DOKUZ EYLÜL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DETECTION OF DEGRADATION LEVELS IN FOOD INDUSTRY BY USING e-NOSE SENSORS

by

Özgün Boray YURDAKOŞ

January, 2019

İZMİR

DETECTION OF DEGRADATION LEVELS IN FOOD INDUSTRY BY USING e-NOSE SENSORS

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"DETECTION OF DEGRADATION LEVELS IN FOOD INDUSTRY BY USING e-NOSE SENSORS"** completed by **ÖZGÜN BORAY YURDAKOŞ** under supervision of **ASSOC. PROF. DR. ÖZGE CİHANBEĞENDİ** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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DETECTION OF DEGRADATION LEVELS IN FOOD INDUSTRY BY USING e-NOSE SENSORS

ABSTRACT

The need for high level of understanding of storage conditions for food products have become mandatory with enhanced population and demand for the freshness and quality of food. Even with advanced preservation methods, a very large amounts of foods are lost because of microbial spoilage. As the very first step of the preservation process, the microflora that grows during the storage time and in spoiling foods should be well-known to identify the critical levels. In this point of view, gas measurement systems used to detect the existence of such microflora with a specific methodology can be easily referred to as electronic nose or e-nose. Odour and taste sensations emerging from a range of compound identification can be used in many field of industries to determine the quality of such structures taking part in foods or drinks. Considering the comparison techniques with the others, electronic nose and gas chromatography analysis systems are quite simple to perform and can ensure sensitive and promising results since they can verify each other practically. The aim of this thesis is to determine the degradation levels for some foods under different environmental conditions such as temperature and duration by keeping the others stable and to compare with other measurement techniques for evaluating the verification of data. In order to test sensory measurements during the period, GC-MS (Gas chromatography and mass spectrometry) and microbial measurements are used.

Keywords: Food quality, microbial spoilage, electronic nose, GC-MS

YİYECEK ENDÜSTRİSİNDE ELEKTRONİK-BURUN SENSÖRLERİ KULLANARAK BOZULMA SEVİYELERİNİN BELİRLENMESİ

ÖZ

Gıda ürünleri için depolama koşullarının yüksek düzeyde anlaşılması ihtiyacı, artan nüfus ve gıda kalitesi ve tazeliği için artan talep ile zorunlu hale gelmiştir. Günümüz koruma teknikleriyle bile mikrobiyal bozulma nedeniyle çok büyük miktarlarda yiyecek kaybedilmektedir. Koruma sürecinin ilk adımı olarak, depolama sırasında ve yiyeceklerin bozulmasında gelişen mikrofloranın kritik seviyelerin belirlenmesi için iyi bilinmesi gerekir. Bu bakış açısından hareketle, belirli bir metodolojiyle bu tür mikrofloraların varlığını tespit etmek için kullanılan gaz ölçüm sistemleri rahatlıkla elektronik burun ya da e-burun olarak adlandırılabilir. Bir bileşik tanıma serisinden oluşan koku ve tat hisleri endüstrinin birçok alanında, yiyecek ve içecek içinde yer alan yapıların kalitelerinin belirlenmesinde kullanılabilir. Diğer karşılaştırma teknikleriyle beraber düşünüldüğünde, elektronik burun ve GC-MS analiz sistemlerinin pratikte birbirlerini doğrulama imkanları olduğundan uygulamaları oldukça kolaydır ve hassas, güvenilir sonuçlar sağlarlar. Bu tezin amacı, sıcaklık ve süre gibi farklı çevre koşullarında bazı gıdaların bozulma düzeylerini, diğer parametreleri sabit tutarak saptamak ve verilerin doğrulamasını değerlendirmek için diğer ölçüm teknikleriyle karşılaştırmaktır. Bu periyotta algılayıcı ölçümlerini test etmek için, GC-MS (Gaz kromotografisi ve kütle spektrometresi) ve mikrobiyal analiz sistemleri kullanılmıştır.

Anahtar sözcükler: Yiyecek kalitesi, mikrobiyal saptama, elektronik burun, GC-MS

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

Smart odour sensing is a complex research subject and involves a comprehensive analysis regarding many steps such as mainly signal processing, types of sensors, advanced pattern recognition methods. An electronic nose is composed of an array of chemical sensors whose internal resistance value rise or fall in the event of exposed to the specific gasses. The fields of the application of commercial electronic noses are widely expanding in ordinary to various areas such as environmental monitoring, medical instrumentation, and food industries (Hussein, Luo, Liu & Xu, 2007).

Electronic noses are used in a number of industries by smell recognition being capable of dangerous chemical gasses as well as analyzing wide range of food products beside evaluating the quality inspection. The process of recognition of an odour starts by obtaining the responses of each sensor, which creates electrical change as a signal through the chemical reactions odours caused. The choice of number of and type of these sensors taking part in the array highly determines the selectivity grade in the process.

A typical electronic nose compose of firstly array of sensors which called metal oxide gas sensors based on semiconductor, secondly digitization unit of sensor data and depending on application type pattern recognition for the discrimination of samples depending on the odour type (Botre, Gharpure & Shaligram, 2009).

As the pattern recognition and data processing techniques are directly applied to the sensor signals to analyze substances or train a system, a classification based on the collection of known responses from the array is provided. (Hussein, Luo, Liu & Xu, 2007).

Electronic noses and electronic tongues mimic the mammalian smell and taste sensing method and their communication with the mammalian brain (Baldwin, Bai, Plotto & Dea, 2011).

The advantage of the mammalian sensory system is that the brain can obtain signals from both olfactory and taste receptors and combine both sets of data to form classifications. On the other hand, the electronic nose and tongue are not combined since each has its own software package, however, the data from both tools could be imported into another program and combined. Another disadvantage for the e-nose and e-tongue systems is that they are also affected by the environment including temperature for both e-nose and e-tongue and humidity for e-nose, which can cause sensor drift, although calibration systems and built-in algorithms help compensate for this (Perri, Benincasa, Muzzalupo, 2012). Nevertheless, the advantages are not only in favour of mammalian sensory system. There are several different angle of view regarding the opposite circumstances between mammalians and electronic nose and tongue. For instance, the disadvantage of the mammalian sensory system is that no two brains are alike and the same brain may react differently from one day to the next, depending on an individual's health, mood or environment, making the data subjective. Such changes are directly related to stability and repeatable of a system. On the contrary, e-nose and e-tongue instruments can be calibrated to be reliably consistent and can give objective data for important functions like freshness control and quality. These tools can also perform samples whether those are available for mammalian consumption (Perri, Benincasa, Muzzalupo, 2012).

Since the primary sensory method used by mammalians to sense flavor is olfaction, the use of smell can often provide us with suitable information provided that the flavor of a specific substance is to be characterized, (Dodd, Bartlett & Gardner, 1992).

Electrical nasal techniques allow the identification of complex odors using various chemical gas sensors with appropriate statistical methods. In the evaluation of volatile compounds in food, cosmetics and other daily life, the availability of

commercial facilities has led to a significant increase in research on the application of electronic noses. Recently, piezoelectric crystal, organic conductive polymers, metal oxide semiconductor field effect transistors, metal oxide semiconductors sensors are among the types of industrial gas sensors. Analysis that have many variables such as radial basic functions, artificial neural network and simple graphical evaluation can be named as statistical analysis techniques. Electronic nose application areas include freshness determination, process monitoring, quality control, originality analysis and shelf life calculation. In addition to this, there are many studies on meat, fish, cheese, mushrooms, sugar, apple, coffee and other beverages have already been performed. (Schaller, Bosset & Escher, 1998).

In the design of these systems, repeatability and stability should be ensured at a high level, based on long-term use. Therefore, the ability of analyzing on same sensor array for a sample along the duration that measurements are obtained and the ability of various sensor groups or tools to built the same sample pattern with the previous one should be adequately high. Since sensor analysis is a key factor to achieving the desired analytical results, the current state of the sensor analysis method and statistical data analysis depending on those will be examined in the following section.

1.2 The Aim of the Thesis

It was considered as the aim of the thesis is to determine the degradation levels for some foods under different environmental conditions such as temperature and duration by keeping the others stable and to compare with the other measurement techniques for evaluating the verification of data. In order to test sensory measurements during the period, GC-MS (Gas chromatography and mass spectrometry) and microbial measurements are used.

1.3 Historical Background of e-Nose

When the historical process of e-Nose is examined, it seems that the earliest study on the improvement of such tool specifically to sense smells probably dates back to very early in 60's belong to Moncrieff in 1961. This design was quite similar to a nose yet looking like more mechanical one, less electronic one. In this context. The first electronic noses in the real sense, as the formation of redox reactions of a substance of odorants were publicated by Wilkens and Hatman in 1964, Buck et al. as modulation of the odorants' conductivity odourants and Dravieks and Trotter as the modulation of odorants' contact potential, both in 1965 were designed successfully.

On the other hand, the term of an electronic nose for smell categorization by using smart sensor arrays including gas sensors did not appeared throughout approximately 20 years after these studies until the publication of Persaud and Dodd in 1982. Artificial nose system was adduced the same year. As a theory, the basically such systems is based on gas sensors progressed 30 years ago.

NATO organized Advanced Workshop on Chemosensory Information Processing and designed an artificial scent system in 1989. After then, the initial conference in the area of electronic nose was organized in 1990. At the first years of this decade, with the spread of the term artificial electronic nose, many devices started to be used commercially and as the researches in this subject increased. These devices were tested especially in the food industry.

1.4 Outline Of The Thesis

In the following sections there will be experimental layout including various steps of the study. The goal of this study was determined to detect the degradation levels of a punch of minced beef meat and flaked poultry meat between the intervals consequently 1 to 7 days under different environmental conditions such as temperature 4 and 22 °C by using a sensor array. Additionally, GC-MS and

microbial analysis were conducted at the very beginning and the last phase of the study to verify the sensory outputs during this period.

CHAPTER TWO
ARTIFICIAL OLFACTION RECOGNATION

2.1 Biological Olfaction Principle

The olfactory system of mammals is very complex and difficult to understand. The ordinary mammalian nose structure includes the olfactory epithelium and olfactory receptor cells consequently. These nerve cells are directly responsible to sense the smell, by interacting with the source of smell.

G protein-coupled receptors or receptors with seven transmembrane fragments are a broad family of receptors. They detect extracellular molecules and, accordingly, activate intracellular signal transduction pathways. The olfactory cell contains a large number of cilia that are placed over G receptors. The receptor binding stimulates these proteins neurons. They have the sensitivity to segmentary overlap with the sources of odor. The signal is amplified here and sent to the following unit as a message.

Therefore, the sensory cells in the epithelial tissue transmit signals along the axon in the olfactory bulb and terminate in the region called the glomeruli, which is the set of neural networks. These signals are processed in mitral cells and transmitted to the brain and they are decoded in the brain by using a kind of modeule of pattern recognition. Thus, the mammalian olfactory system separates the compounds in their fragrances easily without mixing them. In the improvement of artificial olfactory devices, these three units are basically taken as basis; brain, olfactory receptor cells and olfactory bulb (Kroeze, Sheffler & Roth, 2003).

Considering all this comprehensive approach, in the design of olfactory technologies, mammalian olfactory systems are taken as reference. Thus, mammalian olfactory systems should be well analyzed to better understand the nature of these measurements using a sensor array. To reveal this mechanism, Figure 2.1 clearly demonstrates this similarity of such systems.

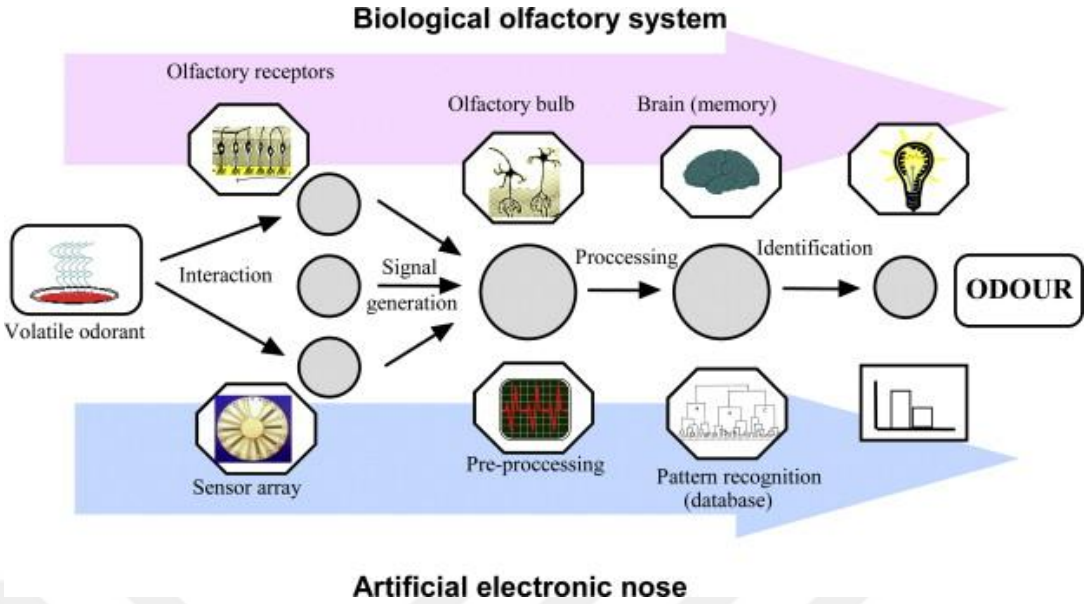

Figure 2.1 Similarity of biological olfactory system and artificial electronic nose (Varnamkhasti et al., 2011)

2.2 E-nose System Properties

Technically, the electronic nose can be called as an artificial olfaction system described on previous subtitle, replaces the odor field receptor cells with the chemical sensor array. The operating principle of the sensor group is to produce a time-dependent electrical signal in response to the odor sensor interacting with itself.

The olfactory bulb is a unit that improves noise and sensor shift and characterizes the steps taken before the main processing unit. This system is the pattern recognition part in the human brain and can be defined as the last step for an artificial olfaction (Gardner & Bartlett, 1999)

The electronic nose aims to mimic the mammalian olfactory system by modeling the responses to odorants with a sensor array. Methods such as bubbles, pre-concentrators, diffusion methods or headspace sampling are applied until this odor is transmitted into the nose and sent to the next unit. The odour source is processed through the sensor array and a reversible chemical reaction occurs by altering the conductivity as one of the electrical properties.

As in the olfactory systems, every single cell located in sensor array attitudes like a single receptor by responding to different odourants with the different levels. These differences can be converted to electrical signals by pre-processing and defined by the model recognition system. This response from the array is formed as an electronic nose to allow for a specific odor in each odor group (Arshak, Moore, Lyons, Harris & Clifford, 2004).

