Original Article

Gas Array Sensors based on Electronic Nose for Detection of Tuna (*Euthynnus Affinis*) Contaminated by *Pseudomonas Aeruginosa*

Suryani Dyah Astuti[1], Achmad Ilham Fanany Al Isyrofie[2], Roichatun Nashichah[2], Muhammad Kashif[2], Tri Mujiwati[3], Yunus Susilo[4], Winarno[3], Ardiyansyah Syahrom[5]

[1]Forensic Sciences Studies Research Group, Magister of Forensic, Post Graduate School, Airlangga University, [2]Department of Physics, Magister of Biomedical Engineering, Faculty of Science and Technology, Airlangga University, [3]Department of Physics, Faculty of Science and Technology, Airlangga University, [4]Faculty of Engineering, Universitas Dr Soetomo, Surabaya, Indonesia, [5]Medical Devices and Technology Centre, Universiti Teknologi Malaysia, Johor, Malaysia

Abstract

Background: Fish is a food ingredient that is consumed throughout the world. When fishes die, their freshness begins to decrease. The freshness of the fish can be determined by the aroma it produces. The purpose of this study is to monitor the odor of fish using a collection of gas sensors that can detect distinct odors. **Methods:** The sensor was tested with three kinds of samples, namely *Pseudomonas aeruginosa*, tuna, and tuna that was contaminated with *P. aeruginosa* bacteria. During the process of collecting sensor data, all samples were placed in a vacuum so that the gas or aroma produced was not contaminated with other aromas. Eight sensors were used which were designed and implemented in an electronic nose (E-nose) device that can withstand aroma. The data collection process was carried out for 48 h, with an interval of 6 h for each data collection. Data processing was performed by using the principal component analysis and support vector machine (SVM) methods to obtain a plot score visualization and classification and to determine the aroma pattern of the fish. **Results:** The results of this study indicate that the E-nose system is able to smell fish based on the hour with 95% of the cumulative variance of the main component in the classification test between fresh tuna and tuna fish contaminated with *P. aeruginosa*. **Conclusion:** The SVM classifier was able to classify the healthy and unhealthy fish with an accuracy of 99%. The sensors that provided the highest response are the TGS 825 and TGS 826 sensors.

Keywords: *Electronic nose, food security, principal component analysis, Pseudomonas aeruginosa bacteria, tuna*

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Introduction

Tuna (*Euthynnus affinis*) is a seawater fish that has high economic value. It contains high protein content and is rich in omega 3 fatty acids. Every 100 g has a chemical composition consisting of 69.40% water, 1.50% fat, 25.00% protein, and 0.03% carbohydrates. One of the causes of fish damage is high water content (70%–80% of the weight of the meat) which makes it easy for microorganisms to breed. Fish damaged by microorganisms will produce volatile nitrogenous bases, also known as total volatile nitrogen bases, which mostly consist of trimethylamine (TMA), dimethylamine, and ammonia. TMA is an organic compound containing nitrogen, carbon, and hydrogen atoms, with the formula of NR3. These compounds can be used to determine the freshness of fish.[1]

The poor process of storing fish will cause the fish to rot quickly. According to Jay (2005), bacteria that cause fish to rot include *Pseudomonas* (32%–60%) and *Bacillus* sp. (<18%). The bacterium *Pseudomonas aeruginosa* as one of the bacteria that causes fish spoilage is a Gram-negative, rod-shaped, movable, aerobic bacterium that is commonly found in water, soil, plants, humans, and animals.[2] *P. aeruginosa* is a pathogenic bacterium in humans. It is invasive and toxigenic, so patients who have low immune systems can get infections.[3] Besides that, *P. aeruginosa* can interfere with the human digestive tract by enterotoxins, resulting in food poisoning.

Research on the quality of fish with preservation has been carried out by Alfiana Fadhilatul Nisa.[4] The research method used was a completely randomized design with two factors. Factor 1 is the concentration ratio for Physalis leaf extract

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Address for correspondence:
Dr. Suryani Dyah Astuti, Campus C Jl. Mulyorejo, Surabaya, Indonesia.
E-mail: suryanidyah@fst.unair.ac.id

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and distilled water (1:4); and the factor of 2 is immersion time (60s, 100s). The results showed that the best quality of albacore tuna was in the L1C3 treatment (*Physalis* leaf extract 50 ml +200 ml distilled water and soaking time 60') with the number of bacterial colonies being 16×10^5 cfu/g. The results also revealed a water content of 41.33% and pH of 6 with a less bright appearance, flexible dense texture, flat eyeballs, fresh smell or smell, and bright red gill color.

The assessment of fish-quality degradation still uses sensory methods such as appearance, texture, smell, and color.[5] So far, to clarify the level of freshness of fish, the human nose is used as an odorant in addition to physical detection. However, in reality, human olfaction has weaknesses, especially in standardization because of the subjective assessment of each human being. One of the efforts for early detection of fish quality is to use an electronic nose (E-nose).[6]

