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Rapid Detection of *Escherchia coli* and *Salmonella Typhimurium* Using Lab-Made Electronic Nose Coupled with Chemometric Tools

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***Abstract*—This study aims to investigate the performance of a lab-made electronic nose coupled with chemometric tools for detecting *Escherichia coli* (*E. coli)* and *Salmonella Typhimurium* (*S. Typhimurium*) inoculated in media. The pathogenic *E. coli* and *S. Typhimurium* play a significant role as the agent causing food-borne diseases, posing a threat to human health worldwide. Some advanced analytical instruments like RT-PCR and GC-MS are often used for detecting such pathogenic bacteria. Unfortunately, they are not suitable for rapid and routine measurements because of time-consuming, require experts, and complicated sample preparation. Otherwise, electronic nose (e-nose) has been reported to be successful for profiling volatile compounds released by various biological materials. The e-nose comprised eight types of metal oxide gas sensors connected with a data acquisition system and chemometric tools. For this purpose, Fast Fourier Transform (FFT) was applied for signal pre-processing and feature extraction to all datasets collected by the sensor array in the e-nose. Furthermore, chemometric tools are used for classification models of all extracted features, including linear and quadratic discriminant analysis (LDA and QDA) and support vector machine (SVM). As a result, SVM showed the highest performance, enabling identifying *E. coli* and *S. Typhimurium* inoculated TSB with an accuracy of 99% and 98%, respectively. Among the chemometric tools, the e-nose-SVM also resulted in the highest accuracy in differentiating *E. coli* from *S. Typhimurium* of 84%. These results motivated e-nose to have a high prospect to rapidly detect such bacteria for food safety and quality control inspection, particularly potential quarantine products.**

***Keywords*— Electronic nose; *Escherichia coli; Salmonella Typhimurium;* chemometric; metabolic volatile organic compounds.**

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I. INTRODUCTION

Contaminated food by pathogenic bacteria has been posing a crucial issue due to the threat to human health and life worldwide. Millions of people suffered, and thousands died every year due to these food-borne disease outbreaks, even though certain bacteria such as *Bacillus subtilis natto* can degrade insoluble fibrin fibers of thrombosis [1]. Food is one of the media that facilitates the growth of pathogenic microorganisms to become an agent of food-borne diseases. More than 90% of food contamination cases in developing countries are caused by bacteria, such as *S. Typhimurium* and *E. coli* [2]–[4]. *E. coli* produces verotoxigenic, the primary source of food-borne diseases in humans. Meanwhile, *S. Typhimurium* is classified as a nontyphoidal serovar *Salmonella* (NTS) which can cause fever, nausea, cramps, headaches, diarrhea, and vomiting in humans [3], [5]. Also, food changes its taste and aroma when it is contaminated with bacterial like *E. coli*, decreasing its quality [6].

Contamination of *Salmonella sp.* in food is prohibited by the Indonesian standard of food safety (SNI), while *E. coli* contamination in food is allowed up to a maximum of 10 CFU/g. Conventional methods such as bacterial count calculations, growing on selective media, and simple biochemistry analysis methods are usually used to identify

bacteria and other pathogenic microorganisms in food samples. Unfortunately, these conventional methods are not suitable for rapid testing because of taking a long time to grow microorganisms on selective media. Moreover, the biochemistry analysis requires various media and reagents and takes about overnight to complete the procedure. Polymerase Chain Reaction (PCR), on the other hand, has been employed to overcome the limitation; but it takes about 18 hours of pre-enrichment step to obtain high accuracy. Furthermore, both methods require experts and have not provided a real-time result yet [6], [7].

Gas chromatography-mass spectrometry (GC-MS) can detect and identify the forming gas from microorganisms through sample headspace gas analysis. Here, a microorganism produces metabolic volatile organic compounds (MVOCs). These volatile compounds serve as a unique biomarker for the presence of each pathogenic microorganism in the media. In addition, MVOCs produced depend on the media for growing the microorganism [6]–[10]. However, GC-MS analysis is limited by a long time for sample extraction and analysis [11].