Gas chromatography and mass spectrometry (GC / MS) are used as a conventional method for odor detection systems due to the intensive use of chemical analysis methods. This conventional approach enabled the modeling of such a system called E-nose with rapid advances in smell sensing technologies in the literature. The fundamental source of this improvements have come across as the large number of the studies performed with many different motivations along the period, and the obstacles emerged on various steps, consequently, new solutions related to these problems.

In addition to improvements another fundamental issue based on motivation behind this, analysis of odours and volatile compounds in a correct, reproducible manner; is the use of non-fixed, inexpensive and reliable devices. The working principle of the e-nose is based on biology, which is a fundamental science. The physiology of the sense of smell can be explored in detail and the e-nose systems that are working analytically and faithfully can be produced.

The e-nose is originally designed to simulate the other livings. However, given the fact that the system is a multi-sensor array, this should be assumed that it is far below the odor characteristics of a mammal. For these reasons, the purpose of the e-nose is not to completely replace other analytical techniques or the human nose. The desired products to be detected are identified by using sensor arrays, and hence the system is trained whether to run correctly or not.

Compared to the other designs, electronic noses only perform with logic 1 or logic 0, yet, after the quantization operations, necessary grading levels can be provided.

This can enable the detection of odorless and irritating gases to better perform the function of the sensor analysis. Due to this feature, the electronic nose is characterized as a tool for rapid quality control in the food industry. Nevertheless, many improvements are necessary to be achieved in order to ensure that the system is sufficiently reliable and becomes a common industrial tool. These improvements include the creation of appropriate calibration methods and reduction of dead volume. Since today's technological advances cannot interfere with human nose sensitivity, designers are striving to use sensor technologies developed in a single structure instead. On the other hand, gathering these properties in a single structure has some difficulties, such as the long duration of statistical analysis and the use of a large number of sensors. Despite all these challenges, the overall trend encourages a system-specific design. This approach points a portable and compact design (Schaller, Bosset & Escher, 1998).

2.3 The Electronic Tongue

In the light of recent studies, the artificial sensory technique developed for the electronic nose has begun to be applied in the sense of taste as well. In this point of view, taste sensors, to be formed or worked as electronic tongue, have been studied.

The first notification come across to this field of such studies as electronic tongue described by D'Natale et al., in 2000. They made a description to electronic tongue as a tool contains ion sensors, data collectors, transducers and analysis instrument. This design was developed in order to classificate the liquid samples and the amount of chemical variety taking part of this solutions.

2.4 Application to Food

Since 1993, the amount of publications in the area of electronic olfaction is more than 12,000 articles. The main application areas related to the food industry have been: fish, meat, milk, wine, coffee and tea, and constitute approximately 5000 publications since 1993. The number of the food applications reveal that main

investigation area of electronic olfaction is food analysis having approximately half of total publications (Loutfi et al., 2015).

Identification of sensory, detection of chemical and physical, methods of microbial appraisal etc. are the main traditional determination techniques of meat freshness. There are many drawbacks of these traditional methods, especially in the long analysis period. It is highly crucial to be quickly understood in the control of meat freshness. For this reason, the rapid and reliable detection of food freshness can be made with the technical development of gas sensors. The meat, which is composed of basic constituents of living such as fat, water, protein and some carbohydrates, is converted into volatile gases by bacteria and enzymes during the decay process. During degradation process, protein decomposes into ammonia, ethanethiol and hydrogen sulfide; carbohydrates decomposes into ketones, alcohol and carboxylic acids; fats decompose into aldehydes and aldehyde acid; while whose concentrations decrease, in the opposite way, concentration level of such volatile gasses show a rise exponentially. Therefore, the determination of such gasses is vital. In order to determine these volatiles and hence the freshness of food samples could be provided by using related gas sensors. While humidity and temperature sensors are indispensible for analyzing microbial growth, in addition to these, gas sensors are also key tool to obtain accurate results (Eom et al., 2014).

2.4.1 Red Meat

Red meat is one of the main consumption foods. A significant amount of red meat is consumed worldwide. Certain processes are applied before red meat is consumed. Especially in the storage method known as aging, the meat is exposed to low temperature during long storage and thus becomes suitable for consumption. During storage, there is also bacterial growth as well as aging. If bacterial growth and aging are controlled simultaneously, aging meat is obtained which is suitable for consumption (Ghasemi-Varnamkhasti et al., 2009).

The use of e-nose in the meat sector is one of the basic, common and reliable methods in the food industry. In the past 20 years many studies have been published in this field. E-nose studies related to meat have been concentrated in the following; identification of spoilage, taste and flavor status, bacterial strains categorization, quality of assessing. In addition, categorization and processing methods of meat products are studied using this method. The E-nose created using 38 sensors together with the LDA algorithm, the recognition of the freshness of the meat and the success of determining the degradation of the meat by using various variables (Loutfi et al., 2015).

2.4.2 Poultry Meat

Some studies on poultry meat applications are given in historical order. In 1998, cultured bacterium gropes isolated from poultry were examined. Arnold and Senter, then unlike the previous study in 1999, Siegmund and Pfannhauser, in a chilled environment, observed a difference in volatiles observed on cooked chicken meat. In 2002, Boothe and Arnold, revealed that some changes on the samples of chicken meat can be determined depending on the duration of storage and temperature with the help of an electronic nose. (Rajamaki et al., 2006).

2.5 Data Analysis

Data analysis methods used in e-nose application are numerous. There are listed a few analysis tools to show that what types of analysis methods exist. These type of analysis methods are vital especially in dealing with not only one-unique data content but also two or more. As provided theoretical background such methods below should be well-known.

2.5.1 Principal Component Analysis (PCA)

Principal component analysis, a size reduction tool, converts a large set of variables into a smaller set containing many of the information in the set. This

analysis is a mathematical process that converts possibly correlated variables into fewer uncorrelated variables.

In this process, the first main component controls the changes in the data and each subsequent component controls the exchange of other remaining components. In this process, the first major component controls the variability in the data and each subsequent component controls the variability of the other remaining components. In fact, this method is in common with Factor Analysis. The matrix in which principal component analysis is applied is a square symmetric type. If the variance of the individual variables or the units of measurement vary greatly, the analysis matrix should be used in the analysis of such data (Statgen, 2018).

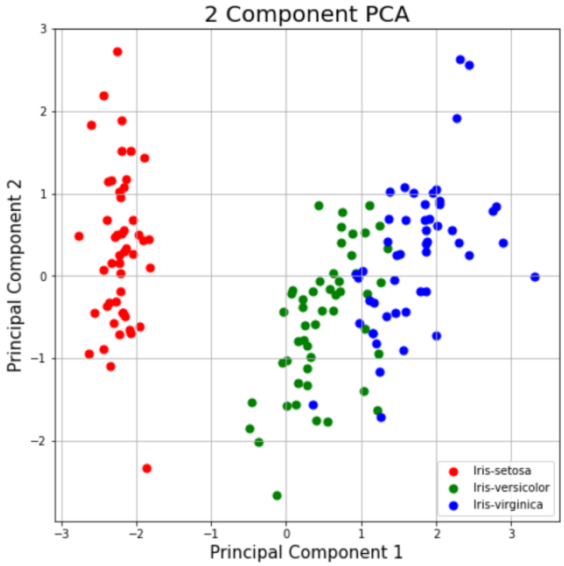

Figure 2.1 An example of a PCA Analysis showing the distribution of each sample (Towardsdatscience, 2018)

2.5.2 Partial Least Squares Regression (PLS)

In the simplest case of this regression method, which is an extension of a multiple linear model, a linear model indicates the relationship which is between a dependent variable Y and a vector of X in the followings:

$$Y = b_0 + b_1X_1 + b_2X_2 + ... + b_pX_p \quad (2.1)$$

As seen in equation 2.1, For variables from 1 to p, b_0 and b_i regression coefficients, b_0 values are calculated for intersection, b_i values are calculated by means of data

In other words, the least restrictive regression of the multiple linear regression model is the partial least squares regression. Thanks to this advantage of the method, the use of the partial least squares regression is provided when the use of multivariate methods is very limited. Also, this method can be utilized as a exploratory analysis tool by determining outliers before classical linear regression and selecting appropriate predictor variables (Statsoft, 2018).

This type of regression has been used in many areas (pharmacy, chemistry, medicine, psychology, economics, etc.), especially where predictive linear modeling is required, with a lot of predictors. Partial least squares regression is considered as a standard method for modeling linear relationships between multivariate measurements, especially in chemometry (de Jong, 1993).

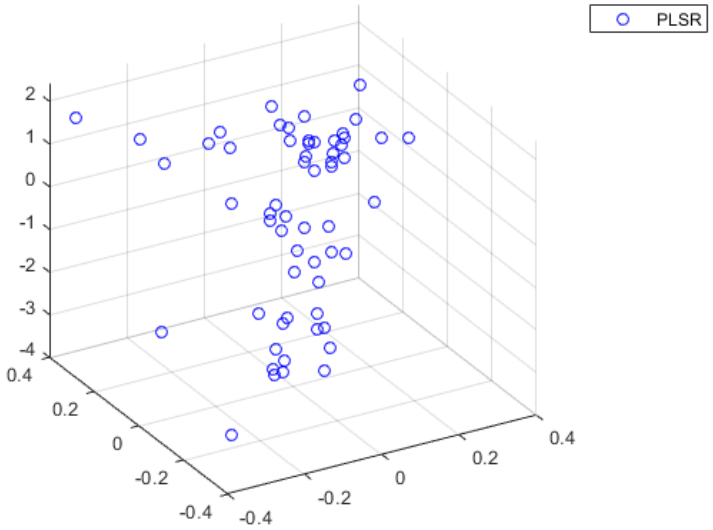

Figure 2.2 Schmatic view of a random PLS (or PLSR) Analysis (Mathworks, 2018)

2.5.3 Support Vector Machines (SVM)

In defining decision limits based on the principle of decision plans support vector machines are used. In support vector machines, a group of objects with different class memberships is called a decision plane that makes the allocation process. Objects belonging to a green or red class are shown in the following schematic example. As seen in Figure 2.3 (a), all objects on the left of the line separating objects are red while the ones on the right are green and this line is defined as the separation line. White circle which one is a new object, is categorized in red because it falls on the left side. If this object fell to the right side would be defined as green.

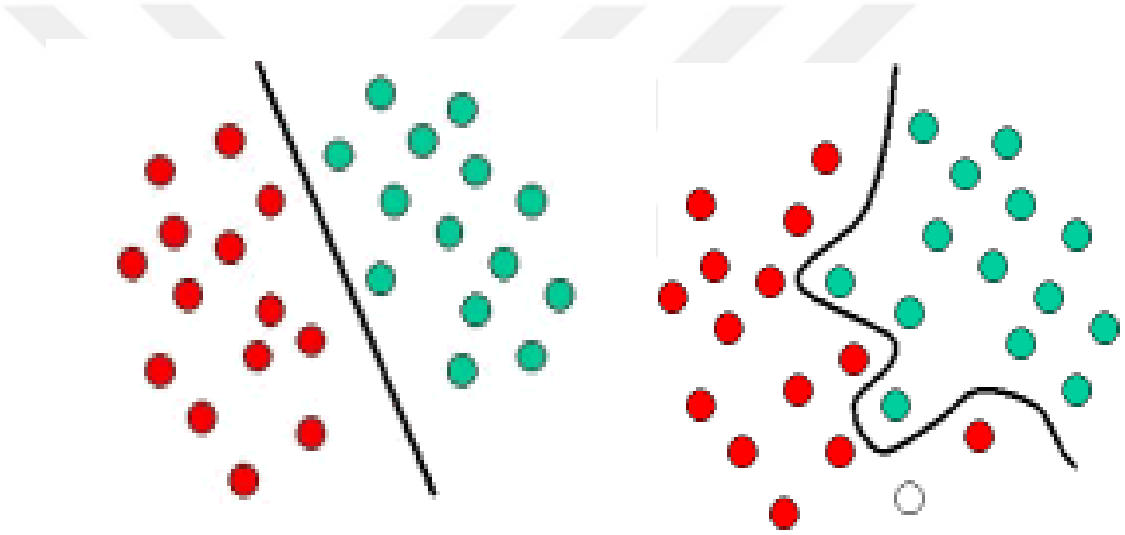

Figure 2.3 A SVM output imformation illustration corelation between measurements. (a) Type 1 and (b) Type 2 (Statsoft, 2018)

As shown in both examples in Figure 2.3, there is a classifier that separates objects into their groups by a line. This is a typical example since the linear classification has a distribution as in the figure. On the other hand, making the distinction of objects in the most accurate way is not always as easy as Figure 2.3 shows, and referring to existing ones which are described as train, more complex systems are needed to classify the new objects described as test. In comparison of (a) and (b) tasks of Figure 2.3, it is obviously seen that a curve should be drawn rather than a straight line for a sharp, better separation. If multiple lines need to be drawn to better distinguish objects in different groups, such classifiers are called hyper-

directory. Especially in such examples, support vector machines are preferred (Statsoft, 2018).

CHAPTER THREE
MATERIALS & METHODS

3.1 Block Diagram of the Study

The study included a few steps seen on the block diagram in Figure 3.1 demonstrating the principle of the e-nose system designed according to sensor array outputs to appoint the sample freshness. In the beginning, samples were brought closer enough to sensor array by helps of an injector obtained from the chamber in order to detect what type of gas content exist. This mixture of gasses was separated according to sensor type for each sensing among all the gas content. Then this value was calculated over analog outputs whether the critical value was exceeded or not in terms of determined values for each ones before. According to this exceeded limit, the system gave deterioration warning number of 1 to 4. If such value is not observed over the sample then logic-0 is produced to carry on measurements.