E-nose is an instrument that works to imitate the working principle of the sense of smell.[7] In the mechanism of the biological nose, there are mucus and vibrissae in the nasal cavity which serve as a filter and concentration of odorant molecules. Aroma molecules are carried to the epithelial tissue due to the passive pressure exerted by the lungs. The glucose epithelium contains millions of sensory cells and olfactory receptors located in the membranes of these cells. Receptors convert chemical signals into electroneurographic signals. This unique pattern of electroneurographic signals is decoded by a craft neural network.[8] In the general design of the E-nose, the pump functions as a lung, the sampling system acts as mucus and vibrissae in the nasal passages, sensor arrays act as olfactory receptors, and a signal processing system using a computer functions as the processing of the olfactory neural network.[9] E-nose consists of an array of gas sensors as a substitute for olfactory receptors that function to detect odors or scents. The aroma detected by several gas sensors will then form a certain pattern.[10] The detection of freshwater fish quality has been carried out by Lintang *et al*.[11] The study used three kinds of freshwater fish samples. The results of this study indicate that the E-Nose system can cluster the aroma of freshwater fish using the PCA method with the percentage of the first main component, namely 98.7% (onion), 98.8% (catfish), and 99.5% (tilapia). Sensors that gave a high response to each sample were the TGS 2620 and TGS 2600 sensors. The TGS 822 sensor gave a high response to fish when they were not fit for consumption. Furthermore, research done by Fachri Rosyad and Danang Lelono classified the purity of beef based on the E-nose by using the principal component analysis (PCA) method.[12] They used mixed beef samples with variations in pork content of 20%, 40%, 60%, and 80% of the total sample mass, and the data were collected for 10 days.

The E-nose used in this study has eight sensors consisting of sensors TGS 2620, TGS 2611, TGS 822, TGS 832, TGS 2602, TGS 2600, TGS 826, and TGS 825. Each sensor has sensitivity to a certain type of gas. When interacting with volatile compounds from a sample, each sensor responds in the form of different voltages and forms a unique pattern for each detected sample.

Hidayat (2015) suggested that the TGS gas sensor consists of three parts, namely the sensing element, the sensor base, and the sensor cap.[13] The gas-sensing element of the TGS sensor uses metal oxides, such as SnO_2.[14] The heater on this sensor functions as a trigger for the sensor to be able to detect the expected gas target after being given a 5 V voltage. Two metal elements are spaced at a predetermined distance. If the sensor detects gas, the density of the space between the metals will increase or decrease. When the resistance gets smaller, the current will flow so that the sensor voltage output will be large. The TGS gas sensor-sensing element material uses metal oxides, such as SnO_2. The heater which is used as a heating element for the sensing element works optimally with temperatures between 300°C and 550°C.[15] At low temperature, the reaction rate in the metal oxide surface is very slow. When the metal oxide grains are heated at high temperatures in free air, oxygen will be absorbed by the surface of the metal oxide grains, resulting in a negative charge. The donor electrons in the surface of the metal oxide grains are sent toward the adsorbed oxygen. This event leaves a positive charge in the layer. Therefore, a barrier potential is formed which can hinder the flow of electrons.[16]

When there are another gas and gas reduction, deoxidation reaction will occur which leads to the concentration of oxygen gas on the surface of the sensing material decreasing. This causes a decrease in the barrier potential so that the electrical resistance will also decrease and electrons will easily flow through the potential barrier.[17] The mechanism of increasing the concentration of charge carriers resulting from the interaction between the semiconductor material and the reduced gas is described in the following equation[18]:

$$e + \frac{1}{2}O_2 \Rightarrow O^-(s) \quad (1)$$

$$X(g) + O^-(s) \Rightarrow XO(g) + e \quad (2)$$

The above equation shows the oxygen adsorbed on the empty lattice of the sensing material. Oxygen is absorbed on the surface of the metal oxide causing the electrons in the conduction band to decrease and a depletion region is formed so that the electrical resistance is higher than when no oxygen is absorbed.[19] The electrons produced by the reduced gas are the result of the reaction of oxygen ions to the reduced gas X(g). As a result of this event, electrons will return to the conduction band and the depletion region is reduced, then the electrical resistance will decrease as the number of carrier concentrations increases.[20]

The E-nose system has four main components, namely the gas sensor array, headspace system, data acquisition, and pattern recognition.[21] Gas sensors used in making E-noses include conductive polymer gas sensors, quartz-micro balance, surface acoustic waves, and metal oxides. The headspace system has two processes, namely the sensing and purging processes. The data acquisition system can be performed by using a microcontroller. Methods commonly used to read certain patterns include PCA, linear discriminant analysis, partial least squares, multiple linear regression, cluster analysis, along with network methods such as artificial neural network, such as multi-layer perceptron, fuzzy inference systems, self-organizing map, radial basis function, genetic algorithms, neuro-fuzzy systems, and adaptive resonance theory.[22,23]

In the food industry, E-nose can be used as odor identification to monitor production processes, such as detecting pathogenic fungi that attack strawberry crops.[24] Arshak *et al*.'s research in 2004 proved that E-nose is able to sense the existence of microorganism pollution in food products, by sensing the odor patterns result coming from the organism's metabolic processes.[16] In 2015, Triyana *et al*. succeeded in making a gas sensor that detects the aroma of tempeh during fermentation to verify the tempeh aroma profile related to microorganisms growth.[17] Based on its advantages, which are rapid and nondestructive detection, the E-nose has been widely used in many types of meat evaluation.[25] However, in medical field, E-nose is also able to detect bacterial biofilms that cause many oral diseases, such as *Streptococcus mutans*.[26]

In recent years, the development of electronic sensor technology such as electronic tongue and E-nose has shown favorable application for pattern detection in daily life.[27] The present study aims to characterize fresh tuna and *P. aeruginosa* bacteria contaminated tuna based on the shelf time by using the pattern of gas sensor array system on the E-nose.

