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Original Article

Electronic Nose (E-Nose) for Quality Detection of Tuna *(Thunnus thynnus)* Contaminated Bacteria

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ABSTRACT

Tuna *(Thunnus thynnus)* is a food that is often consumed raw to support raw food diet activities, so it has the potential to be contaminated with *Salmonella typhi* bacteria. Fish can be contaminated by bacteria due to their high water and protein content. Indonesia is the world's main tuna producer. Salmonella typhi detection in fresh tuna in Indonesia must be negative for Salmonella microbial contamination in order to meet food safety requirements. Microbial testing has drawbacks, such as long delays. An electronic nose was used to detect *Salmonella typhi* bacteria in tuna fish. The sample used consisted of 3 kinds of samples: *Salmonella typhi* bacteria, tuna, and tuna with *Salmonella typhi* contamination. The research was conducted with a shelf life of 48 hours and a sensing period every 6 hours with a sensor array of 8 sensors. The sensor output data is processed using the PCA (Principal Component Analysis) method. Through the PCA method, each variation of bacterial treatment can be classified. The result of the cumulative percentage variance of the two main components (PC) in the classification test between *Salmonella typhi*, tuna, and tuna with *Salmonella typhi* bacteria contamination was 90.5%. The most influential sensors in this study are TGS 825 for PC1 with a loading value of 0.625 and TGS 826 for PC2 with a loading value of -0.753. Therefore, it can be concluded that an electronic nose can classify between pure tuna and tuna contaminated with *Salmonella typhi* bacteria.

Keywords: array gas sensor; electronic nose; principal component analysis; *Salmonella typhi*; Tuna *(Thunnus thynnus)*

Highlights: The most influential sensors in this study are TGS 825 for PC1 with a loading value of 0.625 and TGS 826 for PC2 with a loading value of -0.753. Therefore, it can be concluded that an electronic nose can classify between pure tuna and tuna contaminated with Salmonella typhi bacteria.

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INTRODUCTION

People's habits and behavioral patterns have changed as a result of the times. Knowledge of something, including information about food, enables a person to alter his behavior. Humans require food to survive. Food is therefore crucial for humans. The raw food diet is one change in lifestyle that can be influenced by food knowledge.

A raw food diet is a way of eating that involves only consuming unprocessed, uncooked, or unheated food. Due to the growing propensity of people to eat healthy foods and create a society that values health, this trend has spread more widely in recent years. This is in line with research on the perceptions of the Surabaya population towards organic food.[1] By using a quantitative exploratory research method and a multidimensional scaling technique, it was discovered that respondents' perceptions of the quality and safety of their food were, on average, 3.26, with the highest values occurring between the intervals of 2.6 and 3.4. This suggests that the Surabaya population, or respondent, has perceptions of food quality that are very favorable.

Despite having a higher nutritional value than processed food, raw food has the potential to be contaminated with pathogenic bacteria, according to microbiological hazard identification research by the Foodborne Illness Investigation (FII). The cleanliness and absence of pathogenic microorganisms that could potentially cause disease are indicators of good food quality. This illness is referred to as a foodborne illness.

The majority of the bacteria identified as histamine producers are gram negative (87% of isolates), and the majority of these isolates (80%) are members of the *Enterobacteriaceae* family. *Morganella morganii* was the organism most frequently and actively producing histamine in canned tuna fish. Along with several strains of *Enterobacter cloacae* and *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and other potent histamine-producing bacteria were also discovered during the canning process. Some workers have previously experienced similar outcomes. 73% of the former histamine-producing strains that were isolated and identified were from *Morganella* and *Enterobacter spp*.

Salmonella typhi bacteria is one of the microorganisms that frequently contaminate raw food. The maximum contamination limit for *Salmonella sp* is negative per 25 grams of food. *Salmonella typhi* is a gram-negative bacterium that causes typhoid fever. The disease may occur anywhere in the world, but is most prevalent in developing countries, including in Indonesia. The incidence of typhoid fever in Indonesia is thought to be between 300 and 810 cases per 100,000 people per year, with a range of 600,000 to 1,500,000 cases per year. Effective prevention measures are required because this disease has a 1-5% patient mortality rate.[3]

Salmonella typhi bacteria may be present in tuna (*Thunnus thynnus*), which is one of the foods frequently consumed in a raw state to support raw food diet activities. Additionally, due to the high water and protein content of fish meat, bacteria can easily contaminate fish.[4] Indonesia is the primary producer of tuna in the world, according to the Food Agriculture Organization (FAO).

