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Electronic Nose Integrated with Chemometrics for Rapid Identification of Foodborne Pathogen

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1. Introduction

Diseases caused by foodborne pathogens have been a serious threat to public health and food safety for decades and remain one of the major concerns of our society. There are hundreds of diseases caused by different foodborne pathogenic microorganisms, including pathogenic viruses, bacteria, fungi, parasites, marine phytoplankton, and cyanobacteria, etc (Hui, 2001). Among these, bacteria such as *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Campylobacter coli*, *Bacillus cereus*, *Vibrio parahaemolyticus* and *Listeria monocytogenes* are the most common foodborne pathogens (McClure, 2002), which can spread easily and rapidly under requiring food, moisture and a favorable temperature (Bhunia, 2008).

Identification and detection pathogens in clinical, environmental or food samples usually involves time-consuming growth in selective media, subsequent isolation and laborious biochemical and molecular diagnostic procedures (Gates, 2011). Many of these techniques are also expensive or not sensitive enough for the early detection of bacterial activity (Adley, 2006). The development of alternative analytical techniques that are rapid and simple has become increasingly important to reduce sample preparation time investment and to conduct real time analyses.

It is well known that microorganisms can produce species-specific microbial volatile organic compounds (MVOCs), or odor compounds, which characterize as odor fingerprinting (Turner & Magan, 2004). Early in this research area, the question arose as to can we use odor fingerprinting like DNA fingerprinting to identify or detect microbe in pure culture or in food samples. To date it is still a very interesting scientific question. Many studies (Bjurman, 1999, Kim et al., 2007, Korpi et al., 1998, Pasanen et al., 1996, Wilkins et al., 2003), especially those using analytical tools such as gas chromatography (GC) or gas chromatography coupled with mass spectrometry (GC–MS) for headspace analysis, have shown that microorganisms produce many MVOCs, including alcohols, aliphatic acids and terpenes, some of which have characteristic odors (Schnürer et al., 1999).

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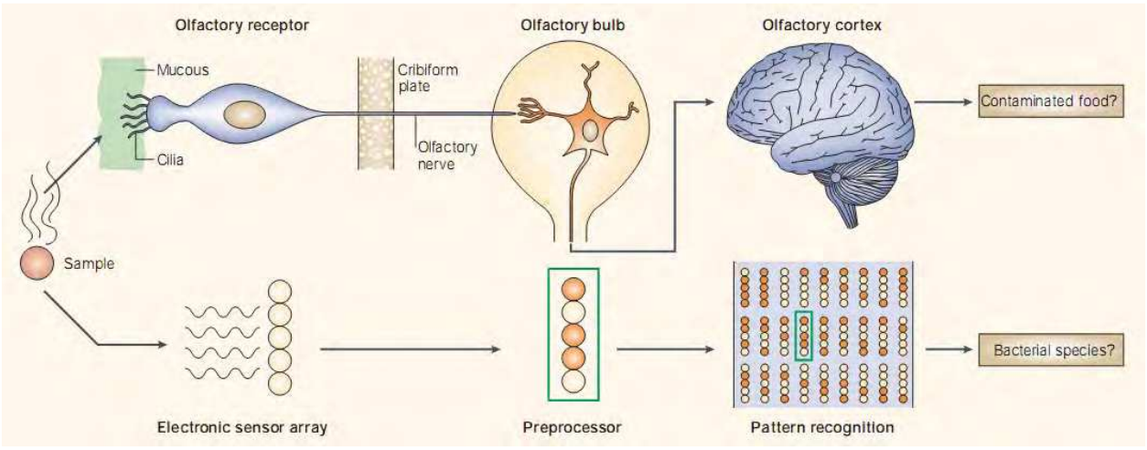


Fig. 1. Electronic nose devices mimic the human olfactory system.

The electronic devices simulate the different stages of the human olfactory system, resulting in volatile odor recognition, which can now be used to discriminate between different bacterial infections. (Turner & Magan, 2004)

During the past three decades there has been significant research interest in the development of electronic nose (E-nose) technology for food, agricultural and environmental applications (Buratti et al., 2004, Pasanen et al., 1996, Romain et al., 2000, Wilkins et al., 2003). The term E-nose describes a machine olfaction system, which successfully mimics human olfaction and intelligently integrates of multitudes of technologies like sensing technology, chemometrics, microelectronics and advanced soft computing (see Fig. 1). Basically, this device is used to detect and distinguish complex odor at low cost. Typically, an electronic nose consists of three parts: a sensor array which is exposed to the volatiles, conversion of the sensor signals to a readable format, and software analysis of the data to produce characteristic outputs related to the odor encountered. The output from the sensor array may be interpreted via a variety of chemometrics methods (Capone et al., 2001, Evans et al., 2000, Haugen & Kvaal, 1998) such as principal component analysis (PCA), discriminant function analysis (DFA), cluster analysis (CA), soft independent modelling by class analogy (SIMCA), partial least squares (PLS) and artificial neural networks (ANN) to discriminate between different samples. The data obtained from the sensor array are comparative and generally not quantitative or qualitative in any way. It has the potential to be a sensitive, fast, one-step method to characterize a wide array of different volatile chemicals. Since the first model of an intelligent electronic gas sensing model was described, a significant amount of gas sensing research has been focused on several industrial applications.