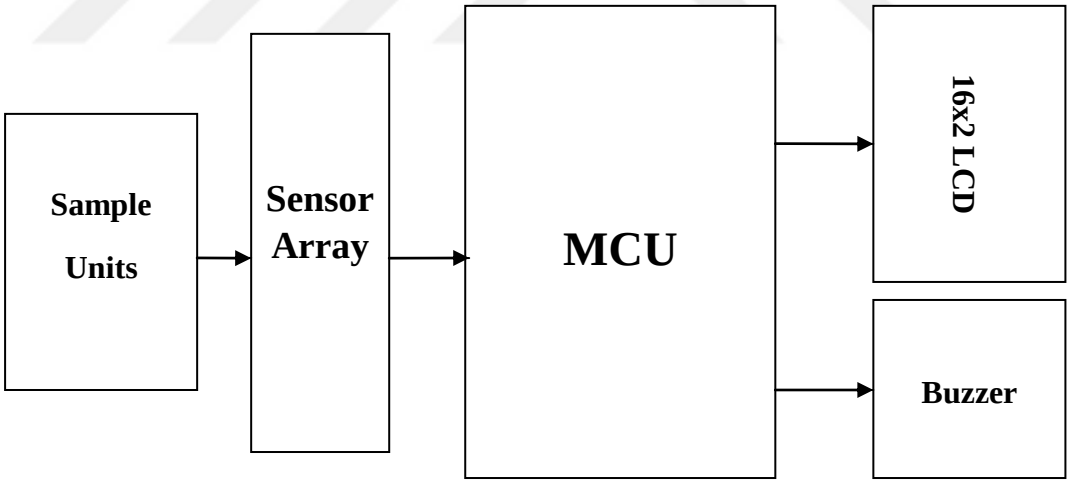

Figure 3.1 Block diagram examining how the selective software of the thesis works

3.2 Sensor Types

Main part of MQ is a semiconductor metal oxide. MQ sensor has a resistance sensor that is dependent to oxygen concentration contacting directly with

semiconductor metal oxide. The higher level of oxygen, the more potential level of the barrier. This creates an increase of the resistance of sensor material. If there exists any chemical matters observed or measured by the sensors, oxide intensity is reduced as the concentration of oxygen. This situation causes the decrease of the potential intergain of the barrier and decreases the resistance of the resistor. The relation between emerged gas concentration and sensor resistant can be basically found in Equation 3.1.

$$S = A\,[C]^{-\alpha} \tag{3.1}$$

where C is the gas concentration on measurements are taken, S is response value of metal-oxide sensor, A is the coefficient response which changes specifically depending on some gases, and α is sensitivity power. As it is stated, A and α totally depend on material type of sensor. The response of sensor is also defined in Equation (3.2).

$$S = R_s / R_o \tag{3.2}$$

where R_o and R_s are the resistance values of the sensor in reference air and in the presence of gas contamination, respectively. Sensor MQ has two main parts; first part is tin oxide (SnO_2) as sensor material. This material is connected to pins 2 and 3. Second part is the heater for heating sensor material. This heater is connected to pins 1 and 4. The pin connections mentioned above is seen in Figure 3.2 where H representing heater pins oppositely and A and B as input and output pins regardless of which one. In this circuit model, the key circuit element is to determine the load resistance since it is vital parameter to set the optimum sensivity.

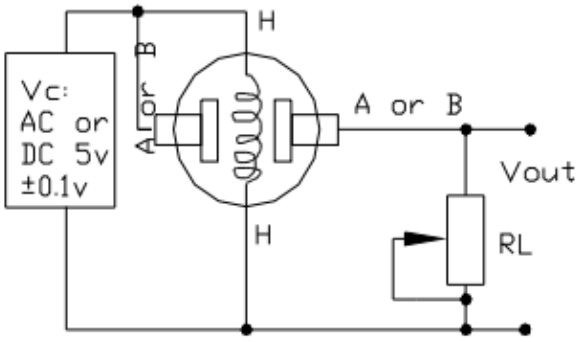

Figure 3.2 Circuit model of a standard MQ series (Wisense, 2018)

3.2.1 Sensor Selection

In sensor selection for this thesis, some parameters are considered. These parameters are also determined so that the product has low volume to use as portative, becomes easy to obtain for daily use of ordinary users and is available in multi use mode in order to be worked with several sensors. Related parameters listed below.

- Small size
- Availability
- Multi Detection

3.2.2 Metal Oxide Sensors

In operational mode, the main response of a metal oxide sensor heavily depends on the alteration in value of conductance of the oxide as the interaction occurs with an external excitation such as a gas. This change is usually proportional to the concentration of the gas since the number of molecules bound to the compound increases. Basically, there are two types of metal oxide sensors; n-types and p-types. This discrimination is made depending on their response type. N-type respond to reducing gases while p-type respond to oxidizing gases.

Having low response time in addition to recovery time can be considered as one of the advantages of metal oxide sensor, directly depends on relationship between gas and sensor and the temperature. Provided that thin film metal oxide sensors are preferable, being smaller compared to the others with the same mission. Therefore they could be directly interconnected into the circuit. This is a key factor to built larger systems including multi sensor array. Beside this, easy to obtain low prices respectively. On the other hand, one of the disadvantage of these type of sensors is their high level of consumption since they can work on high temperatures could be appointed. Another side effect of this sensors is that they are in danger in performing of sulfuric compounds to poisoning risk whose mean in sensor language, being blind to the other volatiles after that time for long periods (Arshak, Moore, Lyons, Harris & Clifford, 2004).

3.2.3 Gas Types Detected by Sensors

As the elements of MQ series of HANWEI company, MQ7 is used to detect carbon monoxide (CO), MQ4 is for methane (CH_4), MQ3 is for ethanol (C_2H_5OH) and MQ137 is for ammonia (NH_3) are used for the samples during the period of 7-days.

3.3 Design of the e-Nose System

Designing an e-Nose system requires many tools. In this study there are several modules such as the sampling unit, sensor array, microcontroller and LCD in addition to the buzzer which can be seen with the connections in detail in Figure 3.3. The sampling unit where the samples are located as the input of the sensor array module. In this transition of analog data from the source to the sensory inputs, the efficiency of related transfer is not %100 naturally. Considering the gas forms as the storing element, the highest level of the loss occur here. Nevertheless, the adequate effort is shown to transfer this data into the next module. The sensor array is another analog data part of the design which sense and send the data into MCU. In this module, analog data is converted into digital data so that MCU could only process

the outputs data in this way. Finally the decision (logic 1 or 0) is shown at these outputs.

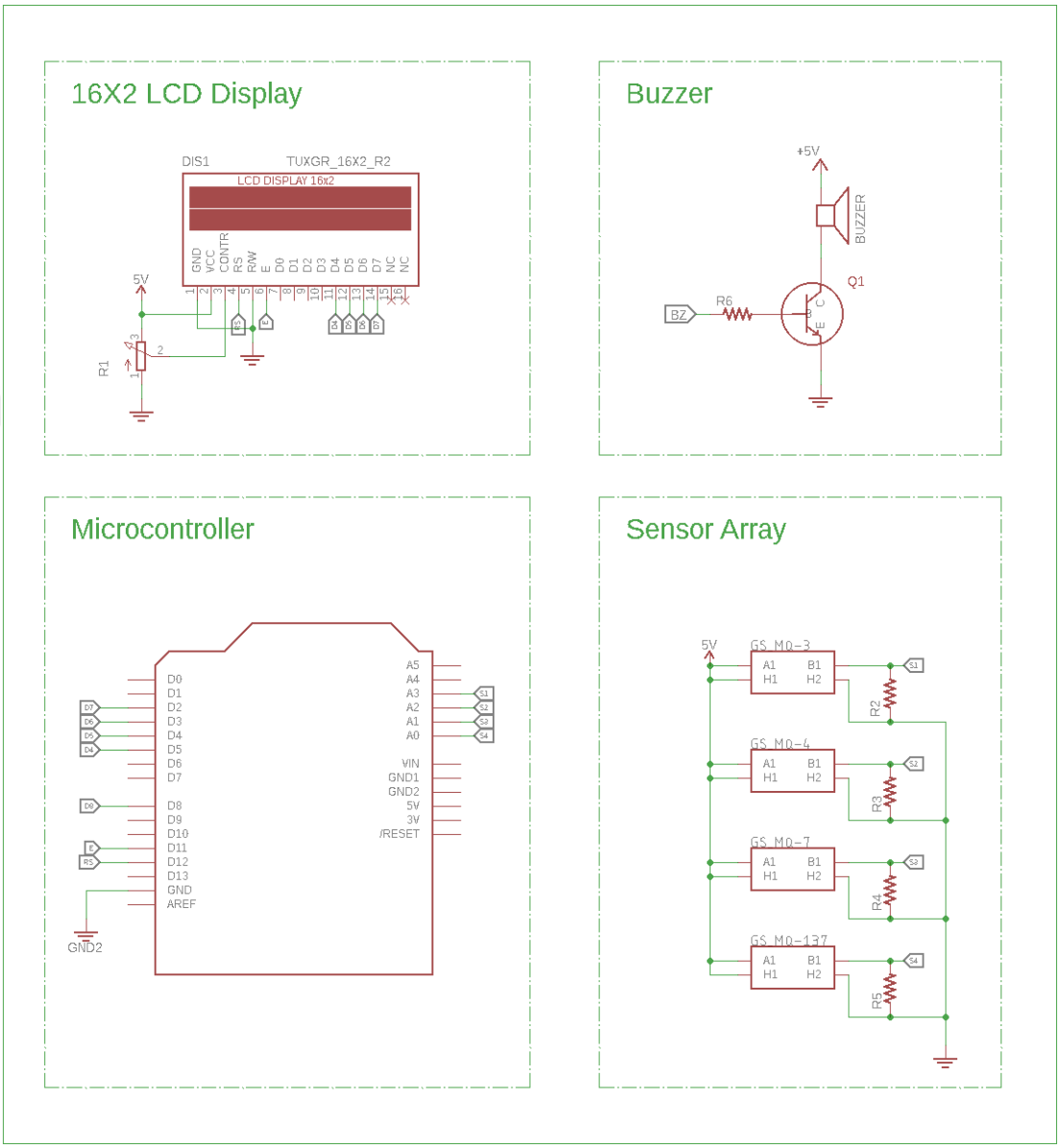

Figure 3.3 Detailed circuit schema of the e-Nose system

3.4 Sample Preparation

Samples were kept in close chamber under same condition for the duration time. Sensory measurements were recorded for every single day through an injector over the chamber with neglected gas leakage from one measurement to the another. The samples were purchased from a local butcher and they were stored at 4°C and 22°C

during 7 days. Sample size was determined for red meats as minced shaped so that there can be observed microbial contamination obviously. The other sample was selected as its original size in order to simulate the environmental conditions more realistic.

Figure 3.4 The samples are captured on day-7 after whole measurement (Personal archive, 2018)

Figure 3.4 includes four different measurement conditions such as temperature for 4°C and 22°C, and meat types such as poultry and meat. In addition to this, the samples pictured in Figure 3.5 is selected as the maximum size between 1.5 and 2 gram in order to detect the released gasses for chemical analysis.

(a) (b) (c)

Figure 3.5 The samples are weighed on a precision scale (a) for minced red meat (b) for flaked poultry meat (c) for the total poultry meat weight with the tare (Personal archive, 2018)

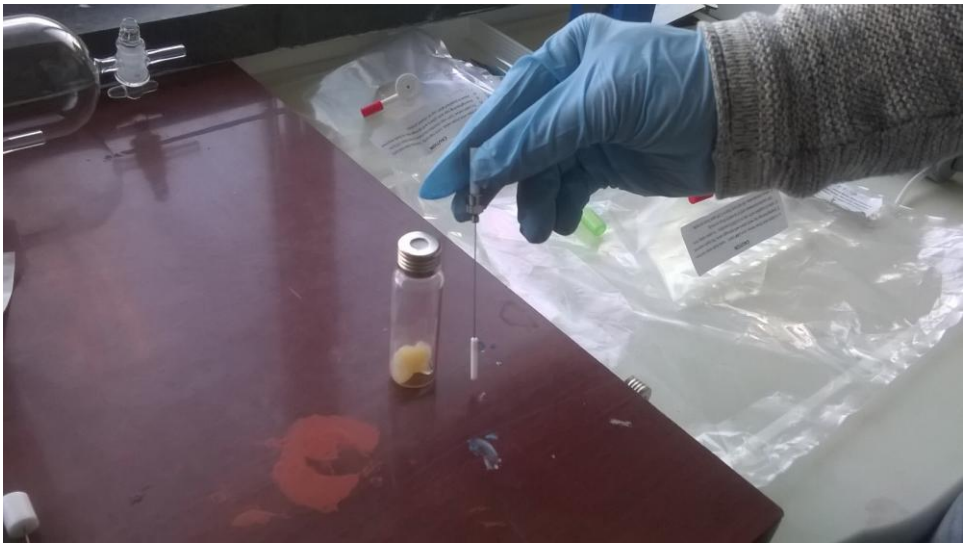

Figure 3.6 One of the prepared sample with the injection pin before GC-MS Analysis starts (Personal archive, 2018)

To analyze any material by using an E-nose, the sample must be kept into the sensor chamber that the sensor array is able to be put into or smaller sized which is also possible to inject the released gasses as it is seen in Figure 3.6. The main role of the sampling unit is to collect the sample data mostly as the form of gas phase and transfer it into the sensor unit located inside the same chamber or in an another form. In designing of the sampling unit, all factors such as mainly temperature and humidity at least that are directly capable of influencing sensor responses by causing the internal resistive changes must be maintained. These factors are kept stable as far as possible under required parameters, so that only the composition of odour is observable in the sample. This type of selection of sampling unit provides the high level of stability and repeatability, and of course additionally fast sensor responses and high amplitude signals which then could be set desired sensivity. These factors are of great importance, since sampling is the first step as fundamental among the other processes of data acquisition, hence its achievement highly impresses all upcoming steps in a good or bad way in terms of determining the key parameters of such design. One important factor which should also be paid attention is the preparation of the same conditional samples in order not to increase the number of the parameters that is compulsorily essential to focus. For instance, provided that great changes exist between the samples, it cannot be regarded that the sampling method as comparative.

3.4.1 Head Space Sampling

The method of head space sampling is necessarily as a separation technique where volatile organic compounds are decomposed from a heavier sample by mass and hence they can be injected into a gas chromatograph input for analysis. The composition of the sample should not be altered by any manipulation and should be provided maximum efficiency during the sampling. The measurement of head space can be used directly by the chemical gas sensors instead of injecting into gas chromatography colon to detect the volatile gas composition released over regardless of mass or liquid form of the materials.

The sampling methods especially in application for gas chromatography also prevents nonvolatile residue accumulation in the head of column beside simplifying sample preparation at the same time. Many samples include substantially amounts of non-analyte or highly complex materials containing water alcohol, essential oils etc. in the sample matrix for gas chromatography. With direction injection into a typical gas chromatography injector, non-volatile residual materials and very strongly retained solutes will be transferred into the post analysis system of gas chromatography and hence waste a lot of time by eluting compounds that is not desired. In order to prevent such circumstances, a headspace sampling system improves this process by extracting a small volume of the accumulated headspace gas from the vial seen in Figure 3.6 and then it is transferred to the column input of gas chromatography.

3.4 Microbiological Population Enumeration

Counts of the microbial population should be accurate data to have an idea about the verification of the gas measurements. If the expectation can be made before the process for what type of bacterial growth would be observed, the other measurements would be more meaningful in terms of emerged gasses over the sample. As it is known this, amount of gasses results in almost completely from the respiratory mechanism of such bacteria. The higher number of bacteria groups, the more verified

the environmental gas detection. The measurements were conducted in Microbial Laboratory in Ege University, Izmir by the device represented in Figure 3.6 with the model STOMACHER 400.