Salmonella sp must test negative in every gram of fresh tuna for the specific type of *Salmonella* microbial contamination test in order to meet quality and food safety requirements in Indonesia. An organization called BKIPM (Fish Quarantine Agency, Quality Control and Safety of Fishery Products) is in charge of vetting the safety and quality requirements for fishery products.[5] Microbiological tests in accordance with Indonesian National Standard (INS) are used as a detection, isolation, and confirmation mechanism for *Salmonella bacteria* in *tuna*. However, due to the lengthy turnaround time (1-3 days) required for test results and the high absorption capacity of both the domestic and international tuna markets, there are a number

of weaknesses with the microbiological method of detection.[6] The United States was Indonesia's primary export market for fisheries products. The quantity of tuna products exported by Indonesia to the United States from 8,504 tons in 2015 to 10,788 tons in 2016, the number of states increased. As of May 2017, Indonesian tuna exports were registered at 65,875 metric tons, valued at USD 226 million. It was anticipated that the volume of fishery goods exported would grow, possibly leading to more products being returned. The primary cause of the rejection of shrimp commodities was microbial contamination. The most frequently reported microorganisms that caused the illnesses were Salmonella, E. coli, and Vibrio cholerae. The challenge faced by exporters from Indonesia, the occurrence of variations in sample collection techniques and testing microorganisms between Indonesian laboratories and destination nations. Testing technology and laboratory infrastructure (methods and tools) change in order to provide results with varying degrees of precision.[7]

As anticipated, precooking tuna fish significantly reduced the numbers of all bacterial groups under investigation. However, the bacterial burden in tuna fish continued to rise following the precooking stage. For precooking tuna fish, high heat (100–105 °C for 110 min) is typically employed. The time-temperature link is sufficiently strong to significantly lower the bacterial load. After precooking, tuna was allegedly left at room temperature, allowing damaged germs to quickly recover, multiply, or get decontaminated by the environment. *Mesophilic* and *psychrotrophic* bacteria counts rose concurrently during canning, but with a modest advantage for the latter. The temperature of the water where the tuna fish was caught (between 8 and 15 °C) and the length of time it was kept frozen are likely to be to blame for the larger number of *psychrotrophic* organisms. In frozen tuna fish, *Enterobacteriaceae* and coliform counts have always been low and only made up 0.34 percent of the overall bacterial burden. But as the tuna was handled during the canning process, the number of *Enterobacteriaceae* grew until it made up 2.18 percent of the bacterial load.

An instrument called the *Electronic Nose* (*E-Nose*) mimics how the sense of smell functions. As an alternative to olfactory receptors, which are responsible for detecting smells or scents, the *E-Nose* is made up of a variety of gas sensors. The aroma picked up by numerous gas sensors will then take the form of a specific pattern.[8] *E-Nose* has applications in the areas of microbiological detection and food safety.[9,10] *E-Nose* has the benefits of being non-destructive, real-time, quick, and inexpensive.

According to the research's conclusions, E-Nose could differentiate between samples of beef, pig, or a combination of the two based on the fragrance pattern each sample created. *E-Nose* has been extensively used in a variety of fields and industries, including those related to food, drink, chemicals, defense, health, etc.[11] Research on early detection and classification of pathogenic fungi that attack strawberry farming is one application of *E-Nose* in the food industry for monitoring production processes.[12]

Principal Component Analysis (PCA) is one technique for analyzing the data produced by Electronic Nose. By using the PCA method, it is possible to replace some of the original, correlated variables with a new, smaller set of uncorrelated variables. In order to make it simpler to interpret the data, the main goal of this method is to reduce the dimensions of the interconnected and numerous variables. The authors intend to conduct research on the pattern of data generated by the *E-Nose* gas sensor array in an effort to detect the content of *Salmonella typhi* in tuna *(Thunnus thynnus)* using PCA method because the national standard for microbial testing used to detect the content of *Salmonella typhi* in *tuna* (Thunnus thynnus) has a drawback, namely it takes a long time.

By using a gas sensor that can react to specific scents, the *E-Nose* device imitates how a mammal's nose detects smells.[13] As an alternative to olfactory receptors, which are responsible for detecting smells or scents, the *E-Nose* is made up of a variety of gas sensors. The aroma picked up by various gas sensors will then take on a particular pattern. In order to analyze and identify the signal response produced by the *E-Nose* to a specific scent, pattern recognition software will be used. *E-Nose* has a wide range of uses, such as assessing food quality, tracking air pollution, and identifying various gases and toxins.[14]

E-Nose uses the biological nose's operating principle to characterize various gas mixtures. The human smell system is divided into three layers, namely.[15]

1. A layer of approximately one billion olfactory cells
2. Olfactory vesicles have three main functions: to control, regulate, and amplify messages from olfactory cells.
3. The brain's olfactory center, which defines signals and organizes the different types of smells that can be detected.

The *E-Nose* has three main components, sample handling, detection systems, and data processing systems.[16] The *E-Nose* operates on a similar principle to the human nose, which contains a variety of receptors for identifying scents. The sensors on the *E-Nose* serve as a replacement for these receptors, and each remaining receptor reacts differently to the same aroma vapor.