Subjects and Methods

Sample preparation

1–2 oz of *P. aeruginosa* was taken from oblique agar and then put into 9 ml of TSB and homogenized. Bacterial cultures were incubated for 2 h. Furthermore, 1 ml of culture was taken and put into a cuvette to calculate its optical density by using a spectrophotometer. After that, the culture solution was added with 2 ml of 2% sucrose and vortexed to make it homogeneous. 2 ml of samples was taken using a micropipette to be put into a 10 ml beaker glass. Then, the bacteria were incubated for 48 h at 37°C. The treatments were administered to the bacteria after going through the incubation process. The fresh tuna fish meat sample, which had been cut weighing 3 g, was then contaminated with *P. aeruginosa* bacteria.

Preheating time sensor

All sensors were warmed up first for 30 min so that they were stable and could work properly. The sample used in this process is clean air or commonly known as the baseline.

Normalization of sensors

The stability test of eight gas sensors was carried out, each of which has sensitivity to a certain gas. This process was done to equalize the baseline of each sensor to make further data processing easier. A baseline is a sensor response to reference substances, for example, clean air or nitrogen gas.[11] Baseline normalization is done by reducing each datum value by the first value.[28]

$$Y_n = Y_n - Y_1 \quad (3)$$

where Y_n is the value of sensor data and Y_1 is the first or lowest value of the data obtained.

Sensor response test to H_2S

The sensor response test to H_2S was done by using a concentration of 1–5 ppm. Furthermore, H_2S gas was sensed to obtain the stress results for each test.

Sample testing

After the samples were prepared, they were placed in a closed sample room. Next, the repetition test was conducted by taking four peaks of the E-nose response signal for each sample. The odor on was set at 180 s, while the odor off was set at 160 s. Each test was carried out three times with odor off and odor on cycles on the sample. The sample sensing process uses sampling rate of 17 Hz After data were collected, the sensor was left exposed to free air for 5 min before continuing to the next sample measurement.

Principal component analysis

The data result from the sample testing was processed with an average of two repetitions. The correlation between the sensor voltage output value of two times the type and concentration of gas can be used to obtain information about the freshness of the fish to be tested. Fish meat will produce a different sensory response to fish meat that begins to rot. The data analysis was performed by using the PCA method. PCA is a method that involves a mathematical procedure that transforms a large number of correlated variables into a small number of uncorrelated variables, without losing important information in it.[14] The PCA procedure aims to simplify the observed variables by shrinking or reducing their dimensions, which is done by eliminating the correlation between the independent variables through the transformation of the original independent variable to a new variable that is not correlated at all or commonly called the principal component. PCA transforms data into new coordinates, where the first coordinate is the first principal component obtained from the first largest eigenvalue and the second coordinate is the second principal

component obtained from the second-largest eigenvalue, and so on. After several components of PCA results that are independent of multicollinearity are obtained, these components become new independent variables that will be regressed or analyzed for their effects on the dependent variables using the regression analysis.

In this study, the range values of obtained data from E-nose were too high. Hence, before applying PCA, we did data normalization to scale our data into (0, 1). In machine learning, the data normalization is compulsory to get more higher accuracy (new).[29] The responded from E-nose was normalized using min-max scaler on python. The formula of min-max scaler is given below:

$$x_{sclaed} = \frac{x - x_{min}}{x_{max} - x_{min}} \tag{4}$$

After applying the min-max scaler, the data were scaled between (0, 1). Then, the feature extraction process was performed using PCA.

Here are some mathematical steps of PCA algorithm implementation.

1. Given a data matrix (X= [x_1, x_2,…, x_N]), where N represents the total number of samples and x_i represents the i^{th} sample
2. Calculate the mean of all samples as follows:

$$\mu = \frac{1}{N}\sum_{i=1}^{N} x_i \tag{5}$$

3. Subtract the mean from all samples as follows:

$$D = \{d_1, d_2, \ldots, d_N\} = \sum_{i=1}^{N} x_i - \mu \tag{6}$$

4. Calculate the covariance matrix as follows:

$$\sum = \frac{1}{N-1} D \times D^T \tag{7}$$

5. Calculate the eigenvectors V and eigenvalues λ of the covariance matrix (∑)
6. Sort eigenvectors according to their corresponding eigenvalues
7. Select the eigenvectors that have the largest eigenvalues $W = \{v_1, \ldots, v_k\}$. The selected eigenvectors (W) represents the projection space of PCA
8. All samples are projected on the lower dimensional space of PCA (as follows:
   $Y = W^T D$

After implementing the PCA with three components, the labeling process was performed. The sample of extracted features and labels are given in Table 1.

Table 1: The sample of extracted features and labels

PCA1	PCA2	PCA3	Labels
−1.087954807	−0.093784117	0.132708408	Healthy
−1.087934025	−0.093618344	0.13262034	Healthy
−1.087952939	−0.093595661	0.132604618	Healthy
−1.087992484	−0.093592535	0.13259655	Healthy
−1.087972791	−0.093611652	0.13258083	Healthy
0.262794185	−0.330256941	−0.028058147	Unhealthy
0.262527436	−0.330263216	−0.028029919	Unhealthy
0.262355096	−0.330239634	−0.028016373	Unhealthy
0.262162986	−0.330059406	−0.028052282	Unhealthy
0.261878639	−0.33020223	−0.027875204	Unhealthy