Figure 3.7 Microbial enumeration device in detecting the number of the total bacteria (a) frontal view (b) upper-side view (Personal archive, 2018)

3.5 Gas Chromatography Topology

Gas chromatography/mass spectrometry is a combined device of which gas chromatography and mass spectrometry units are able to work together where is used for structure analysis and amount detector. The device can be used lonely as gas chromatography and gas chromatography/mass spectrometry units. Gas chromatography/mass spectrometry is commonly used for the detection of substances where separated in gas chromatography pillar. During gas chromatography/mass spectrometry processes mass spectrometry plays a role as detector. The chromatographs of the compounds which are sent to mass spectrometry after leaving gas chromatography, can be obtained and qualitative appointment can be easily done with more accuracy by getting mass spectrum of every compound. Having high

power of separation, the skill of quantative and quality analysis and high level of sensitivity are the basic advantages of the device.

Basically, gas chromatography can be divided into two subtitles, depending on the type of the stationary phase. One of these subtitles is GSC (gas–solid chromatography) where used in the separation of low-boiling hydrocarbons and permanent gases. The second one, key process especially for lupine alkaloid analysis and food freshness applications, is gas–liquid chromatography (GLC) (Aniszewski, 2007).

In chromatographic separation, substances are distributed between the two phases. One of these phases is active while the other is stable. The movement rate of each ingredient in the mixture is determined by the coefficient of dispersion. Substances that are more dispersed in the mobile phase move more rapidly, while the substances with higher distribution in the stationary phase move more slowly. Pours particles close to each other which are named colon are also known as stationary phases while the active phases fill the gap between such particles. The active phases drag the substances along the colon. The concentration profile of each substances that come out of the colon are referred to as peak and the table formed by these peaks is called a chromatogram.

The type of chromatography where gasses are used as active phases while liquids are used as stationary phases is called gas chromatography. Gas chromatography is widely accepted for such analysis as a suitable method and the separation of volatiles and other gasses in the field of chemistry and related research topics.

Figure 3.8 Gas Chromatography and Mass Spectroscopy Analysis device at GC Laboratory in Ege University Chemist Department (Personal archive, 2018)

Figure 3.8 displays the GC-MS Analysis device, SHIMADZU brand with the model GCMS-QP2020 used for the measurements in ambient gasses of food samples throughout the interval.

3.5.1 Principle of GC-MS:

The main working principle of GC/MS instrument is separating chemical mixtures (the GC component) and defines the components according to their molecular mass level (the MS component). It is one of the most accurate tools to analyze the environmental mixture of samples. The GC works on the principle that a mixture will separate into individual substances when heated. As in many chemical devices, the heated gases are carried through a column with an inert gas. As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry is responsible for identifying compounds in the mixture by the mass of the analyte molecule. A library of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector. The first general application of molecular mass spectrometry occurred in the early 1940s in the petroleum industry for quantitative analysis of hydrocarbon mixtures in catalytic crackers. Recently, manufacturers of

GC/MS instruments have significantly reduced their overall size and increased durability. This allows what was once a laboratory bench-top instrument to perform field analysis (Cpeo, 2018).

3.5.2 Main Components of GC-MS

Oven: The gas chromatography device is designed to deliver the gas sample directly from the injector aid or from the tube to the device. The amount and composition of adsorbed gases and free gases can be determined. There exists a oven having high range level of temperature in GC-MS's. The column oven ensures homogeneous distribution for temperature with high thermal stability, rapid heating, cooling and air circulation. Thus, the accuracy of the chromatographic peaks and the performance of the analysis increase. This oven are temperature adjustable like many of them, the typical gas chromatographic ovens temperature can be set up from -5 °C to 400 °C depending the sample requirements. Therefore the minimum value of temperature is able to reach to as low as -25 °C with the special cooling method named 'cryogenic'.

Colon: Colons are designed as where chromatographic distinction occurs. Many of them exist for different types and lengths according to areas of use.

Carrier Gas: Selecting high level of purity of carrier gas improves the sensivity and lifetime of the detector. Additionally, the flow rate of carrier gas is determined by the colon diameter.

Detector: There exists two types of systems such as Flame Ionization Detector (FID) and Electron Capture Detector (ECD). FID is the detector type that is the most preferred due to high stability, repeatable and sensivity. It is quite sensitive to high flow-rate. Organic matters which come from the colon, are ionized by burning onto hydrogen flame and positive ions emerged in this process, cause the current changes in the detector. The resulting current is directly proportional to the total amount of carbon passing through the flame in unit time. While FID is sensitive to all

compounds containing the C-H bond, it is insensitive to only sulfur compounds, water, ammonia and nitrogen oxides.

ECD: Electron capturing detector measures the signal loss instead of signal increase. Carrier gas, examined previously, is ionized when it passes through a radioactive region. It has high sensivity to nitrogenous compounds and halogenated compounds, in contrast, low against alcohols and hydrocarbons. They are devices in which the sensed current in the detector is recorded on a scale striped paper so that calculations can be carried out after converting to an electrical signal. The temperature of the following three parts must be controlled separately for the purpose of the discrimination to be repeated in exactly the same way. The parts are detector temperature, column temperature and injection part temperature.

3.5.3 The Advantages of Gas Chromatography

One of the most important aspects of chromatography is that each peak obtained during the determination of the number of volatiles in a sample analyzed indicates a different substance. Another important benefit is that it can be understood whether a substance is pure or not. For instance, if the substance that is for purity-controller gives more than one peak, hence impurity is present. The ratio of areas under the peaks to each other is the ratio of substance and foreign matter to the mixture.

3.5.4 Reasons of Frequently Use of Gas Chromatography in Industry

Many advantages are obtained by using gas chromatography in industry. For example, the results are quickly obtained during the analysis. Another advantage is that very complicated samples can be divided into components (including isomers) and little sample volume is needed when analyzing (microliter). Another important advantage of gas chromatography is that very low vapor pressure samples can be used. According to other methods, qualitative and quantitative results are obtained more sensitively.

GC-MS applications in the food industry are one of the fastest developing fields. The need for accurate, fast and stable molecular characterization of the food, demanded both by consumers and regulatory agencies, is of importance the food industry to apply such advanced methods for detailed analytical evaluation of food (Hussain & Maqbool, 2014).

Over the past 100 years, many techniques were applied in several scientific fields to recognize the aroma-active compounds in food. Among the various techniques, GC-MS is an effective and commonly used technique for aroma analysis such as odour of foods (Song & Liu, 2018).

CHAPTER FOUR
RESULT AND DISCUSSION

4.1 Bacterial Analysis

Bacterial analysis is an essential technique for the evaluation of the emerged gasses during the process. The counts are made in the beginning and last part of the degradation as t_0 and t_1 in order to determine a reference level of bacterial growth for the minced beef meat and flaked poultry meat. Figures 4.1 and Figure 4.2 captured during the time where counts are made for the both samples inside the petri dishes.

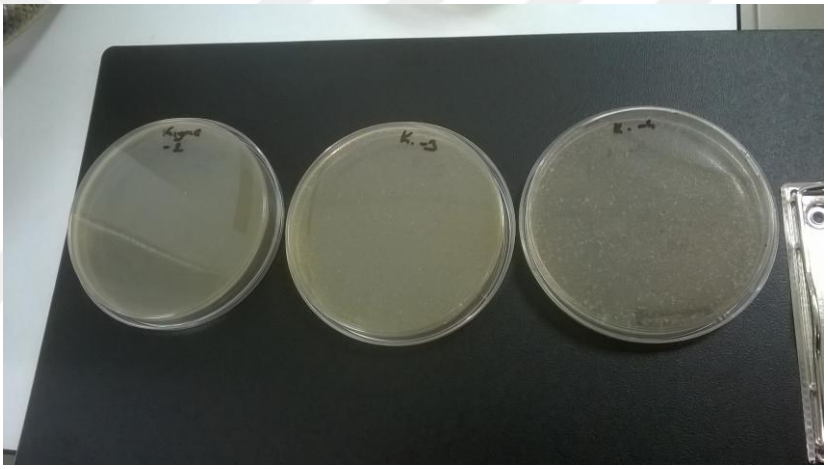

Figure 4.1 Representing the minced beef meat bacterial count at 4°C for the first day (Personal archive, 2018)

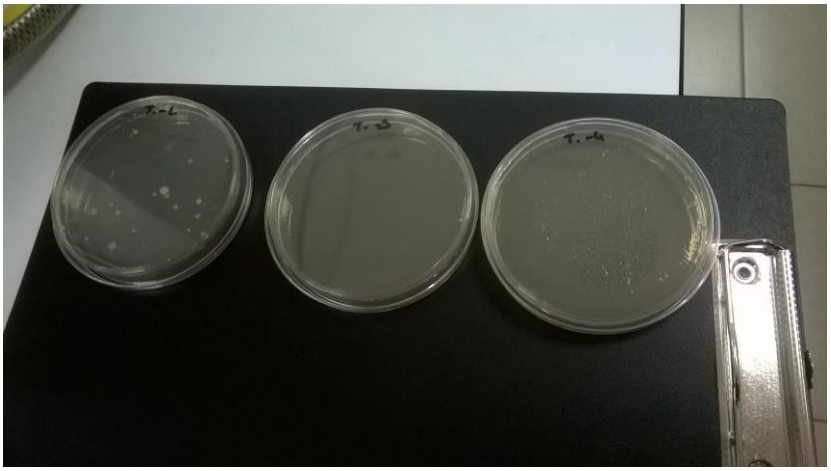

Figure 4.2 Representing the flaked poultry meat bacterial count at 4°C for the first day (Personal archive, 2018)

Table 4.1 Displays total number of living or occurring bacteria over the samples at 4°C for t_0

Sample Type	Scaled Calculation	Multiplication	Exponential Factor	Total Counts of Bacteria
Poultry meat	220 x 2	440	(-2)	4.4×10^4 cfu/g
Red meat	31 x 2	63	(-4)	6.3×10^5 cfu/g

The total number of aerobic mesophilic bacteria was analyzed in the microbiology laboratory. The total amounts of bacteria are observed consequently poultry meat and beef meat with sample propagation cultural counting method. The aim of this experiment is to determine the 7-day change (t_0 and t_1) in total number of microorganism in poultry meat and red meat. For this purpose, total aerobic mesophilic bacterial count test was performed on days on which samples were purchased (t_0) and after 7 days (t_1). Plate Count Agar (PCA, MERC) was used in the experiment. Incubation degree is 30°C and incubation period is 24-48 hours. Results and other information of total number of aerobic mesophilic bacteria analysis were given in between Table 4.1 and Table 4.2 for poultry meat and red meat. Total number of microorganism in poultry meat were calculated 4.4×10^4 cfu/g at t_0 and 7.2×10^6 cfu/g at t_1. Total number of live in red meat were calculated 6.3×10^5 cfu/g at t_0 and 3.7×10^7 cfu/g.

Table 4.2 Displays total number of living or occurring bacteria over the samples at 4°C for t_1

Sample Type	Scaled Calculation	Multiplication	Exponential Factor	Total Counts of Bacteria
Poultry meat	220 x 2	72	(-5)	7.2×10^6 cfu/g
Red meat	185 x 2	370	(-5)	3.7×10^7 cfu/g

4.2 GC-MS Analysis

As it is mentioned in previous chapter, GC-MS detects substances in liquid or gas phase over the samples. In our laboratory, it was used gas-phase GC-MS. Hence, the samples ought to release such gasses or at least have these gasses inside. The GC-MS results prove that at both samples, the expected gas phase peaks are obtained seen on

the figures, the total spectrum of, consequently, a piece of flaked poultry meat including the other compounds and minced beef meat including others as well.

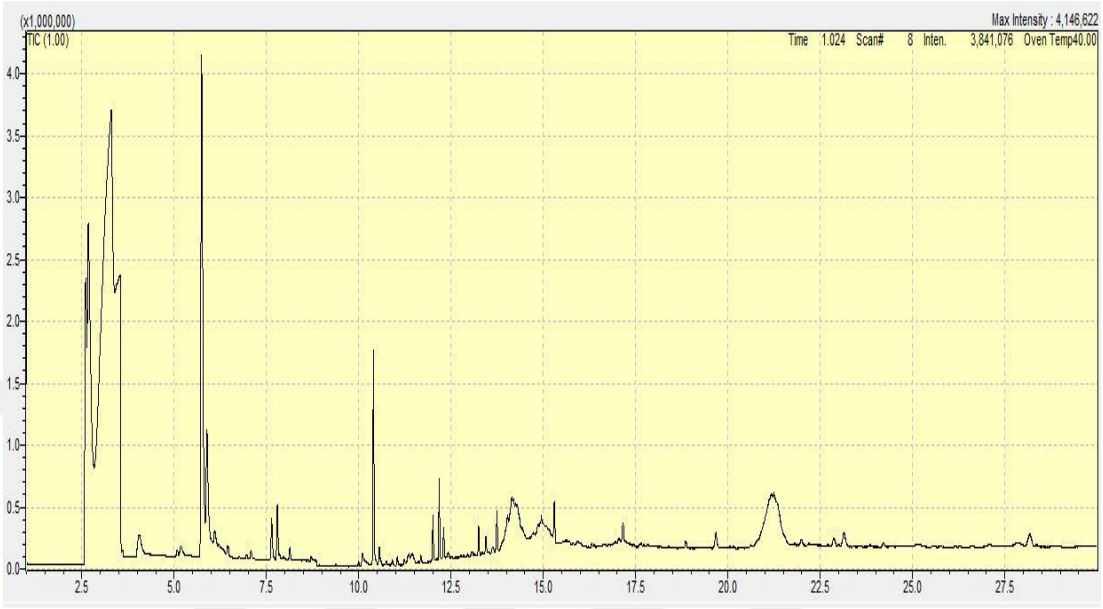

Figure 4.3 GC-MS analysis containing the total spectrum of minced beef meat

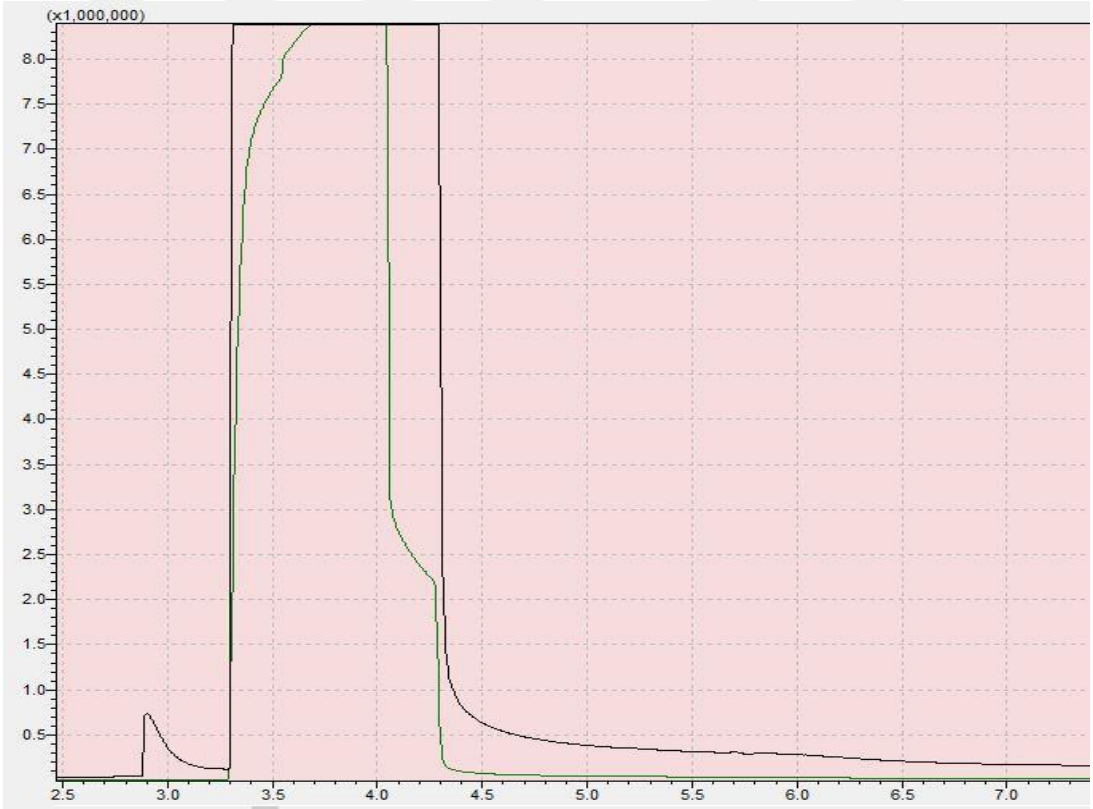

Figure 4.4 Specialized spectrum containing the only desired compound(s), NH_3

Figure 4.3 represents the GC-MS analysis result of the minced red meat sample in 4 °C arranging the molecular mass range due to the desired or questioned compound. In GC-MS analysis, ammonia (NH_3) was selected as the major gas type compound of red meat contamination. Also from the Figure 4.4, as zoomed in a smaller interval, it can be seen clearly the existence of ammonia compound over the minced red meat sample.