The stages in the *E-Nose* system are signal pre-processing, signal processing, and pattern recognition system processing. The sensor array is initially exposed to the scent that needs to be detected. These sensors perform nearly as well as olfactory cells in humans. An *analogue* to *digital converter* (ADC) will convert the *analogue* data from the sensor into *digital* data, which can then be saved to a computer and used for further analysis. Preprocessing will be done on the ADC data first. Processing is used to get the signal ready so that a pattern recognition machine can process it quickly. Similar to the vesicle layer in the human sense of smell, this stage performs almost the same functions. The pattern recognition system processes the data in the final step. This section seeks to categorize and forecast unidentified samples. This component's function is comparable to that of the brain's olfactory center.[17]

The following list of necessary components is provided by Gardner and Bartlett as a definition of an electronic nose device's fundamental requirements:

1. A sensor array system with an aroma delivery system that transfers volatile aromatic molecules from the source material.[18]
2. The environment in which the sensor is located: normally, the temperature and humidity are fixed, as this would prevent the aroma molecules from being absorbed otherwise.
3. Electrical signals are transformed into chemical signals by electronic transistors.
4. A *digital converter* that transforms electrical (*analogue*) signals into *digital*.
5. A computer microprocessor that reads the digital signal and outputs it after statistical analysis is carried out to classify or identify a sample.

Each of the gas sensors in the *E-Nose* will react to changes in smell or aroma. Each gas sensor will respond to aroma or odor by changing its resistance.[19]Each gas sensor's resistance will fluctuate, changing the voltage as a result. This voltage changes yielded data in the form of *digital* computer data. From this point forwards, a data processing device will be used to process the data. Figure 1 displays the block diagram for the *E-Nose*.

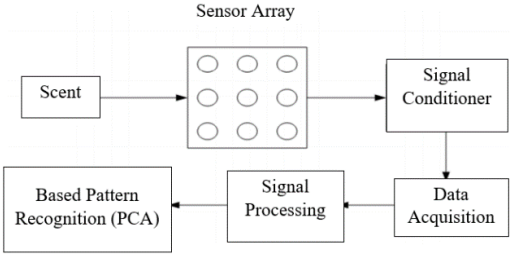


Figure 1. Electronic Nose Block Diagram

A sensor is a piece of technology used to identify symptoms or signals brought on by changes in energy, including electrical, mechanical, chemical, biological, and other types of energy. A transducer is a device that, when powered by an energy in a transmission system, transmits the energy to the following transmission system in the same form or in a different form. This energy transmission may be thermal, optical (radiation), mechanical, chemical, or electrical (heat). In other words, the sensor is a part that can be used to transform a certain quantity into an analogue unit so that an electronic circuit can read it. The sensor is the main part of a transducer, and the transducer is a supporting system that enables the sensor to have the desired output and to be directly readable at the output.

Principle component analysis (PCA) is a mathematical technique that transforms a set of potential correlated variable observations into a set of principal components, which are linearly uncorrelated variable values.[10] Data from *electronic nose* output is processed using PCA, which can classify data based on the type and concentration of bacteria.

In order to obtain a smaller amount of data with the greatest data variation in the new coordinate system, PCA reduces variable data by transforming it linearly. Figure 2 displays thePCA transformation's outcomes.

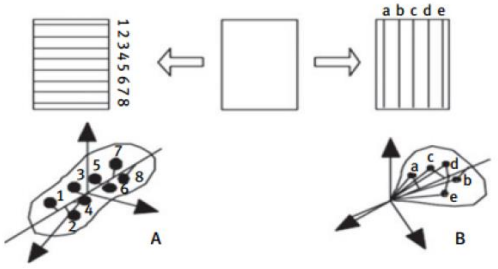


Figure 2. Results of PCA transformation.[20]

The PCA method aims to reduce the dimensions of the observed variables, thus simplifying them. This is accomplished by changing the original independent variable into a new variable that is completely uncorrelated, thereby removing the correlation between the independent variables. These components become new independent variables once several components of the PCA results that are independent of multicollinearity are obtained. One benefit of the PCA method is that correlations can be effectively eliminated without reducing the number of initial variables.

Direct observation of the *E-Nose* sensor output makes it challenging to distinguish between different samples. Since the gas sensors used by the *E-Nose* are non-selective and cross-sensitive, multivariable pattern recognition techniques like PCA are required to represent the data for simple analysis.

MATERIALS AND METHODS

Materials

The materials used by the bacteria in this study were *Salmonella typhi* isolates, tuna fish (*Thunnus thynnus*), cotton, plastic wrap, physiological graphic water, TSA, TSB, 70% alcohol, tissue, distilled water, aluminum foil.

Methods

The first sample, specifically *Salmonella typhi bacteria*, will be cultured and incubated for 48 hours until it forms a biofilm; once a biofilm has formed, the bacterial culture will release a more overpowering odor. Both the second and final samples of tuna contain *Salmonella typhi* bacteria. The electronic nose functions as a "sensing system" made up of three components: a sampling system, a chemical gas sensor array that produces a range of signals when exposed to gases, vapors, or scents, and a system for classifying the resulting pattern. The sensor in the *E-Nose* will generate a voltage that varies depending on the sample time and the sensor's sensitivity in order to detect odors from the sample. At each data retrieval, a voltage will be measured and sent to a computer for analysis.