Consequently, an alternative tool capable of detecting disease agents from various samples, responding rapidly and inexpensively, such as an electronic nose, is needed [12]. The electronic nose device has a multisensorial system that promises to carry out such diagnoses non-invasive. Furthermore, various non-specific sensors and statistical approaches can be used in data processing, which are important steps for assembling functional devices in multisensorial systems [13].

The working principle of the electronic nose (e-nose) is inspired to mimic the olfactory system of a mammal, which was developed to identify the specific aroma of a sample [14]. The e-nose is capable to correctly analyze, identify, and recognize a blend of aroma and MVOCs since it is built from a gas sensor array with global sensitivity [12]. The e-nose is a much faster characterization system compared to GC-MS in detecting aroma. Besides providing results in real-time, the electronic nose can also operate at room temperature and use air as a carrier gas. The electronic nose comprises various chemical sensors, where each sensor can widely detect different gases or vapors. When complex samples are presented to a chemical sensor array, because each sensor is different, the response of each sensor to the sample is unique. However, the response of all sensors can be put together to make a fingerprint on the sample [15]. Some researchers have reported the success of e-nose in identifying or differentiating the types of bacteria employing various gas sensors, bacteria growth mediums such as *L monocytogenes* and *B cereus* [16], and type of analysis [17]–[19]. They use microbial detection in various fields such as medicine and the food industry. Some groups have used different electronic noses to classify and quantify bacteria and fungi to get an accurate medical diagnosis and control of food quality. So far, the detection and identification of volatile bacteria and fungi have been achieved using e-nose, which offers a different percentage of correct classification [20]. The satisfying performance of the e-nose device for analyzing pathogenic microorganisms has motivated this work.

This study aims to investigate the performance of a lab-made electronic nose coupled with chemometric tools for *E. coli* and *S. Typhimurium* inoculated in media. Here, the lab-made e-nose comprised an array of eight metal oxide gas sensors. Before the analysis using chemometric tools, the pre-processing method of Fast Fourier Transform (FFT) was applied to all the raw data (time-domain) to convert them to a frequency domain. The fast Fourier Transform (FFT) method is commonly used to extract features of an e-nose application [21]. The main purpose of FFT is to reduce dimension and electrochemical marker complexity by maintaining relevant information. Besides, FFT also provides an advantage during training, avoids data input redundancies, and acquires the correct general model [22], [23]. Tian *et al*. [11]also employed the FFT method to extract features of e-nose for grouping seven types of bacteria. The detection of such bacteria was carried out employing classifying the datasets into the respective class using supervised multivariate classification models (linear discriminant analysis, LDA; quadratic discriminant analysis, QDA; and Support Vector Machine, SVM).

II. Material and Method

A. E. coli and S. Typhimurium Stock

Escherichia coli (ATCC 25922) and *Salmonella enterica subsp enterica serovar typhimurium* (ATCC 14028) were obtained from MBRIO Food Laboratory, Indonesia. Both types of bacteria were then grown in *Tryptic Soy Broth (TSB)* (CM1029, Oxoid®) at 37 °C for 24 h, followed by storing in 30% glycerol at -20 °C before analysis.

B. Identification of Bacteria

Identification of bacteria was performed by Gram staining to identify cell morphology. For this purpose, *E. coli* was cultured in selective media of Brilliant Green (BG) Agar, Eosin Methylene Blue Agar (EMB) Agar, and Brilliant *E. coli* (BEC) Agar. Meanwhile, *S. Typhimurium* was cultured in selective media: BG Agar, *Salmonella Shigella* (SS) Agar, and Xylose Lysine Deoxycholate (XLD) Agar. Then, both types of bacteria were also biochemically tested, such as applying the indole test, the methyl red-Voges test was analyzed (MR-VP) test, citrate test, urease test, sulfur-indole-motility (SIM) reduction test, sugar fermentation ability, and hydrogen-sulfide production on Triple Sugar Iron Agar (TSIA), carbohydrate fermentation test (glucose, sucrose, and mannitol), and lysine decarboxylase and ornithine test.