Recently, some novel microbiological applications of E-nose have been reported, such as the characterization of fungi (Keshri et al., 1998, Pasanen et al., 1996, Schnürer et al., 1999), bacteria (Dutta et al., 2005, Pavlou et al., 2002a) and the diagnosis of disease (Gardner et al., 2000, Pavlou et al., 2002b, Zhang et al., 2000). It is more and more clear that E-nose techniques coupled with different chemometrics analyses of the odor fingerprinting offer a wide range of applications for food microbiology, including identification of foodborne pathogen.

2. Detection strategies

Several E-nose devices have been developed, all of which comprise three basic building blocks: a volatile gas odor passes over a sensor array, the conductance of the sensors changes owing to the level of binding and results in a set of sensor signals, which are coupled to data-analysis software to produce an output (Turner & Magan, 2004).

The main strategy of foodborne pathogen identification based on E-nose, which is composed of three steps: headspace sampling, gas sensor detection and chemometrics analysis (see Fig. 2).

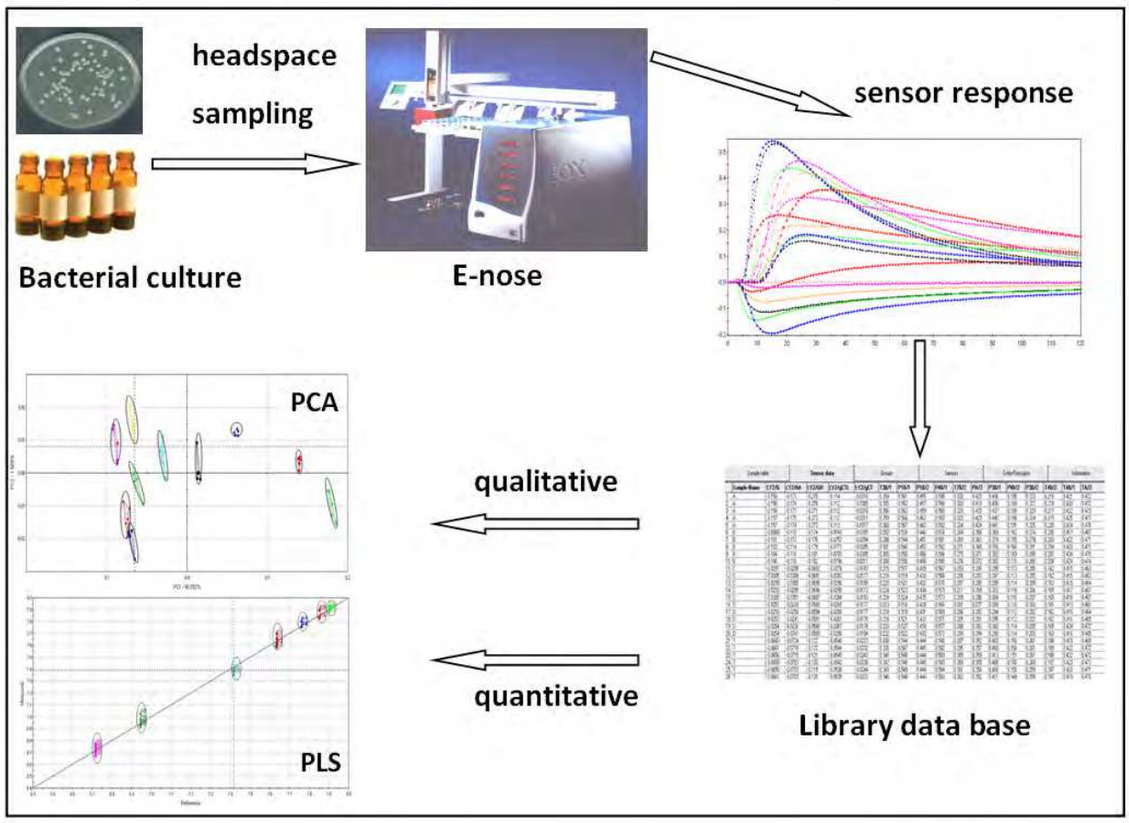


Fig. 2. Electronic nose and chemometrics for the identification of foodborne pathogen. The main strategy of foodborne pathogen identification based on E-nose.

2.1 Headspace sampling

Before analysis, the bacterial cultures should be transferred into standard 20 ml headspace vials and sealed with PTFE-lined Teflon caps to equilibrate the headspace. Sample handling is a critical step affecting the analysis by E-nose. The quality of the analysis can be improved by adopting an appropriate sampling technique. To introduce the volatile compounds present in the headspace (HS) of the sample into the E-nose's detection system, several headspace sampling techniques have been used in E-nose. Typically, the methods of headspace sampling (Ayoko, 2004) include static headspace (SHS) technique, purge and trap (P&T) technique, stir bar sorptive extraction (SBSE) technique, inside-needle dynamic

extraction (INDEX) technique, membrane introduction mass spectrometry (MIMS) technique and solid phase micro extraction (SPME) technique.

