E-Tongue Based Measurement of Chemical Residue in Broccoli using Principal Component Analysis

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CHAPTER 1

INTRODUCTION

Broccoli or *Brassica oleracea var. italica*, a vegetable that belongs to the family of *Brassicaceae* and sometimes called *Cruciferous* vegetables, is considered to be one of the highest sources of vitamin C among fruits and vegetables which makes it an ideal source of nutrients. In the Philippines, broccoli has a total land area of 282 hectares which are commonly found in the Cordillera Administrative Region (CAR) and Northern Mindanao. Development of chemicals called pesticides are used in order to help protect and improve the crops in the agricultural industry to avoid significant losses due to the proliferation of unwanted plants (weeds), insects, mites, and fungi. However, there are health risks involved in the usage of pesticides in various plantations, specifically to the health of its consumers. Laboratory results showed that 31 out of 96 sample specimens contained residues above the normal reporting level of pesticide residue contamination. Hazardous chemical residue from pesticide causes several diseases, disorders, infections, and different genetic problems in human health. Raw, uncut, and pre-cut vegetables are prone to pathogens and pesticide residue contamination. In a laboratory experiment, almost 53.33% of samples tested positive in pesticide residues, 59.06% are pre-cut vegetables and 47.68% are uncut vegetables specimens. Measurement and classification chemical residue drawbacks from being laborious and time-consuming in terms of sample preparation, needs skilled personnel instrument operation, and flawed flavour assessment by a human due to the standardization of measurements, training, the stability and the reproducibility of the evaluation. The main problem is that, consumers are not knowledgeable of the presences of chemical pesticide residue.

Traditionally, food quality detection methods are mainly done in conventional techniques. However, there are groups of researchers who used Gas Chromatography, Spectrometry, and E-tongue. Use of Varian chrompack CP–3800 gas chromatography is utilized in the analysis of vegetable samples for pesticides residues by the introduction of Solid Phase Extraction cartridges in order to remove the interferences before gas chromatography analysis as a good clean-up method that allows the detection of pesticides at part per billion levels (Farina, Abdullah, Bibi & Khalik, 2017). In a different approach, Waring blender and freezing in gas chromatography is introduced in preparing the samples before being analyzed through Multi-Residue Method or a modified QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method (Zheng et al., 2013). Another method that researchers used in acquiring the chemical residues of vegetables is by using Thermo Scientific Q-Exactive and Exactive Orbitrap Mass Spectrometers. They used two sets of sample experiments where the first was by using the Q-Exactive spectrometer and the second set used the Exactive Orbitrap Mass Spectrometer (Kern, Lin & Fricke, 2014). Researchers used electronic tongue device to analyze the foods and drinks consumed by humans. Research applied in the Learning Vector Quantization (LVQ) algorithm, which is an Artificial Neural Network (ANN) based pattern recognition technique which can help provide a lower costing yet more efficient device in providing accurate results for liquid identification. By utilizing the LVQ algorithm, more accurate results of up to 95% can be achieved (Sharma & Ugale, 2015).

Researchers have used the traditional method, which requires a laboratory to test different samples in order to determine and measure the contents of chemical residues of food products. Gas chromatography and Spectrometry are being utilized in this laboratory experiments. The cost and the labor proves to be time consuming when undergoing a laboratory testing. The use of electronic tongues are for analysis of different characteristics of foods and drinks but are not yet applied in measuring the amount of chemical residues found in broccoli. The application of principal component analysis will minimize the amount of unnecessary information that the device and algorithm may gather which in turn will also increase the accuracy of the accumulated data sets.

The main purpose of this study is to detect the presence of chemical residue in broccoli using MATLAB based E-tongue sensors. Significantly, the study aims: (1) to develop an electronic tongue capable of detecting chemical residue in broccoli; (2) to characterize chemical residue found in broccoli using the electronic tongue and principal component analysis; (3) and to conduct tests and validations of results in comparison with the samples that undergo standard laboratory testing.

Many of the consumers are not informed that not all the vegetables that are available in the market are organically farmed and harvested. Numerous amounts of the available fruits and vegetable are subjected to prior pesticide control and acquired different chemicals from this treatments. The study paves a way to test, determine, and measure the amount of chemical residue present in the broccoli to be able to distinguish organically grown broccoli from the ones that are afflicted with different chemicals that can harm and pollute the human body. Broccoli are often washed and blanched before serving. This method of preparing broccoli does not serve as a preventive measure to diminish the amount of chemical residue present in the vegetable. The study will enable farmers and consumers to gain knowledge about the classification and amount of chemical contaminants present on the specific vegetable sample.

The study limits its coverage to broccoli found locally in the Philippines. It would only detect the amount of pesticide chemical residue that can be found in a certain sample of broccoli. The study will focus on three types of chemical residue found in broccoli, such as C*hlorpyrifos*, *Paraoxon*, and *Malaoxon*. Other crops or chemicals are not included in this study. An electronic tongue sensor will be used together with principal component analysis with the application of Linear Vector Quantification to quantitatively collect and measure data. The sensor would be limited to chemicals that would only affect humans when consumed. Side effects caused by the chemicals to the environment or other species are not included in this study. The broccoli samples to be analyzed will be from a producer or manufacturer that claims that the vegetable was grown organically or plantations that uses different chemical pesticide. This study will not show how the pesticide residues would affect the quality of the broccoli.

CHAPTER II

REVIEW OF RELATED RESEARCH

2.1 Broccoli (*Brassica oleracea var. italica)*

Figure 2.1 Broccoli

Broccoli is correlated to cauliflower for which they belong to the same family and are characterized to have grown with unopened flower buds and tender flower stalks. High quality broccoli can be classified to either have dark or bright green with closed flower buds. Vegetable crops grown with the help of chemical fertilizer increases the yield while organic manure enhances the quality.

The combination of optimum level of organic manure and chemical fertilizer needs to be explored and standardized. A study conducted at the Institute of Agriculture and Animal Science, Rampur, Chit wan district in Nepal, during the span of October 2007 to February 2008 the production of broccoli was observed as the farm used by appropriate levels of farm yard manure and inorganic nitrogen in combination and an increase in the yield and quality of broccoli was experienced. A suitable combination of organic manure and various chemical fertilizers are recommended for better yield and quality of broccoli all throughout the span of growth [1].

2.2 Pesticides

Figure 2.2 Chemical Pesticides

The presence of pesticides in agricultural crops continues to be an important issue as many are concerned with the potential health risks, which are small yet costly, posed by the presence of chemicals in their food as it has been difficult to identify the appropriate action for this issue. Pesticides can affect the environment (water, soil, and air) which may include non-target organisms such as animals, insects, and microorganisms. These are made from different chemical ingredients and are used to help protect agricultural crops against pests and unwanted plants such as weed. The chemical residues are then absorbed by the plants which may have adverse effects to humans when consumed regularly or in large quantities. The neglect of proper usage of pesticides stems from the proverb of “if a little is good, a lot more will be better” which results to the overuse of pesticides in agriculture leading to some consumers preferring little to no chemical on the foods they eat [2]. Pesticides are also used to improve crop yield. However, these may enter into food chains through air, water, and soil which eventually leads to health problems when consumed by humans as pesticides can be carcinogenic or citogenic which produces bone and heart diseases, infertility, nerve disorders, and immunological and respiratory diseases to name a few [3].

2.3 Benefit and Risk of Pesticide

Pesticides are commonly used in plantations and farms for crop production and specifically for pest control. In agriculture, the use of pesticide such as insecticide, herbicides and fungicides is in the beneficial side, but considering the properties and physiognomies of such product can cause various hazardous effects on every living organism including humans. Pesticide contains toxic chemicals that are harmful to human health, these agents can cause possible diseases and disorders, such as cancer, infertility, cytogenetic effects, neurological disorders, bone deformation, immunological diseases, and respiratory problems. Pesticides may also be characterized to be non-biodegradable, this affects the environment and may cause problems in the ecosystem and other living organisms [4].

2.4 Pesticide Dependence of Southeast Asian Vegetable Farm Lands

Most farmers are aware of the adverse health risks of pesticide use but considering the effect of various weather in Asia, climate change, and global warming, pesticides are considered to be highly effective and indispensable in terms of plantation outputs. Vegetable farmers in Laos, Cambodia and Vietnam highly depend on agrochemicals for managing pests and diseases. Annual growth in pesticide imports in Cambodia is estimated to be 61%, Laos having 55%, and 10% for Vietnam [5]. Leafy vegetables are the most common crops that needs pesticide treatments in order to speed up the growth process and diminish pest and crop diseases. Due to the vast usage of chemical pesticides in different farms and household plantation, pesticide residues found in Vietnam exceeds above the maximum residue limits (MRLs) for about 33% of the samples and 9% of the specimen from Thailand [6].

2.5 Three Types of Chemical Residue Intake Caused by Pesticide

Poisoning from chemical residue of pesticides is a global health problem and every year this records approximately 300,000 deaths worldwide. There are different ways in which humans get exposed to Agrochemicals, the intentional exposure, occupational exposure, and the non-occupational exposure. Intentional exposure are characterized to be the most common case of chemical intake. Pesticide intake rank 3rd as the most commonly used suicidal attempts in most countries. Another type is the occupational exposure, this is experienced by farmers, pesticide dealers and makers, transporters, sellers and applicators. Most farmers used pesticides in order to propagate fruit and vegetable farms and plantation, but this causes health issues and in copious intake caused death. The last form is the non-occupational exposure, this type was the most unnoticed and understated form of pesticide intake but is also considered as a dangerous type of toxicity due to the suspicious contamination. Consumers do not have the knowledge that the fruits and vegetable that they are eating contains various amounts of chemical pesticide which causes different effects in the human body. The human body does not immediately release out the chemicals consumed by the person after eating a certain type of food that is contaminated by pesticide and this causes long-term diseases and body disorders such as cancer, Non-Hodgkin lymphoma (NHL), Parkinson's disease, Alzheimer's disease, oxidative stress, and other genetic and hereditary damages [7].

2.6 Environmental and Health Related Problems Due to Chemical Residue

Broccoli contains high levels of phytochemicals including vitamins and minerals. The properties of vegetables does not only focus on nutritional properties, but also in the agricultural practices, reduced amount of pesticide residues, and moderated environmental impact. Contamination and impact of chemical pesticide residues in soils, aqua and marine systems, and the whole ecosystem cause massive destruction and diseases to many living organisms including biodiversity [8]. In other cases, farmers themselves are affected by pesticide intoxication and approximately 25 million agricultural workers experienced worldwide pesticide poisonings each year. In this range of hazardous effects on environmental and human health many consumers are in doubt in buying fruits and vegetable that are not organically cultivated. Many cities are conducting agricultural control in order to diminish health problems acquire in chemical intake [9].

Chemical residue from pesticide can cause skin disorder, eye failure, gastronomical and intestinal problems, urinary tract infection, and sexual dysfunctions. In different studies, consumption of fruits and vegetables with pesticide contamination can also contribute in fertility and reproduction problems. Among men, consumption of fruits and vegetables with high levels of pesticide residues can be associated with decreasing total sperm count, lower semen quality, and a lower percentage of morphologically normal sperm [10].

2.7 Pesticide Food Safety Standards

Pesticide residue have certain amount and percentage in order to be considered safe in terms of food contamination. Maximum residue limits (MRLs) are permissible levels of pesticide residues in foods, and is widely used worldwide in the determination of safety level concern. A pesticide food safety standard (PFSS) level can be established for any combination of certain pesticide and commodity and can be useful in the health significance assessment and pesticide residue control. Tolerances and MRLs for pesticides commonly range from 3 to 20 mg kg–1, and acts as a significant role in pesticide residue monitoring and pesticide application directions [11].

Vegetables that are the likes of salads, dish garnishes, and another dish recipes that are simply washed, blanched and is eaten raw are considered to have higher risk of contamination by foodborne pathogens and pesticide residues. Raw, uncut, and pre-cut vegetables are prone to pathogens and pesticide residue. In an experiment, different set are subjected to prior testing which resulted to a study in which 1% of the given samples tested positive in pathogens detection and 53.33% of samples tested positive in pesticide residues, and 59.06% of the samples are pre-cut vegetables and is more often to be contaminated than of the uncut vegetables that contains 47.68% pesticide level [12].

2.8 Pesticide residue in broccoli

The use of synthetic and chemical based pesticides is a conventional way in agriculture, as for plant protection measures and for cultivation of food storage. The monitoring and determination of pesticides aim to detect samples and other food specimen that exceeds the maximum residue level (MRL), and provides data for the consumer’s assessment in an address to the exposure to pesticide residues.

A research was conducted between the months of January to November 2015, where 96 samples were tested for the obtainability of over 339 pesticide sample residues. Fresh and frozen broccoli are laboratory tested in order to acquire valuable results and conclusions. Several results are found in the conducted experiments, reports tallied that 65 of the given broccoli samples contained no residues, 31 of the following specimens contained residues above the normal reporting level, and none of the samples contained residues above the MRL. Tests subjects that 17 of the following samples were organic and several samples are found to have multiple chemical residues as acquired in the experiment where 13 samples contained residues of more than one pesticide, 6 of those samples contained 2 residues, the other 6 samples contained 3 residues and a specimen that contained 4 residues. In this study, percentage values of the results have concluded that none of the residues detected by the laboratory would have an expected influence and effect on human health [13].

2.9 Insecticide Residue Found in Broccoli Grown in Fields

High temperature and humidity contribute to the rapid spread of pests and various diseases. Pest control requires spraying insecticides as a preliminary precaution during the cultivation of vegetables. On various vegetable plantations insecticides are commonly used for controlling aphids, diamondback moths (Plutella xylostella), and striped flea beetles (Phyllotreta striolata). Double doses of chemicals are used in a pesticide mix which then composed of acetamiprid 20% SP, chlorpyrifos 22.5% EC, deltamethrin 2.4% SC, and methomyl 40% SP. In Chinese broccoli, pesticides use high doses of chemicals and then produce residues of chlorpyrifos and deltamethrin that reaches the maximum residue limits (MRLs) at recommended pre harvest intervals (PHIs) [14].