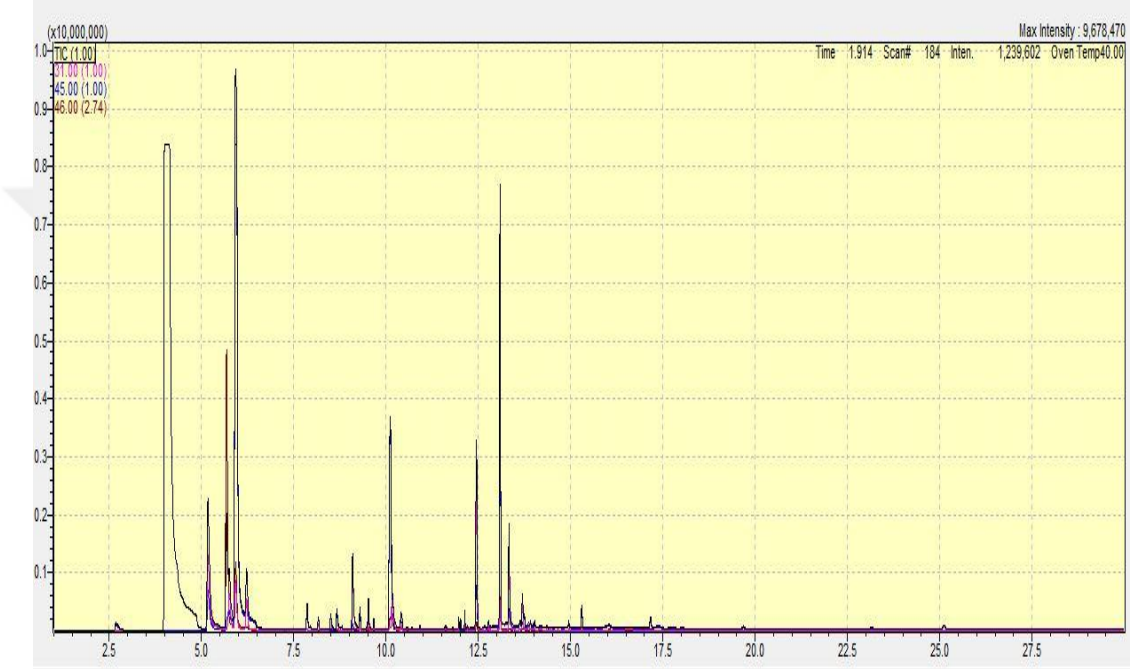

Figure 4.5 GC-MS analysis containing the total spectrum of flaked poultry meat

The same analysis was conducted for the poultry meat sample as well. The total molecular mass spectrum is shown in Figure 4.5 while Figure 4.6 examining the focused interval of total spectrum including only C_2H_5OH compounds.

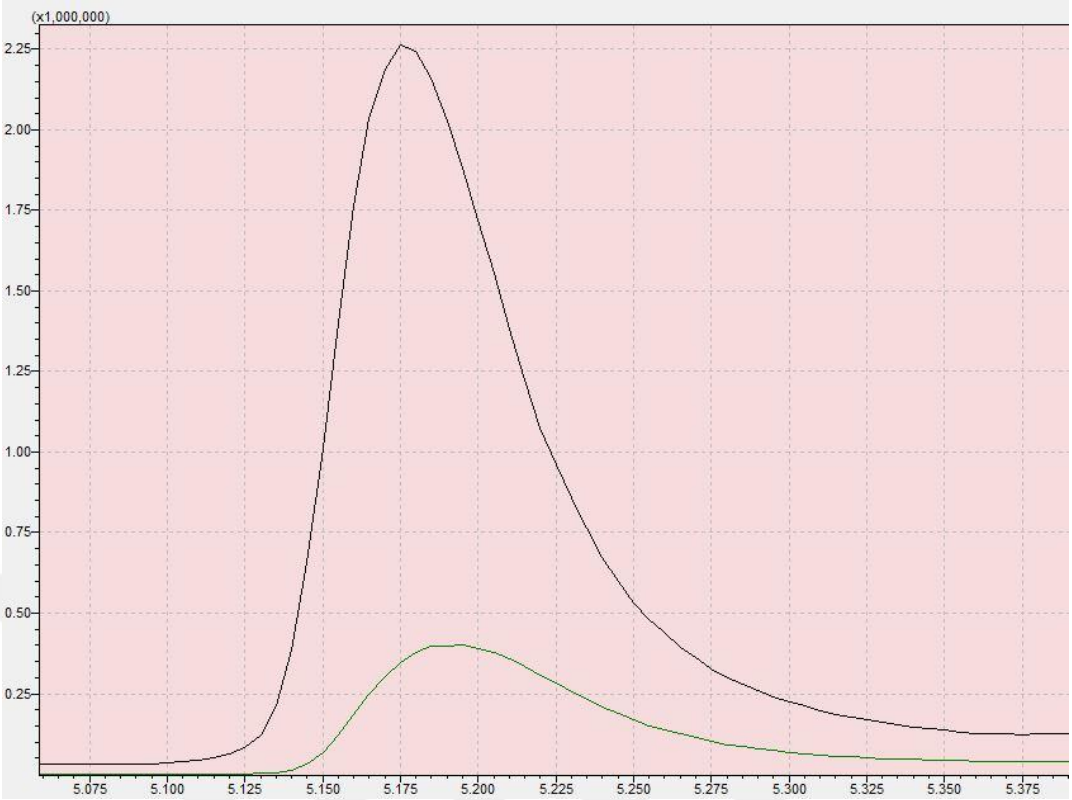

Figure 4.6 Specialized spectrum containing the only desired compound(s), C_2H_5OH

4.3 E-nose Analysis

The sensory results were obtained under different temperatures and durations. Each result emerged on the output pins of the sensor array is shown in Figures 4.7 to 4.23 below.

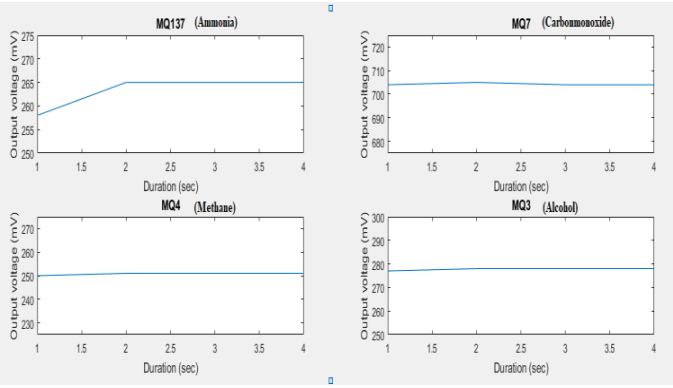

Figure 4.7 Reference sensory outputs at open air condition at 4 °C

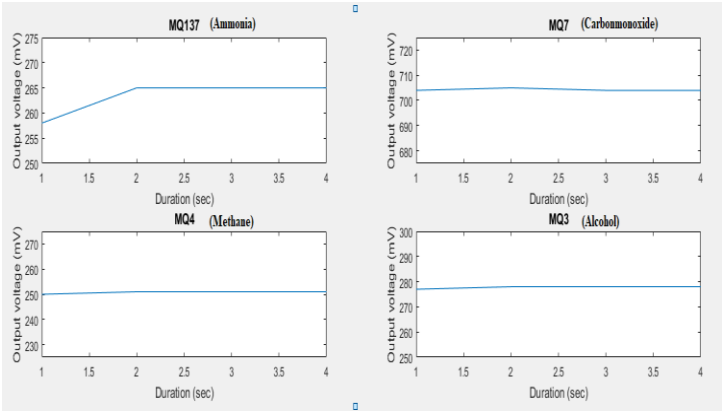

Figure 4.8 Reference sensory outputs at open air condition at 22 °C

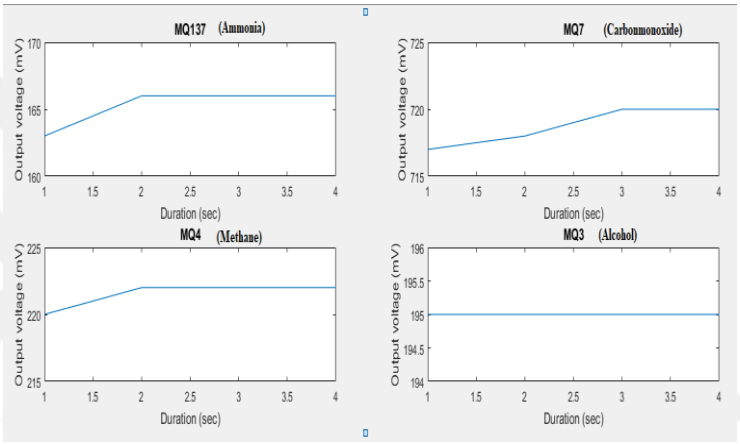

Figure 4.9 Sensor array values for the poultry meat at 4 °C, day-1

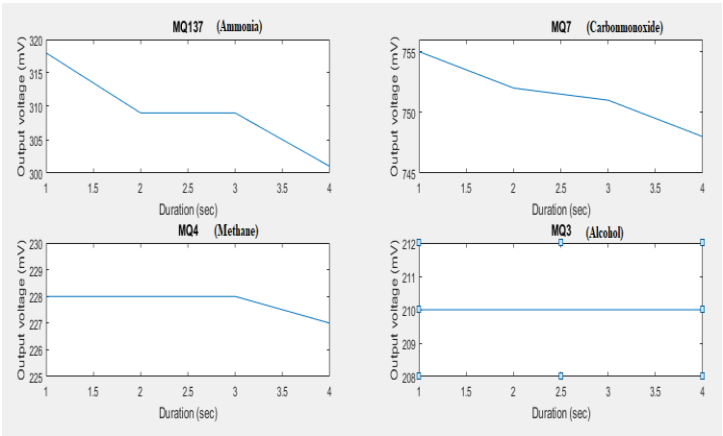

Figure 4.10 Sensor array values for the red meat at 4 °C, day-1

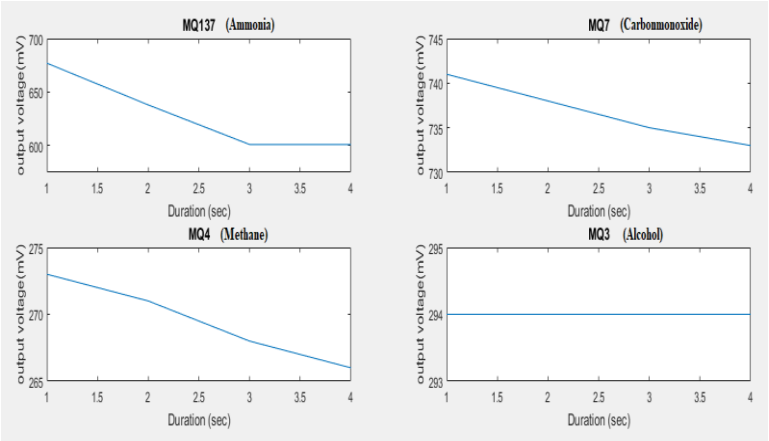

Figure 4.11 Sensor array values for the poultry meat at 22 °C, day-1

The sensory measurements were recorded for only specialized circumstances at 4 and 22 °C. Figure 4.7 demonstrates the isolated measurement under the air condition that is effected by any samples in terms of being a reference value to the following steps of study. And the other measurements are related to the specific conditions described formerly.

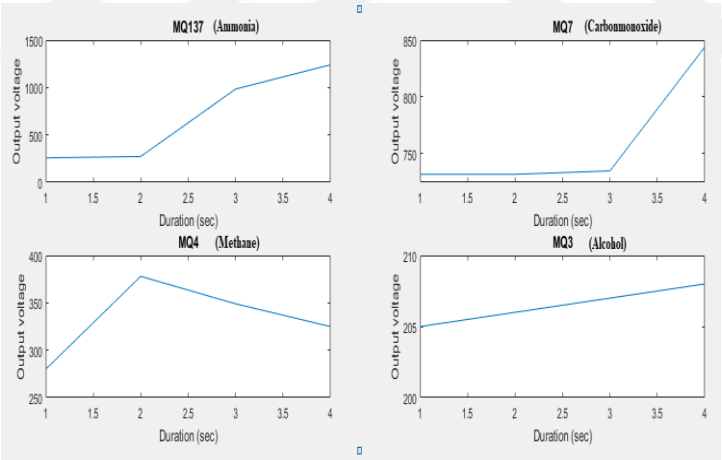

Figure 4.12 Sensor array values for the red meat at 22 °C day-1

After given graph above (Figure 4.11), the measurements were recorded in day-2.