The process for using the gas sensor array system and its basic operation is as follows: after turning on the power source, the tool

warms up the sensor for a minute before the sensor can be used to detect reactants. Eight sensors are used, and each one will send a response voltage that is converted to digital form. There are 8 TGS sensors in this sensor array. A 10 ml beaker was used to hold the sample. Eight gas sensors will then enter and pick up the aroma from the sample. Excel will be used to plot the output voltage that is produced. In Figure 3, systematic data collection is displayed.

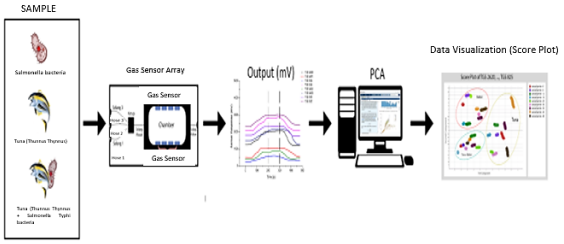


Figure 3. Systematics of data collection

The target clean air will be inhaled by hose 3 during the preheating process as a control, and it will flow through the inlet hose into the chamber with the valve shutting off hoses 2 and 1 to prevent the clean air from mixing with the smell of the sample. Because all sensors are in a steady state during that time, the preheating process takes 60 seconds. The valve closes hose 1 and opens hoses 2 and 3 during the sensing process to allow the target odor to enter the chamber. As the smell of the sample gradually fills the chamber, the sensor responds by outputting a specific voltage.

The sensing process takes 300 seconds to complete. The valve closes hoses 1 and 2 and opens hose 3, draining the desired clean air into the chamber where it will be expelled through the outlet hose during the purging process. The gas inside the chamber is supposed to be cleaned with fresh air during the purging procedure. 120 seconds pass during this process. alternately flowing the target gas into the chamber through a number of processes. The sensing mechanism by the gas sensors kicks in when the target gas is in the chamber, allowing each gas sensor to generate an output in the form of a voltage.

Sensor Response Test

Tuna (Thunnus thynnus), Salmonella typhi biofilm, and a combination of the two were tested for sensor response with each sample being given a time variation of 0, 6, 12, 18, 24, 30, 36, 42, and 48 hour.

Sensor Validity Test

The output data from the sensor is then tested to prove the validity of the data. The data validity test includes sensor precision tests and sensor accuracy tests. Accuracy is the degree to which the results of a measurement closely resemble the actual value of the quantity being measured. In order to assess the correctness of the findings from the analytical tests that have been conducted, it is required to evaluate the percentage recovery (% recovery). At 10% recovery tolerance, or between 90% and 110%, accuracy is regarded as being good.

Data analysis

The results of the sample test using the array of sensors are then processed using a personal computer, and the data is stored as a spreadsheet table in the form of a voltage value obtained from the output of the sensor series. The following are the steps in data processing:

1. Feature extraction is the process of obtaining the most pertinent and instructive values that can represent the general characteristics of the sensor response.
2. Data representation using radar graphs can show differences in the shape of the web between one and the other and serves to display data from 8 sensors in the gas sensor row. This type of radar chart displays a graph with the appearance of a spider's web. in comparison to other samples. The average value of the feature extraction results is used to create the radar graph.
3. Using the Principal Component Analysis (PCA) technique, data on variations in the aroma of tuna *(Thunnus thynnus)* were categorized. By reducing the number of variables, the PCA method is used to

reduce the dimensions of the data. Next, the variance value of each principal component (PC) is obtained. The initial data set used to create PCA is the value obtained from feature extraction. Orange Data Mining and Minitab were the two pieces of software used in this study's PCA analysis. PC data, eigenvectors, eigenvalues, and cumulative proportion of PCA data are obtained using Minitab software.

The score plot graph is used as the final classification outcome and is used to represent the data using the principal component graph of the first and second principal components' values.

RESULTS AND DISCUSSION

Gas Sensor Response Test Results

The goal of the sensor response test is to ascertain the *E-Nose* sensor's response value when testing samples. In comparison to samples or compounds with weak aromas or low concentrations of gaseous compounds, E-Nose sensors respond more strongly (signal amplitude) to samples with stronger aromas and higher concentrations of gaseous compounds.

Preheating Gas Sensor Array

Before the sensor is used to detect and respond to gases that alter the output's resistance value, it is heated. A stable output during data collection is achieved by optimizing the preheating time of each sensor. To get the sensor ready for a steady state condition, preheating treatment is applied. Preheating is done in a clean environment with a room temperature. Figure 4 depicts the preheating process graph.