PCA – Principal component analysis

Support vector machines

Support vector machine (SVM) classifier is one of the machine learning techniques that can help solve big data classification problems.[30] Through kernel trick, the SVM classifier can separate data among higher feature space. The SVM kernel can be represented by the following formula:

$$K\left(\vec{x_i}, \vec{x_j}\right) = \varphi\left(\vec{x_i}\right) \times \left(\vec{x_j}\right) \tag{8}$$

In the above equation, $\varphi(x)$ is referring to a function that can shift the features vector x_i and x_j and then merge both features into a single feature. To classify different domains of data, many kernel functions of SVM have been developed. Linear SVM classifier does not affect the transformation of data. The polynomial SVM kernel using degree d transforms the data by adding simple nonlinear. A radial base kernel is another type of SVM kernel that can classify different types of data efficiently.[31,32]

In the current study, we aim to classify fresh and contaminated tuna. Hence, SVM is categorized into supervised learning algorithm in machine learning that analyze the given dataset and find out the patterns in data. Here are some mathematical steps of SVM algorithm implementation.

1. SVM algorithm usually determines the regression model function by using following minimization function.

$$min \frac{1}{2} w^2 + c\sum_{i=1}^{m} \xi_i^* + \xi_i$$
$$s.t. \begin{cases} y_i - w.\xi(x) - b \le \varepsilon + \xi_i^* \\ w.\xi(x) + b - y_i \le \varepsilon + \xi_i \\ \xi_i^* . \xi \ge 0 \ (i = 1,2,3,\ldots,m) \end{cases} \tag{9}$$

   In above equation, w represents weight of the vector, c represents penalty factor, ξ_i^* and ξ_i represent the relaxation component, $\xi(x)$ indicates linear transformation function, b represents the offset, and ε represents the upper limit of error.
2. The Lagrange multiplier is initiated now, which can be represented by a_i^* and a_i. The following equations are showing the optimization model.

$$\max -\frac{1}{2}\sum_{i,j=1}^{m}\left(a_i^*-a_i\right)\left(a_i^*-a_i\right)k\left(X_i,X\right)+\sum_{i=1}^{m}a_i^*\left(y_i-\varepsilon\right)-\sum_{i=1}^{m}a_i\left(y_i-\varepsilon\right) \quad (10)$$

$$s.t.\begin{cases}\sum_{i=1}^{m}a_i=\sum_{i=1}^{m}a_i^*\\ 0\le a_i,a_i^*\le c(i=1,2,3,\ldots,m\end{cases}$$

3. While the SVM function for regression model can be applied by solving above equations

$$f\left(x\right)=\sum_{i=1}^{m}\left(a_i-a_i^*\right)k\left(X_i,X\right)+b \quad (11)$$

$$k\left(X_i,X_j\right)=\exp\left(-\frac{X_i-X_j^2}{2\sigma^2}\right)=\exp\left(-\gamma X_i-X_j^2\right),\gamma>0 \quad (12)$$

In SVM algorithm, two parameters are crucial to adjust. The first parameter is penalty factor which is represented by c, and the second parameter is kernel which is represented by γ.

Results

Gas sensor series heating results

The heating of each sensor (preheating) had been carried out before the sensors were used so that the reactions with gases cause a change in the resistance value at the output. This initial treatment was done to prepare the sensor in steady-state conditions. Preheating was carried out at room temperature and in clean air conditions. Each sensor has a different standard of preheating time according to the datasheet of each sensor published by the sensor manufacturer. The heating time of the sensor to stabilize is shown in Figure 1. It can be seen that the preheating of each sensor was stable at 60 s with the assumption that at that time all sensors were stable and ready for use.

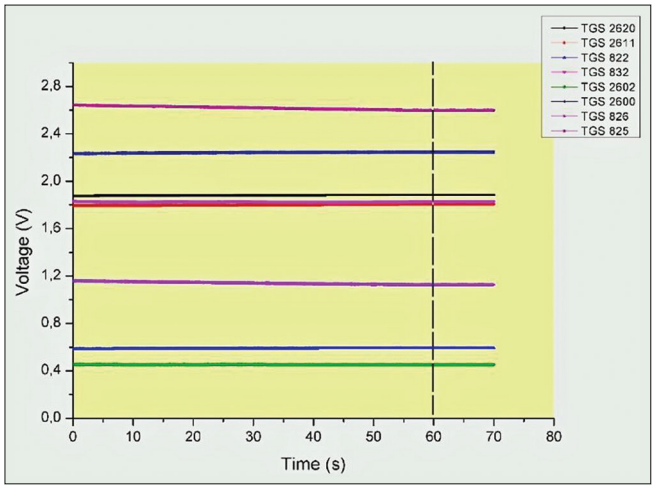

Figure 1: Preheating of each sensor

Electronic nose H_2S sensor response

H_2S gas is an indicator of the odor produced by spoilage samples of tuna. Therefore, variations in the concentration of H_2S were carried out including 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm. The sensor response to changes in H_2S concentration is shown in Figure 2. It can be seen that each sensor reacted to H_2S with different sensitivities. This was indicated by the increasing value of the voltage output to each sensor along with an increase in the amount of concentration.

From the datasheet, it is known that the sensors that were sensitive to H_2S include the TGS 2602 and TGS 825 sensors. Therefore, a test for the TGS 2602 and TGS 825 sensors was carried out based on the shelf-life of the sample. These test results are seen in Figure 3.