Unlike the other techniques, SPME has a considerable concentration capacity and is very simple because it does not require especial equipment. The principle involves exposing a silica fibre covered with a thin layer of adsorbent in the HS of the sample in order to trap the volatile components onto the fibre. The adsorbed compounds are desorbed by heating and introduced into the detection system. A SPME sampler consists of a fused silica fiber that is coated by a suitable polymer (e.g. PDMS, PDMS/divinylbenzene, carboxen/PDMS) and housed inside a needle. The fiber is exposed to headspace volatile and after sampling is complete, it is retracted into the needle. Apart from the nature of the adsorbent deposited on the fiber, the main parameters to optimize are the equilibration time, the sample temperature and the duration of extraction. Compared with other sampling methods, SPME is simple to use and reasonably sensitive, so it is a user-friendly pre-concentration method.

In our studies, the headspace sampling method of E-nose was optimized for MVOCs analysis. The samples were placed in the HS100 auto-sampler in arbitrary order. The automatic injection unit heated the samples to 37°C with an incubation time of 600 seconds. The temperature of the injection syringe was 47°C. The delay time between two injections was 300 seconds. Then the adsorbed compounds are desorbed by heating and introduced into the detection system (Yu Y. X., 2010a, Yu Y. X., 2010b).

2.2 Gas sensor detection

The most complicated part of electronic olfaction process is odor capture and sensor technology to be deployed for such capturing. Once the volatile compounds of samples are introduced into the gas sensor detection system, the sensor array is exposed to the volatile compounds and then the odor fingerprint of samples is generated from sensor respond. By chemical interaction between the volatile compounds and the gas sensors, the state of the sensors is altered giving rise to electrical signals that are registered by the instrument of E-nose. In this way the signals from the individual sensor represent a pattern that is unique for the gas mixture measured and those data based on sensors is transformed to a matrix. The ideal sensors to be integrated in an electronic nose should fulfill the following criteria (Barsan & Weimar, 2001, James et al., 2005): high sensitivity toward the volatile chemical compounds, that is, the chemicals to be detected may be present in the concentration range of ppm or ppb, and the sensor should be sufficiently sensitive to small concentration level of gaseous species within a volatile mixture, similar to that of the human nose (down to 10^{-12} g/ml); low sensitivity toward humidity and temperature; medium selectivity, that is, they must respond to a range of different compounds present in the headspace of the sample; high stability; high reproducibility and reliability; high speed of response, short reaction and recovery time, that is, in order to be used for online measurements, the response time of the sensor should be in the range of seconds; reversibility, that is, the sensor should be able to recover after exposure to gas; robust and durable; easy calibration; easily processable data output; and small dimensions.

The E-nose used in our studies is a commercial equipment (FOX4000, Alpha M.O.S., Toulouse, France), with 18 metal oxide sensors (LY2/AA, LY2/G, LY2/gCT, LY2/gCTl, LY2/Gh, LY2/LG, P10/1, P10/2, P30/1, P30/2, P40/1, P40/2, PA2, T30/1, T40/2, T70/2,

T40/1, TA2), and this sensor array system is used for monitoring the volatile compounds produced by microorganism, and so on. The descriptors associated with the sensors are shown in Table 1. FOX4000 E-nose assay measurements showed signal with maximum intensities changing with the type of samples, which indicate that discrimination is obtained.

Sensors	Volatile description	Sensors	Volatile description
LY2/LG	Fluoride, chloride, oxynitride, sulphide	P30 /1	Hydrocarbons, ammonia, ethanol
LY2 /G	Ammonia, amines, Carbon oxygen compounds	T70 /2	Toluene, xylene, carbon monoxide
LY2 /AA	Alcohol, acetone, ammonia	T40 /1	Fluorine
LY2 /GH	Ammonia, amines compounds	P40 /1	Fluorine, chlorine
P40 /2	Chlorine, hydrogen sulfide, fluoride	LY2 /gCTL	hydrogen sulfide
P30 /2	Hydrogen sulphide, ketone	LY2 /gCT	Propane, butane
T30 /1	Polar compound, hydrogen chloride	T40 /2	chlorine
P10 /1	Nonpolar compound: hydrocarbon, Ammonia, chlorine	PA /2	Ethanol, ammonia, amine compounds
P10 /2	Nonpolar compound: Methane, ethane	TA /2	ethanol

Table 1. Sensor types and volatile descriptors of FOX4000 E-nose.

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

$$\text{Relative electrical resistance change} = (R_{max} - R_0) / R_0$$

where R_0 is the initial baseline electrical resistance of the sensor and $R_{max} - R_0$ is the maximum change of the sensor electrical resistance. The baseline of the sensors was acquired in a synthetic air saturated steam at fixed temperature. The relative electrical resistance change value was used for data evaluation because it gives the most stable result, and is more robust against sensor baseline variation (Siripatrawan, 2008).