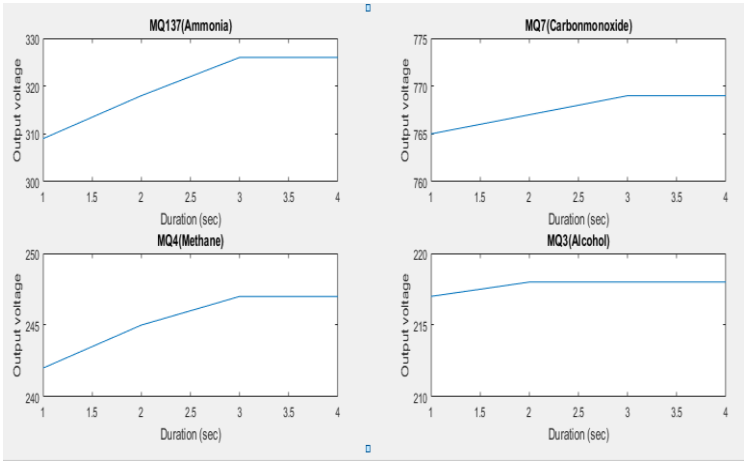

Figure 4.13 Sensor array values for the poultry meat at 4 °C day-2

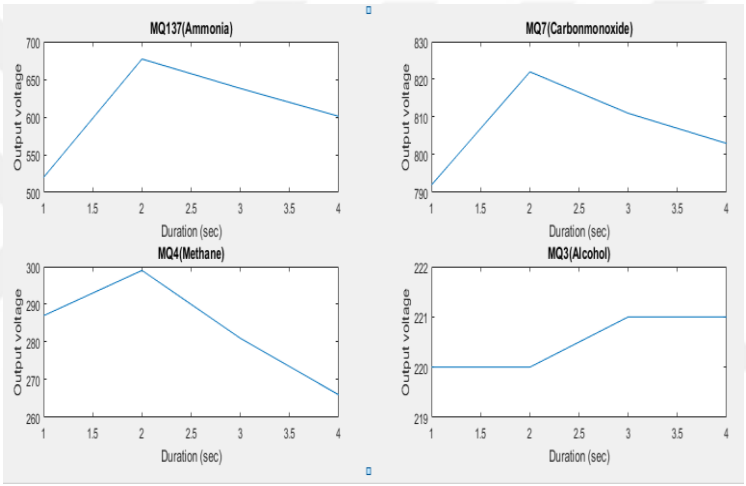

Figure 4.14 Sensor array values for the red meat at 4 °C day-2

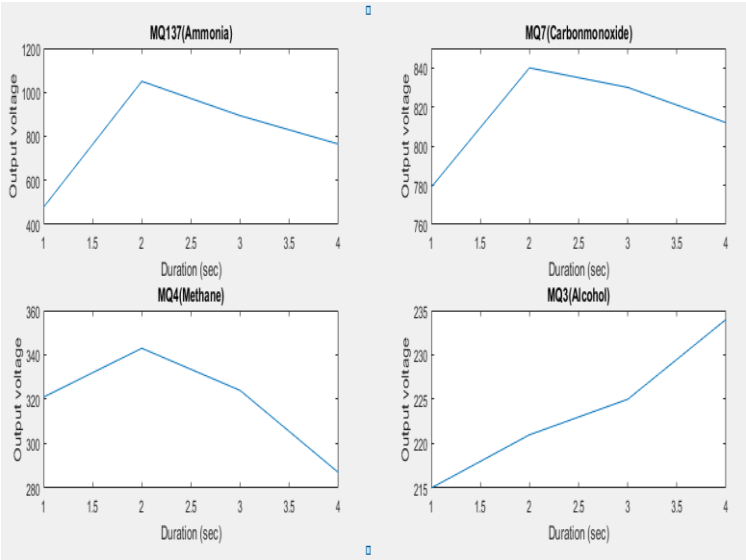

Figure 4.15 Sensor array values for the poultry meat at 22 °C day-2

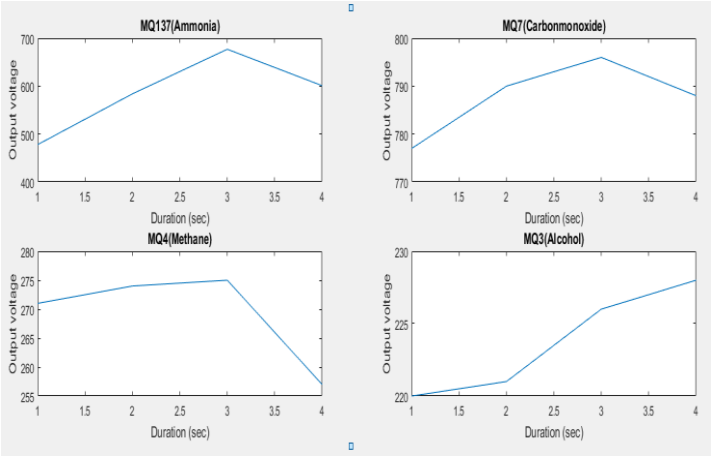

Figure 4.16 Sensor array values for the red meat at 22 °C day-2

After given graph below (Figure 4.16), the measurements were recorded in day-3. Though the changes positively on the other sensor outputs were observed, the main difference occurred MQ137 sensor for red meat samples in contrast to poultry meat sensor responses as occurred mainly for MQ3.

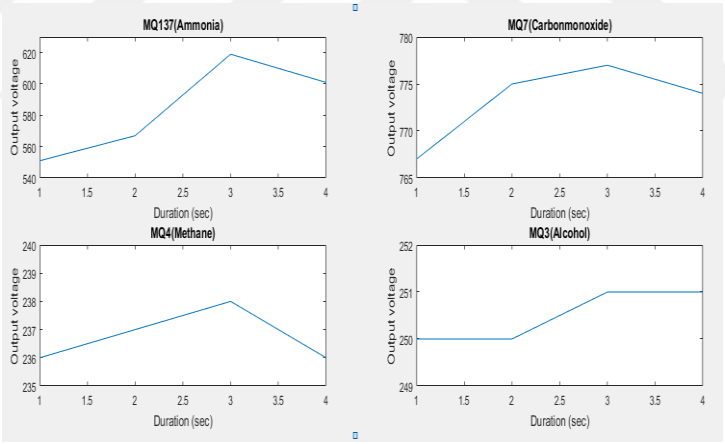

Figure 4.17 Sensor array values for the poultry meat at 4 °C day-3

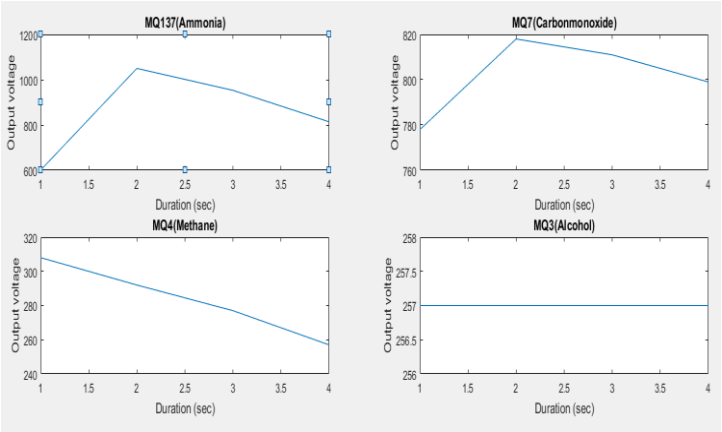

Figure 4.18 Sensor array values for the red meat at 4 °C day-3

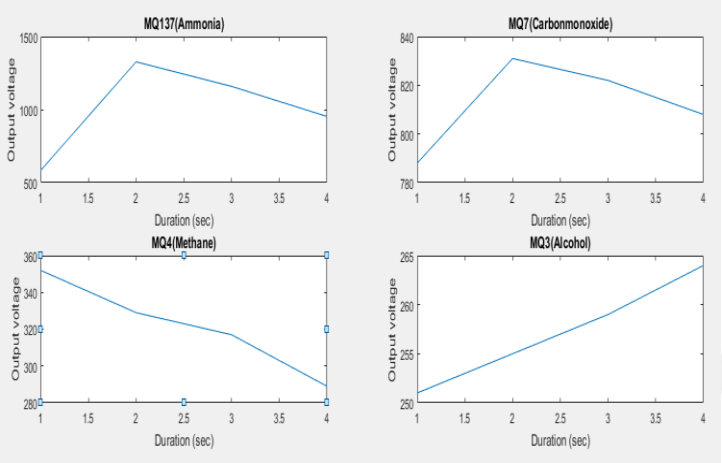

Figure 4.19 Sensor array values for the poultry meat at 22 °C day-3

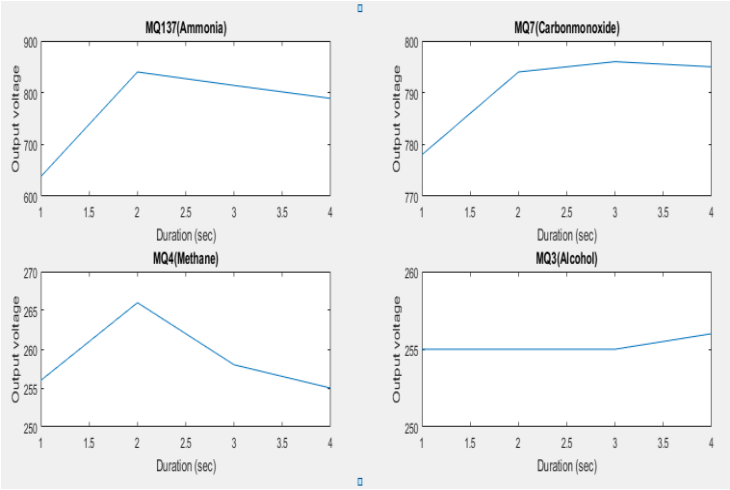

Figure 4.20 Sensor array values for the red meat at 22 °C day-3

In the fourth day measurements, regardless of sensor output amplitude value, the increase was also observed. This different amplitude values sourced from different ambient conditions which also changes considerably according to heater time. Nevertheless, waveforms show extremely rise as the previously measurements for the red and poultry meat seen clearly in Figure 4.20 and 4.21 with the other gasses changes.