2.10 Gas Chromatograph

Gas chromatograph is equipped with a 63Ni μ-electron-capture detector (μ-ECD) and a fused silica capillary column DB-608 (30 m × 0.25 mm i.d., 0.25 μm film thickness) and is used in pesticide residue extraction. The extraction procedure of chemical residue found in broccoli is a laboratory based test and procedures. In order to start the procedure, a portion of vegetable sample was weighed and blended with a homogenized acetone for 1 minute. The macerate was then filtered under vacuum through a funnel using Advantec no. 2 filter paper. In this way, the chemical was then computed in certain percentage, the specificity of the analytical method was confirmed by analysis of blank vegetable samples in triplicate and confirmed through the extraction procedures [14]. Another way for preparing for gas chromatography is by the use of Waring blender and freezing. In a laboratory test, part of a subsample was homogenized in a Waring blender, immediately after processing, all the given specimen samples were blended and then submitted to a -20°C freezing until given analysis of gas chromatography [15]. Another instrument used is the Varian chrome pack CP–3800 gas chromatography was utilized in the analyzation of different vegetable samples for pesticide residues. The instrument was furnished with 63Ni electron capture detector (ECD) and a 30 m x 0.32 mm i.d (0.25 µm film thickness) HP 5ms fused silica capillary column [16].

2.11 Micro-Solid Phase Extraction

Also known as porous membrane protected micro solid phase extraction is a simple and also effective method for the isolation and enrichment of a compound that are found in complex samples. Small sorbent bags made of a membrane, commonly polypropylene, were filled with a small amount of sorbent but bags were also made of composite materials of polyamide and nylon fibers. Micro-Solid phase extraction is successfully commonly used to extract several organic pollutants such as organochlorine pesticides, polychlorinated biphenyls, and fungicides from waters. Also, Micro-SPE is applicable to detection of trace metals, such as Calcium, Lead, Selenium and Chromium, in different kinds of environmental sample. The benefits of Micro-Solid Phase Extractions compared to the conventional Solid Phase Extraction includes: (1) It is easier to handle and also less time consuming which makes it more convenient for daily use and application. (2) It costs less because less organic solvent is required and each device can be used again for approximately 20 more times. (3) It provides high Enrichment Factors. (4) It is very effective in extracting specimen from semi-solid or solid samples because of the porous membrane preventing some particles from contaminating the sorbent base [17].

2.12 Measurement of Chemical Residue using Spectrometry paired with DART

There are two ways types of pesticide residue contamination violation. The first occurrence is when a pesticide residue present exceeds the level of tolerance that is established for a specific food commodity. The most common violation occurs when a chemical residue is detected in a product has not been established and should not be used in any edible commodity. Gas chromatography with mass spectral detection has been the standard technique for chemical residue determination, although liquid chromatography with MS detection has become a corresponding technique for highly polar and thermally unstable types of pesticides. Thermo Scientific Q-Exactive and Exactive Orbitrap Mass Spectrometers are used in obtaining the pesticide residue in commodities investigated that ranges from smooth-skinned produce to rougher surfaces like broccoli. Direct Analysis in Real Time (DART) is versatile ionization technique that lets the researcher save time and resources on solvents because it often requires little to no sample preparation and thus eliminates the need for gas chromatography. It offers ease of handling and increased output. With the assistance of a mass spectrometer, the samples that gets screened by the DART ionization source would provide a better mass accuracy as long as the mass spectrometer was calibrated daily. The procedure for this setup on broccoli was first they did not wash the broccoli because it was difficult to dry and that the remaining water would saturate the foam. If the foam remained wet, it would affect the signal intensity while undergoing the process. Also, the broccoli was not swapped by the same manner as some soft skinned fruits. The foam was instead pressed gently on the florets of the broccoli to remove the pesticide standards [18].

2.13 QuEChERS (Quick Easy Cheap Effective Rugged and Safe) method

Determination of 10 chemical pesticide residues in vegetables was established by coupling modified QuEChERS to gas chromatography and mass spectrometry analysis. QuEChERS is a type of sample preparation method which involves an initial extraction with acetonitrile followed by an abstraction and partitioning after the addition of a salt mixture. The QuEChERS method provides high recovery and reproducibility, and this method requires less cost than other sample preparation methods. Pesticide residue analysis based on QuEChERS method have been widely studied and applied and adapted by different government organizations and private researches for laboratory preparations and procedures in the determination of chemical pesticide residues in fruit and vegetables that contain complicated matrices [19].

2.14 Electronic Tongue Sensors

Traditionally, electronic tongue is utilized testing food products, pharmaceuticals, fruits, vegetables, fluids, biomedical and water samples. Electronic tongue sensors are also applied in water quality, pH recognition, conductivity, organic acid, sweet perception, and acid flavour [20]. Electronic tongue contain arrays of electrochemical sensors, electrodes and integrated circuits. The first E- tongue was used for recognition of five basic taste; sweet, salty, sour, umami, and bitter. Nowadays, E-tongue is used in food quality, freshness detection, and detection of chemical contaminants. Different analytical methods have been developed in determining chemical compounds, such as chromatographic and spectroscopic techniques however, this laboratory tests require professional equipment and pre-treatment of given specimen. Electronic tongue systems offer a nanomolar level of approach in chemical contamination detection. There are a multitude of types of electronic tongue sensors such as potentiometric, amperometric, voltammetric, impedimetric, and conductometric. The use of electronic tongue would be beneficial in gaining information without risking the safety and health of the individuals that would otherwise digest or taste unhealthy samples.

A rough summary of the multiple applications of electronic tongue would first start with Foodstuffs. Foodstuffs would be related to the food recognition and origin tracing or for evaluating the food quality and for detecting possible contamination of food. Analysis of different types of water samples which would help researchers develop reliable and low-cost methods and apparatus for quality assessment and authentication. Taste masking for pharmaceutical products, biomedical research such as analysis of biological fluids and plenty more applications [21].

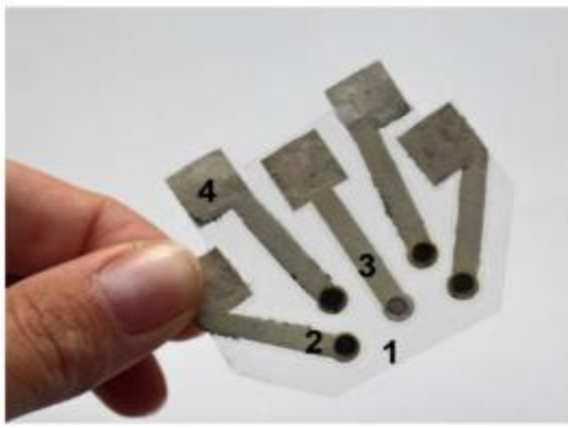

Figure 2.1 Paper-based electronic tongue system, (1) base of the system, (2) ion-selective electrode, (3) reference electrode, (4) electrical connections, illustrated in [21, Fig. 3]

Figure 2.2 Common Application of Electronic Tongue in different fields of science and research, illustrated in [21, Fig. 4]

In order to calibrate the processes of the voltammetric electronic tongue sensors, it must have a compression stage (PCA) to reduce useless noise information and focus on the valuable data, then it passes through the pre-processing stage (LVQ-ANN) to convert the amount of compressed data information into a more identifiable and observable data. The process of cleaning the electronic tongue sensors involves a cleaning solution as well as polishing methods by using sand cloth or filter paper if necessary [22].

2.15 Voltammetric Biosensors

Voltammetric electronic tongue is technology for advanced sensing and measurement applications. The application of electronic tongue is for monitoring of the processes, substances, origin of samples, and the quality of the given specimen. Biosensor approach offers high resolving capabilities for detection of a specific chemical properties found in the sample. Biosensors is a very sensitive tool for determining and observation of variety substance. Biosensors are widely used due to the fact that it is a low cost material and is accessible in field during analysis. It is suggested that instead of using a specific sensor in measuring single parameters, using arrays of cross-sensitivity sensors to gather desirable data would generate better quality parameters with the assistance of multivariate pattern recognition techniques and methods. Voltammetric sensors are one of the classic electrochemical sensors. The first Voltammetric E-Tongue was comprised of two working electrodes made up of gold and platinum was used for detecting various beverages. Principal Component Analysis was used to create the model for the data obtained from the voltammeter to classify beverages of different qualities. Voltammetric Electronic Tongues are classified into three types which are classified according to how the electrodes are used: (1) Bare Electrodes, (2) Modified Electrodes, and (3) Biosensors. The concept of Voltammetric E-tongue biosensors revolve around creating a field of biosensors where each biosensor is tasked to recognize one or group of analytes and the quantity of the analytes in a sample. Then, through the use of advanced statistical methods, the result would be a very accurate characterization of the sample which means voltammetric biosensors can provide very high resolving power for detection of a specific analytes especially for organic compounds.

Voltammetric Electronic Tongue Biosensors are wildly used for their low cost and portability to use them on field analysis. However, fabricating a biosensor would need days because it would undergo rigorous processes because the reproducibility of the sensor is a key factor. Injecting biological reagents show a higher selectivity and specificity in determining a substance [22].

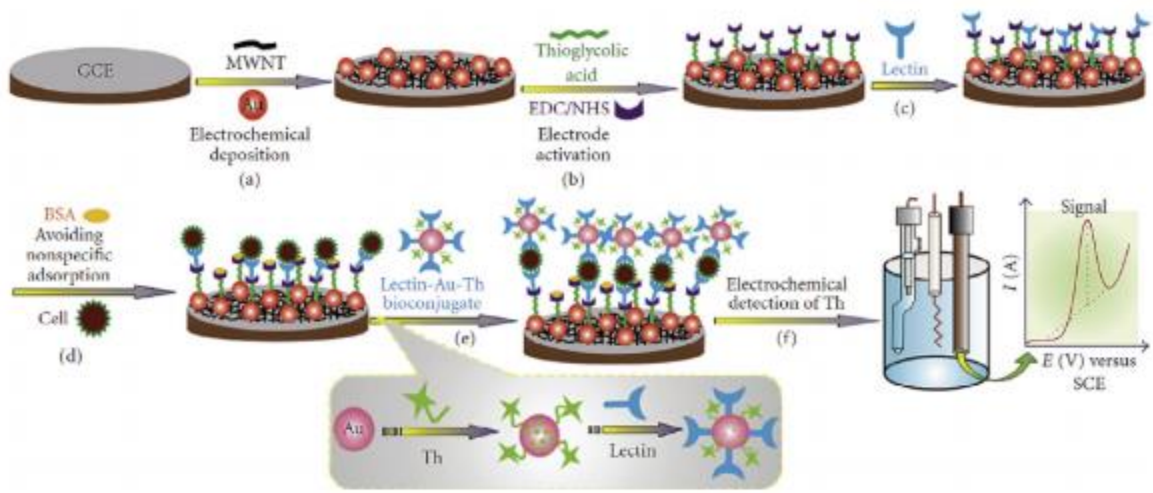

Figure 2.3 Schematic of a sandwich-type lectin-based sensor for electrochemical analysis of glycan expression on cells, presented in [22, Fig 3].

2.16 Linear Vector Quantization (LVQ) algorithm of artificial neural networks (ANN)

The tongue is one of the most significant sensory organs in the human body. The present work uses an electronic tongue that consists of an array of sensors as an efficient way of identifying the characteristic and components of liquids. Electronic tongue, or E-tongue is used to classify the liquids by sensing different parameters, as the sensors generate the data in the form of electrical signals which then processed for pattern recognition with the application of Linear Vector Quantization (LVQ) algorithm of artificial neural networks (ANN) [23].

The LVQ neural network is a three-layered neural network which consists of the input layer, the competing layer, and the output layer. An LVQ network can eliminate the explicit need to tune multiple parameters while training. The classification results are transmitted to the output layer where each neuron represents one data. The competing layer is used to classify samples by calculating the average distance between the input sample vectors. Its competitive layer demands less hidden layer neurons as well as less computation time. The algorithm for the LVQ goes by 1) Initialization of the weight value and its learning rate. 2) Calculate the distance between a data neuron in the competing layer and an input vector. 3) Select a correspondent neuron from the competing layer and use it as the winning data neuron. 4) Adjust the weights of the winning data neurons in accordance with the set classification results [24].

2.17 Principal Component Analysis

In the field of science and research, the amount of large datasets is increasing. As time progresses, people learn new things and understand new concepts and data. Because of this, methods and techniques were invented in order reduce the dimensionality of the information of large datasets, but at the same time maintaining the information in the data so that it can be interpreted and analyzed in an easier process. Principal Component Analysis (PCA) is one of the oldest and widely used method for configuration of large data sets. The idea of PCA is to reduce the dimensionality of a given data set while preserving as much variability or statistical information as possible. Finding new variables that are linear functions of those in the original data set that will help in maximizing the variance and these new variables should be uncorrelated with each other. These new variables which can be called as the Principal Components (PC) will be reduced into solving for the eigenvalue and eigenvector problems. PCA is used as an explanatory tool for analysis of data which involves a dataset with observations on p numerical variables, for each of n entities or individuals. These data values define p n-dimensional vectors x1, ..., xp or, equivalently, an $n \times p$ data matrix X, whose jth column is the vector xj of observations on the jth variable. PCA as a descriptive tool does not need any distributional assumptions and it is an adaptive exploratory method which can be used on numerical data of various types of fields and researches. There are four common adaptation made on Principal Component Analysis that can be used on many fields of research and discipline: (1) Functional Principal Component Analysis which emphasizes on samples at which observations are functional in nature that changes with some continues variable which is commonly time. (2) Simplified Principal Component Analysis which maximizes the variance in dimensions by applying rotation or by adding a constraint in order to give the best possible representational dataset. (3) Robust Principal Component Analysis stemmed from the need to for PCA to deal with very large datasets in areas like image processing, machine learning, Web Data Analysis, or bioinformatics. (4) Symbolic Data Principal Component Analysis which is a general term for dealing with more complex data structures such as histograms and intervals. Interval datasets arise when the researcher wishes to retain a measure of underlying variability in the observations [25].