H_2S gas occurs due to natural processes as a bond product from the decomposition of organic substances by bacteria or because it is intentionally made. The formation of H_2S gas was obtained from the following reaction equation:

$$FeS + 2HCl \rightarrow H_2S\ FeCl_2 \quad (13)$$

The procedure for making H_2S gas refers to research done by Prasetyo (2002) using FeS and 1M HCl which were then reacted with a mass composition of 0.0001 g and 10 ml.[33] The gas formed was then stored in a 600 ml tube and immediately tested into the E-nose to obtain a voltage value that responds to H_2S gas. After that, the voltage value was converted to ppm so that the results were obtained as shown in the picture.

Electronic nose response to sample

As can be seen in Figure 4, E-nose produced different sensor responses in each test of three sample types, namely *P. aeruginosa* bacteria, tuna, and tuna contaminated with *P. aeruginosa* bacteria. The sensor response results revealed a typical response value for each sample so that it has a different type of sensor with the highest output.

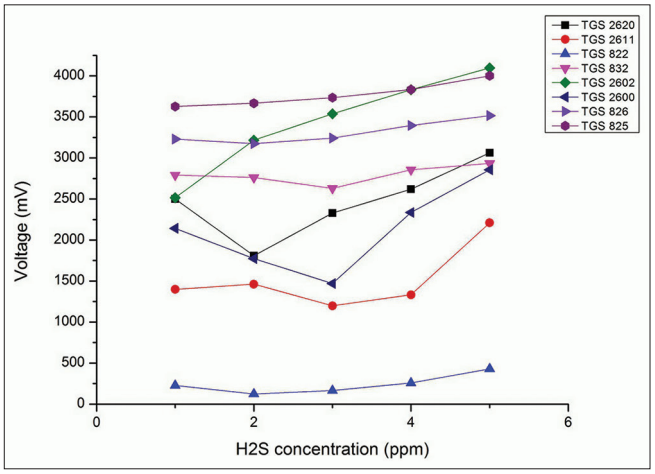

Figure 2: The graph of sensor react to H_2S concentration

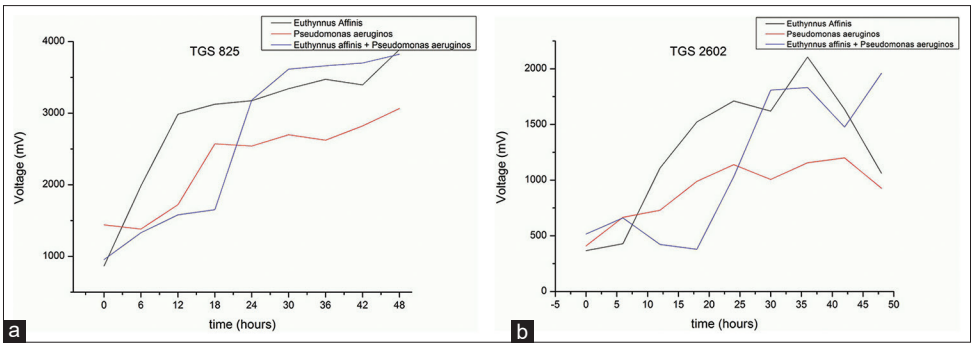

Figure 3: (a) Graph of sensor response to H_2S based on sample shelf-life for the TGS 825 sensor, (b) graph of sensor response to H_2S based on sample shelf-life for TGS 2602 sensor

Accuracy test

An accuracy test was performed to determine the closeness of the measurement results to the actual value. Accuracy is a close match between the results of a measurement and the correct value of the quantity being measured. It is necessary to test the percentage of recovery (% recovery) and to measure the accuracy of the test result. Accuracy is considered to be either within the recovery tolerance (% recovery) of 10% or within the range of 90%–110%. The results of the accuracy test on H_2S gas detected by the TGS 2602 and TGS 825 sensors are shown in Table 2.

Table 2: Sensor accuracy test results

Sensor	Percentage recovery				
	1 ppm	2 ppm	3 ppm	4 pmm	5 ppm
TGS 2602	95.899	99.297	102.594	101.263	98.534
TGS 825	101.037	100.716	99.268	98.984	100.756

TGS – Taguchi Gas Sensor

Principal component analysis score plots

PCA was done to analyze gas sensor series data for detection tests and classification of samples so that the ability of the gas sensor series can be known and the optimal type of sensor for this study can be determined. The PCA score plot graph can be used to determine the existence of groupings, clusters, and trends. The existing data grouping indicates the existence of 2 or more data distributions. Figure 4 shows PCA plot scores that were differentiated based on (a) sample storage time and (b) types of samples.

Figure 5 presents a graph of the score plot that was tested based on the aroma of fish that is fit for consumption. It is found that fish that is suitable for consumption had a storage period of 0–18 h. Meanwhile, fish that is not fit for consumption gathers elsewhere. This means that the PCA method is able to distinguish between the aroma of fish samples that are fit for consumption (fresh) and not (rotten).

PCA method can obtain the data variation of *P. aeruginosa*, tuna fish, and *P. aeruginosa* bacteria-contaminated tuna fish. Eigenvalue that generated from PCA score plot explained the difference of data information in the new coordinate of principal component.

Interpretation of principal component analysis loading plots

The results of the loading plot for all samples are shown in Figure 6. It is found that the variables with values close to 1 or −1 are the TGS 825 and TGS 826 sensors. This shows that the TGS 825 and TGS 826 sensors are the most influential sensors and the most responsive to the sample. Loading plots shown in Figure 7 are used to identify the most influential variables on the PCA component. If the loading plot value of a variable is 0, then that variable is considered to have the least effect on component analysis. Meanwhile, if the variable has a value close to 1 or −1, it indicates that the variable has the most influence on component analysis on PCA.