Data of the relative electrical resistance changes from the 18 sensors can combine with every sample to form a matrix (see Fig. 2: The library data base) and the data is without preprocessing prior to chemometrics analysis. The sensor response is stored in the computer through data acquisition card and these data sets are analyzed to extract information.

2.3 Chemometrics analysis

The matrix of signal is interpreted by multivariate chemometrics techniques like the PCA, PLS, ANN, and so on. Samples with similar odor fingerprinting generally give rise to similar sensor response patterns, while samples with different odor fingerprinting show differences in their patterns. The sensors of an E-nose can respond to both odorous and odorless volatile compounds.

These various chemometrics methods are used in those works, according to the aim of the studies. Generally speaking, the chemometrics methods can be divided into two types: unsupervised and supervised methods(Mariey et al., 2001). The objective of unsupervised methods is to extrapolate the odor fingerprinting data without a prior knowledge about the bacteria studied. Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) are major examples of unsupervised methods. Supervised methods, on the other hand, require prior knowledge of the sample identity. With a set of well-characterized samples, a model can be trained so that it can predict the identity of unknown samples. Discriminant analysis (DA) and artificial neural network (ANN) analysis are major examples of supervised methods.

PCA is used to reduce the multidimensionality of the data set into its most dominant components or scores while maintaining the relevant variation between the data points. PCA identifies the natural clusters in the data set with the first principal component (PC) expressing the largest amount of variation, followed by the second PC which conveys the second most important factor of the remaining analysis, and so forth(Di et al., 2009, Huang et al., 2009, Ivosev et al., 2008). Score plots can be used to interpret the similarities and differences between bacteria. The closer the samples are within a score plot, the more similar they are with respect to the principal component score evaluated(Mariey et al., 2001). In our studies, each sample data of 18 sensors is then compared to the others in order to make homogeneous groups. A scatter plot can then be drawn to visualize the results, each sample being represented by a plot.

3. Application of E-nose and chemometrics for bacteria identification

With the success of the above applications of the E-nose have been published, the authors were interested in determining whether or not an E-nose would be able to identify bacteria. A series of experiments were designed to determine this. In this part, bacteria identification at different levels (genus, species, strains) was cited as an example to illustrate using this integrated technology to foodborne bacteria effective identification.

3.1 At genus level

In this study, three bacteria, *Listeria monocytogenes*, *Staphylococcus lentus* and *Bacillus cereus*, which from three different genus, were investigated for the odor fingerprint by E-nose. The result of PCA (Fig.3a) shows that, the fingerprints give a good difference between the blank culture and the bacterial culture, and the three bacteria can be classified from each other by the odor fingerprints. Using the cluster analysis to represent the sensor responses (Fig. 3b), it is also possible to obtain a clear separation between the blank control and culture inoculated with bacteria. And the CA result also reveals that successful discrimination between the bacteria at different genus is possible(Yu Y. X., 2010a).

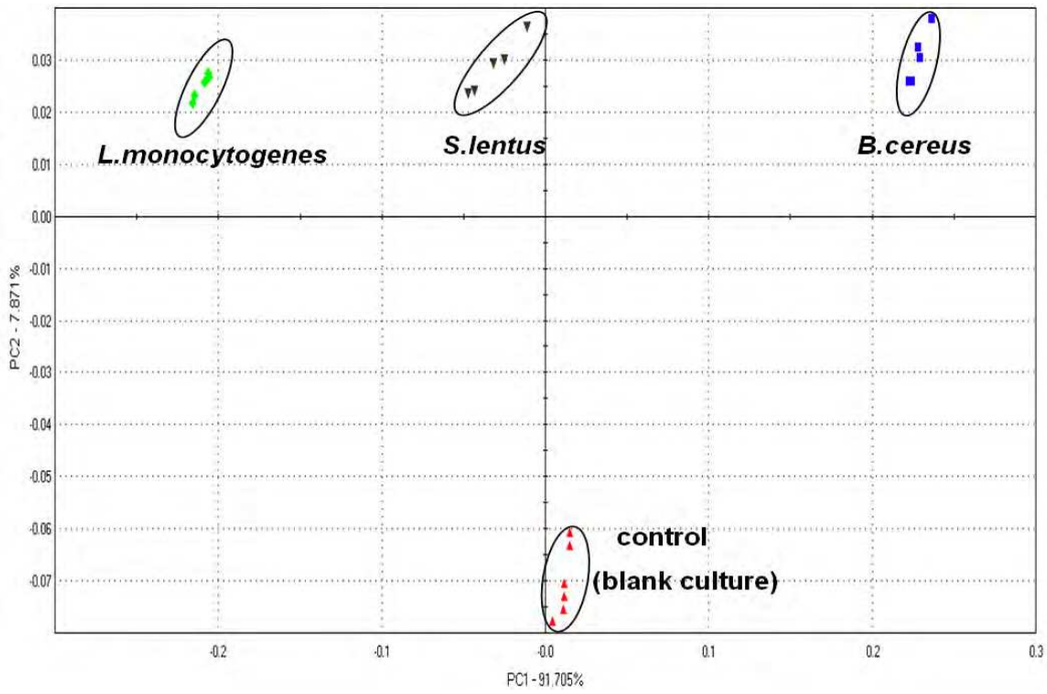


Fig. 3(a). Principal components analysis (PCA) for the discrimination of three bacteria from different genus on the basis of E-nose. The plot displays clear discrimination between the four groups, accounting for nearly 99% of the variance within the dataset.

