were set in Table 1.

Table 1: Parameters settings of the Cyranose-320

Cyranose 320	Time	Pump Speed
Baseline Purge	10 s	Low
Sample Draw	35 s	Low
Sample Draw 2	0	N/A
Snout removal	0	N/A
1st Sample Gas Purge	0	N/A
1st Air Intake Purge	35 s	High
2nd Air Intake Purge	0	N/A
Digital Filter ON		
Substrate Temperature 35 ℃		
Algorithm	Canonical	
Preprocessing	Auto scaling	
Normalization	Norm1	

The e-nose was trained with two classes having ten exposures each. "Class 1" was meat with no bacteria and named "good" and "class2" was meat inoculated with bacteria named "bad". The algorithm was set at "canonical", preprocessing in "auto-scaling" and normalization was set in "none". The sensors were set at 42°C during data acquisition. All 32 sensors were active for these experiments. Cross validation was performed by removing one exposure from each class in canonical algorithm. Six to seven exposures were used to get a cross validation of 91-100%, which is the percentage of the sample correctly identified. After cross validation, the e-nose was used to identify the contaminated and non-contaminated samples.

2.3 Data Analysis Method

Principal component analysis (PCA) and Canonical Discriminant Analysis (CDA) were used to investigate whether the electronic nose was able to distinguish among different types. PCA is a multivariate technique used for reducing the dimensionality of the data while preserving the structure. PCA uses eigenvectors and eigenvalues to define the reduced subspace, which is a representation of the original N-dimension space. The principal components are linear combinations of interrelated variables. Coefficients of the linear combinations are the eigenvectors of the covariance or correlation matrix. A correlation matrix was used to enhance the influence of small spectral features. The PCA score plot can provide information on the clustering of data, while the PCA loading plot can be used to investigate the contribution from each sensor. The CDA method was used to assign a new sample to a class. The selection criterion was the class with the shortest Mahalonobis distance between its centroid and the sample in canonical space. With CDA we are interested in the principal component score matrix (Tp), which is input to the CDA instead of the response matrix for the 32 sensors that is of concern in PCA. After cross-validation, the optimal number of factors was determined. The canonical scores, Tc, were calculated with the optimal number of principal component factors as input to the CDA. The canonical factors were pulled from the Cyranose-320 and graphically displayed on the PC. Data analyses were made by the Polymer Composite Sensor (PCS) technology, which consists of polymer composite sensors that are composite materials of conductive carbon black blended throughout a non-conducting polymer.

Before a model was made, the cross-validation technique was applied to determine the optimum number of principal components. This later was used as an input in calculating the model. In cross-validation, the "leave one out" technique was used where one exposure was taken out from each class. A canonical model was made with the remaining exposures and applied to predict the exposure left out. Each exposure was predicted correctly if the distance from its known class was the smallest and it had a *P*-value (probability) of 0.01 or higher. During cross-validation, after the formation of each partial training set (by removing one exposure from each class), the partial training set remaining and the removed exposures were dependent upon the pre-processing selection as if the removed exposures were unknown. The results of this operation are displayed in the cross validation view in the PC-nose software.

RESULTS AND DISCUSSION

Preliminary experiments indicate that the e-nose can detect bacteria but not with 100% accuracy. The correct identification varied from 18 to 77% in different batches with 300 μl of the culture and 2h head space accumulation time (Table 2).

The increase in time, for the accumulation of gas, to 3 and 4 h did not yield much difference in the results. However, the e-nose once trained could be used for identification without updating the training set for at least 2 weeks.

The results from the experiments indicate that e-nose has a potential for being used as a tool for rapid detection of contamination, however more experiments need to be done before any conclusion is drawn.

The data and the Principal Component Analysis (PCA) for both contaminated and non-contaminated meat indicate that they either overlap or are very close making it very difficult for the machine to correctly identify them. It seems that the samples were so similar that they were used interchangeably during normal

operation of the equipment. The interclass Mahalanobis distance (M-distance) even after 100% validation could not exceed 6.

Table 2: Identification of meat contaminated by E.coli using Cyranose 320

S.N	Cross validation (%)	Total of samples	Identification		Correct Identification (%)	Culture used (µl)	Time (h)
			Correct	incorrect			
1.	93	8	5	3	62	300	2
2.	93	24	10	14	41	300	2
3.	93	20	5	15	25	300	2
4.	100	9	7	2	77	300	2
5.	100	10	3	7	30	300	2
6.	100	11	8	3	72	300	2
7.	100	11	2	9	18	300	2
8.	100	6	3	3	50	300	2
9.	100	6	2	4	33	300	2
10.	100	16	7	9	43	300	2
11.	100	9	1	8	11	300	3
12.	100	10	3	7	30	300	3
13.	91	11	5	6	45	500	4
14.	91	12	7	5	58	300	4
15.	91	13	2	11	15	500	4

* Time= duration of time the samples were left for head space accumulation.

