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Rapid differentiation between *E. coli* and *Salmonella* Typhimurium using metal oxide sensors integrated with pattern recognition

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ABSTRACT

A rapid method to differentiate between *E coli* and *Salmonella* Typhimurium was developed. *E. coli* and *S.* Typhimurium were separately grown in super broth and incubated at 37 °C. Super broth without inoculation of *E. coli* or *S.* Typhimurium was used as control. Numbers of *E. coli* and *S.* Typhimurium were followed using a colony counting method. Identification of the volatile metabolites produced by *E. coli* and *S.* Typhimurium was determined using solid-phase microextraction coupled with gas chromatography/mass spectrometry. An electronic nose with 12 non-specific metal oxide sensors was used to monitor the volatile profiles produced by *E. coli* and *S.* Typhimurium. Principal component analysis (PCA) and back-propagation neural network (BPNN) were used as pattern recognition tools. PCA was used for data exploration and dimensional reduction. PCA could visualize class separation between sample subgroups. The BPNN was shown to be capable of predicting the number of *E. coli* and *S.* Typhimurium. Good prediction was possible as measured by a regression coefficient (R^2 = 0.96) between true and predicted data. Using metal oxide sensors and pattern recognition techniques, it was possible to discriminate between samples containing *E. coli* from those containing *S.* Typhimurium.

1. Introduction

Detection and differentiation of foodborne pathogens is a major concern of the food industry. The public health implications of failing to detect certain pathogens can cause disease or even death [1,2]. Classical methods for the detection and differentiation of microorganisms have been based on traditional plate count methods. These methods are both tedious and time consuming because they usually require a series of tests with the incubation of the microorganisms. The limitations of these methods have led to the research focusing on development of rapid and accurate techniques to identify pathogens in food products [3]. Microorganisms can be characterized by identification of specific metabolites generated by specific biochemical pathways [4,5]. However, many metabolites may be common to several microorganism species. Therefore, the differences between samples often relate to a complex balance between patterns of volatiles rather than to a major change in one or two constituents [6,7].

Electronic nose technology has been shown to provide rapid, continuous monitoring of a wide array of different volatile chemicals [8]. A considerable number of electronic nose applications have been reported, including evaluation of the off-odor in wine [9], detection of *Salmonella* Typhimurium in fat free milk [10], classification of milk [11], and detection of foodborne pathogens in vegetables [12]. The electronic noses can be used for the specific analysis, identification, and recognition of complex odors and volatile organic compounds [9]. However, the electronic noses generated multi-dimensional data that was difficult to handle and visualize. This underlines the importance of using multivariate data analysis to extract the specific information necessary to target a specific microorganism. Pattern recognition interpretation techniques, such as principal component analysis (PCA), linear discriminant analysis (LDA) and artificial neural network (ANN), provide complementary information which was simply unachievable by conventional data analysis [13,14].

PCA is an unsupervised technique commonly used in signal processing and pattern recognition [15,16]. PCA is used to reduce the dimensionality of multivariate data while preserving most of the variance, and thus is an excellent technique for observing the natural relationships between samples [17–19]. Artificial neural network has been proved to be powerful tools for data processing [11,20]. The advantages of the artificial neural network approach to the multivariate calibration of sensor arrays are well known. The most common neural network approach to regression-type problems is multilayer perceptrons with back-propagation neural network (BPNN). The objective is to identify a model that will correctly associate inputs with outputs. The learning data is used to train the system and to develop the calibration model. The

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test data are then evaluated by the calibration model in order to obtain the predicted results. This technique has the advantage of producing low prediction errors [20,21].

The objective of this work was to develop a method to identify and differentiate between *E. coli* and *S.* Typhimurium using 12 non-specific metal oxide sensors integrated with PCA and BPNN. In this approach, identification and differentiation was based on the determination of the volatile metabolites that signify a particular microorganism. *E. coli* and *S.* Typhimurium were used as the target microorganisms in this study because outbreaks of foodborne illness caused by *S.* Typhimurium have been repeatedly reported [1,22,23], and the presence of *E. coli* in foods indicates fecal contamination and the presence of pathogenic microorganisms.

2. Methodology

2.1. Preparation of stock culture and test solution

The nonpathogenic strain *E. coli* ATCC 25922 obtained from the American Type Culture Collection (ATCC, Rockville, MD) was inoculated into Luria Bertani broth consisting of 10 g Bacto tryptone (Becton, Dickinson and Company, Sparks, MD), 5 g Bacto yeast (Becton, Dickinson and Company, Sparks, MD), and 5 g NaCl, and incubated at 37 °C in a gyratory shaker (G-25 New Brunswick Scientific Corporation, New Brunswick, NJ) at 100 rpm. The *E. coli* suspension was then dispensed into sterile 125 ml polypropylene centrifuge bottles, and centrifuged (Rotor-GSA model RC 5 C Superspeed Centrifuge, Sorvall Instruments, Dupont Co., Hoffman Estate, IL) at 1600 × *g* for 10 min. The supernatant was decanted, and the resulting cell pellets were resuspended in a sterile 15% glycerol solution. One ml aliquots of bacterial suspension were transferred into 1.5 ml microcentrifugal tubes and frozen using liquid nitrogen. The frozen cultures were stored at −80 °C.