Interpretation with support vector machine

SVM is categorized into supervised learning model; it can be used for both regression and classification problems. For the binary classification tasks, SVM is the most commonly used method in machine learning. SVM grew in popularity becoming one of the most widely used machine learning algorithms. SVM is being used in a variety of disciplines, such as biomedicine and handwriting recognition problems.[34] Clinical diagnosis, weather forecasting, stock exchange analysis, and image analysis are among applications that employ SVM. SVM is the most commonly used machine learning algorithm that learns from experience and assigns the targets to the objects. For instance, in order for SVM to differentiate among real and fake credit cards, it must examine a huge number of actual and fake credit card pictures. SVM's primary role is to distinguish binary-tagged data based on a line that achieves the largest gap between the labels.[29]

Most of the supervised machine learning algorithms suffer with the curse of dimensionality. When a machine learning method retrieves a small number of instances and has little expertise in the context of many features, it suffers from the curse of dimensionality. The efficiency of a model may be harmed as a result of such constraints. The SVM classifier was shown to be vulnerable toward the dimensionality

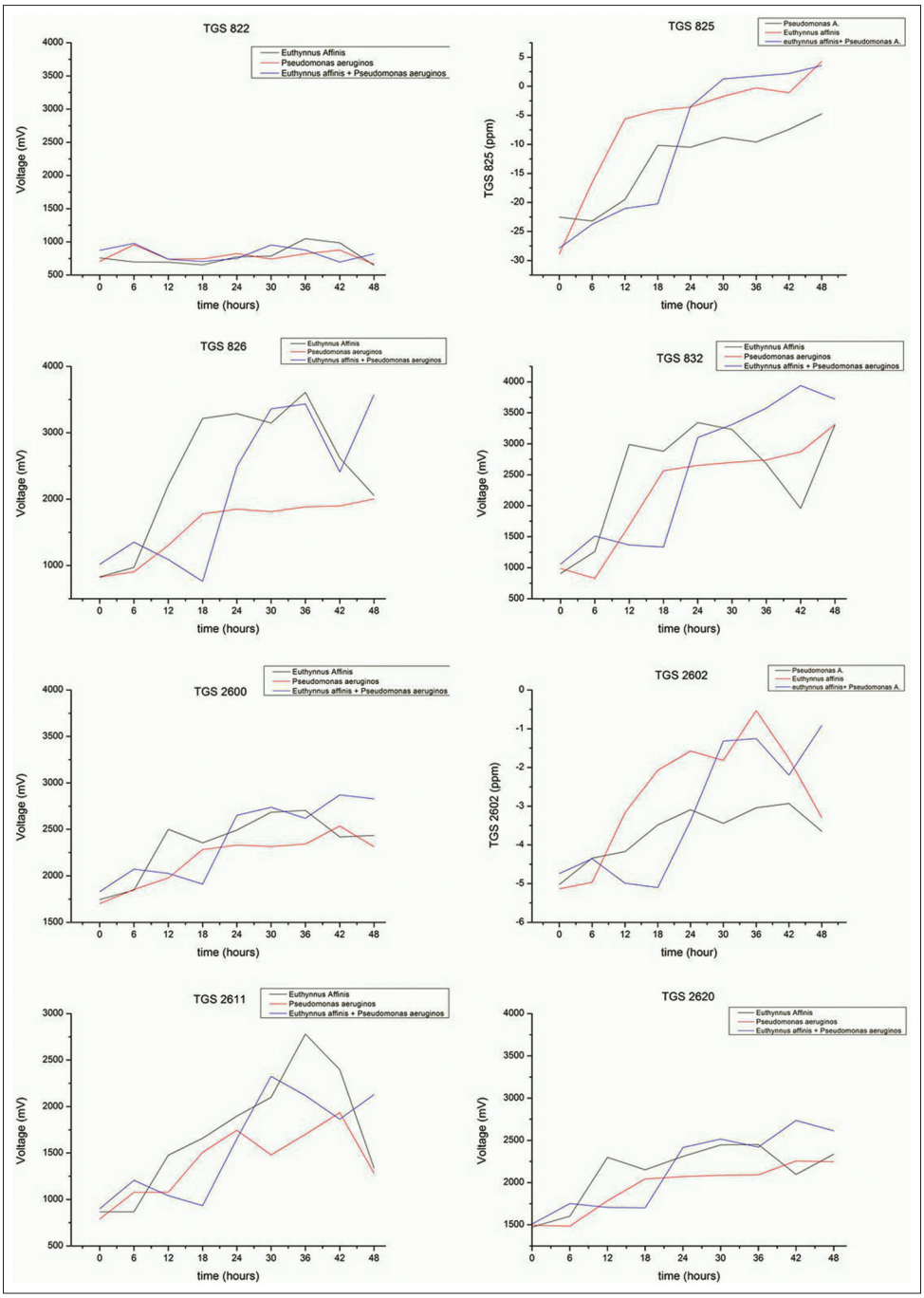

Figure 4: Graph of sensor sensitivity to the sample

curse.[35] Because of these advantages, we used SVM classifier to classify the healthy and unhealthy fish meat. We analyze the data using Weka tool (ref.weka), which is publicly available. After getting PCA features with three components, the data were divided into two parts. 80% of data were used to train the model while 20% of data were used to test the model. We used 10-fold cross-validation method to evaluate our SVM model. The performance metrics of SVM classifier are given in Table 3. The SVM classifier was able to classify the data with 99.50% of accuracy.