Experiments with different concentration of solutions as samples were done. However, no noticeable difference could be observed in the results (results not shown). More experiments with the increase in the time for sample intake, excluding some of the sensors which are very sensitive need to be done to check if this would affect the results.

Data available in this research have shown that bacteria (*E. coli*) cultured in goat meat can be detected according to their volatile compounds profile obtained by electronic nose. Therefore, to validate the applicability of the same method on the bacteria differentiation, each goat meat type in this research was considered separately. In that way, differences caused by the bacteria level were neutralised. PCA analysis of data set obtained for bacteria volatile compound profiles by electronic nose resulted with large number of principal components. All samples form compact cloud in the centre of a plot, with some samples displaced, but there is no grouping that can be connected with their com.

CONCLUSIONS

Results presented in this work indicate that electronic nose could be useful in detection for the determination of goat meat contaminant case if combined with simple statistical analysis. Bacteria profile data obtained by electronic nose analysis and analysed using statistic analysis method showed that samples contaminated level. Therefore bacteria profile of contaminated goat meat obtained by electronic nose can be used for bacteria level. However, 100% identification could not be obtained even with higher concentration or density. A change in the vessel size did not seem to make much difference, as there was no improvement in the results.

Since there are a lot of factors affecting the results, more experiments need to be done before the accuracy can be predicted. It seems that the e-nose is not able to detect very low concentration of the contaminant.

REFERENCES

Banwart G J. (1979). *Basic Food Microbiology*, AVI Publishing Company, Inc., Westport CT.

Bartlett P N, Elliot J M, Gardner J W. (1997). Electronic noses and their application in the food industry. *Food Tech, 51*:44-48.

Boothe D D H, Amold J. (2002). Electronic nose analysis of volatile compounds from poultry meat samples, fresh and after refrigerated storage. *Sci Food, 82:* 315-322

Braggin T J, Frost D A, Agnew M P, Farouk M M. (1999). Evaluation of an electronic nose for use in meat industry. In *Proceedings of the 5th International Symposium of Olfaction and Electronic nose,* Baltimore, USA.

Braggin T J, Frost D A. (1997). The effect of extended chilling storage in CO_2 atmosphere on the odor and flavor of sheep meat as assessed by sensory panel and electronic nose. In *Proceedings of the 43rd international congress of meat science and technology*, Auckland, New Zealand, 198-199.

Bruce J. (1996). Automated system rapidly identifies and characterizes microorganisms in food. *Food Tech, 50*: 77-81.

Dutta R, Hines E L, Gardner J W, Boilot P. (2002). *Bacteria classification using Cyranose 320 electric nose. 1*:4, http://www.biomedical-engineering-online.com/content/1/1/4.

Gardner J W, Craven M, Dow C, Hines E L. (1998). The prediction of bacteria type and culture growth phase by an electronic nose with multi-layer perceptron network. *Meas Sci Technol, 9*:120-127.

Hobbs S. [2003-09-02]. *A tour of Cyranose 320*, http://cyranosciences.com/technology/tour.html.

Holmberg M, Gustafsson F, Hornsten E G, Winquist F, Nilsson L E, Ljung L, Lunstrom I. (1998). Bacteria classification based on feature extraction from sensor data. *Biotech Tech, 12*:319-324.

Kudva T, Blanch K. (1998). Analysis of *Escherichia coli* O157: H7 survival in ovine or bovine manure and manure slurry. *Applied and environmental microbiology, 64:*3166-3174.

Pierce J, Knight K. (1996). *President Clinton announces new food safety rules to protect consumers*. Press release, No. 0362.96, USDA.

Powell T B, Heinmann P, Demirici A. (2002). Electronic nose detection of *E. coli* on the surface of apples. In *2002 ASAE Annual International Meeting*, Chicago, USA, 03-3025.

Rossi V, Talon R, Berdague J. (1995). Rapid discrimination of *Micrococcacea*e species using semiconductor gas sensors. *Journal Micro Met, 24*:183-190.

Spanier A M, Braggin T J. (1999). Electronic noses: what are they and what are their potential uses in meat science. In *Proceedings of reciprocal meat conference, 52*:103-112.

Winquist F, Hornsten E G, Sungren H, Lundstrom I. (1993). Performance of an electronic nose for quality estimation of ground meat. *Meas Sci Technol, 4:*1493- 1500.