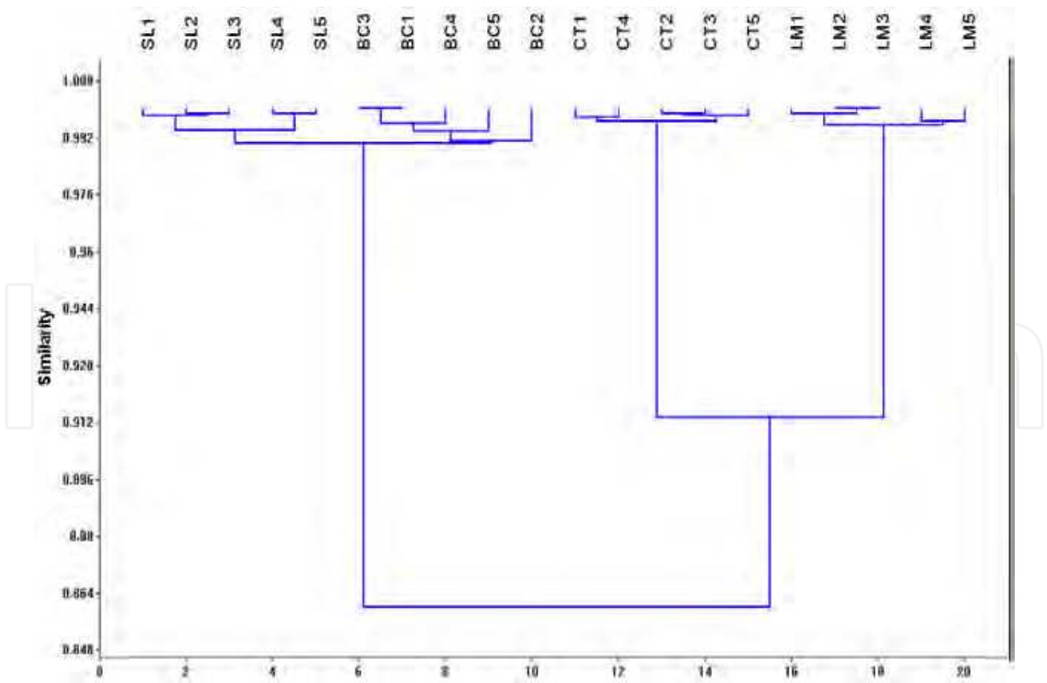


Fig. 3(b). Cluster analysis (CA) for the discrimination of three bacteria from different genus on the basis of E-nose. (*S. lentus*: SL1-SL5, *B. cereus*: BC1-BC5, *L. monocytogenes*: LM1-LM5, control blank culture: CT1-CT5).

3.2 At species level

In this study, using the same collection methodology, the E-nose was tested for its ability to distinguish among bacterial pathogens at species levels. Four species bacteria selected from *Pseudomonas* sp, named *Pseudomonas fragi*, *Pseudomonas fluorescens, Pseudomonas putida* and *Pseudomonas aeruginosa*, were investigated for the odor fingerprint by E-nose. It is clear that the E-nose was able to distinguish amongst all specimens tested. The PCA result in Fig.4(a) shows a representative experiment, where individual species of bacteria clustered in individual groups, separate from each other and the bacteria *Pseudomonas fragi* is given a great difference form the three other bacteria by the odor fingerprints. The result of cluster analysis in Fig. 4(b) also reveals that successful discrimination between the different bacteria at strains level is possible.