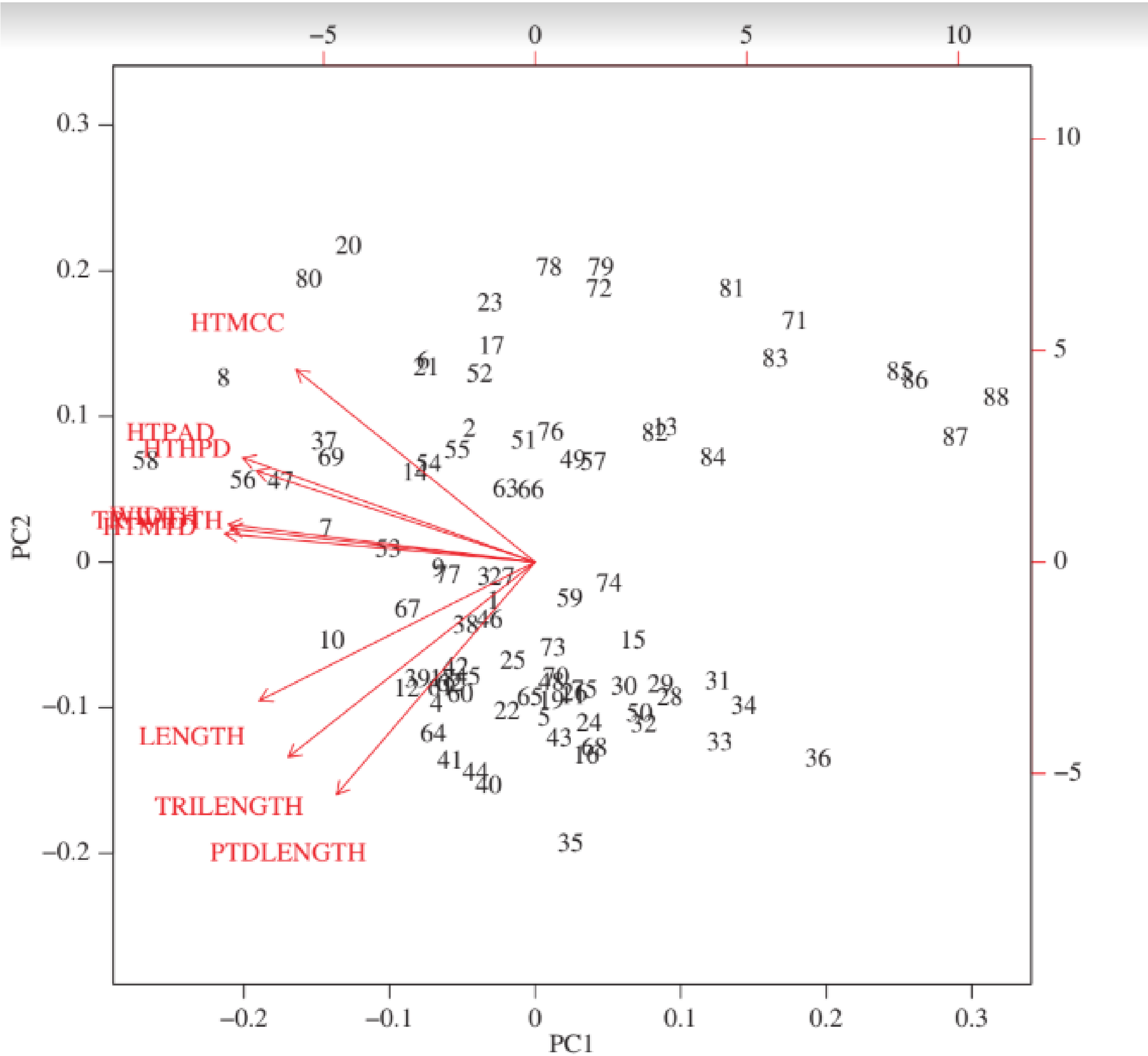

Figure 2.4 Example of Biplot for fossil teeth data using correlation matrix PCA, obtained by using R's biplot command, illustrated in [25, Fig. 2]

Principal Component Analysis is known for as a statistical technique which converts a set of correlated data variables into a set of linear uncorrelated data variables with minimum loss of original information.

2.18 Carbamate insecticides

Carbamate pesticides are derived from carbamic acid and kill insects in a similar fashion as organophosphate insecticides. They are widely used in homes, gardens, and agriculture. Like the organophosphates, their mode of action is inhibition of cholinesterase enzymes, affecting nerve impulse transmission. The signs and symptoms of carbamate poisonings are similar to those caused by the organophosphate pesticides. The carbamate's principal route of entry is either by inhalation or ingestion or secondarily by the dermal route. Dermal exposure tends to be the less toxic route than inhalation or ingestion. The carbamates are hydrolysed enzymatically by the liver; degradation products are excreted by the kidneys and the liver. Respiratory depression combined with pulmonary dilemma is the usual cause of death from poisoning by carbamate compounds. As with organophosphates, the signs and symptoms are based on excessive cholinergic stimulation. Unlike organophosphate poisoning, carbamate poisonings tend to be of shorter duration because the inhibition of nervous tissue acetyl cholinesterase is reversible, and carbamates are more rapidly metabolized. Muscle weakness, dizziness, sweating, and slight body discomfort are commonly reported early symptoms. Headache, salivation, nausea, vomiting, abdominal pain, and diarrhea are often prominent at higher levels of exposure. Contraction of the pupils with blurred vision, incoordination, muscle twitching, and slurred speech have also been reported [26].

2.19 Raspberry Pi 3

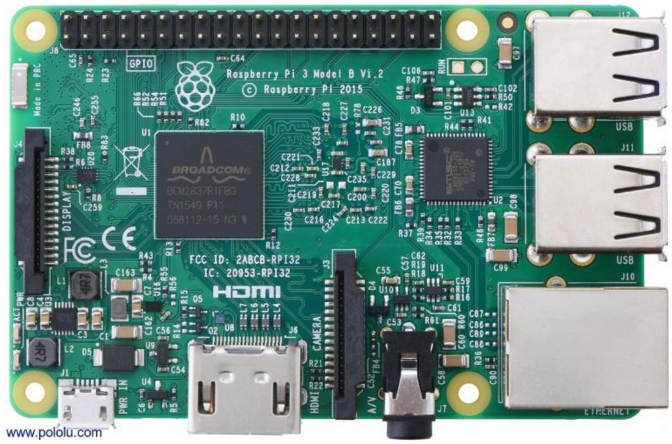

Figure 2.6 Raspberry Pi 3

The RPi 3 has 1GB 900 MHz RAM and 4× ARM Cortex-A53, 1.2GHz CPU. It has a Broadcom BCM2837 System-On-Chip (SOC) hardware, High-Definition Multimedia Interface (HDMI), 3.5mm analogue audio-video jack, 4× USB 2.0, Ethernet, Camera Serial Interface (CSI), and a Display Serial Interface (DSI. One powerful feature of the Raspberry Pi is the row of 40 General Purpose Input Output (GPIO) pins along the edge of the board as pins are the physical interface between the Pi and the outside world such as the electrochemical sensor connected to this module [27].

2.20 Battery Module

Figure 2.7 Battery Module

The system is assumed to be used mostly on remote locations wherein electricity is limited. The Raspberry Pi 3 needs a power supply of about 5V-2A. In order to make the proposed device portable, RPi 3 is supplied power through an Anker Power Core 26800 having a 26,800 mAH capacity and 3A output. The battery device is durable as well as portable to be included in the main device. It has dual micro-USB input for faster charging, Power IQ Technology that automatically identifies any connected device and deliver an optimal charge to the device, Voltage Boost Technology that determines when charging output is encountering cable resistance which then compensates for this resistance to ensure charging speeds are unaffected by long or old cables, as well as multiple safety features [28].

2.21 Secure Digital Card

Figure 2.8 Secure Digital (SD) Card

The Raspberry Pi microcontroller uses a micro traditional hard drive to store files. Instead, it uses a micro Secure Digital (micro-SD) memory card, a solid-state storage system typically used in many electronic devices such as digital cameras, tablets, and smartphones. Almost any SD card will work with the Raspberry Pi, however, at least 8GB of space is required for the files of the operating system. Storage can be extended through many types of USB connected peripherals.

When the Raspberry Pi is 'switched on' or connected to a power supply, piece of code called the boot loader is executed, which reads more special codes from the SD card to start up the microcontroller. However, if there is no SD card inserted, it will not start [29].

2.22 Raspberry Pi 3.5 inch Thin-Film-Transistor Liquid-Crystal Display Touch Screen

Figure 2.9 Raspberry Pi 3.5 inch Thin-Film-Transistor Liquid-Crystal Display Touch Screen

Resistive LCD is one of the major touch screen technologies. Its popularity is supported by stability, usability, and low cost. Resistive technology is employed in wide range of applications. A resistive touch screen panel comprises of several layers, the most important of which are two thin, transparent electrically-resistive layers separated by a thin space. It consists of top and bottom transparent sheets facing each other with a gap between them. The top and bottom sheets have uniform resistance value over its surface. As the top sheet gets pressed, the pressed point of the top sheet physically yields and contacts the bottom sheet. As the layers of the top and bottom sheets contact, electricity gets conducted at the contacted point, and the location of the conducted point is detected. The panel then behaves as a pair of voltage dividers, by rapidly switching between each layer, the position of a pressure on the screen can be read one axis at a time [30].

2.23 pH Sensor

The pH sensor is equipped with Indium-tin-oxide based electric double layer (EDL) and also think-film transistors (TFTs). This sensor uses low operation voltage thanks to the EDL making it cheaper. The transfer and output characteristics of this device were recorded by a semiconductor parameters characterization key. It uses the standard 3 electrode system with the reference electrode being treated with pre-processing procedures. The sensor also exhibits high pH sensitivity [31].

2.24 Chlorine Sensor

Chlorine is mostly used for disinfection in water treatment facilities and also inactivation of pathogens. The sensor is built with a three electrode setup. This sensor is used to carry out graphite modification as well as free-chlorine detection researches. Its basis is graphite from pencil lead after it is being cleaned and treated. This test was conducted using normal tap water. The output unit of measurement is ppm or mg/kg [32].

2.25 Phosphorus Sensor

Phosphorus is a main nutrient in living cells to perform their necessary actions. Some applications of phosphorus is for formulations of fertilizers to enrich their nutritional content or the use of phosphorus in detergents which eventually leads to natural waters because of sewage disposal methods. Phosphorus has usually been measured through the use of optical research processing techniques such as colorimetric which is based on the color of a complex compound resulting in the reaction of two ions while adding in another two kinds of chemicals to act as reducing agents. This standard recommended way of detection consumes valuable time because of converting phosphorus into its subcategory forms using sample digestion and the cost of doing these methods are not cheap. The sensor to be used introduced is by using screen-printed graphite macro electrodes using electrochemical technique cyclic voltammetry. Screen-printed three electrode setup was used where a graphite ink formulation was applied onto the SPE and as an electrode material for the counter and working electrodes. Afterwards, Silver/silver chloride reference electrode was screen-printed onto a flexible polyester film. The measurements provided by the research was in µmg/L which can also be called as ppb and can then be converted into ppm by using a relationship scale of 1ppm equal to 1000ppb [33].

2.26 Chlorpyrifos

Chlorpyrifos is an organophosphate insecticide. Chlorpyrifos (IUPAC name: O, O-diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate). Operational applications of pesticides can be hazardous to humans and other non-target organisms. Chlorpyrifos acts on the nervous system of insects by inhibiting acetyl cholinesterase. Chlorpyrifos is an organophosphate, with potential for both acute toxicity at larger amounts and neurological effects in fetuses and children even at very small amounts [34]. Chlorpyrifos is widely applied as an organophosphate pesticide mostly on the insecticidal aspect. Has a large array of insects and mites that it can deal with usually through contact or through ingestion. The chemical formula for Chlorpyrifos is $C_9H_{11}Cl_3NO_3PS$. Chlorpyrifos comes in the forms of granular types or spray types for applying the pesticides which means it can be applied in many ways such as during pre-plantation, post-plantation and on the soil, dormant trees and foliage. Chlorpyrifos inhibits the action of the enzyme acetylcholinesterase in the junctions of the nervous systems which could disrupt the nervous system and can lead to the death of the animal or human. Ingestion or exposure to high concentrations in the air could contribute to the damage in the nervous systems and thus, in humans can prove to be very fatal since it could lead to neurological disorders and even brain damage [35].

2.27 Paraoxon

Paraoxon is known to be a metabolite of the pesticide Parathion. It is an organophosphorus chemical which can easily be absorbed mucous membranes and skin. Paraoxon has a chemical formula name of $C_{10}H_{14}NO_6P$. Paraoxon being a –oxon metabolite of the parathion is more toxic than its parent compound because the toxicity of organophosphorus pesticides largely depends on the bioactivation of their –oxon metabolites. Exposure to paraoxon especially in large amounts would lead to damage to the lung systems resulting in diseases and inflammations in the lungs. Paraoxon dosage of as low as 0.25 mmol/L could result in apoptosis which means the death of cells in a controlled manner because paraoxon induces caspase activity which activates a process of programmed cell death. Although paraoxon, when tested in higher concentrations, induced more into the necrotic cell death which means the death of cells because of injuries, or failure in blood supply. Paraoxon is slightly more toxic in large airway cells than smaller airway cells [36].

2.28 Malaoxon

Malaoxon is an organophosphorus chemical and it is also a metabolite of the chemical pesticide compound malathion. Malatoxon can easily enter our body's system through inhalation, dermal contact, ingestion and sometimes intravenous means. Malaoxon has an empirical formula of $C_{10}H_{19}O_7PS$. Excess exposure to malaoxon can lead to pulmonary injuries and pulmonary toxicity. At a dosage of as low as 1 mmol/L would be enough to induce cell death in through caspase activation in small airway epithelial cells as compared to its parent compound malathion. This means that malaoxon is more toxic than its parent compound which should be the case since malathion being a organophosphorus compound means that its toxicity highly depends on the amount of bioactivation of its –oxon metabolite [36].

CHAPTER III

METHODOLOGY

3.1 Introduction

As discussed in the previous chapters, the aim of this research is to utilize E-tongue sensors with the application of Principal Component Analysis method to be able to detect and measure the amount of chemical residues that are found in a sample of broccoli obtained in the Philippines. The amount of volume of imports rose from 20 metric tons to an enormous 180 metric tons from the year 2013 to the year 2017 respectively as recorded by the Philippine Statistics Authority. It is also recorded that the value of imports of broccoli also escalated from 0.05 million US$ in the year 2013 to 0.21 million US$ in the year 2017 which shows that broccoli is rising in value and produce. The rise in value and produce implies that the quality and quantity of broccoli in the Philippines should be kept in a steady pace, thus the use of modern day techniques in farms such as chemical fertilizers and chemical pesticides are used to increase the growth and reduce the chances of plants being damaged by insects and pests.

The pesticides used for protecting the plants from pests and insects could be absorbed by the plants themselves. It is the duty of the seller to ensure the safety and the real quality of their products as what they have stated to their customers. The prototype device that will be developed will detect and measure the amount of chemical residue that are present in broccolis found in markets and after gaining this information, customers will learn whether some broccoli have chemicals that passed their Maximum Residue Limit which may prove harmful to human health. The producer will also have to take proper action in ensuring that the next batches of broccoli will be safe to consume. The farmers will also be able to use the device on the field to regularly update and monitor the chemical residues on their broccoli in order to ensure the customers safety as early as possible.

3.2 Research Methodology

Device and Environment Setup

A reasonably large clean room to accommodate the device and avoid contamination of samples taken for testing. To be done at normal room temperature.