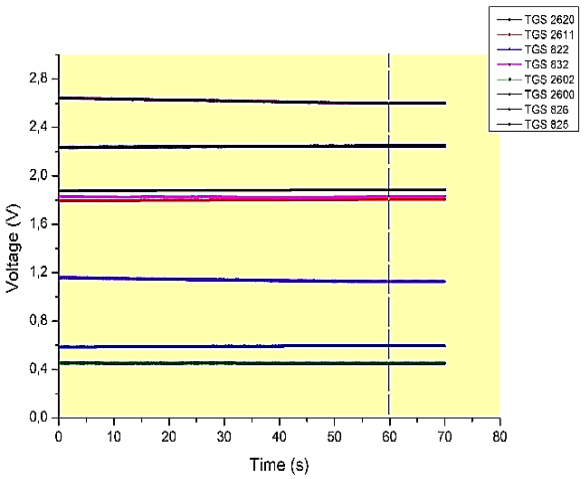


Figure 4. Graph of Preheating Sensor

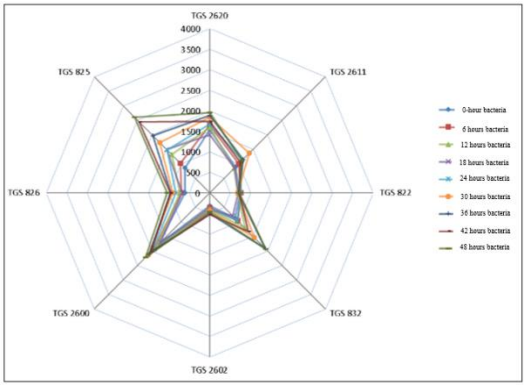
Time sensing is performed after 60 seconds because the sensor is ready to use at that point, as shown in Figure 4 where all sensors produce a stable voltage output at 50–60 seconds.

Sensing the Gas Sensor Array to the Sample

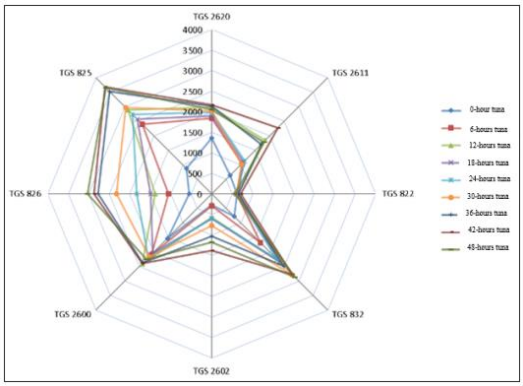
The *E-Nose* device's sample sensing treatment was performed based on the shelf life, which was as follows: 0 hours, 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, and 48 hours. Two replications of each sensing data collection procedure were run on each sample for a total of 5 minutes. The information derived from the *E-Nose* output includes stress on the smell of *tuna (Thunnus thynnus)* with varying shelf life, stress on the smell of *Salmonella typhi bacteria* with varying shelf life, and stress on the smell of *tuna (Thunnus thynnus)* contaminated with *Salmonella bacteria*. variations in the shelf life of typhi.[21] The figure depicts the sensor array's response to varying time variations during the preheating, sensing, and purging processes for each type of sample.

Following observation, it was determined that the sensor output response is stable between 100 and 200 seconds, so the output data between 100 and 200 seconds was used to analyze using PCA after first being visualized as a line plot graph. The line plot graph is useful for displaying the range of data from each sensor. Line plots with time variations are created in this study so that the range of data can be seen during each sensing period.

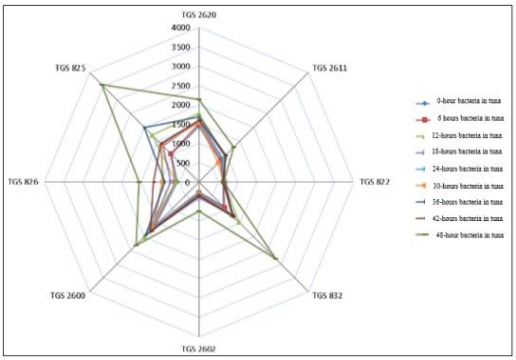
Each sample generates a unique voltage output, which results in a unique graphic pattern. Radar graphs were used to visualize the data based on the sample type and shelf life. The radar graph interprets the sensor array's response for each sensing period. It is evident that the *E-Nose* generates various sensor outputs for various sample types, resulting in various radar graphic patterns. The radar chart pattern of the three samples, however, is not noticeably different at 0 hour. The radar graph in Figure 5 interprets the sensor array response for each type of sample.



(a)



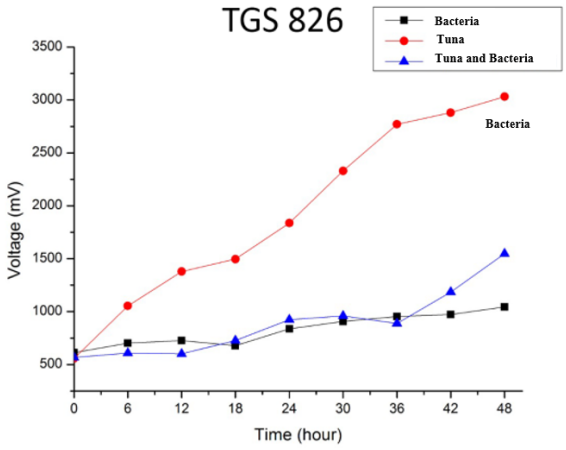
(b)