C. Preparation of Sample Solution

TSB was used as basal media, where TSB without bacteria inoculation (blank) was used as a negative control. Before analysis, the bacteria stock was thawed in a water bath at 37 °C followed by transferring to TSB broth. Then, *E. coli* (100 – 1000 CFU/ml) was inoculated into TSB. A total of 3 mL of TSB (as a sample solution) was transferred into a 5-ml closed sterile glass vial bottle. Next, an amount of 3-ml TSB (as a sample solution) was transferred into a 5-ml closed sterile glass vial, and the sample was incubated at 37 °C. Analysis of the sample using the e-nose was performed every 8 hours for 48 hours incubation. The same treatments and analysis were applied to *S. Typhimurium* culture stock.

D. Bacteria Enumeration Method

For calculating the bacterial colony, all bacterial culture stock solution samples were diluted using 0.9% sterile liquid of sodium chloride (Otsu-NS®). Then, 1 mL of the diluted product was transferred to sterile Petri dish, followed by adding 15-20 mL of sterile Plate Count Agar (M019S, Himedia®), where each liter of the Plate Count Agar contained Casein Enzymic Hydrolysate 5 g, Yeast Extract 2.5 g, Dextrose 1 g, Sodium Chloride 6.5 g, and Agar 15 g. Petri disk shaker was used to homogenize the mixture between the media and the sample. When the sample and media mixture became solid, it was incubated at 37 °C for 24 hours. The number of colony-forming units (CFU) per mL growing during incubation is then calculated using the Quebec colony counfter.

E. Volatile Analysis by Electronic Nose

The e-nose used in this study comprised eight metal oxide-based sensors, namely TGS813, TGS822, TGS823, TGS826, TGS2600, TGS2603, TGS2612, and TGS2620 [22]–[24]. The e-nose was also equipped with SHT31-D sensor to monitor the temperature and humidity in the sample chamber (Fig. 1). Briefly, the e-nose consisted of sampling and sensor array systems (in the sensor chamber) controlled by a data acquisition system (DAQ). In addition, the output of the e-nose was then analyzed using signal processing and chemometric systems. In the sampling system, two valves (valve-1 and valve-2) were controlled for the sensing process and purging process. During the sensing process, MVOC from the sample was sucked by the small pump into the sensor chamber. Oppositely, during the purging process, reference air (dry and clean) or inert gas was sucked into the sensor chamber to remove the MVOC so that the sensor response returned to its initial condition. For measurement, the vial containing the sample was placed hot plate at a temperature setting of 47 °C to maintain the temperature inside the vial was 37 °C (incubation temperature). A more detailed description of the two processes is explained in Hidayat *et al*. [22]. As shown in Fig. 2, the typical response of a gas sensor in the e-nose and the time configuration sets are as follows: 10 seconds for the delay, 60 seconds for sampling, and 60 seconds for purging.

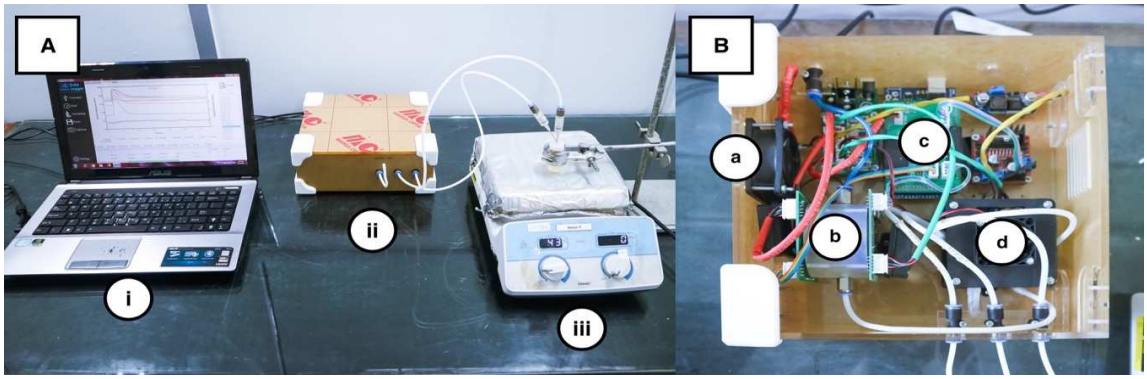


Fig. 1 A. A lab-made electronic-nose device. i: a computer equipped with chemometric tools, ii: the main part of e-nose, and iii: sample on the hot plate; and B. Part of e-nose device. a: power supply, b: sensor chamber, c: DAQ and controller, and d: sampling system