What to Test

Broccoli (*Brassica oleracea var. italica)* that is found in Philippine local supermarkets, farms, groceries and open markets. To be conducted on 100+ samples.

3.2.1 Conceptual Framework

INPUT		**PROCESS**		**OUTPUT**
Prepared samples of broccoli entering prototype	→	• Characterization of the chemical residues using E-Tongue Sensors. • Analysis of chemical residue using the application of Principal Component Analysis	→	Names of chemical residues, amount in mg/kg and percentage level acquired in the broccoli sample

Figure 3.1 Conceptual Framework

The diagram represents the input to output processes. The input will first undergo a process of blending until such time the broccoli sample turns to a liquid state. The study will use a liquefied broccoli in order to measure, classify and compute for the chemical residue present in the given sample. The sample will then undergo process and classification using the voltammetric electronic tongue sensors for the detection of the chemical pesticide residue acquired by the sample. The given data and results will then be uploaded to the raspberry pi where codes and linear vector quantization (LVQ) algorithm and neural network will be used in MATLAB applications for the given sample to undergo principal component analysis (PCA). After going through all the processes, the device will output of the study shows different classification of chemical residue and the amount of chemical found in the broccoli samples in terms of parts per million (ppm) or milligram per kilogram (mg/kg) and the a percentage of the acquired contamination of samples will be the output of the given test experiments and study.

3.2.2 Process Flow Diagram

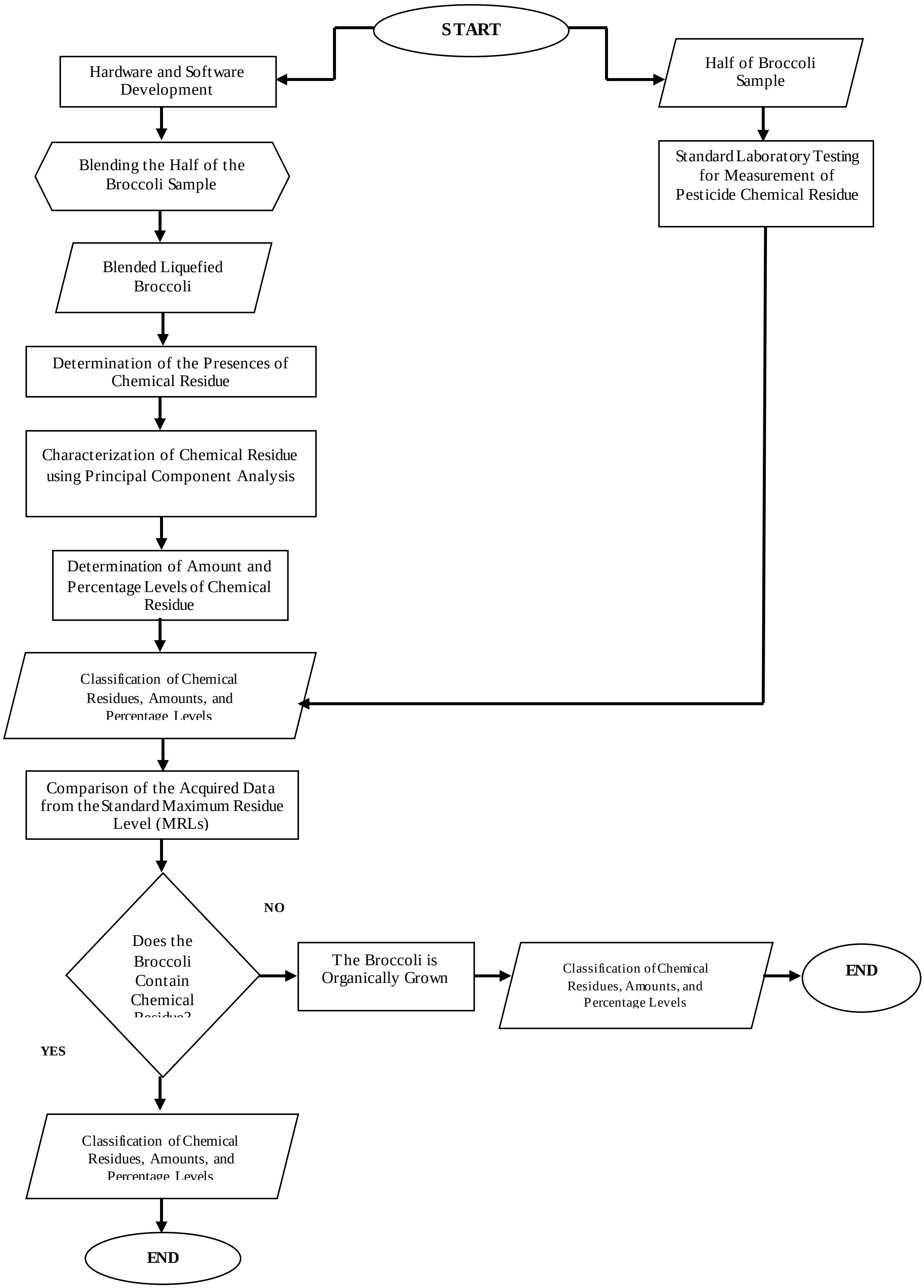
START
Hardware and Software Development
Half of Broccoli Sample
Blending the Half of the Broccoli Sample
Standard Laboratory Testing for Measurement of Pesticide Chemical Residue
Blended Liquefied Broccoli
Determination of the Presences of Chemical Residue
Characterization of Chemical Residue using Principal Component Analysis
Determination of Amount and Percentage Levels of Chemical Residue
Classification of Chemical Residues, Amounts, and Percentage Levels
Comparison of the Acquired Data from the Standard Maximum Residue Level (MRLs)
NO
Does the Broccoli Contain Chemical Residue?
The Broccoli is Organically Grown
Classification of Chemical Residues, Amounts, and Percentage Levels
END
YES
Classification of Chemical Residues, Amounts, and Percentage Levels
END

Figure 3.2 Process Flow Diagram

The diagram shows that a given sample will be split in half. The first half of the specimen will undergo series of laboratory testing in order to determine the amount and characterize the chemical residue found in the given broccoli sample. The other half will undergo the blending process using a normal blender. The system flow diagram starts with the hardware and software development. The hardware comprises of different parts which includes the casing, 3.5 LCD display raspberry pi, and set of voltammetric sensors, power source, and specimen holder. The given software will be comprised of MATLAB codes and linear vector quantization (LVQ) algorithm neural network. In this process, the liquefied broccoli will be the input, the electronic tongue voltammetric sensor will then be submerge to the given liquid sample in order to measure and identify different chemical residue present in the specimen. After the determination comes the characterization, this is where the data will then be classified using principal component analysis (PCA). The amounts are then computed and evaluated in order to test whether the broccoli is contaminated by different chemical residues. Comparing the results to the given standard of the maximum residue limits (MRL), the study will then accounts to an output whether or not the given specimen is naturally grown or contaminated with different chemical pesticide. If the given broccoli sample does not contain any chemical residue then “organically grown” having given percentage will be flashed on the screen for the final output. If the given specimen is then proven to have been contaminated with the chemical pesticide residue, the program will then compare the data acquired with the given normal residue limits. The output will then give a percentage of results of the given data the program will then calculate for the measurement and amount of error difference.

3.3 Main Objective

The main purpose of this study is to measure the amount of chemical residue present in broccoli using MATLAB based E-tongue sensors equipped with Learning Vector Quantity algorithm with application of Principal Component Analysis algorithms.

3.3.1 Objective 1: To design and build a prototype equipped with electronic tongue sensor

Hardware Development

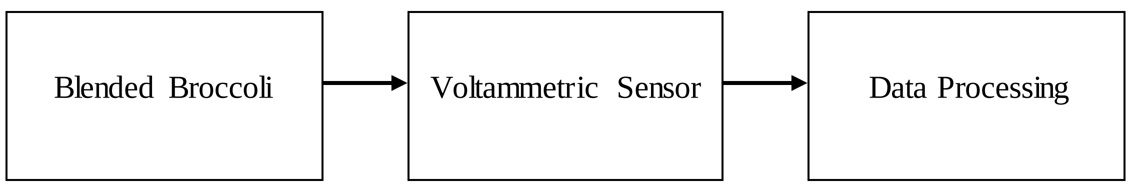

Figure 3.3 Process Flow Diagram of the Hardware Development

The test sample will undergo voltammetric test in the specific voltammetric sensor tube, then the data will then be process to be able to measure the chemical residue from the broccoli sample.

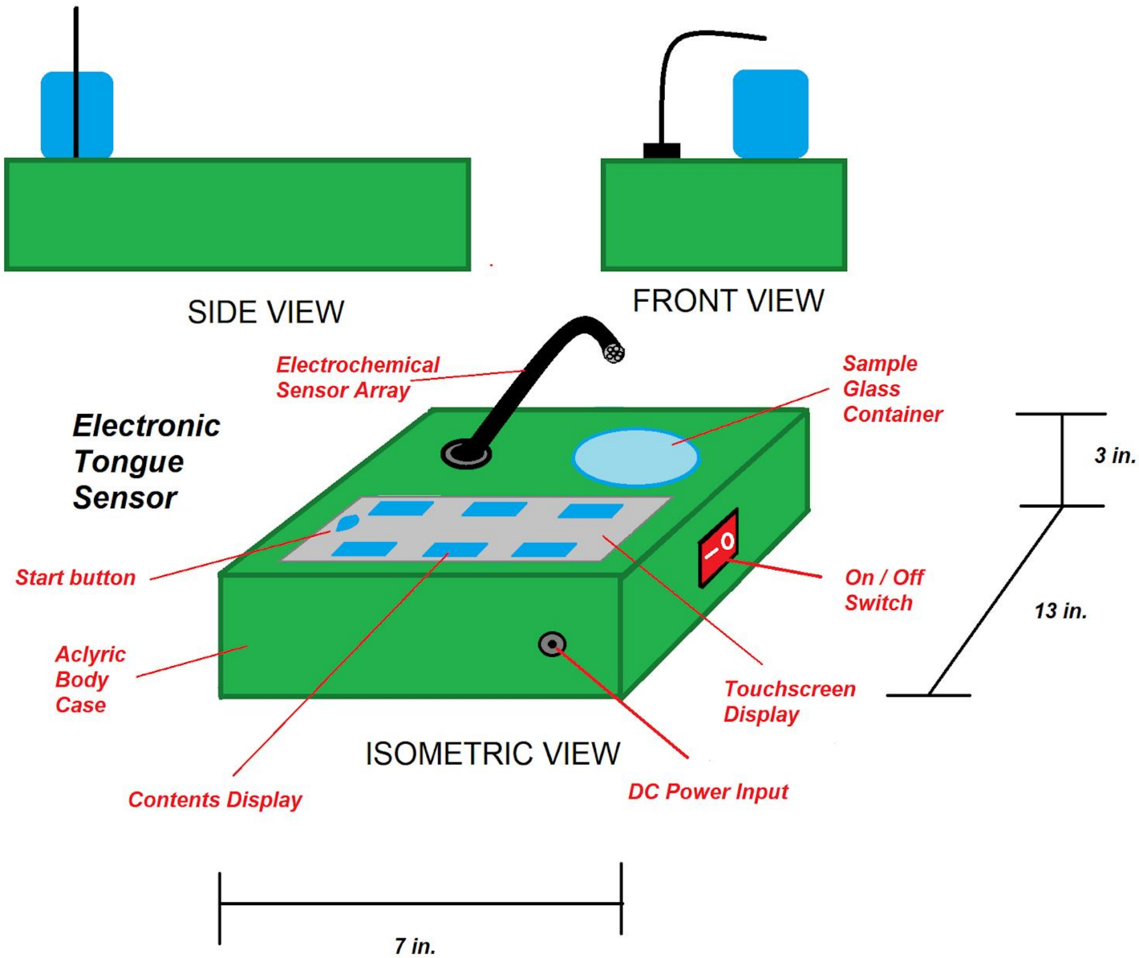

Figure 3.4 Conceptual Prototype Design

Hardware design, procedures and calibrations will serve as research protocols and are made to solve and conduct the research study. The proposed hardware design are composed of different specific parts for sensing and data processing. The voltammetric sensor will have an adjustable tube-like body casing that will contain the wires and the series of sensors. The data will then be stored to a 128GB micro SD card for software and data storage. The data will then be sent to the Raspberry Pi module where the data processing will be done. The data and results will be displayed in the 3.5 inches Raspberry Pi Touch Screen LCD display. The software will be used for the characterization and measurement of the chemical residue found in the sample specimen. The power supply will be the Anker Power Core 26800 power bank with 26,800 mAh capacity in order to meet the specific power need for the sensors and Raspberry Pi module.

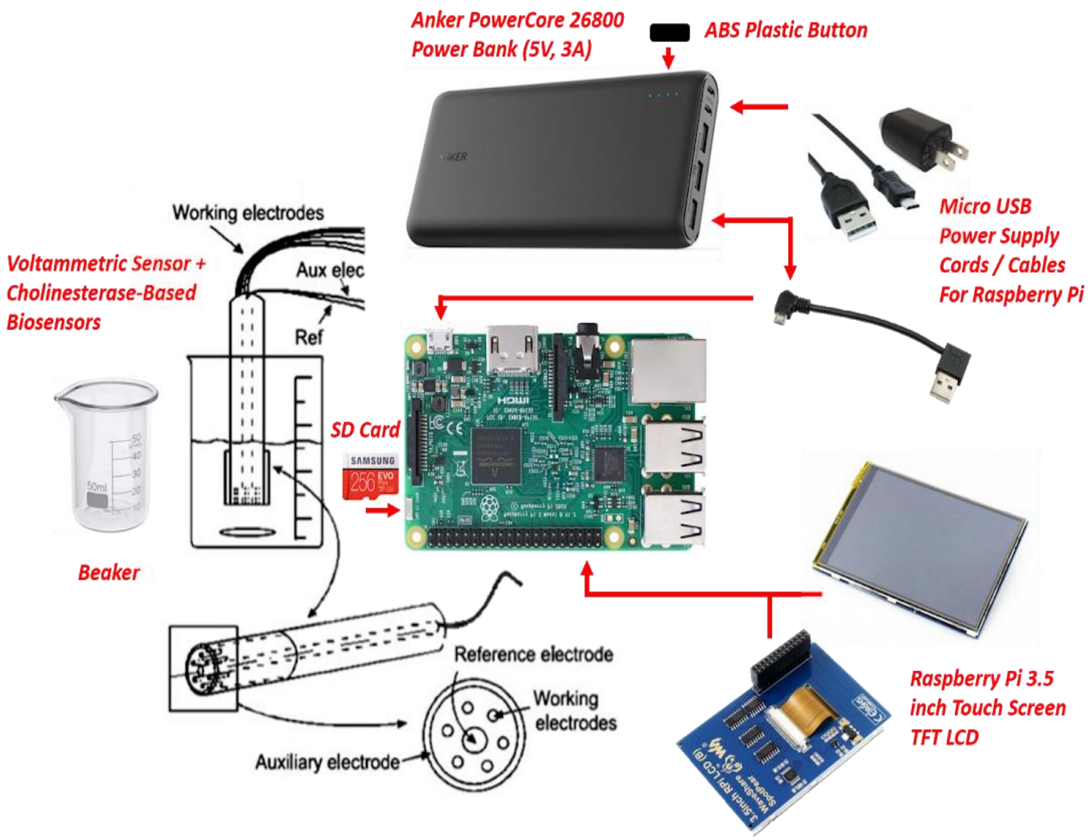

Figure 3.5 Components of the Electronic tongue prototype

Table 3.1 Components Specification and Descriptions

Component	**Description**
Voltammetric Sensor	An amperometric sensor where an analyte (a chemical substance that is the subject of chemical analysis) is obtained by varying a potential and then measuring the resulting current.