(c)

Figure 5. Radar Sensor Graph for Each Sample Type (a) *Salmonella typhi* bacteria; (b) tuna (*Thunnus thynnus*), and (c) tuna (*Thunnus thynnus*) contaminated with *Salmonella typhi* bacteria

Each sensing period saw an increase from the TGS 826 sensor in the *tuna* sample (*Thunnus thynnus*). In the meantime, each sensing period saw an increase in the *Salmonella typhi bacteria* sensor TGS 825 sample. It is clear from the radar graph that the sensor output response to the sample yields various values. Each sample has a unique set of odor characteristics, which accounts for the variation in the radar chart pattern. Because the sensor will produce a higher voltage when reacting to the target gas, which has a higher gas concentration as well, an increase in output voltage is obtained for the same sample with variations in shelf life at each shelf-life period. Figure 6 displays the output value for each sensor in the sample with varying shelf lives.



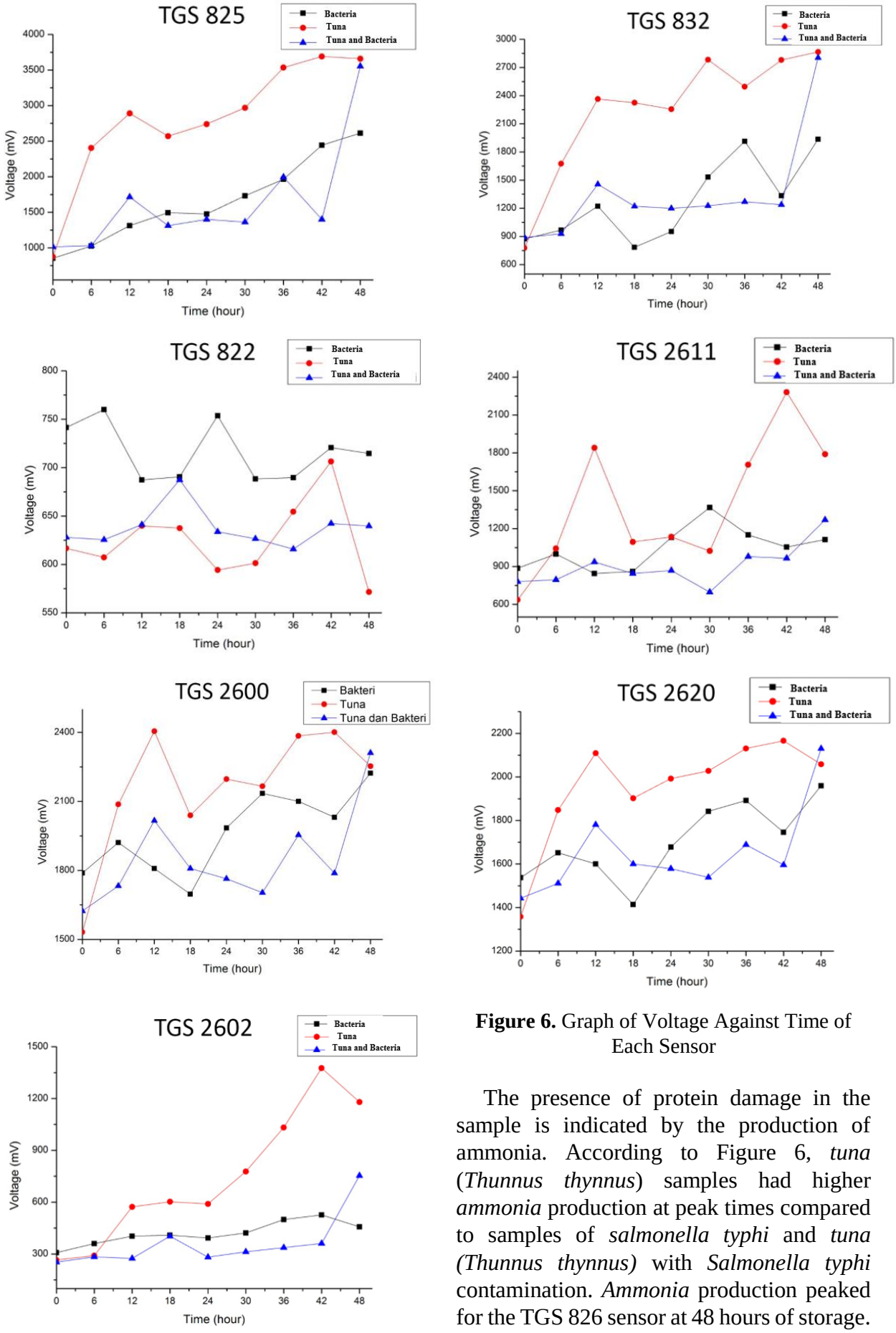


Figure 6. Graph of Voltage Against Time of Each Sensor

The presence of protein damage in the sample is indicated by the production of ammonia. According to Figure 6, *tuna* (*Thunnus thynnus*) samples had higher *ammonia* production at peak times compared to samples of *salmonella typhi* and *tuna (Thunnus thynnus)* with *Salmonella typhi* contamination. *Ammonia* production peaked for the TGS 826 sensor at 48 hours of storage.