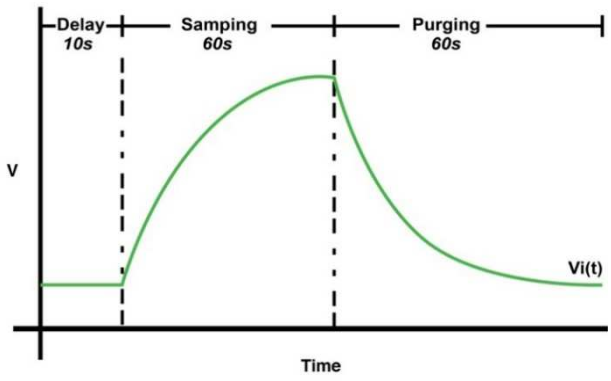


Fig. 2 Typical of response of a gas sensor to a sample. Time configuration setting in this study, for delay, sampling and purging were 10 s, 60 s, and 60 s, respectively.

F. Data Analysis

The total data of measurement are 336 consisted of 7 incubation times × 6 independent replication = 42 data of TBS blank (Neutral (N)), 7 incubation times × 21 independent replications = 147 data of *E. coli* (E), and 7 incubation times × 21 independent replications = 147 data of *S. Typhimurium* (S), as shown in Table 1. In this case, the sample of TBS blank was repeated six times because of very similar measurement results, while the sample of *E. coli* and *S. Typhimurium* were repeated 21 times. The independent replication means the different culture stocks. Each sample was measured using eight types of gas sensors, one temperature sensor, and one humidity sensor so that the sensor responses contained ten sensors x 1301 data lines.

TABLE I
TIME OF MEASUREMENT REFERRED AS TIME OF INCUBATION AND THE LABEL OF EACH SAMPLE

Incubation time (h)	Blank TBS (Neutral (N))	*E. coli* (E)	*S. Typhimurium* (S)
2	N2	E2	S2
8	N8	E8	S8
16	N16	E16	S16
24	N24	E24	S24
32	N32	E32	S32
40	N40	E40	S40
48	N48	E48	S48

Because resulting in big datasets of all measurements, Fast Fourier Transform (FFT) was used as a data pre-processing technique, while robustScaler was applied to the original data for obtaining a relatively similar scale. For data prediction, partial least square (PLS) was applied to the data regarding the incubation time. Supervised chemometric tools included

linear and quadratic discriminant analysis (LDA dan QDA) analysis, and support vector machine (SVM) were used in pattern recognition to classify the samples according to their respective classes. Finally, to define an excellent chemometric tool, the datasets were split into 10 folds and 10 times repeated of training datasets (for optimization and internal validation using K-fold cross-validation procedure) and test datasets (for external validation). The development of a technique or sensing methods for ensuring product quality/safety has become a priority, which will also help the community [25].

III. Results and Discussion

A. Identification of E. Coli and S. Typhimurium in Selective Media

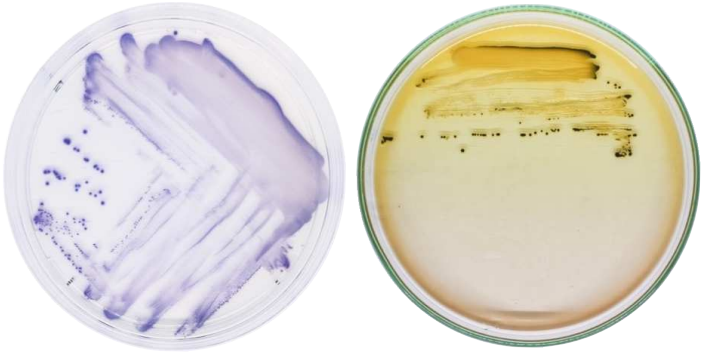
It is important to identify the type of bacteria at the beginning of this study to confirm whether the bacteria used were correct. Figure 3 confirmed that species of *E. coli* grew well, as indicated by purple colonies in Brilliance *E. coli*/Coliform Agar (BECA) media, while species of *S. Typhimurium* grew well as indicated by black center colonies in Salmonella Shigella Agar (SSA) media. Gram stain of *E. coli* described rounded end rod shape, occurred individually and in pairs while *S. Typhimurium* was rod shape, relatively smaller than *E. coli.* Both appeared pink, which indicated Gram-negative bacteria. Biochemistry analyses of both bacteria are summarized in Table 2.