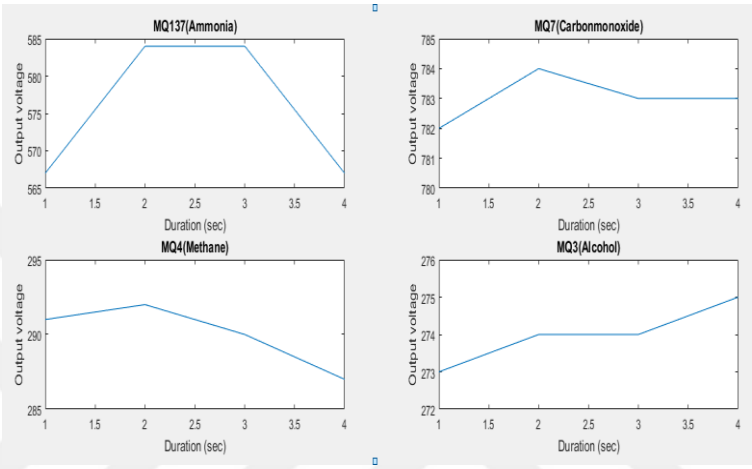

Figure 4.21 Sensor array values for the poultry meat at 4 °C day-4

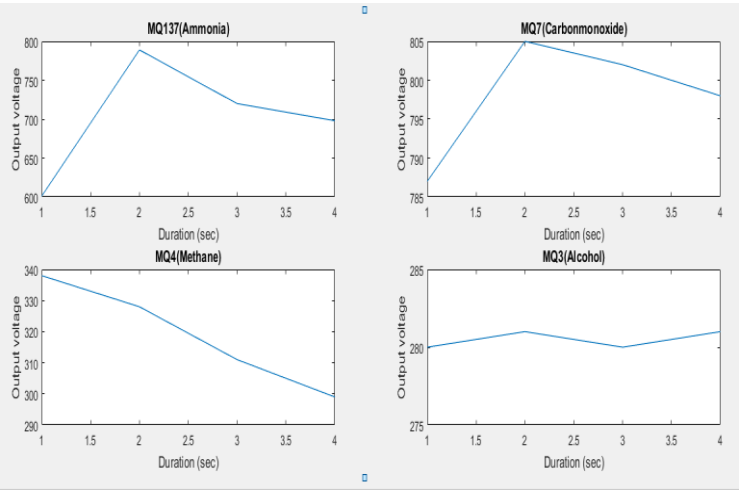

Figure 4.22 Sensor array values for the red meat at 4 °C day-4

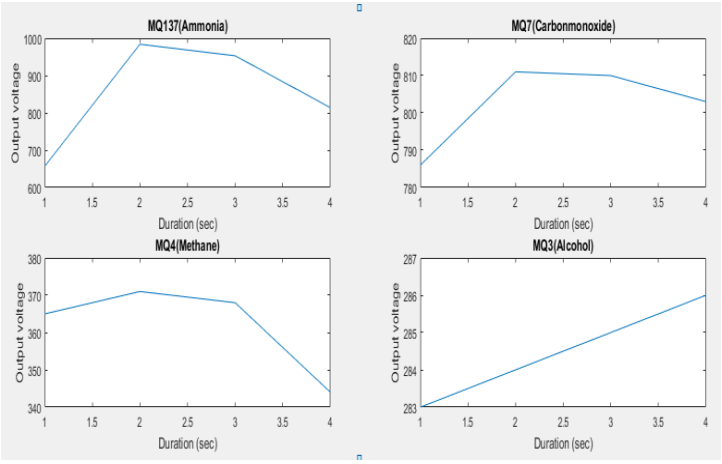

Figure 4.23 Sensor array values for the poultry meat at 22 °C day-4

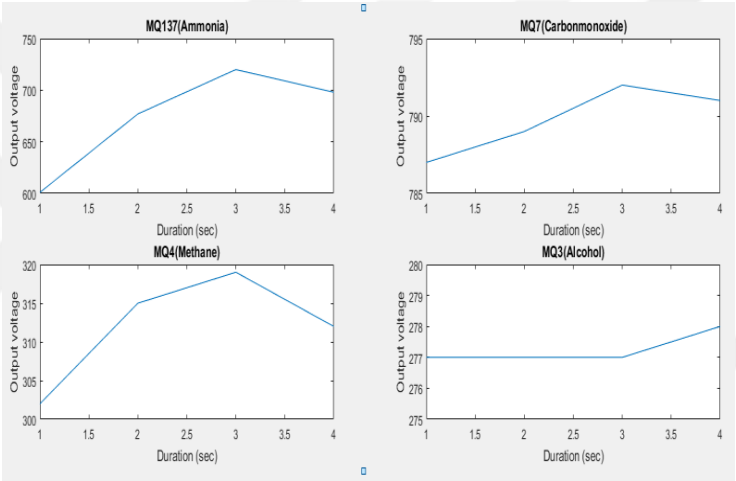

Figure 4.24 Sensor array values for the red meat at 22 °C day-4

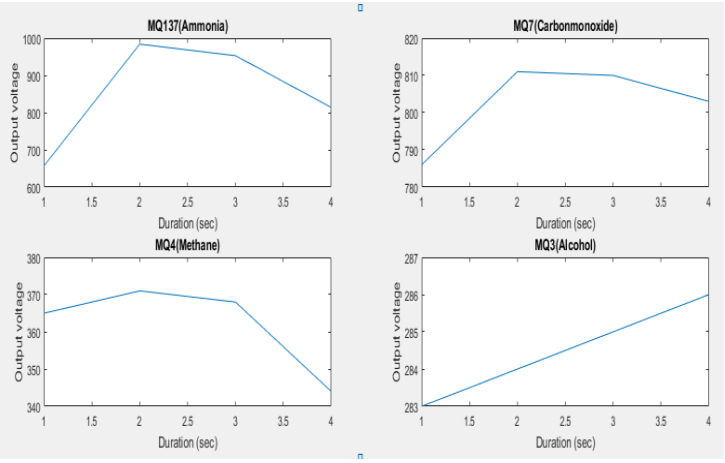

Figure 4.25 Sensor array values for the poultry meat at 4 °C day-5

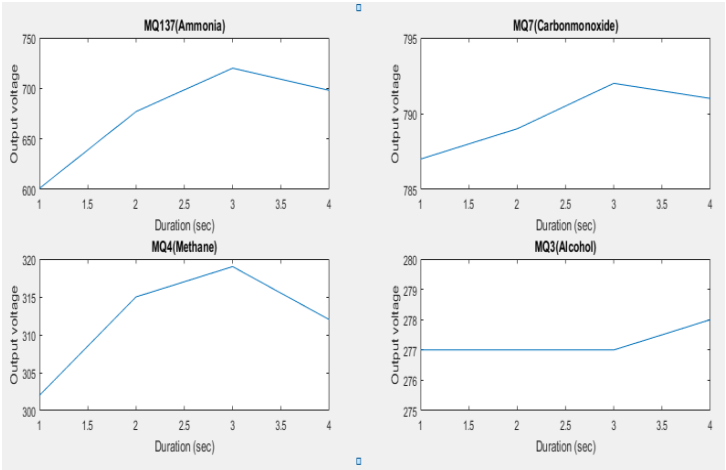

Figure 4.26 Sensor array values for the red meat at 4 °C day-5

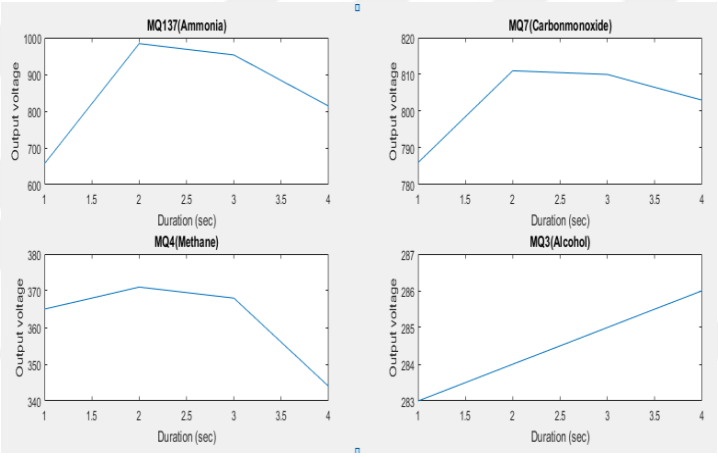

Figure 4.27 Sensor array values for the poultry meat at 22 °C day-5

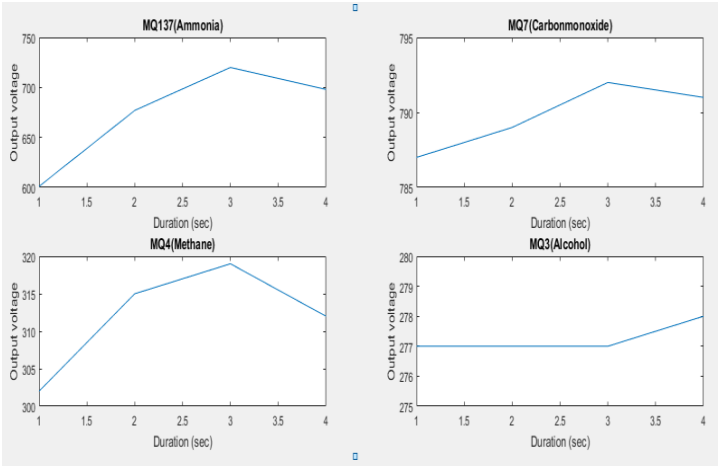

Figure 4.28 Sensor array values for the red meat at 22 °C day-5

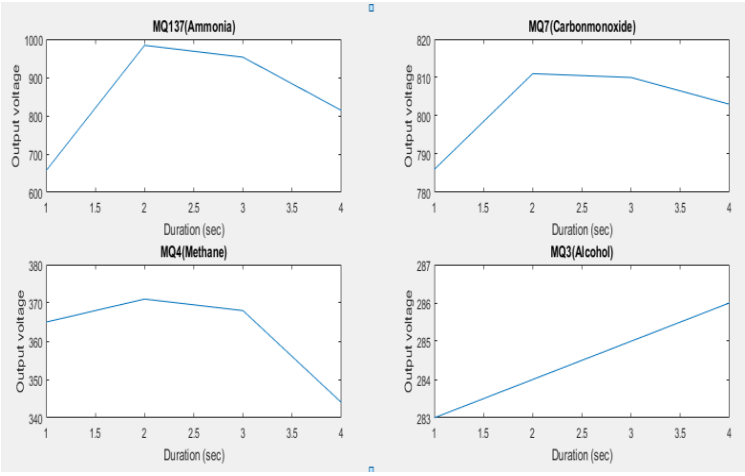

Figure 4.29 Sensor array values for the poultry meat at 4 °C day-6

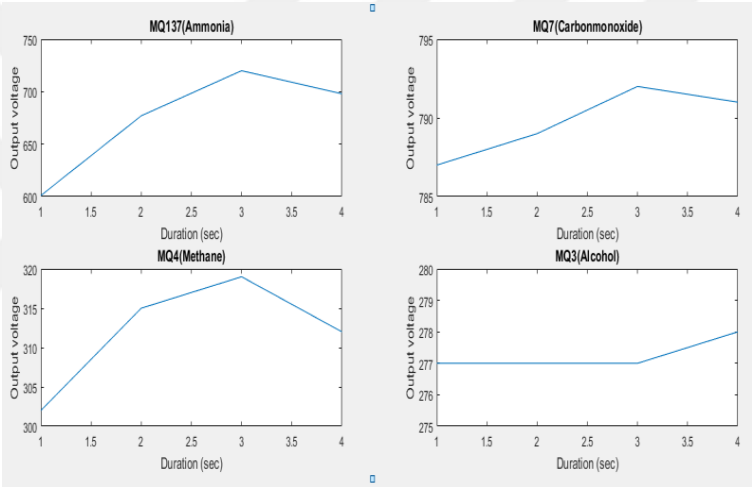

Figure 4.30 Sensor array values for the red meat at 4 °C day-6

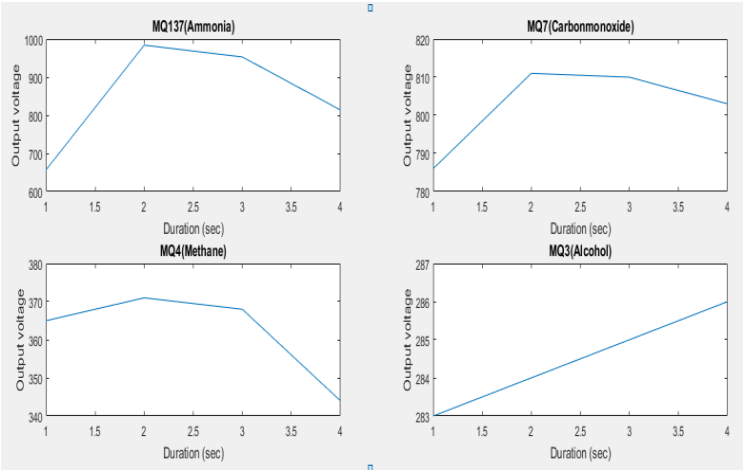

Figure 4.31 Sensor array values for the poultry meat at 22 °C day-6

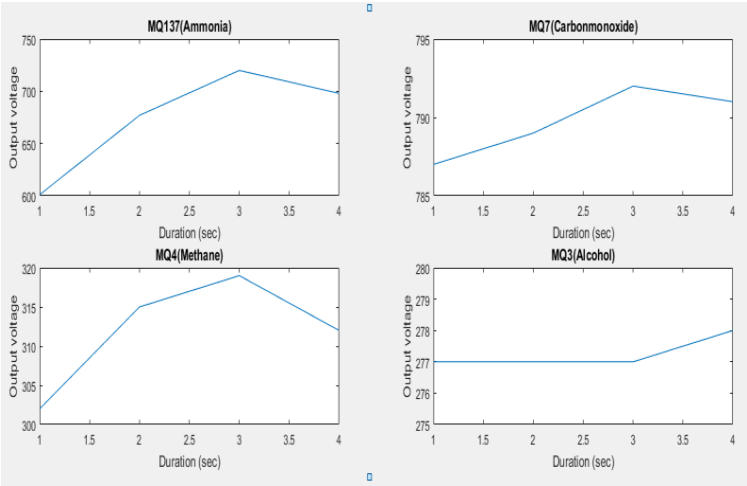

Figure 4.32 Sensor array values for the red meat at 22 °C day-6

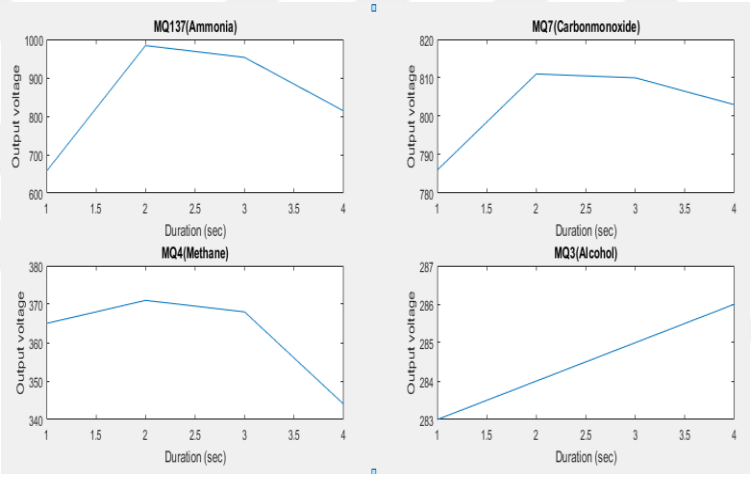

Figure 4.33 Sensor array values for the poultry meat at 4 °C day-7

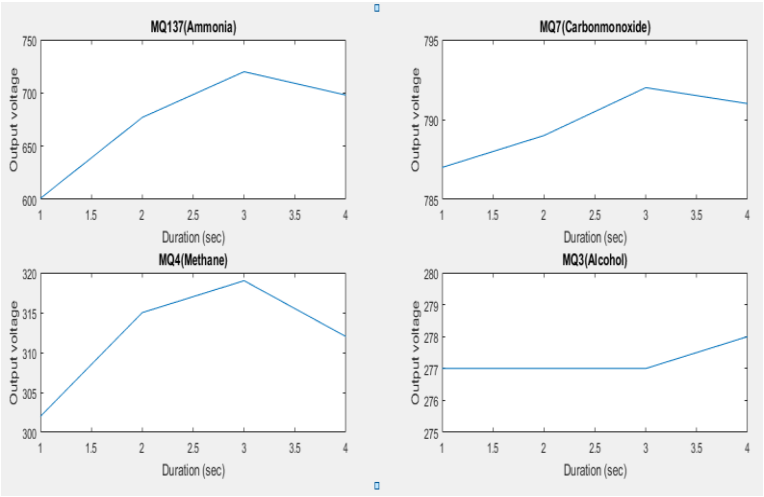

Figure 4.34 Sensor array values for the red meat at 4 °C day-7

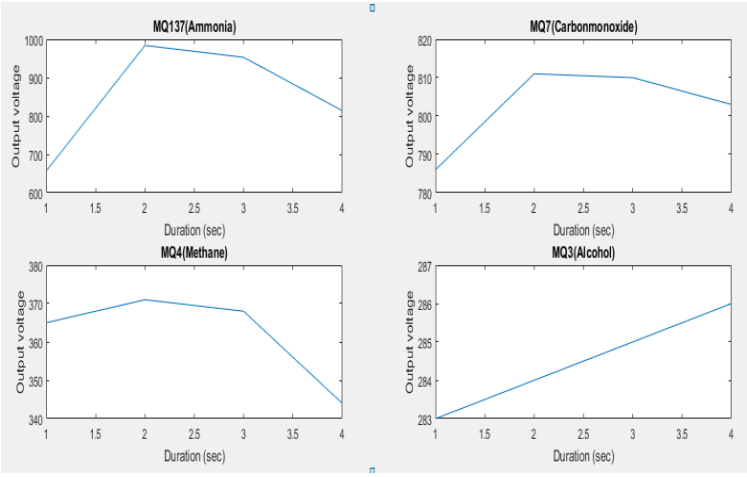

Figure 4.35 Sensor array values for the poultry meat at 22 °C day-7

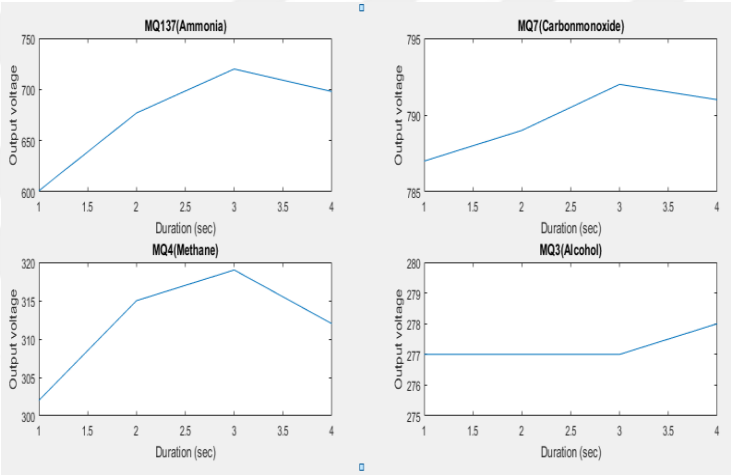

Figure 4.36 Sensor array values for the red meat at 22 °C day-7

As it is expected 4 °C and 22 °C temperature conditions did not cause significant difference in Figure 4.21 and Figure 4.23 in sensor array unlike the other measurements such as microbial counts and chemical analysis.