EDL and TFT pH Sensor	An Indium-tin-oxide-based Electric Double layer (EDL) Thin film transistor (TFT) pH sensor that exhibits high sensitivity as well as low drift rates.
Graphite Based Free Chlorine Sensor	A sensor composed of the same properties as pencil lead with a reference electrode of an initial electrolyte solution buffers.
Electrochemical Phosphorus Sensor	An electrochemical method based sensor with screen-printed electrodes. A normal three electrode setup with a graphite layer and a silver/silver chloride reference electrode was placed on a polyester flexible film
Battery Module	An Anker Power Core 26800 Power Bank will be used to power the device. This durable and portable rechargeable battery-powered device has a 26,800 mAh capacity, dual micro-USB input for faster recharging, Power IQ Technology that automatically identifies any connected device and deliver an optimum, high-speed charge to the device, Voltage Boost Technology that determines when charging output is encountering cable resistance which then compensates for this resistance to ensure charging speeds are unaffected by long or old cables, as well as multiple safety features.
Secure Digital (SD) Card	A Samsung Evo Plus 256GB Micro SDXC U3 Class 10 micro SD will be used card having a 128GB Storage Capacity with read and write speeds of up to 100 MB/s and 90 MB/s respectively.

Raspberry Pi 3.5 inch Touch Screen TFT LCD	A WAVESHARE 3.5inch RPi LCD (B) Module will be used. The module features a 320×480 resolution, resistive touch control, support for any revision of Raspberry Pi, IPS technology that provides high quality display wide viewing angles, and supports Raspbian system enabling the system to use software keyboard (system interaction without keyboard/mouse).
Micro USB Power Supply Cord / Cable For Raspberry Pi	The Raspberry Pi 3 is powered by a +5.1V micro USB supply. This 24 AWG micro USB cable with a length 10 cm should run the necessary power required from the power bank to the controller board.
Acrylic Device Enclosure (13 x 7 x 3 in)	Acrylic is a lightweight, high impact material with excellent transparency, and superior weather ability. Acrylic sheets offer properties similar to glass such as clarity, brilliance and translucence, but is lighter and has better impact resistance. Acrylic is used on a regular basis with enclosure design, to provide clear windows into cases.
ABS Plastic Button (15 mm x 6 mm)	ABS has a strong resistance to corrosive chemicals and/or physical impacts. It is very easy to machine and has a low melting temperature making it particularly simple to use in injection molding manufacturing processes. ABS is also relatively inexpensive.

50 ml Glass Beaker	Is a cylindrical borosilicate glass container with a flat bottom having a small spout or "beak" to aid in pouring. The container is glass based as it is unreactive and inert to avoid any interference with any chemical reactions.

Block Diagram

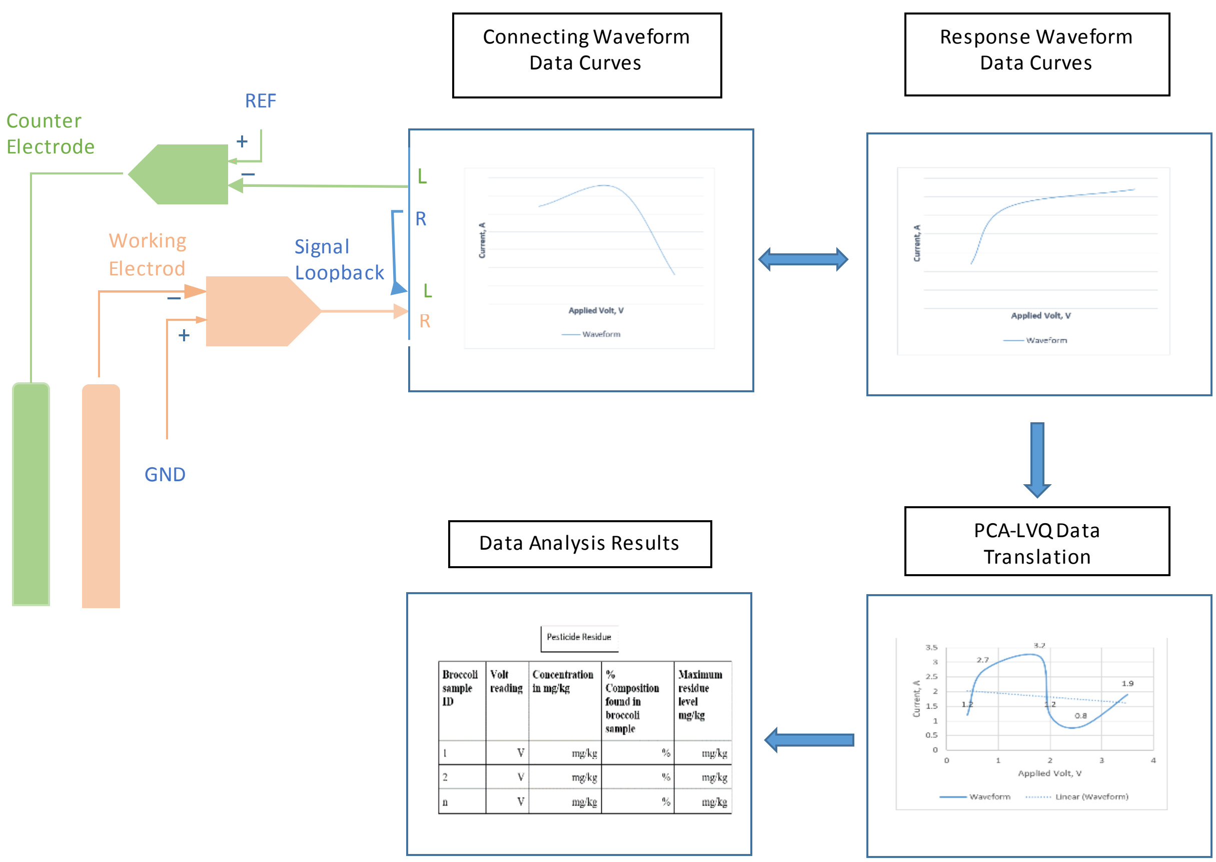

Figure 3.6 Block Diagram for sensor device.

The voltammetric sensor equipped with electrodes would start receiving varying amounts of voltage in order to create “Pulses” that will be formed into data sets which can be plotted into waveforms into scatter plots. Once raw voltammetric data are converted into recognizable plot data, response waveform pulses will be formed from the resulting data and both of these data sets will be scanned and revised by the Principal Component Analysis Data method along with Learning Vector Quantity. These statistical techniques will then convert the voltammetric data into a much simpler and precise data that will provide the necessary information for the pesticide residues found in the broccoli.

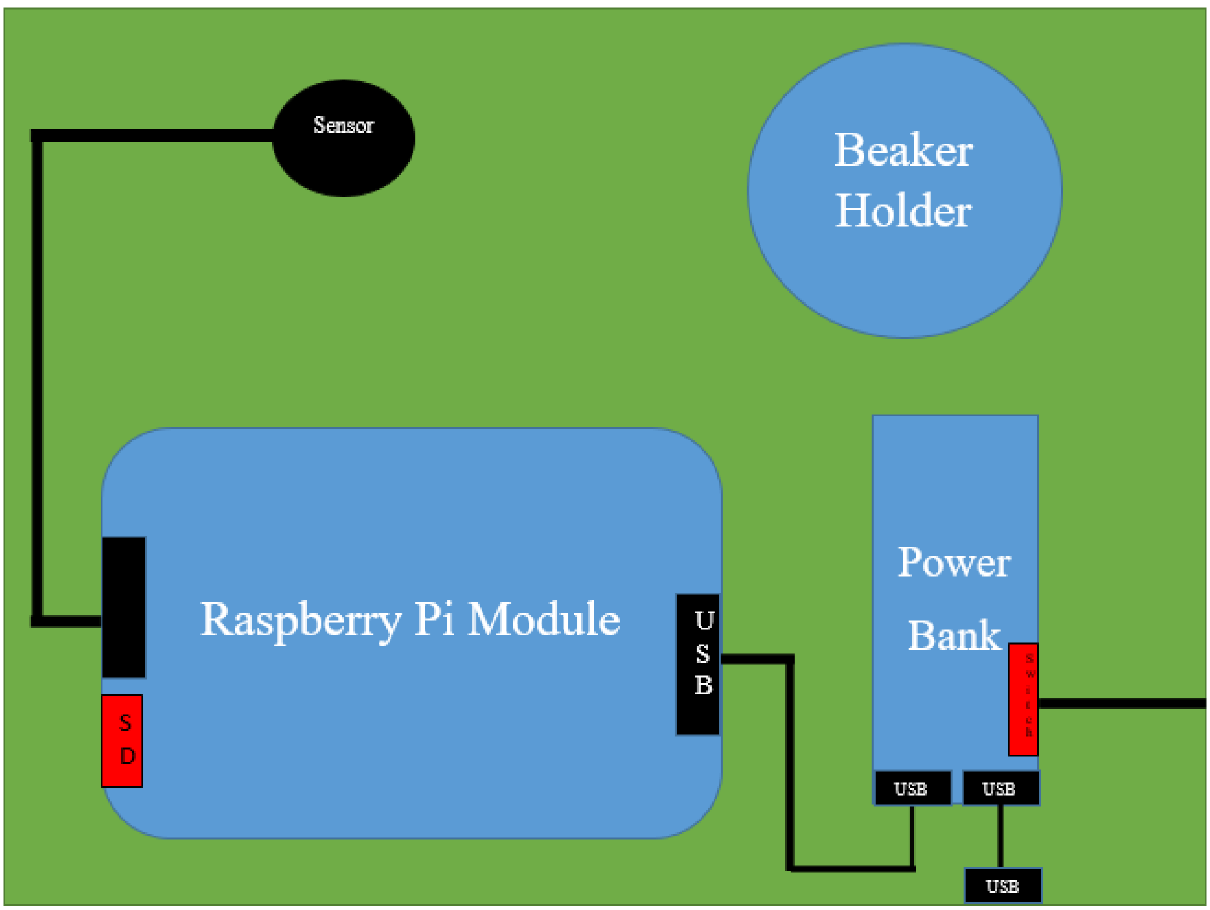

Figure 3.7 Circuit Diagram of the Device

In 3.7 the rough draft of what the circuit diagram of the device would appear as. The sensor would be attached to the R Pi module and it will also be powered by the module. The sensor will send collected data to the R Pi module so that module can then convert it accessible information that can be interacted with by the touchscreen monitor. The device will be powered by a power bank that can sustain all of its components. SD card will be provided along with the whole device.

Graphical User Interface

Figure 3.8 Boot Screen Graphical User Interface

Shown in the figure above is the boot screen user interface where a "Start" button is located. This would be the screen that would be first shown to the user, addition information such as a loading screen or bar, time, date, or micro SD card status can be included before showing the start button.

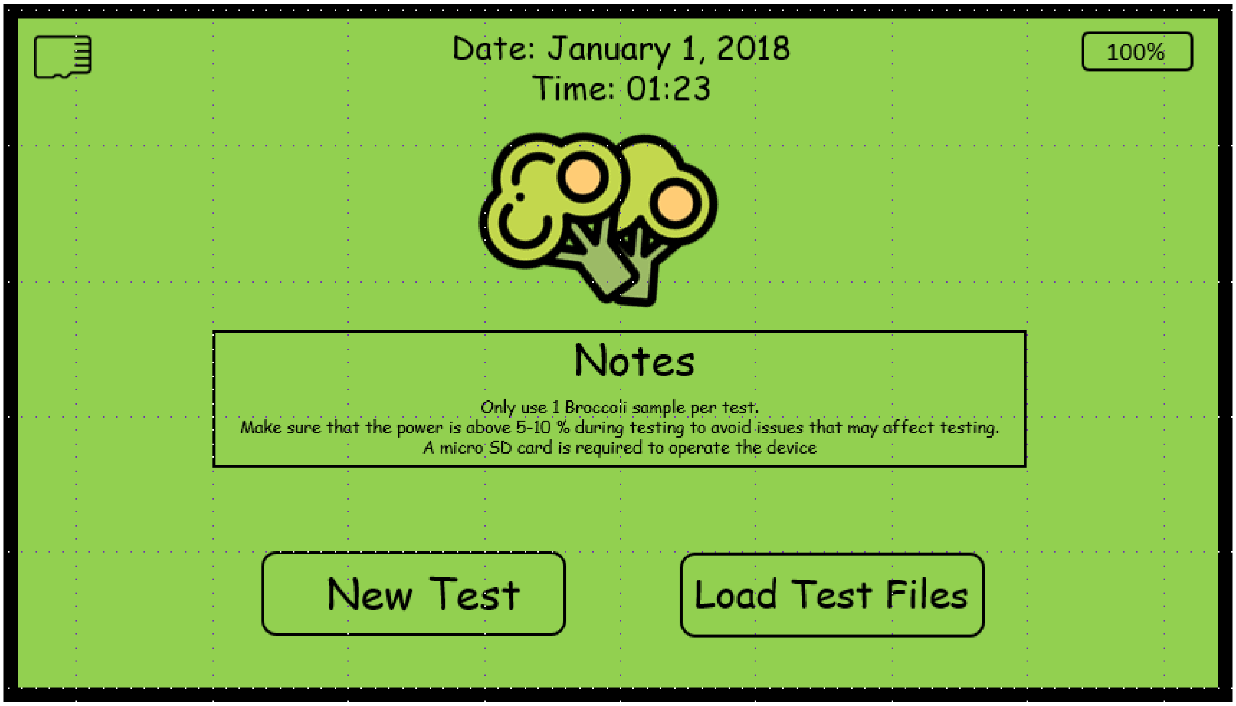

Figure 3.9 Main Menu Graphical User Interface

Shown above in figure 3.9 is the main menu interface for the device. Included here is a note section as well as two functions which are to initiate a new test or to load a record of the results of the previous tests or tests saved on the micro SD card of the device. Included in the note section are precautionary measures to properly operate the device such the number of sample per test, remaining power for optimum operation, as well as a requirement for a micro SD card as this card would serve to be a storage for new and previous tests.