Discussion

The E-nose sensor was preheated before starting the sensing process. This initial treatment was carried out to prepare the sensor in steady-state conditions. Preheating was performed at room temperature and in clean air conditions.[36] Each sensor has a different standard of preheating time according to the datasheet published by the sensor manufacturer.[37] The preheating process was done at an interval of 60 s.

Table 3: Detailed accuracy by class

	TP-rate	FP rate	Precision	Recall	F-measure	MCC	ROC area	PRC area	Class
Weighted	0.985	0.000	1.000	0.985	0.992	0.989	0.993	0.990	Healthy
average	1.000	0.015	0.993	1.000	0.996	0.989	0.993	0.993	Unhealthy
	0.995	0.010	0.995	0.995	0.995	0.989	0.993	0.992	

TP – True positives; FP – False positives; MCC – Matthews correlation coefficient ; ROC – Receiver Operating Characteristics; PRC – Precision-Recall Curve

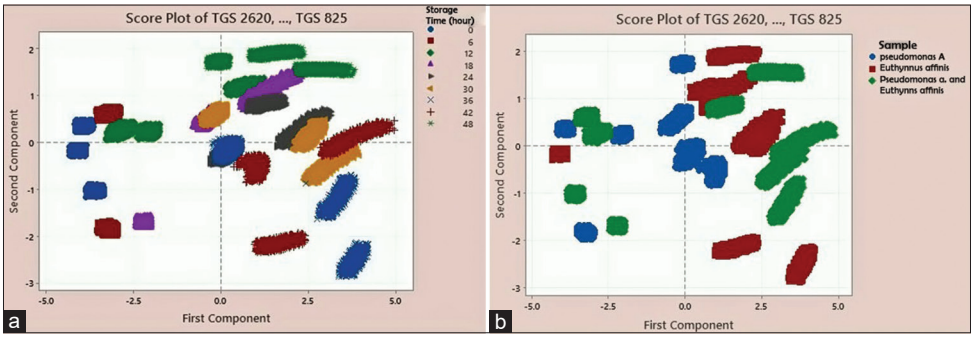

Figure 5: Principal component analysis plot scores were differentiated based on (a) sample storage time and (b) kind of samples

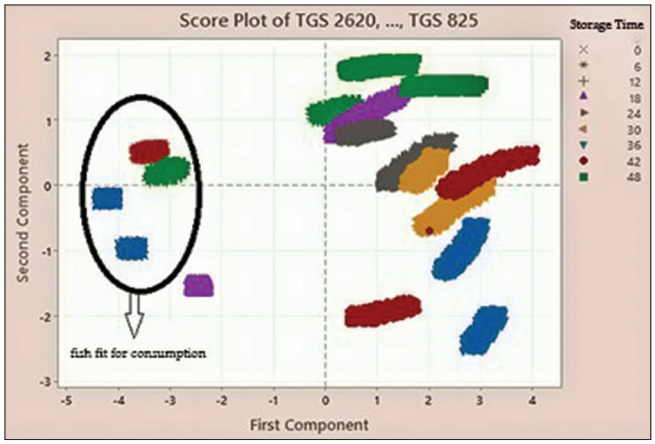

Figure 6: Graph of score plot of tuna and contaminated fish based on hours

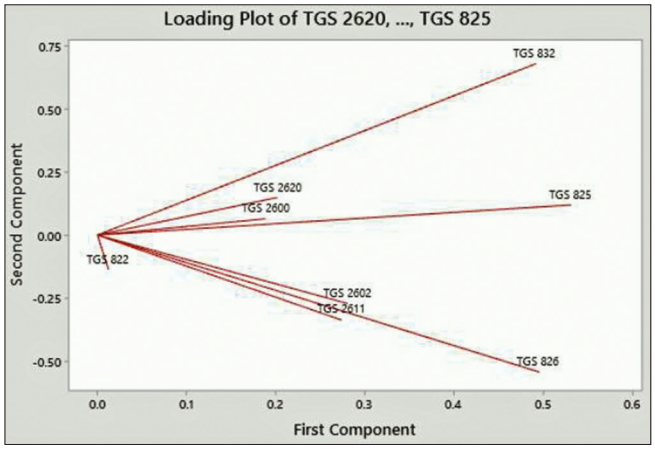

Figure 7: Results of the loading plot for the entire sample

The sensor characteristics test was conducted using H_2S gas. H_2S gas is one of the odors produced by tuna samples. The calibration was carried out with variations in H_2S concentrations, namely 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm. The sensor response to changes in H_2S concentration is shown in Figure 2, where the most responsive sensors to H_2S gas were TGS 2602 and TGS 825. These results are in accordance with the sensor datasheet. Figure 3 shows that the TGS 825 sensor has a sensor response that increases along with increased shelf-life. Meanwhile, the TGS 2602 sensor peaked at the 30th h.

E-Nose sensor testing was carried out on *P. aeruginosa* bacteria, tuna, and tuna contaminated with *P. aeruginosa* bacteria. The tests were carried out based on the samples' shelf-life. The samples that have been made were stored based on variations from 0 to 48 h, and the test was done every 6 h and two times repetitions.