Super broth consisting of 32 g tryptone, 20 g yeast, 5 g NaCl, and 5 ml of 1N NaOH per liter was used as a basal medium. Before being utilized, *E. coli* was transferred from the stock culture to super broth and incubated overnight at 37 °C. *E. coli* (100 CFU/ml) was inoculated into super broth, and 5 ml of super broth were transferred into standard 20 ml headspace vials and sealed with PTFE-lined Teflon caps (Alpha M.O.S., Hillsborough, NJ). The cultures were allowed to grow in vials at 37 °C in a gyratory shaker (G-25 New Brunswick Scientific Corporation, New Brunswick, NJ) at 100 rpm. Test solution of *S.* Typhimurium was prepared in the same manner. Samples were periodically analyzed after incubation at 37 °C for 4, 6, 8 and 10 h using colony counting, solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME–GC–MS) and an electronic nose.

2.2. Colony counting method

Samples were serially diluted in sterile Butterfield's phosphate buffer. A series of dilutions was prepared from the stock suspension. Serially diluted samples were plated in duplicate using 3 M Petrifilm aerobic count plates (3 M Industrial Markets, St. Paul, MN) to determine total bacteria. All plates were incubated at 35 °C for 48 ± 2 h. Plate counts were recorded as CFU/ml.

2.3. Identification of volatile compounds using SPME–GC–MS

Volatile compounds from headspace samples were collected using a polydimethylsiloxane solid-phase microextraction fiber (Supelco, Inc., Bellefonte, PA). For headspace sampling, 5 ml of a liquid sample were placed into a 20 ml vial and the fiber was exposed to the head space of the media solution. Sampling temperature was maintained at 37 °C, and the sampling duration was 10 min, which was sufficient to permit the establishment of a near equilibrium for the compounds tested. A GC (HP-6890, Hewlett-Packard Co., Wilmington, DE) was used for the analysis of the compounds. Volatiles were separated using a capillary column (SPB5, 30 m × 0.1 mm i.d., 0.25 μm coating thickness). The carrier gas used was ultrapurified helium (99.99% purity) at a flow rate of 0.5 ml/min. The temperature program was isothermal for 2 min at 40 °C and raised to 240 °C at a rate of 50 °C/min. Electron impact ionization (FCD-650, LECO Corp., St Joseph, MI) was used by the time-of-flight (TOF) mass spectrometer in evaluation of the volatiles. Mass spectra were collected at a rate of 40 spectra/s over a range of 30–400 *m*/*z*. The ionization energy was 70 eV. Identification of volatile components was determined by comparison of collected mass spectra with those of authenticated standards and spectra in the National Institute for Standards and Technology (NIST) mass spectral library.

Table 1
Sensor types and volatile descriptors

Sensors	Volatile description
P101, P102, SYGCT	Non-polar volatiles: methane, propane
PA2, SYAA, T301	Organic solvents: polar compounds, ethanol
T702	Alcohol and aromatic compounds
SYG, SYGH	Amines and amine containing compounds and ammonia derivatives
SYGCTI	Ammonia and sulfur
P401, SYLG	Fluoride and chloride: fluorinated and chlorinated compounds, aldehydes

2.4. Volatile analysis using metal oxide sensors

An electronic nose (Fox 3000, Alpha M.O.S., Hillsborough, NJ) with 12 metal oxide sensors (SYLG, SYG, SYAA, SYGH, SYGCTI, SYGCT, T301, P101, P102, P401, T702, and PA2) was used for monitoring changes in volatiles produced by *E. coli* in a super broth medium. The descriptors associated with the sensors are shown in Table 1. The volatile analysis system combines a measurement chamber for generating the volatile compounds and a detection system made up of 12 metal oxide sensors. This instrument was linked to an autosampler capable of analyzing a total of 64 samples. Samples were placed in glass vials and sealed with crimped PTFE/metal septa.

Samples were placed in the HS100 auto-sampler in arbitrary order. Prior to analysis, the vial was removed from the sample tray and placed in a temperature-controlled chamber. The vial temperature was held at 37 °C while being spun in order to produce an equilibrated headspace. The time the vial remains in this chamber is the headspace generation time. The automatic injection unit heats the samples to 37 °C using an incubation time of 300 s. The temperature of the injection syringe was 42 °C. The injector needle then removes 2.5 ml of headspace and injects it into the sensor chamber. The delay time between two injections was 300 s. Each injection was repeated, with separate samples (three times for all variations per day) for seven days. The electronic signals from the sensors were digitized and then transferred to the control computer.

Each sensor element changes in resistance (R_{max}) when exposed to volatile compounds. In order to produce consistent data for the classification, the sensor response was presented with a volatile chemical relative to the base resistance in air, which is the maximum change in the sensor electrical resistance divided by the initial resistance, as follows

$$\text{Relative resistance change} = \frac{\Delta R_{max}}{R_0} \quad (1)$$

where $\Delta R_{max} = R_{max} - R_0$ is the maximum change in the sensor electrical resistance and R_0 the initial baseline resistance of the sensor. The baseline of the sensors was acquired in a synthetic air