TABLE II
BIOCHEMISTRY AND GRAM STAIN PROPERTIES OF E. COLI AND S. TYPHIMURIUM

Test	***E. coli***	***S. Typhimurium***
Indole	+	-
MR	+	+
VP	-	-
Citrate	-	+
Urease	-	-
SIM	H_2S : -, Indole : +, Motility : +	H_2S : +, Indole : -, Motility : -
TSIA	Gas : +, acid slant/acid butt.	H_2S: +, alkaline slant/acid butt
Glucose	+	+
Mannitol	+	+
Sucrose	-	-
Lysine decarboxylase	+	+
Ornithine decarboxylase	Are not done	+
Gram stain	Gram-negative	Gram-negative

B. Colony Counting of Bacteria

Before *E. coli* and *S. Typhimurium* were moved into a closed vial containing TSB, the bacterial population was calculated firstly. Data are derived from seven replications of each kind of bacterium. The culture showed that the number of inoculated bacteria was about 10^2 - 10^3 CFU/ml.



(a) (b)

Fig. 3 (a). *E. coli* in BEC media and (b). *S. Typhimurium* in SS media.

C. Data analysis Sensor Response

The *E. coli* and *S. Typhimurium* bacteria have been successfully grown in superbroth (tryptone 32 g, yeast 20 g, NaCl 5g, and NaOH 5ml). The base of the superbroth is similar to TSB. Using solid-phase microextraction analysis, specific compounds of dimethyl disulfide, ethanol, 2-nonanone, 2-heptanone, pentyl-cyclopropane, and indole were detected in *E. coli* hydrogen sulfide, ethanol, carbon disulfide, dimethyl cyclopropane, and 1-propanol were detected in *S. Typhimurium* [17], [18]. It has been reported that *E. coli* and *S. Typhimurium* in TSB produced 1-octanol, 1-decanol, and dodecanol. Specially, *E. coli* also produced indole, 2-undercanone, and 2-tridicanone [26]. From another report, *E. coli* produces a tryptophanase enzyme to catalyze tryptophan into indole and other compounds [27]. Although Salmonella species is indole negative, it produced hydrogen sulfide. Detected alcohol in the samples was likely derived from decarboxylation and deamination of amino acids. The presence of dimethyl disulfide resulted from the degradation of sulfur-contained amino acids such as methionine and cysteine [28]. The microorganism produces various metabolic and intermediate compounds due to metabolic activity during the growing phase. All data matrices collected from three types of samples resulted in 336 sample replications listed in Table 1. The original (time-domain) sensor responses of three types are depicted in Fig. 4. These plots are used to illustrate the variability between sensor responses, enabling the existence of the differences of patterns of three types of samples.

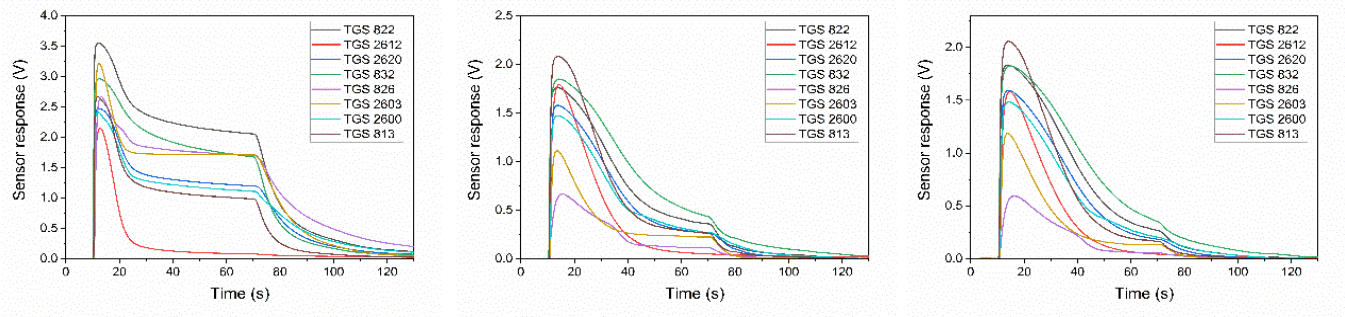


Fig. 4 Examples of the response on sensor array from raw data of measurement. Neutral (left), *E. coli* (middle), and S. *Typhimurium* (right).