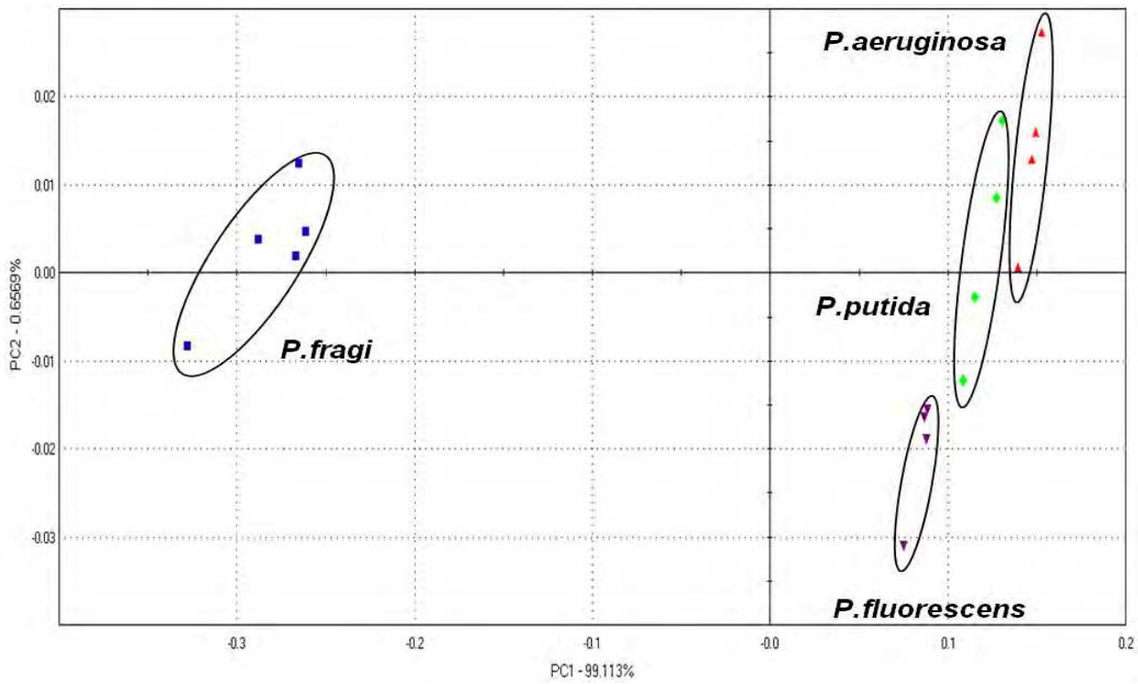
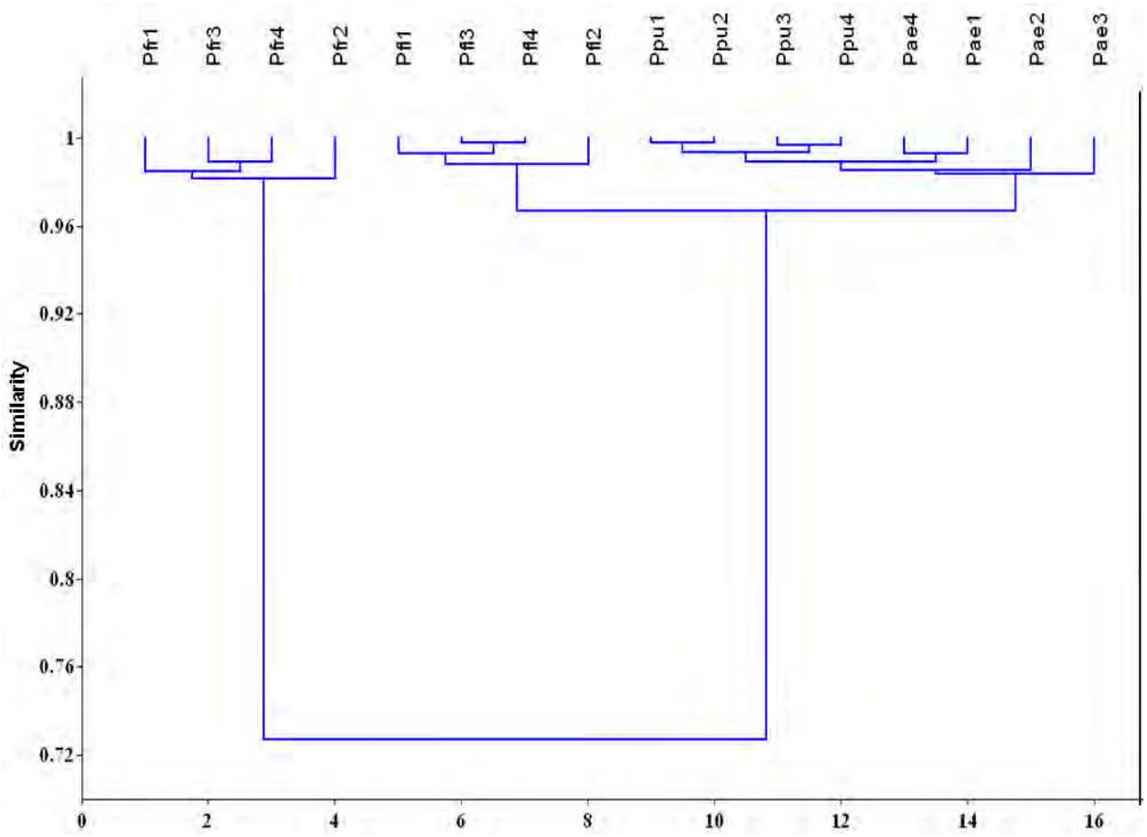


Fig. 4(a). Principal components analysis (PCA) for the discrimination of four different species of Pseudomonas sp on the basis of E-nose. The plot displays clear discrimination between the four groups, accounting for nearly 99% of the variance within the dataset.



(*P. fragi*: Pfr1-Pfr4, *P. fluorescens*: Pfl1-Pfl4, *P. putida*: Ppu1-Ppu4, *P. aeruginosa*: Pae1-Pae4).