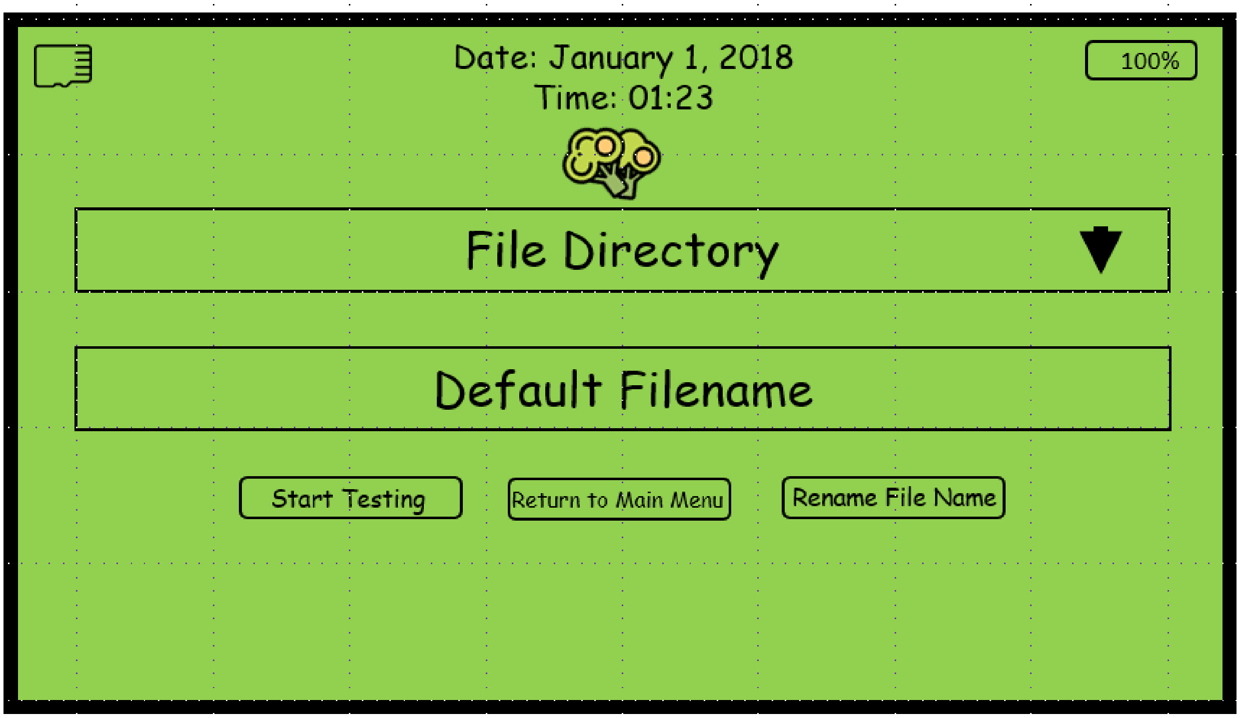

Figure 3.10 New Test Graphical User Interface

Here in figure 3.10, the interface for the new test function is shown. Here, the directory of the file to be created is first chosen should the user chose to save the test results. The filename for the file is also edited in this menu. The user can now initiate testing once the required information are finalized.

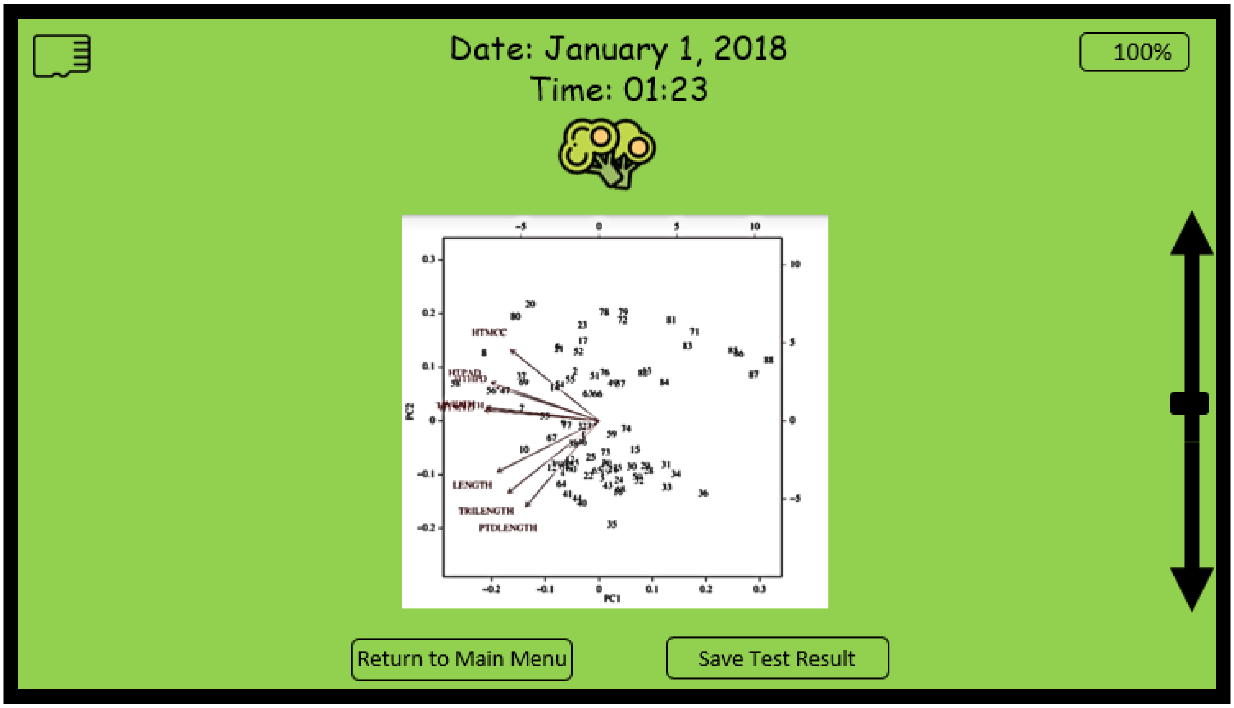

Figure 3.11 PCA Graphical User Interface

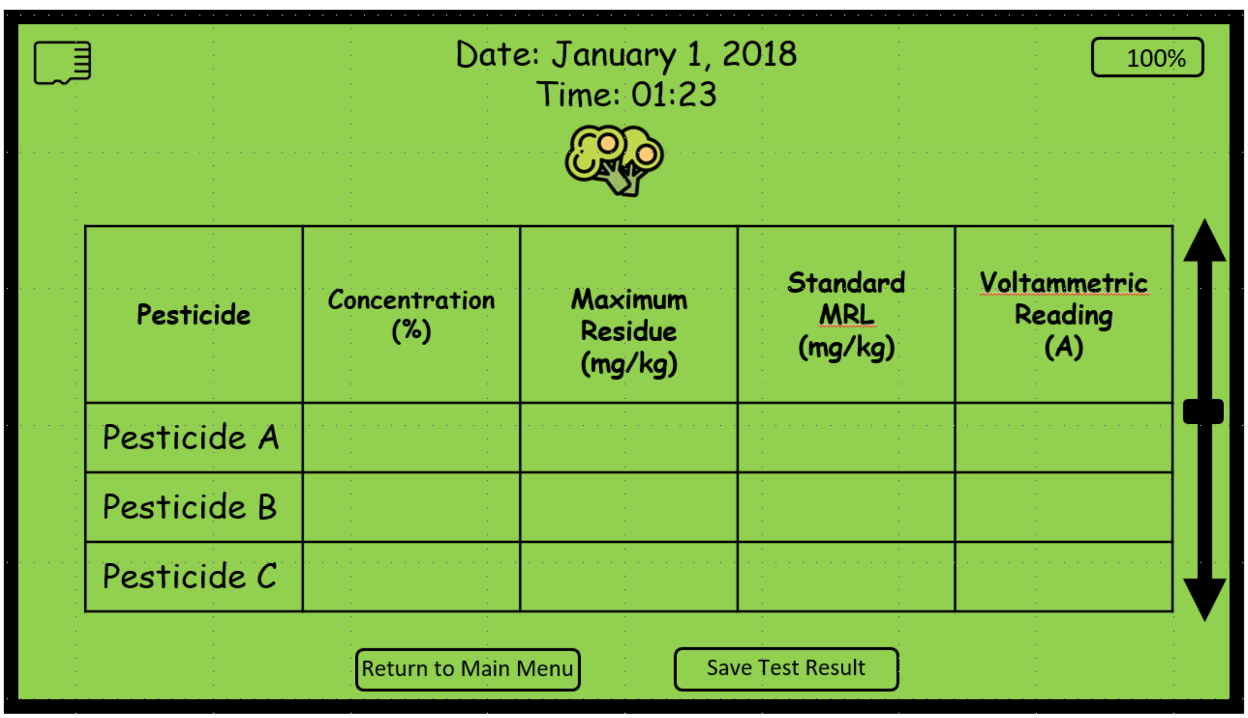

Figure 3.12 Output Graphical User Interface

The test results gained from testing is shown in here in figure 3.12. Parameters such as the type of pesticide, its concentration, its maximum residue, the standard maximum residue limit for comparison, and the voltammetric reading are shown here. The user may use the scroll bar to view more information.

3.3.2 Objective 2: To characterize chemical residue found in broccoli using principal component analysis

Procedures

Preparation before using the device

1. Gathering the samples from their respective suppliers, farmers, and plantations.
2. Each broccoli sample will be cut into half.
3. The first half will undergo standard laboratory testing for the measurement of pesticide chemical residue.
4. The second half will be blended until it reaches the liquefied state using a vegetable blender.

Device Calibration

5. A sample will be injected with known amount of pesticide at mg/kg.
6. The samples will proceed to the E- tongue sensors for testing.
7. The results of the E-tongue would be calibrated in order to be approximately as similar to the actual injected value of the pesticide with known mg/kg.

Device implementation

8. Injecting each sample of broccoli through screening process of voltammetric electronic tongue sensors. Then it will pass through the systems separation module to reduce noise information obtained by the sensors.

   It will then determine and generate an output data through the screen through algorithms of Learning Vector Quantity using the application of Principal Component Analysis. The theoretical basis algorithm for LVQ Neural Network starts by initializing the weight value and the learning rate using equation 1,

   $$d_i = \sqrt{\sum_{j=1}^{n}(X_j - w_{ij})^2}$$ Equation 3.1, as seen in [24, Eq. (5)]

   Equations are then used to calculate the distance between a neuron in the competing layer and a given input vector then followed up by

   $$w_{ij}' = w_{ij} + v(X_j - w_{ij})$$ Equation 3.2, as seen in [24, eq. (6)]

   $$w_{ij}' = w_{ij} + v\left(X_j - w_{ij}\right)$$ Equation 3.3, as seen in [24, eq. (7)]

   that overrun the adjustment the weights of the winning neurons. For PCA, the method basis starts by setting a sample matrix such as X=(X1,X2,... ,Xn) where the size of the samples is n and each of the samples has m characteristic indices which makes the matrix Xi=(Xi1,Xi2,...,Xin) , i=(1,2,3,...m). The correlation coefficient matrix of the sample matrix can then be computed using,

   $$\text{cor(X)} = \sum_{i=1}^{n}[X_i - E(x)] \cdot \left[X_j - E(x)\right]^T / \ (\, n = q \cdot q^t)$$ Equation 3.4, as seen in [24, eq. (1)].

   $$E(x) = \sum_{i=1}^{n} X_i / \sqrt{n}$$ Equation 3.5, as discussed in [24]

   will be the mean value of the sample matrix which is extracted in equation 3.4.

   $$q = \sqrt{\sum_{j=1}^{n}(X_i - E(x))^2/2}$$ Equation 3.6, as seen in [24]

Will then be the sample matrix of the standardized data acquired.

9. The device will show series of data results. The Raspberry Pi will display the list of the characterized chemical residue, the amount of residue in (mg/kg), and the percentage level of the present chemical residue.

Comparison of the Acquired Data from the Standard Maximum Residue Level (MRLs)

10. Laboratory results and data acquired using the E-tongue sensors will then be compared to the Standard Maximum Residue Level (MRLs) in order to determine if the broccoli sample will be considered safe or organically grown or whether the sample exceeds the accepted MRLs.
11. Statistical Testing will be made in order to compare data acquired from standard laboratory test and E-tongue sensors.

Calibration

A common problem when dealing with the use of voltammetric sensors is the incredibly large amount of dimensionality of its generated voltammetric data. This is the main reason why the application of voltammetric sensors are not used in research and literatures. This happens when a complete voltammogram is obtained from each sensor in the array. This becomes more prevalent when Artificial Neural Network is used because of the need to pre-process departure information. Signal pre-processing is not always necessary but it is highly recommended because it will overall improve model predictive behaviours of the systems. This stage is considered necessary because of how the sensor array containing complex voltammetric responses that contains information about the analyzed compounds throughout the whole voltammetric curve. A recommended solution for dealing with this problem is by adding a compression stage such as Principal Component Analysis technique in order to reduce the data and focus on the important voltammetric curve data before passing through the pre-processing method which is the Artificial Neural Network.