D. Partial Least Square (PLS)

The growth of bacteria from 2 h to 48 h with the sequential measurement of every 8 h was shown in Fig. 5. Each data measurement was independently repeated six times for blank samples, and the others were repeated 21 times. The PLS actual data plot showed continuously increasing the number of bacteria E and S; therefore, each measurement time data was strongly correlated and overlapping to dispersed prediction plots. For *E. coli* incubated in the media, a clear distinction of each measurement time plot was observed, i.e., 8 h data compared to 32 h data. For *S. Typhimurium* incubated in the media, however, all predicted data are overlapping indicating that bacterial growth continuously.

In general, bacterial growth is divided into four distinct phases, namely the adaptation phase (lag phase), the growth phase (exponential phase), the balance phase (stationary phase), and the death phase [29]. The lag phase is a period with no growth that occurs when stationary phase bacteria are transferred to a fresh medium. The lag phase is a period of time without growth that occurs when the stationary phase bacteria are transferred to fresh media. The lag or adaptation phase for *E. coli* is 0 to 4 hours, whereas for *S. Typhimurium* the initial growth phase or adaptation occurs more quickly, in the incubation period of 0 to 2 hours. The lag phase of bacterial adaptation occurs, not to reproduce immediately, and the number remains constant; cells are metabolically active and only increase cell size. Then entering the exponential phase, the number of cells increases logarithmically, and each cell generation occurs at the same time interval as the previous one. The exponential phase of *E. coli* is 4 to 10 hours; the stationary phase is at 10 to 23 hours, then *E. coli* enters the death and dead phases. For *S. Typhimurium*, the exponential phase occurs in 2 to 7 hours, the stationary phase in 7 to 10 hours, and then the death phase occurs. PLS figures are seen in the exponential phase of *E. coli* 8 hours and *S. Typhimurium* 2 hours closer than the other phases. During the exponential or logarithmic phase, cells will metabolize optimally, multiplying by the time of manufacture which is determined by the quantity and quality of nutrients and available energy sources. When the nutrients in the medium begin to thin out, the metabolic waste products will accumulate [30], [31].

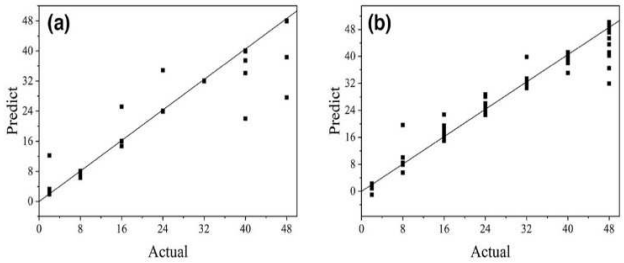


Fig. 5 PLS plot of growing bacteria from 0-48 h of (a) *E. coli* and (b) S. *Typhimurium*

E. Chemometric Tools

In order to evaluate the prediction performance, the e-nose was coupled with chemometric tools. Subsequently, after feature extraction, data of 336 samples (147 E data, 42 N data, and 147 S data) were classified using the chemometric tools of LDA, QDA, and SVM. For cross-validation purposes, 70% of each data class was split for training datasets and internal validation. The remaining 30% was used as external validation or testing datasets. To the extent of internal validation, repeated K-fold-CV Varian (10-repeats x 10-fold) was accounted for resampling to ensure 10% of training data in each run was reserved for internal validation.

The LDA was one of the standard supervised multi-class classification models [32]. In general, LDA also is known as Fisher linear discriminant since Fisher's introduction in 1936.