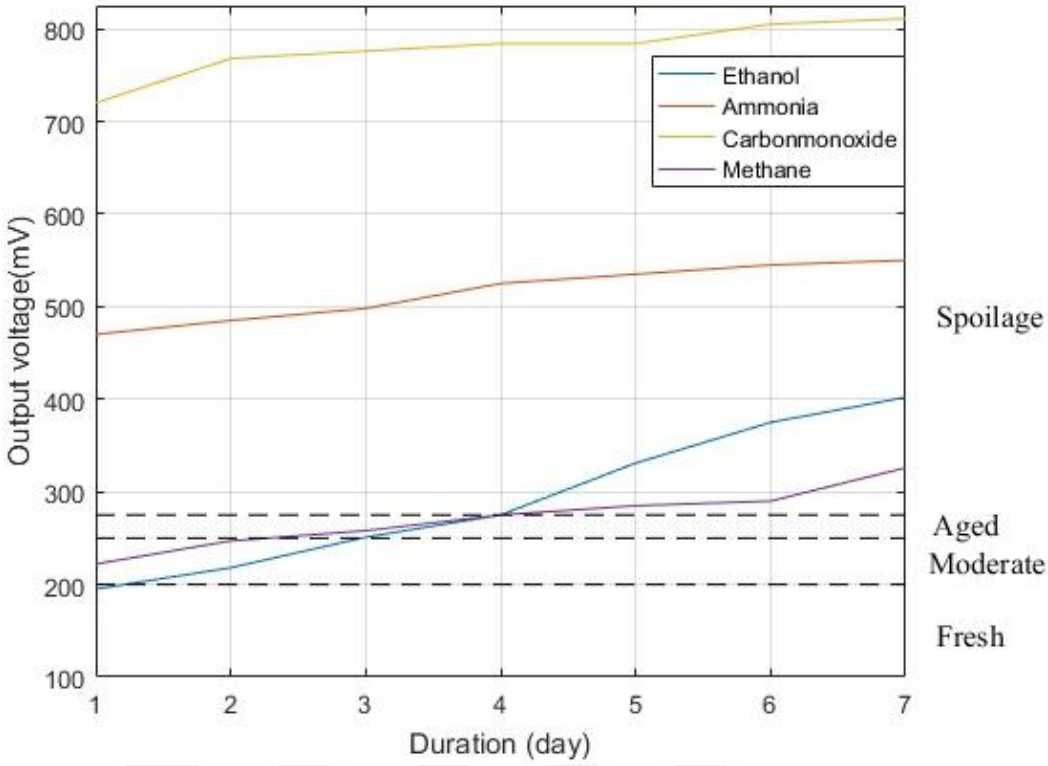

Figure 4.37 Combining the most meaningful discrete values of the poultry meat for each performing day at 4 °C

Figure 4.37, Figure 4.38, Figure 4.39 and Figure 4.40 were drawn as one graph combining of the results of discrete measurements from Figure 4.7 to Figure 4.36. In the process of the detection of the degradation levels for poultry meat, human observation is based regarding smell of it. The degradation levels were determined as 200, 250 and 275 mV depending on the output of ethanol sensor seen in Figure 4.37. Under 200 mV poultry meats considered as fresh up to 1 day after the purchase. Over 275 mV the smell of poultry meat was very poor. Therefore it was taken into consideration that spoilage was realized after this level.

The characteristics of curves belongs to sensor array outputs revealed the similar spread during the time considering the study of Rajamaki et al. (2006). Compared to the others, ethanol sensor response has the largest spread along the time. This is a significant factor to determine the reference value to decide sample freshness provided that is supported by the percentage change. With the increasing rate of over % 100, ethanol ensures the highest increase among these compounds.

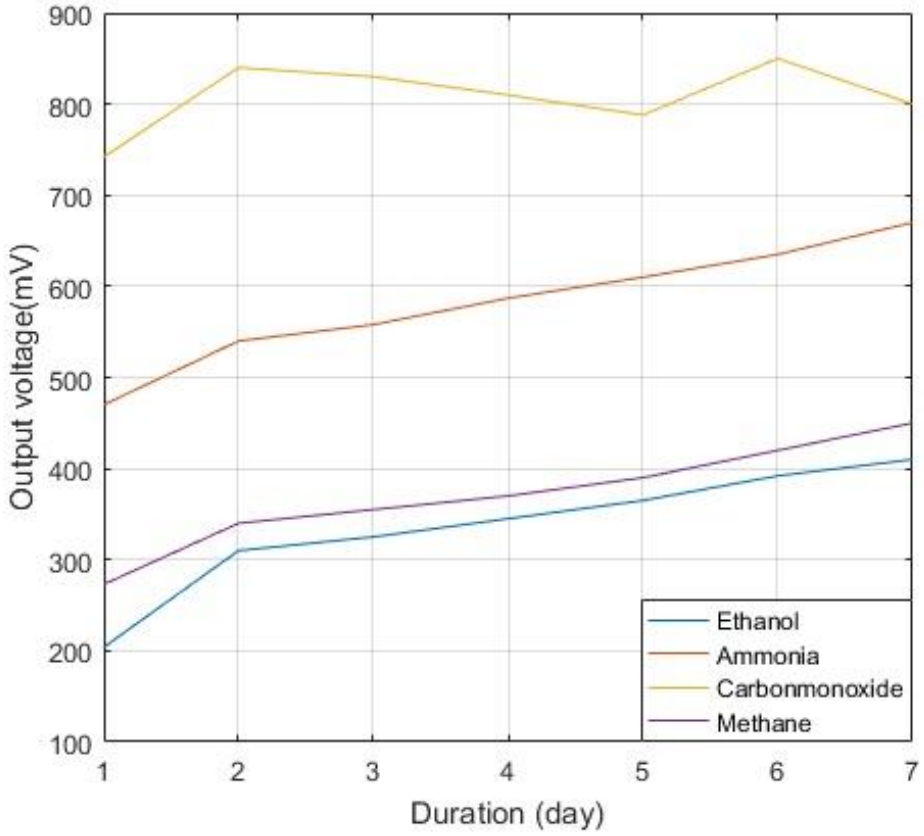

Figure 4.38 Combining the most meaningful discrete values of the poultry meat for each performing day at 22 °C

The degradation levels were not added to the graphic in Figure 4.40 as well as in Figure 4.39 since the spoilage was observed only at the end of first day according to ammonia level while the changes in the other sensor values are negligible.

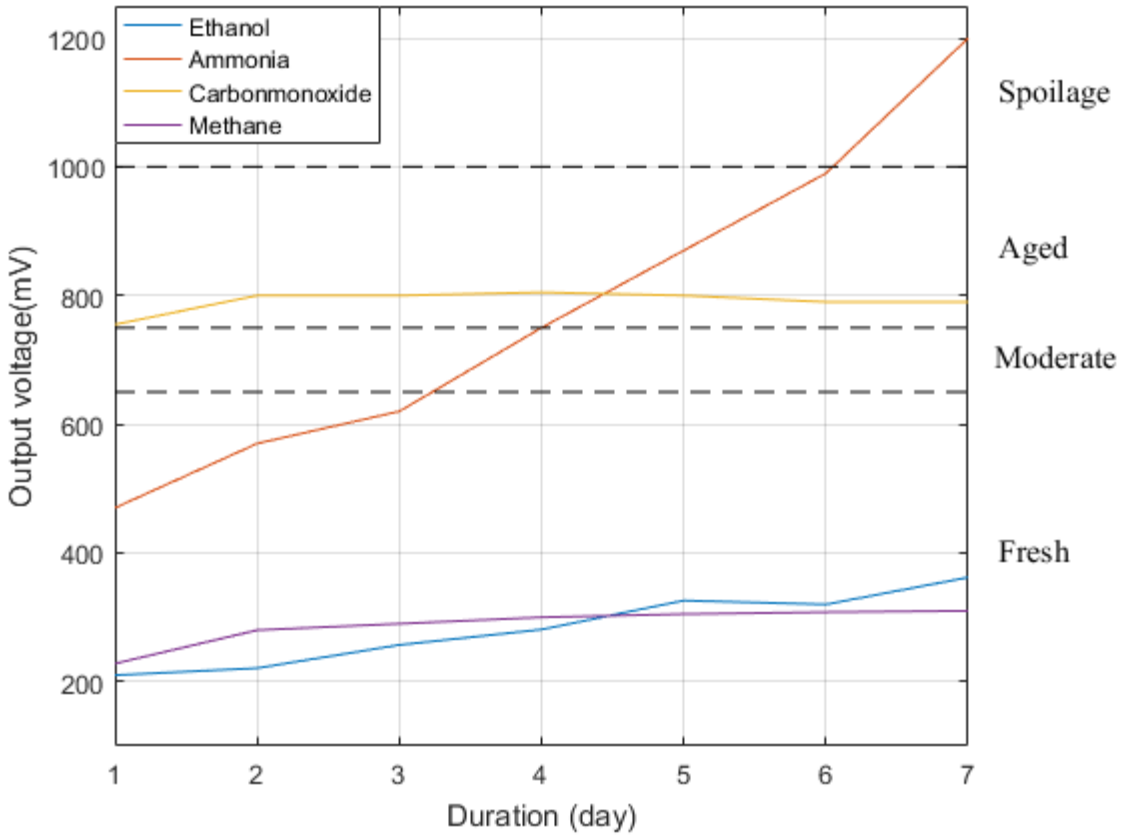

Figure 4.39 Combining the most meaningful discrete values of the red meat for each performing day at 4 °C

The critical levels were determined as 625, 725 and 1000 mV depending on ammonia curve from the study Eom et al. (2014) since scaling would be more observable and measurable among these sensor types range from 450 to 1200 mV with the increase rate of %166 approximately. The freshness is prevented up to 3 days in refrigerator condition (+4°C) from the graph in Figure 4.39 while the spoilage occurs over 6 days.

Interval of Aged between 630 and 730 mV was determined as food poisoning may occur. To summarize, up to 730 mV the samples are accepted as available to consume, in other words logic 0. On the other hand, starting from the value of 1000 mV, red meats can be regarded as spoilage (logic 1).

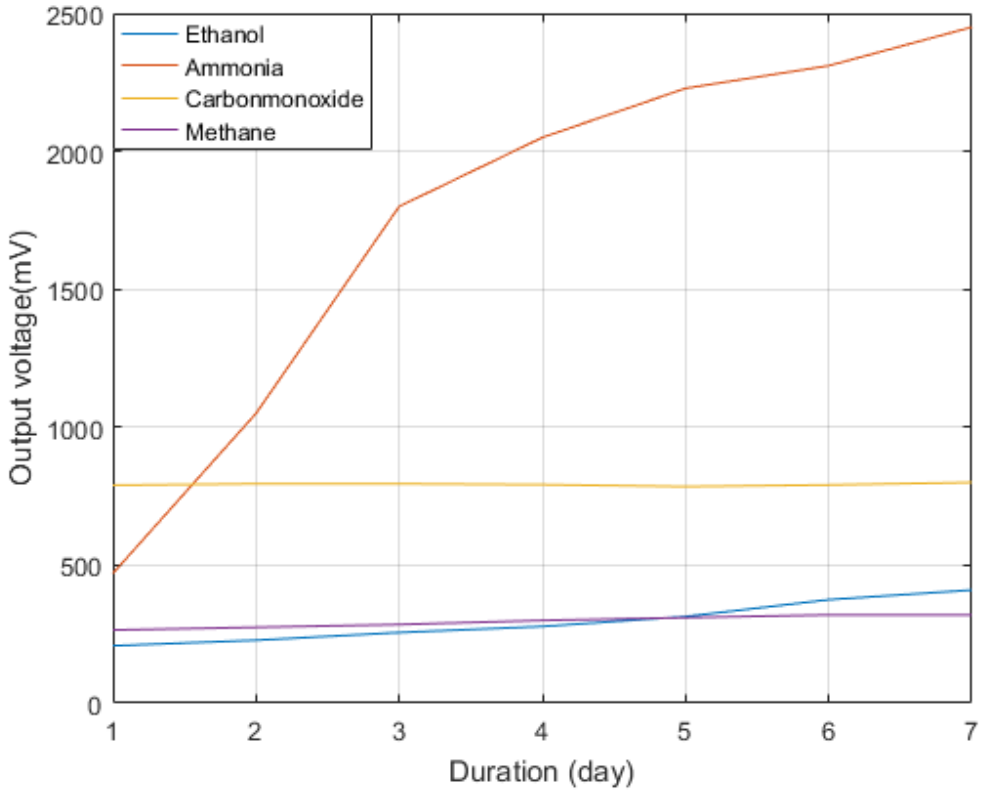

Figure 4.40 Combining the most meaningful discrete values of the red meat for each performing day at 22 °C

4.4 Correlation Between E-nose and the Other Measurement Analysis

As it is thought, there is an obvious relation between e-nose sensor output values and the other analysis methods. However, evaluating the results precisely requires long periods of observations under these circumstances or another. On the other hand, this approach proves that the correlation can be calculated easily according to the measured data. The desired sensory values are obtained as it is expected regardless of considering GC-MS and microbial analysis. Besides, the number of total bacteria increases gradually between intervals as well as GC-MS supports this relation by adding significantly contribution detecting these gasses in head space sampling method.

Calibration is another enhancement issue to obtain better correlation between measurements. The main measurements were performed by sensors to create a reliable data processing system. Hence the sensor values are expected to be decisive

to evaluate the samples are whether available to consume or not. In this context, since it is one of the key point to calibrate the related sensors especially on gas sensors, they should be calibrated depending on what type of conversion is made in software command. In this study ppm measurements were not performed. Hence calibrations were carried out considering the output voltages.

CHAPTER FIVE
CONCLUSION

Traditional techniques such as GC-MS analysis will always be necessary in near future to specify quantitatively or qualitatively the difference among the other food product samples from one to another. The other measurements are conducted only verification of data obtained from the sensor outputs since these process are time-consuming, expensive and requires pre-operational work though they give important data would be needed for the other measurements techniques. Likewise, bacterial analysis is also essential process to determine what kind of living of bacteria groups lead to such gasses emerged in GC-MS analysis during the degradation time. In this point of view, this can be strongly put forward that including the number of higher sensor makes the area of applicable the larger. Although it is thought to have much more space adding numerous different sensors, the total circuit area would not increase linearly in the same way since several output pins are able to connect mutually. This system can easily be made portable taking up such little space. The main measurements are based on sensory.

There could be several improvements to maximize the impact area of the system. As the number of studies in this field increases, the most portable, easy-to-apply and fastest-performing systems will be developed in a similar way. Focusing and performing the developed methods frequently with minor differences will also increase the reliability of such systems and pave the way for their widespread use. Thus, even individually widespread use of similar methods will lower the price and in the near future these measurements could be compulsory for the suppliers. The general trend in this area is to built up a system for many specialized application to measure the freshness quality. This approach puts forward that a portable and compact instrument or an experimental product would be desirable. Nevertheless the thesis in this form is adequately directive in terms of being a guide for new researchers in their road maps having good repeatability and stability.

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