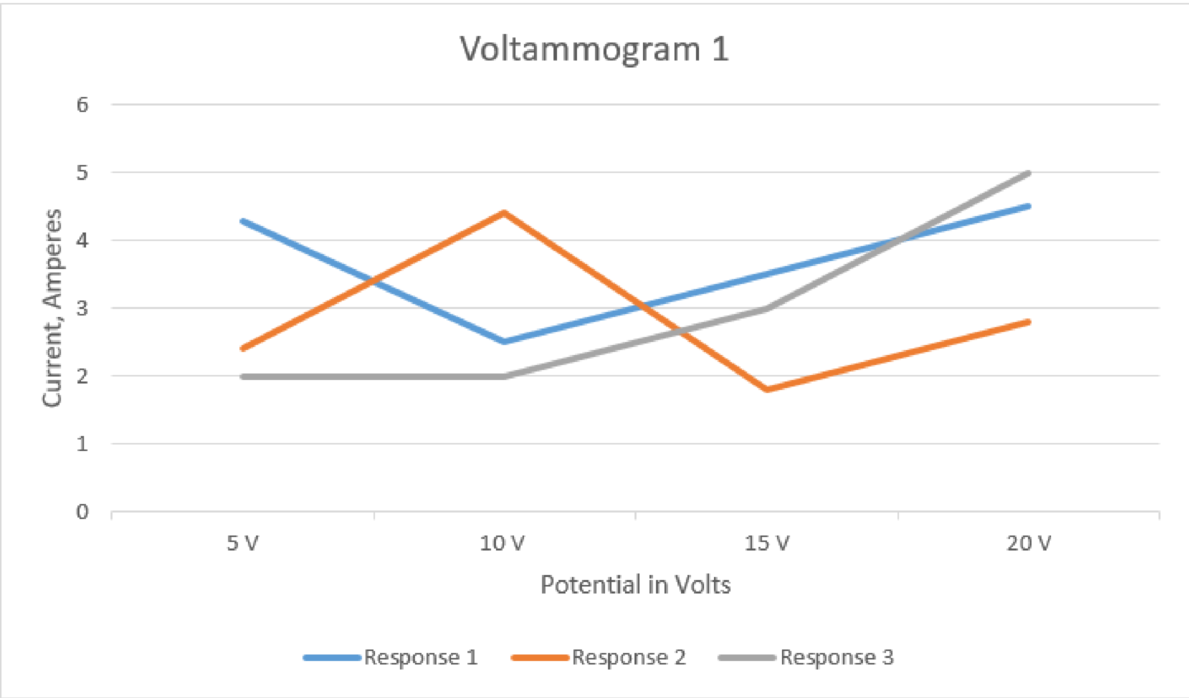

Fig 3.13 Sample Voltammogram data from Voltammetric Electronic Tongue sensors.

An expected resulting voltammogram data from one sample of broccoli that has been detected by the voltammetric electronic tongue sensors. After receiving a voltammogram from wave currents created by applying a potential through the working electrode and the counter electrode, a returning response counter voltammogram formed from the wave currents observed between the working electrode and the reference electrode would provide sets of data that would serve as calibrating variables that the system would compress by applying Principal Component Analysis before pre-process through Artificial Neural Network.

Table 3.2 Calibration basis table for Electronic Tongue

Pesticide Compound	Number of Calibration Trials, n	Injected Amount of Pesticide	Amount of Pesticide Obtained from Electronic Tongue	Remarks
Chlorpyrifos	1	ppm	ppm	
	2	ppm	ppm	
	…	ppm	ppm	
	n	ppm	ppm	
Paraoxon	1	ppm	ppm	
	2	ppm	ppm	
	…	ppm	ppm	
	n	ppm	ppm	
Malaoxon	1	ppm	ppm	
	2	ppm	ppm	
	…	ppm	ppm	
	n	ppm	ppm	

Cleaning Process of Electronic Sensors

Particles that accumulate when testing different molecules on the surface of the working electrode are commonly called as Drifts. The difference in research results from the same samples that are measured repeatedly using Voltammetric Electronic Tongues are attributed to slowly corrode the surface of the working electrode which would cause drifts of signal and eventually leading to loss of activity. Electrochemical cleaning processes are performed with different pulse voltammetry and cleaning solutions. The electrodes are immersed in 0.1 L-1KCl then scanned by CV with a scale of -1.0 up to 1.3 V on a scan rate of 0.1 V* sec-1 . Sometimes, electrodes are just cleaned using cleaning solutions such as 2 M of HNO3 and 10mM of H2O2 and distilled water to clean the electrodes after each measurement. After cleaning and drying, sometimes it is necessary to use mechanical polishing or to cut off the outermost layer of the electrode to regain the correct electrode surfaces. Filter paper, sand cloth and alumina paper are usually the common types of materials used to polish the electrodes then afterwards are rinsed with distilled water.

Algorithm Development

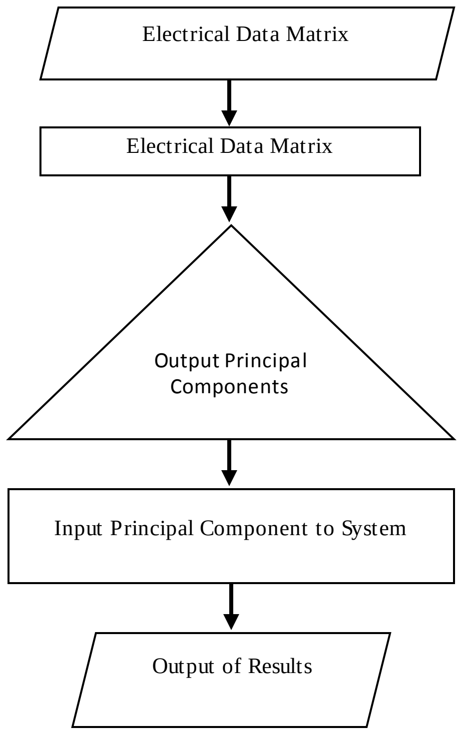

Figure 3.14 Principal Component Analysis (PCA) Flow Chart

The steps of PCA feature extraction method have several sections: first, there are electrical data matrix X as input. Second, through the data dimension reduction, then output the principal components. Third, entering the principal components into online system, then output the result.

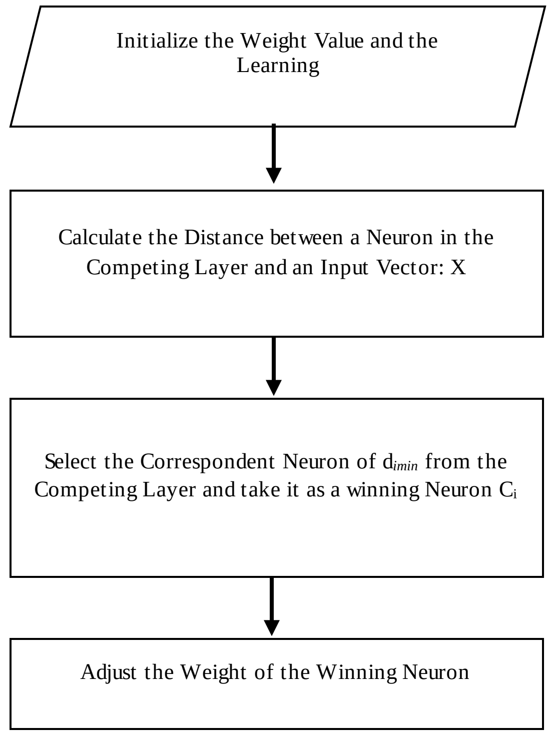

Figure 3.15 Linear Vector Quantity (LVQ) Flowchart

An LVQ neural network is a three-layered supervised neural network. It consists of an input layer, a competing layer and an output layer. The competing layer classifies samples by calculating the distance between the input sample vectors. Its competitive layer demands less hidden layer neurons as well as less computation time. Besides, an LVQ network can eliminate the explicit need to tune multiple parameters while training. The classification results are transmitted to the output layer where each neuron represents one class. Initialize the weight value and the learning rate: wij is the weight value between the jth neuron of the input layer and the ith neuron of the output layer. v is the learning rate. The first step is to initialize wij and v. Calculate the distance between a neuron in the competing layer and an input vector: X stands for a vector of the input samples. di is the distance between a neuron in the competing layer and an input vector. Put X into the input layer, then calculate di. Select the correspondent neuron of dimin from the competing layer and take it as the winning neuron Ci. Adjust the weights of the winning neurons: Adjust them in accordance with the correctness of the classification results.

If Ci=Cx, adjust the weights.

Software Development

MATLAB application will be utilized in the study to be able to acquire values for

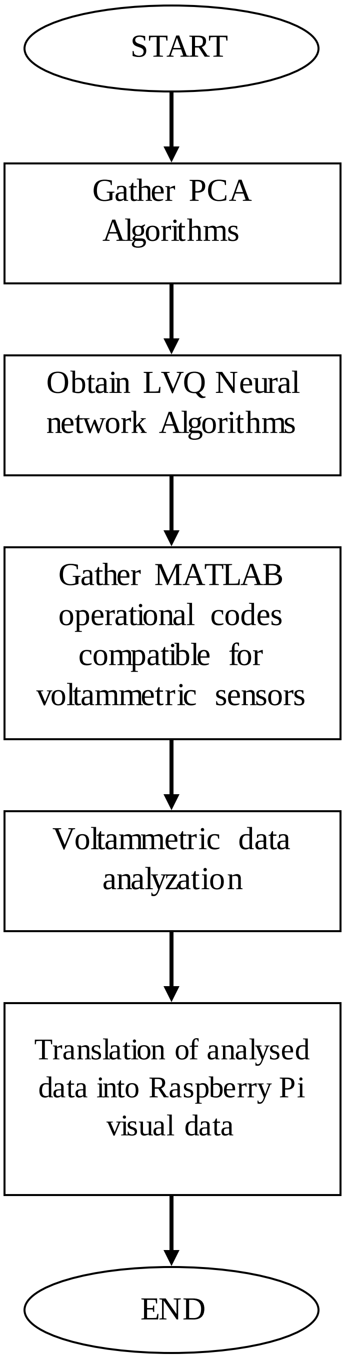

Figure 3.16 Software System Flowchart

Shown in Fig. 3.14 is the software's flowchart where algorithms that are required for the operation of the different fundamental components are prepared for the process. Once the algorithms are compiled and prepared, it is then inserted into the system data handling software and analysis tools which are Principal Component Analysis with Learning Vector Quantity methods. After handling with the software, the datasets obtained from the voltammetric electronic tongue sensors will then pass through the data analyzing tools. The electrochemical voltammetric data will be compiled and reformed into a more simple readable information that can be viewed by the Raspberry Pi visual components. The converted information will then be shown on an interactive graphical user interface by the Raspberry Pi software and components that the researcher or individual can interact with.

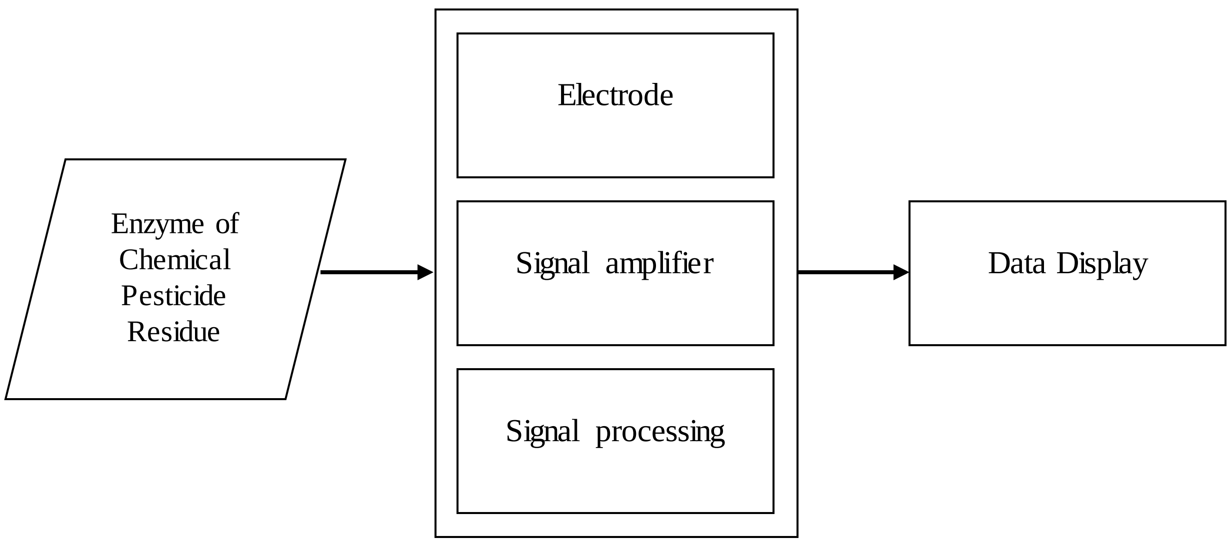

Figure 3.17 Process flow diagram of data processing

Enzyme of pesticide chemical residue will have contact with the electrode of the electronic tongue sensor. The electrochemical sensor that will be utilized in this study is a voltammetric sensor. Signals will pass through the circuit and the software based on MATLAB application. The signals will be process in order to characterize given samples of enzyme. Values and results will be displayed on the touchscreen LCD display for configurations.

Code fragment needed to drive Raspberry Pi

In order to make our Raspberry Pi component piece display the information about the pesticide residues that have been detected. It is then necessary to design it with computer programming languages such as:

```
def ADin():
GPIO.output(AD_Clk,GPIO.LOW)#  set AD_Clk to 0
AD_res=0
for n in range(10):
time.sleep(0.05)
GPIO.output(AD_CS,GPIO.HIGH)#  set AD_CS to 1
MSB=128
time.sleep(0.001)
GPIO.output(AD_CS,GPIO.LOW)#  set AD_CS to 0
time.sleep(0.0005)
AD_value=0
for z in range(8):
if (GPIO.input(AD_Dat)):
AD_value=AD_value+MSB
GPIO.output(AD_Clk,GPIO.HIGH)#  set AD_Clk to 1
time.sleep(0.0005)
GPIO.output(AD_Clk,GPIO.LOW)#  set AD_Clk to 0
MSB=MSB>>1
time.sleep(0.0005)
result=AD_value
```

```
GPIO.output(AD_CS,GPIO.HIGH)#  set AD_CS to 1
AD_res=AD_res+AD_value
AD_res=AD_res/10
result=AD_res
return result
```

This will serve as the working code to make the raspberry pi component work with the voltammeter in incorporating the data sets into the graphical user interface so that the user of the device can read the information and interact with it.

3.3.3 Objective 3: To conduct test and validations of results in comparison with the samples that undergo standard laboratory testing.

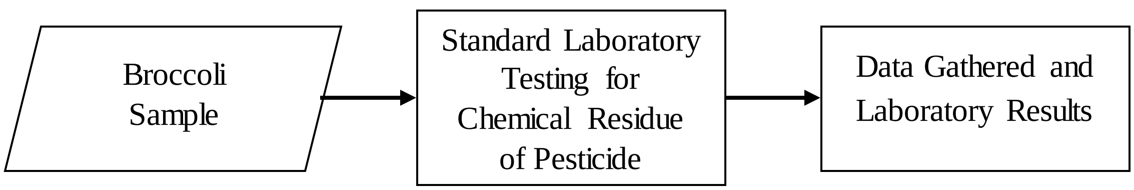

Figure 3.18 Process flow diagram of Standard Laboratory testing

The half of the sample broccoli will undergo laboratory testing of Gas Chromatography with Mass Spectrometry (GC-MS) to be able to identify pesticide present in the broccoli samples.