When using the TGS 825 sensor to detect H_2S, it was discovered that all samples produced the most H_2S after 48 hours of storage, with *Salmonella typhi bacteria* producing the most H_2S overall. Because ammonia and H_2S are the main gases produced by samples of tuna and *Salmonella typhi bacteria*, TGS 825 and TGS 826 also have sensor production peaks.

Sensor Validation Results

Accuracy is the closeness of conformity between the results of a measurement and the true value of the quantity measured. It is necessary to test the percentage recovery to measure the accuracy of the results from the analysis tests that have been carried out. Accuracy is considered good at 10% recovery tolerance, or within the range of 90%–110%. The results of testing the accuracy of the H_2S gas detected by the TGS 2602 and TGS 825 sensors are shown in Table 1.

Table 1. Sensor Accuracy Test Results

Sensor	Recovery (%)				
	1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
TGS 2602	95.89	99.29	102.59	101.2	98.534
TGS 825	100.00	100.00	99.27	98.984	100.756

From Table 1 it is known that the TGS 2602 and TGS 825 sensors, which function to detect H2S gas, meet the validation parameters, which are categorized as good because the percentage of H2S gas recovery ranges from 90% to 110%. The percent recovery value farthest from 100% is produced by the TGS 2602 sensor when it detects standard H_2S gas with a concentration of 1 ppm and a recovery value of 95.899%, and the value closest to the standard concentration is produced by the TGS 2602 sensor when it detects H2S gas with a concentration of 2 ppm and a recovery value of 99.297 percent.

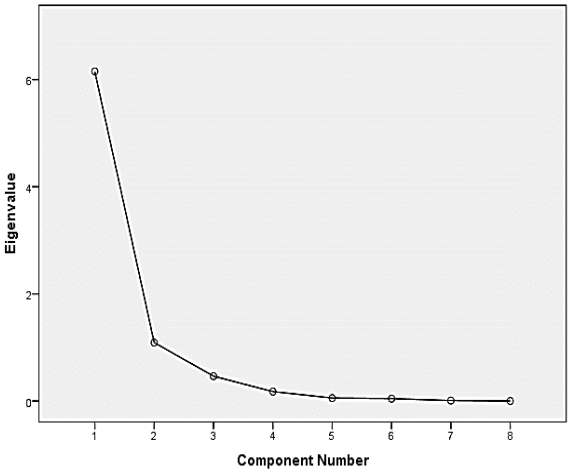


Figure 7. Graph of Eigenvalue Relationship to Principal Component

***Principal Component Analysis* (PCA) Results**

To find the correlation between each variable, the PCA method searches for a covariance matrix. The eigenvalue of each variable is then determined using the covariance matrix. The data information formed at the new coordinates (*principal component*) is described by its eigenvalue. Figure 7 depicts the connection between eigenvalues and principal components.

Score Plot

A graph that displays where data clusters are located is the PCA score plot graph. The similarity of grouped data can be displayed on the score plot graph. Two or more data distributions are present when data are grouped together to form a cluster. The two variables, *Principal Components* 1 and 2, which are not correlated, are substituted for the eight sensor variables that are correlated with one another to create the Score Plot.

The graph shows the score plot of a sample of contaminated tuna (*Thunnus thynnus*), *Salmonella typhi bacteria*, and *tuna fish*. Figure 8 illustrates a time variation of 0-48 hours with *Salmonella typhi bacteria*.

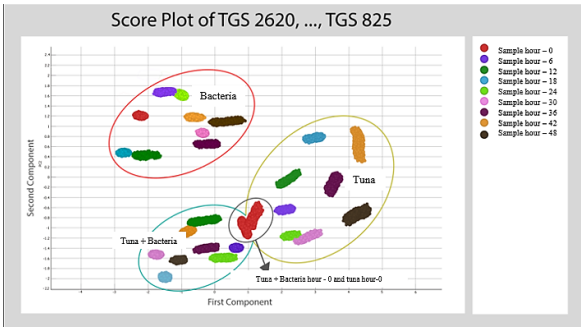


Figure 8. PCA Score Plot Graph

According to the type of sample, Figure 8 depicts clusters forming among the samples. Plotting differs for each type of sample with time variation. However, overlap was seen in samples of *tuna* and *tuna* that had *Salmonella typhi* contamination at the 0th hour of shelf-life variation. This is so because the sample's distinctive odor hasn't yet developed into a distinct or different characteristic. The organoleptic test classified the samples of *tuna* and *tuna* contaminated with *Salmonella typhi bacteria* as fresh. The organoleptic values for pure *tuna* and *tuna fish* with *Salmonella typhi* contamination were 8.4 and 8.3, respectively, making it impossible to tell the two samples apart based on their odor characteristics.