Fig. 4(b). Cluster analysis (CA) for the discrimination of four different species of Pseudomonas sp on the basis of E-nose.

3.3 At strains level

The next set of experiments involved testing the integrated method to see whether it could correctly differentiate bacteria samples as different strains. In this study, four strains of *Vibrio parahaemolyticus*, named *V. parahaemolyticus* F01, *V. parahaemolyticus* F13, *V. parahaemolyticus* F38 and *V. parahaemolyticus* F54, were compared with the odor fingerprint by E-nose. As shown in a representative data set in Fig. 5(a), the four strains of *V. parahaemolyticus* are separated from each other. However, the result from cluster analysis in Fig. 5(b) shows that some overlap appeared between *V. parahaemolyticus* F01 and *V. parahaemolyticus* F13, and it indicate that the odor fingerprints of these two strains may be too similar to identify by this method.

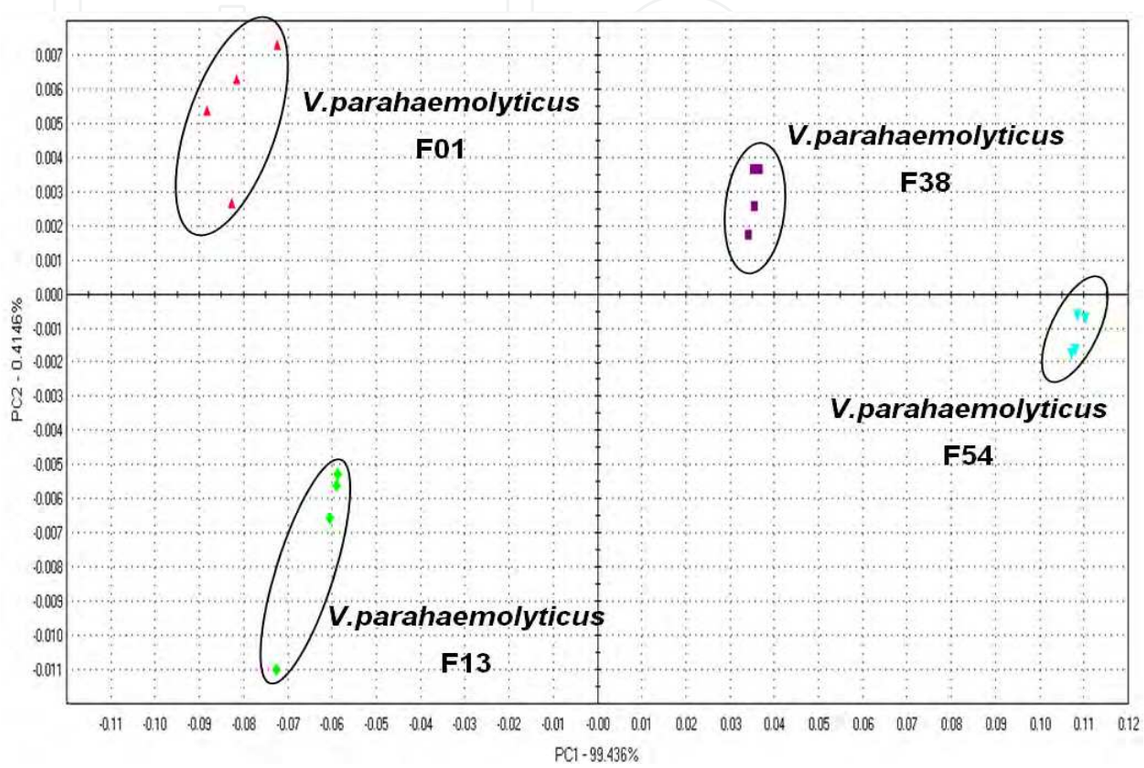
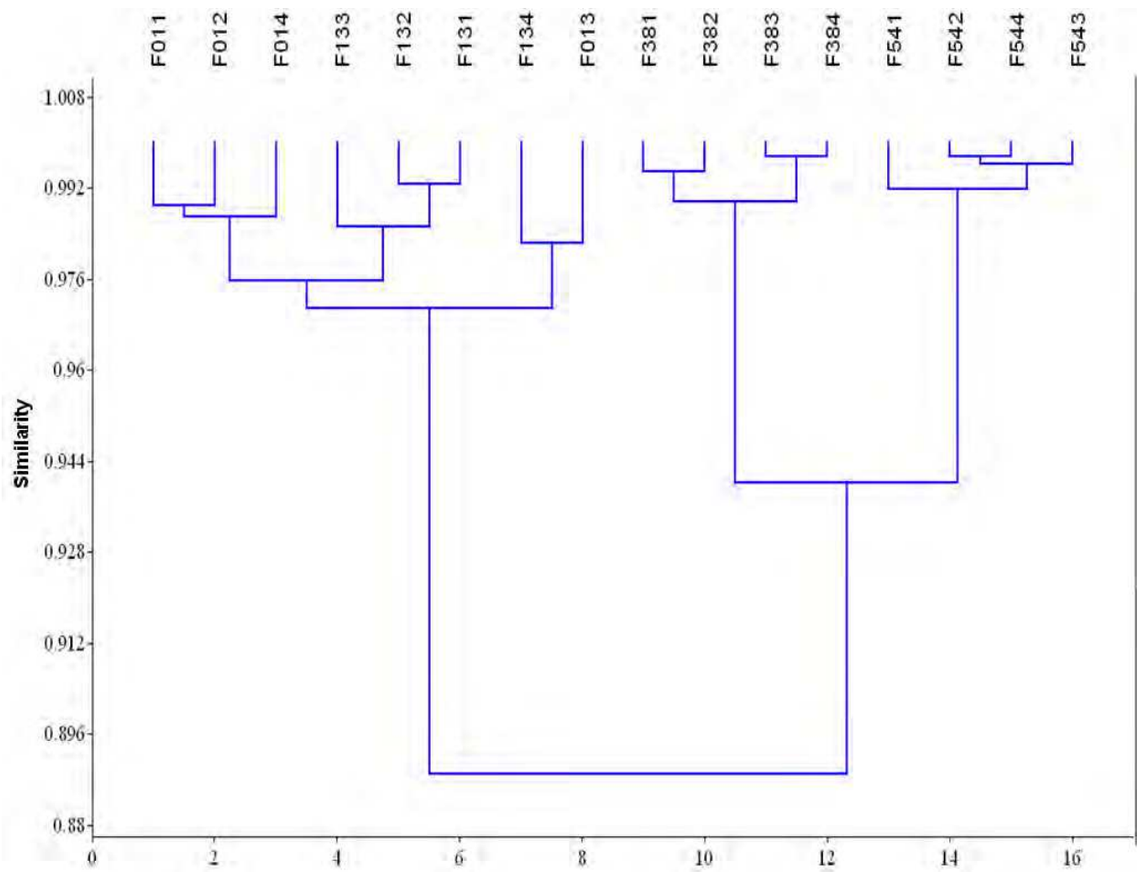