The working mechanism of the electric nose system is detecting aroma by the sensor array, signal preprocessing, as well as processing by pattern recognition system and computational analysis.[38,39] Initially, the odor to be detected is exposed to a sensor array, which functions similarly to the human olfactory cell. Analog data from the sensor will be converted into digital data by an analog to digital converter (ADC) to be saved to a computer and further analyzed. The data from the ADC will be preprocessed first. The processing serves to prepare the signal so that it can be easily processed by a pattern recognition machine. This stage works identically as the vesicle layer in the human sense of smell. The final stage is processing by the pattern recognition system. This section aims to classify and predict unknown samples. The function of this section resembles the function of the olfactory center in the brain.[36] Thus, the E-nose system detects and classifies aromas automatically as a quality controller of aroma recognition, especially for the food industry.

Fish stored at room temperature will experience decay, due to the growth of microorganism activity and foul-smelling enzymes that occur due to the formation of ammonia (NH_3). Ammonia is what causes fish to produce a bad smell. Research on the ammonia content produced per hour continues to increase because the protein in the sample continues to be damaged as the shelf-life increases.[40,41] The mechanism of the E-nose to detect the odor of (a) bacteria and (b) tuna fish is shown in Figure 8.

At first, the smell of the sample is tested on a sensor that has been preheated before. The sensor works by detecting the gases contained in the sample odor. A sensor is a device that functions to detect symptoms or signals originating from changes in energy such as electrical energy. The sensor used in this study is a gas sensor that can respond to the concentration of certain particles such as atoms, molecules, or ions in the gas and convert it into an electrical signal.[42] Commonly, the sensor uses a metal oxide semiconductor material to detect certain gases. Changes in the electrical properties of metal oxide semiconductors are caused by interactions with gas molecules preceded by the absorption of oxygen in the semiconductor. Oxygen molecules are adsorbed on the semiconductor surface and capture electrons from the conduction band.[28]

The formation of H_2S by microorganisms indicates the decomposition of amino acids (the smallest part of a protein) containing sulfur which are produced when proteins are hydrolyzed to meet the nutrient needs of microorganisms.[43] The use of *P. aeruginosa* in this study aims to determine the level of decay of fish contaminated with bacteria. These bacteria generate one or more pigments produced by aromatic amino acids such as tyrosine and phenylalanine.[44,45]

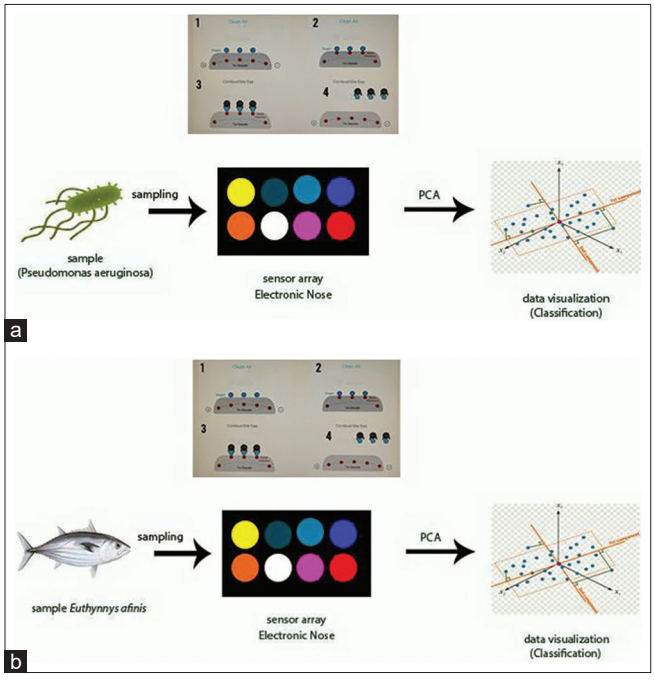

Figure 8: The mechanism of electronic nose to detect the odor of (a) bacteria and (b) tuna fish

Data analysis using PCA aims to reduce the dimensions of the correlated variables into a linearly uncorrelated reduced variable called the principal component to explain profusely the variance that occurs with the minimum number of principal components. The number of input variables in the PCA process is eight variables that represent the number of sensors on the E-nose. These variables will eventually be reduced into two dimensions consisting of the first main component (PC1) and the second main component (PC2), which can represent the percentage of variance values obtained. The significance of the total variance of the data that occurs is used to create a two-dimensional data visualization graph for qualitative analysis and interpretation of information. Figure 4 presents the result of a two-dimensional score plot on the two main components for the samples. The two main components of the score plot graph explain the 95% variance percentage. Figure 5 shows PCA functions in capturing variations in fish and fish contaminated with bacteria.

Conclusion

The electronic nose is able to detect the quality of tuna (*E. affinis*) and tuna contaminated with *P. aeruginosa* based on odor with a percentage of the variance of the two main components of 95%. E-nose which consists of eight gas sensors including TGS 825, TGS 2600, TGS 2620, TGS 832, TGS 822, TGS 826, TGS 2602, and TGS 2611 can identify rotting tuna (*E. affinis*) based on the smell which is indicated by the increasing *g* value of the concentration of gas produced and the increasing value of the voltage received by the E-nose. The results of this study indicate that the electronic nose system is able to smell fish based on the hour with 95% of the cumulative variance of the main component in the classification test between fresh tuna and tuna fish contaminated with *P. aeruginosa*. The SVM classifier was able to classify the healthy and unhealthy fish with accuracy of 99%. The sensors that provided the highest response are the TGS 825 and TGS 826 sensors.

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Conflicts of interest

There are no conflicts of interest.

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