The percentage of variance criterion is used to determine the maximum number of components that can be formed. The *principal component* is a linear combination of variables and a type of variable transformation.[13] The number of main components that have a cumulative percentage of variance of at least 80% will be used in the cluster analysis. Sensor output data can be classified using PCA analysis according to sample type and sample time variation, with a total cumulative variance value of 90.5% and specifics for PC 1 and PC 2 variants of 73.9% and 13.6%, respectively.

Because each sample has different sensor output characteristics, each sensor's significance for the newly formed variable varies, resulting in the cluster on the score plot (*principal component*). The most significant variables are interpreted on the loading plot graph based on the relationship between their principal components.

Salmonella typhi must test negative in every gram of fresh *tuna* for the specific type of *Salmonella microbial* contamination test in order to meet quality and food safety requirements in Indonesia. Microbiological testing for detection has a number of drawbacks, including a lengthy turnaround time (more than 10 days) for test results.[14] Utilizing an *E-Nose* with a gas sensor can solve the issue of lengthy test times.

Each sensing period saw an increase from the TGS 826 sensor in the tuna sample (*Thunnus thynnus*). Given that the TGS 826 sensor measures *ammonia* content and that *ammonia* is one of the odors produced by bacteria that cause rot, it is obvious that the longer *tuna fish* are stored, the rottener tuna fish there will be. Because it contains a lot of free amino acids, which are necessary for microorganism metabolism, ammonia production, biogenic amines, organic acids, ketones, and sulphur components, fish is known as a food that is both high in nutritional value and perishable.[24] Increasing storage time can accelerate bacterial growth. The rate of autolysis and the expansion of spoilage bacteria both decrease with increased handling speed.

An instrument called the *Electronic Nose (E-Nose)* mimics how the sense of smell functions. As an alternative to olfactory receptors, which are responsible for detecting smells or scents, the *E-Nose* is made up of a variety of gas sensors. The aroma picked up by various gas sensors will then take on a particular pattern. Eight semiconductor sensors from the TGS2620, TGS2611, TGS822, TGS832, TGS2602, TGS2600, TGS826 and TGS825 family of E-Nose devices were used in this study.

When SnO_2 (tin dioxide) metal oxide crystals are heated to a high temperature in air, oxygen will be adsorbate on the crystal surface with a negative charge due to the presence of electron donors on the crystal surface. This negative charge is transferred to the adsorbate oxygen, creating a positively charged space layer. As a result, a surface potential will be created that has the ability to prevent the flow of electrons, which causes electrical resistance.

The density of negatively charged oxygen adsorbed on the semiconductor surface of the sensor decreases in the presence of a reducing gas, which lowers the barrier height at the grain boundary. The resistance of the grain sensor in the gas environment decreases as the barrier height is reduced. The resistance value decreases as the gas concentration in free air increases. Additionally, if a lower gas concentration value is detected in free air, a higher resistance value will be detected.[22]

Principal Component Analysis (PCA) is one technique for analyzing the data produced by *E-Nose*. By using the PCA method, it is possible to replace some of the original, correlated variables with a new, smaller set of uncorrelated variables. This method's primary goal is to reduce the dimensions of the variables that are connected and have a sufficient number of variables so that the data will be easier to interpret.[23]

Fish gills and stomachs are where *spoilage bacteria* are most commonly found to accumulate. Because so many organs in a fish's body degrade quickly to rot when it dies, the stomach and gills of fish are parts of the body that are very susceptible to microbial growth[25]. The largest source of microbes in the body is the stomach. The muscles, gills, and guts of fish are likely a source of bacteria because they naturally contain bacteria. With longer storage, there are more bacteria present. an ideal environment for bacterial growth that encourages optimum bacterial growth.

STRENGTH AND LIMITATION

The strength of this study was that the direct observation of the E-Nose sensor output makes it challenging to distinguish between different samples The limitation of this study was Since the gas sensors used by the E-Nose are non-selective and cross-sensitive, multivariable pattern recognition techniques like PCA are required to represent the data for simple analysis

CONCLUSIONS

The classification test between tuna fish, *(Thunnus thynnus)*, and *tuna fish* contaminated with *Salmonella typhi* bacteria yielded results showing a cumulative variance of the two main components (PC) of 90.5%. TGS 825 for PC1 and TGS 826 for PC2 had loading values of 0.625 and -0.753, respectively, making them the most significant sensors in this study. Thus, *E-Nose* can tell the difference between tuna that is pure and tuna that has been tainted with *Salmonella typhi bacteria*.

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CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest.

AUTHOR CONTRIBUTION

Conceptualization, methodology, writing review, validation, editing, funding acquisition, and supervision: SDA. Conceptualization, methodology, validation, original draft preparation: ABM. Writing review and editing, conceptualization, methodology, validation: AR. Writing review and editing, Conceptualization, validation: AKY. Conceptualization, methodology, validation original draft preparation: YS. Conceptualization, methodology, validation: AKA.

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