Fig. 5(a). Principal components analysis (PCA) for the discrimination of four different strains of *V. parahaemolyticus* on the basis of E-nose. The plot displays clear discrimination between the four groups, accounting for nearly 99% of the variance within the dataset.

4. Future perspectives

Electronic nose technology is relatively new and holds great promise as a detection tool in food safety area because it is portable, rapid and has potential applicability in foodborne pathogen identification or detection. On the basis of the work described above, we have demonstrated that the E-nose integrated with chemometrics can be used to identify pathogen bacteria at genus, species and strains levels.

As to know, bacteria respond to environmental triggers by switching to different physiological states. If such changes can be detected in the odor fingerprints, then E-nose analysis can produce information that can be very useful in determining virulence,



(*V.p* F01: F011-F014, *V.p* F13: F131-F134, *V.p* F38: F381-F384, *V.p* F54: F541-F544).

Fig. 5(b). Cluster analysis (CA) for the discrimination of four different strains of *V. parahaemolyticus* on the basis of E-nose.

conducting epidemiological studies, or determining the source of a food poisoning outbreak. Of course the ability to produce information on the physiological state of a microorganism offers many potential benefits. Nevertheless, a variety of different fingerprints, produced under a variety of growth conditions, must be developed for each pathogen, for inclusion in the reference database. To avoid this complication, we should culture the pathogens under controlled conditions. Otherwise, the identification algorithm must be capable of sorting through them all, to find a single, reliable, positive identification for the unknown.

Recently developed chemometrics algorithms are particularly suited to the rapid analysis and depiction of this data. Chemometrics is one approach that may offer novel insights into

our understanding of the difference of microbiology. Adopting appropriate chemometrics methods will improve the quality of analysis.

Odor fingerprinting method based on E-nose is still in its infancy. Many recent technological advances, which are outside the scope of this chapter, can be used to transform the odor fingerprinting concept into user-friendly, automated systems for high-throughput analyses. The introduction of smaller, faster and smarter instrumentation of E-nose to the market could also depend much on the embedding of chemometrics. In addition, more and more classification techniques based on odor fingerprinting may be developed to classify the pathogens into exact levels such as genus, species and stains. Further investigation may contribute to make a distinction between the pathogen and non-pathogen bacterial.

In short, E-nose integrated with chemometrics is a reliable, rapid, and economic technique which could be explored as a routine diagnostic tool for microbial analysis.

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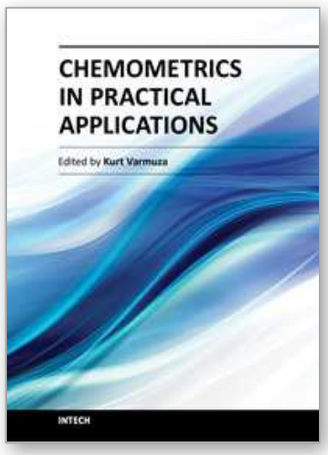

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