The LDA was used to obtained optimum linear combination to discriminate data into the distinct group by projecting data sample into lower dimension chamber. The LDA assumes the data are normally distributed, the class is split in identical covariance, and all features are independent and distributed identically. In order to classify, the LDA measures the distance between the projected objects and uses a scatter matrix to maximize the ratio of sample distances between classes to sample distances within the class. Figure 6 shows a two classes-LDA plot of N with E, N with S, and E with S. Imperfect classification of e-nose LDA was showed in LD-1, indicated by excessive overlapping data between the two classes, particularly E and S class (Fig. 6. (c)).

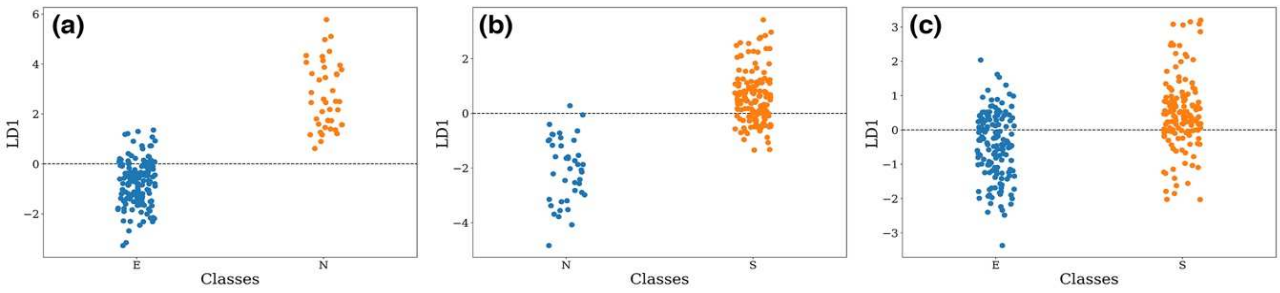


Fig. 6 First LD plots of bacteria incubated for 48 h of (a) N vs E, (b) N vs S, and (c) E vs S.

As listed in Table 3, the three chemometric tools used in this study allow the formation of a satisfying two-class classification modelling between non-inoculated to inoculated sample according to training and internal validation procedures (repeated K-fold-CV). The sensitivity of LDA, QDA and SVM (i.e., the percentage of samples correctly classified, Table 4) between N and E is 95%; 97%; and 99% and between N and S is 90%; 93% and 98%, respectively. Unfortunately, the e-nose capability to different types of bacteria establishing on collected signals by MOS gas sensor array showed relatively low predictive accuracy, i.e. 61% (using LDA), 66% (using QDA) and 84% (using SVM). In addition to that, e-nose SVM is considered superior among all two-class classification modelling.

TABLE III
CONFUSION MATRIX OF TWO CLASSES WITH DIFFERENT CLASSIFICATION MODELS

Actual \ Predicted	LDA N	LDA E	QDA N	QDA E	SVM N	SVM E
N	34	8	38	4	40	2
E	2	146	1	147	0	148

Actual \ Predicted	LDA N	LDA S	QDA N	QDA S	SVM N	SVM S
N	27	15	37	5	38	4
S	4	143	8	139	0	147

Actual \ Predicted	LDA E	LDA S	QDA E	QDA S	SVM E	SVM S
E	89	59	107	41	125	23
S	56	91	58	89	21	126

TABLE IV
THE SUMMARY OF E-NOSE PERFORMANCE COUPLED WITH CHEMOMETRIC TOOLS

Chemometric tools	**N vs E**		**N vs S**		**E vs S**	
	Accuracy	**Standard Error**	**Accuracy**	**Standard Error**	**Accuracy**	**Standard Error**
LDA	0.95	0.01	0.90	0.03	0.61	0.03
QDA	0.97	0.01	0.93	0.01	0.66	0.03
SVM	0.99	0.01	0.98	0.01	0.84	0.02

IV. CONCLUSION

The produced volatile compounds MVOCs by *E. coli,* and *S. Typhimurium* could be utilized as a biomarker to their presence. e-nose was able to sniff the produced MVOCs for detection and differentiation of bacteria type. In this study, SVM is the best method for the detection and differentiation of the two species. In comparison to the conventional method, e-nose showed its capability to detect bacteria without using reagents rapidly. This system is highly applicable for conducting food safety and quality control inspections, particularly potential quarantine products.

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