3.4 Data Gathering

3.4.1 Pesticide Basis Table

Standard measurement for Maximum Residue Level (MRLs) were provided by the EU Commission's Consumers Health, Agriculture, and Food Executive Company. They are an organization that handles the approval of pesticides and the information provided by researchers. The European Food and Safety Administration (EFSA) does the verification of a residue to be considered safe for all European consumer groups, including groups such as babies, children and vegetarians. When a risk is considered for any consumer group, the MRL application is rejected and the pesticide may not be used on that crop. National Authorities are the ones that handle how and when the pesticide could be used. EU Commission has a Pesticide residue library that is accessible to anyone through their website in the internet.

Maximum Residue Level (MRL) acquired from EU Commission:

Table 3.3 Standard MRL from EU Commission

Pesticide compounds	**Standard Maximum Residue Limits in mg/kg**
Chlorpyrifos **C9H11Cl3NO3PS**	0.05 mg/kg
Paraoxon **C10H14NO6P**	0.01 mg/kg
Malaoxon **C10H19O7PS**	0.02 mg/kg

3.4.2 Testing Table

Table 3.4 Training Samples, Pesticide Determination

Training Samples, n	Sensors			REMARKS Chlorpyrifos: $C_9H_{11}Cl_3NO_3PS$ Paraoxon: $C_{10}H_{14}NO_6P$ Malaoxon: $C_{10}H_{19}O_7PS$
	pH Sensor	Phosphorous Sensor (P)	Chlorine Sensor (Cl)	
1				
2				
3				
4				
5				
…				
n				

Table 3.5 Electronic Tongue Testing Table

Test Parameters	Number of Test, n = 10	Training samples		Testing Samples	
		Concentration in mg/kg	Percent Composition found in broccoli sample	Concentration in mg/kg	Percent Composition found in broccoli sample
Chlorpyrifos	1				

C9H11Cl3NO3PS	**2**				
	…				
	10				
Paraoxon **C10H14NO6P**	**1**				
	2				
	…				
	10				
Malaoxon **C10H14NO6P**	**1**				
	2				
	…				
	10				

3.4.3 Parameters to be tested

Table 3.6 Chlorpyrifos (C9H11Cl3NO3PS)

Test Sample, n = 25 broccoli

Broccoli sample ID	pH	Concentration in mg/kg	% Composition found in broccoli sample	Maximum residue level mg/kg
1		mg/kg	%	mg/kg
2		mg/kg	%	mg/kg

...		mg/kg	%	mg/kg
n		mg/kg	%	mg/kg

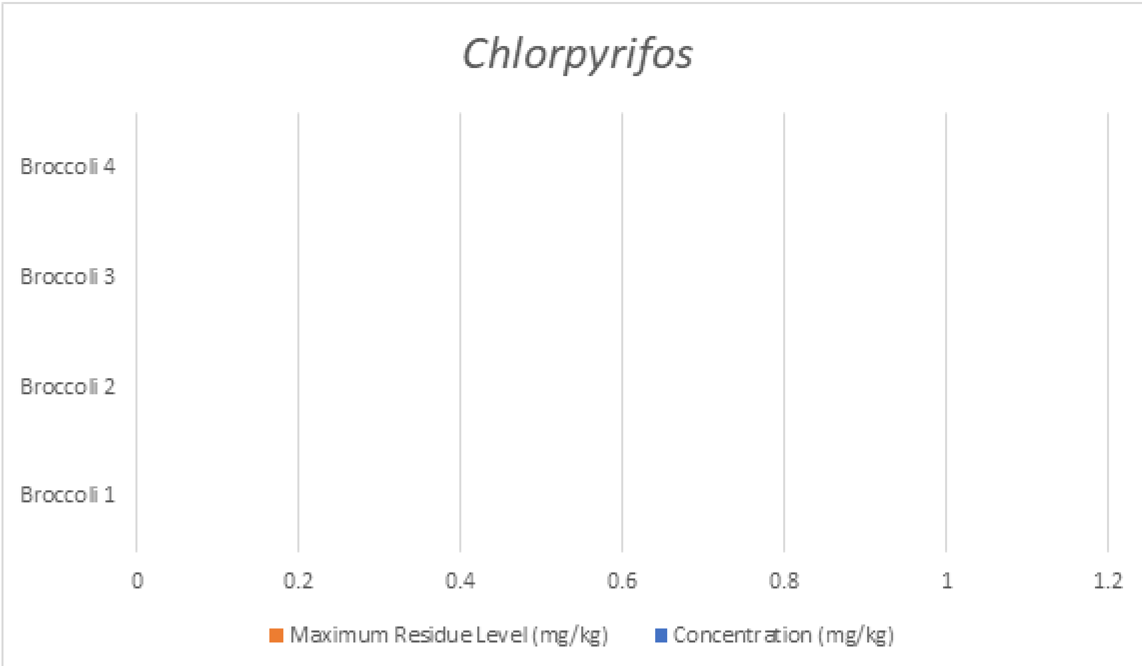

Figure 3.19a Chlorpyrifos Maximum Residue Levels and Concentrations

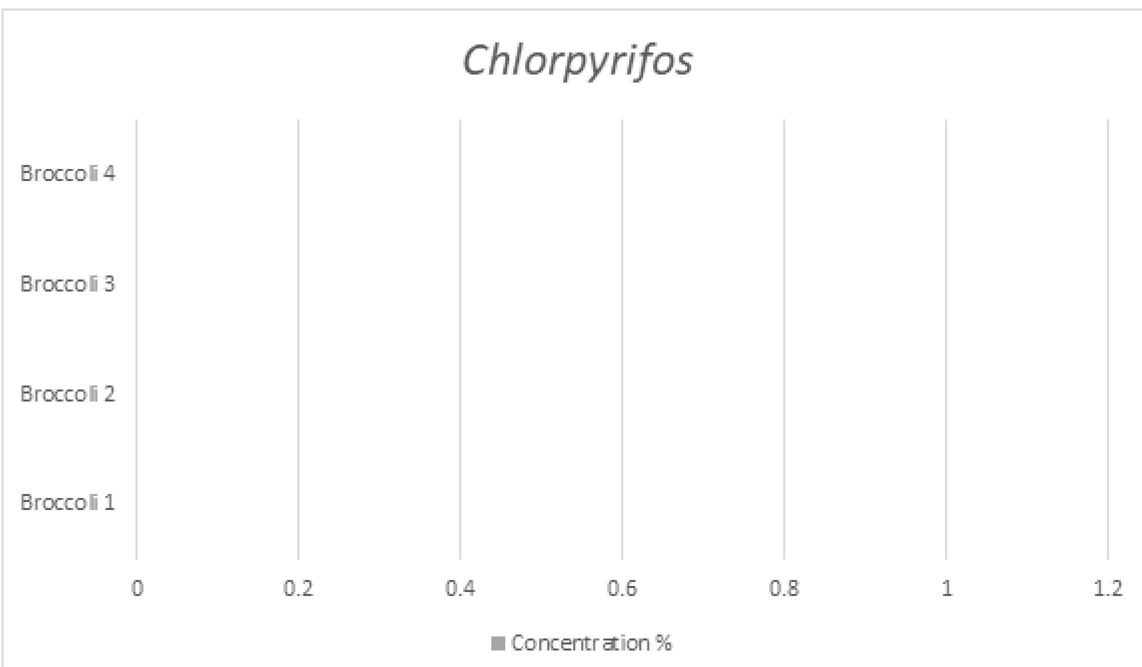

Figure 3.19b Chlorpyrifos Percent Concentrations

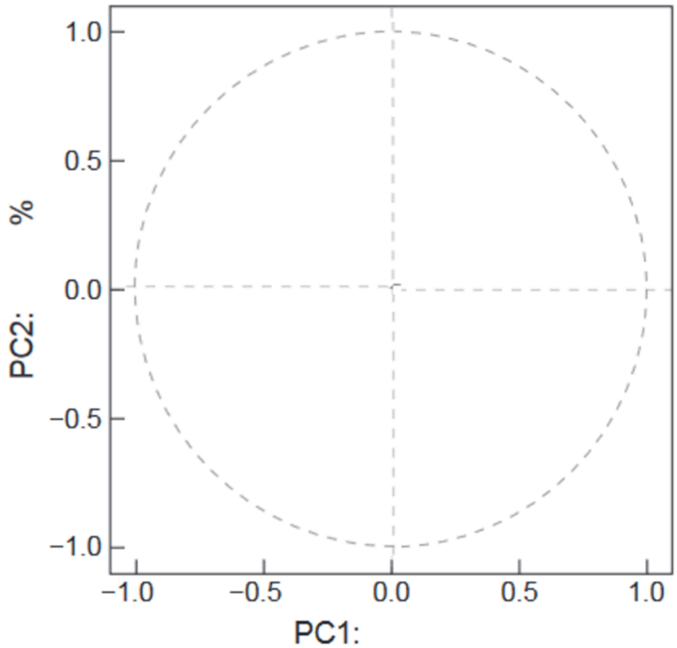

Figure 3.19c Principal Component Analysis for Chlorpyrifos

Table 3.7 Paraoxon (C10H14NO6P)

Test Sample, n = 25 broccoli

Broccoli sample ID	**pH**	**Concentration in mg/kg**	**% Composition found in broccoli sample**	**Maximum residue level mg/kg**
1		mg/kg	%	mg/kg
2		mg/kg	%	mg/kg
...		mg/kg	%	mg/kg
n		mg/kg	%	mg/kg

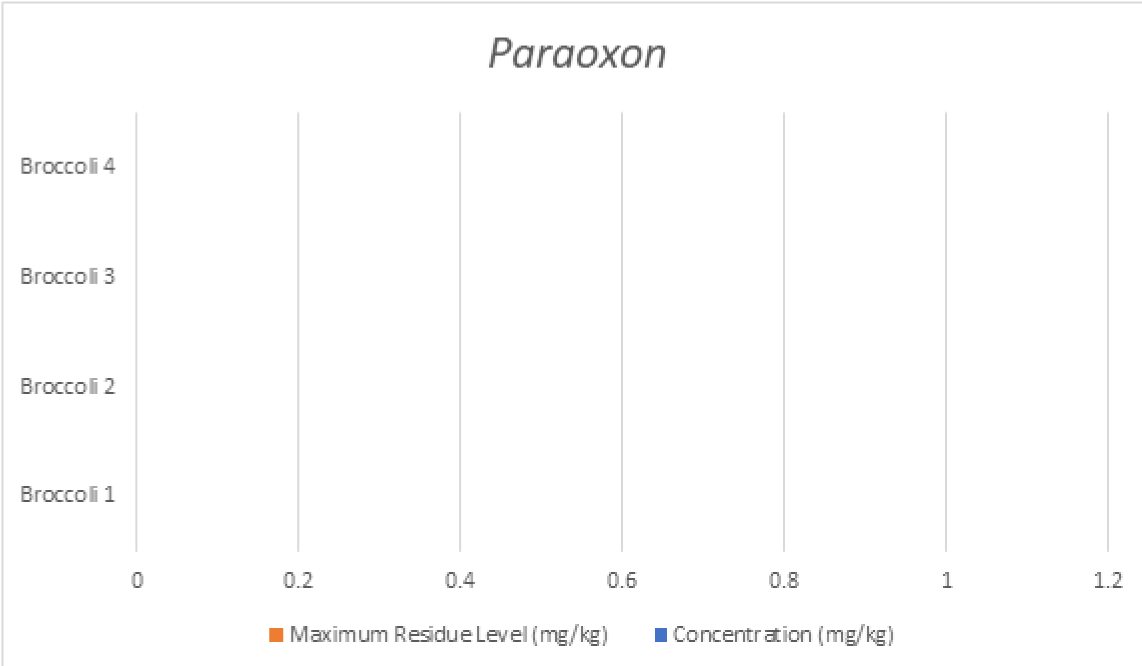

Figure 3.20a Paraoxon Maximum Residue Levels and Concentrations

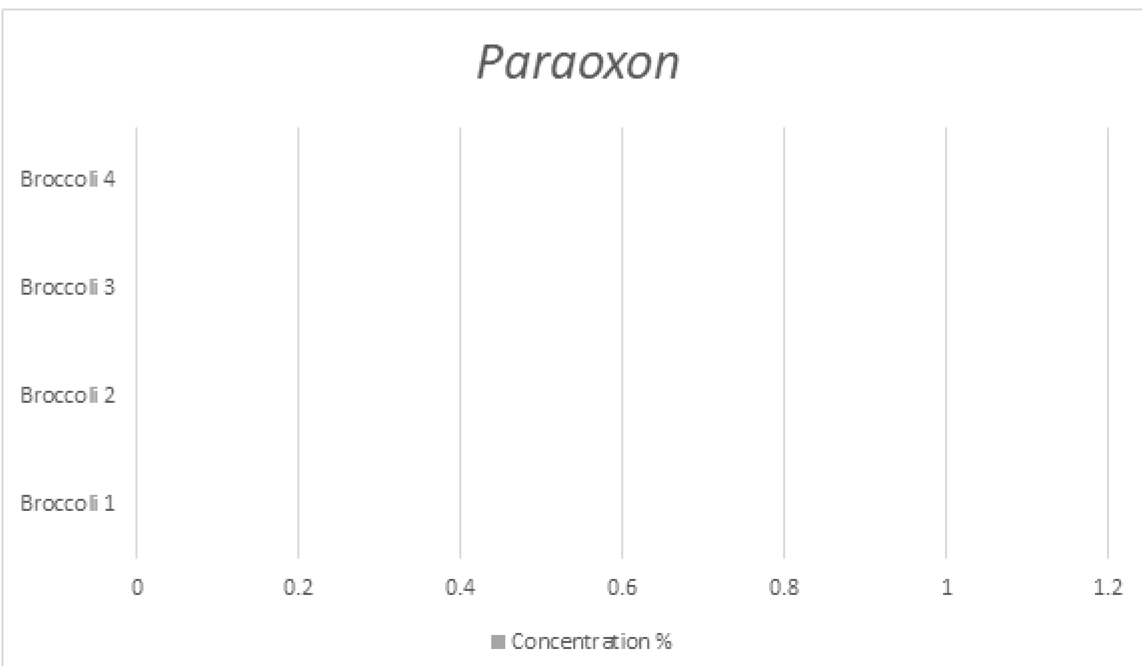

Figure 3.20b Paraoxon Percent Concentrations

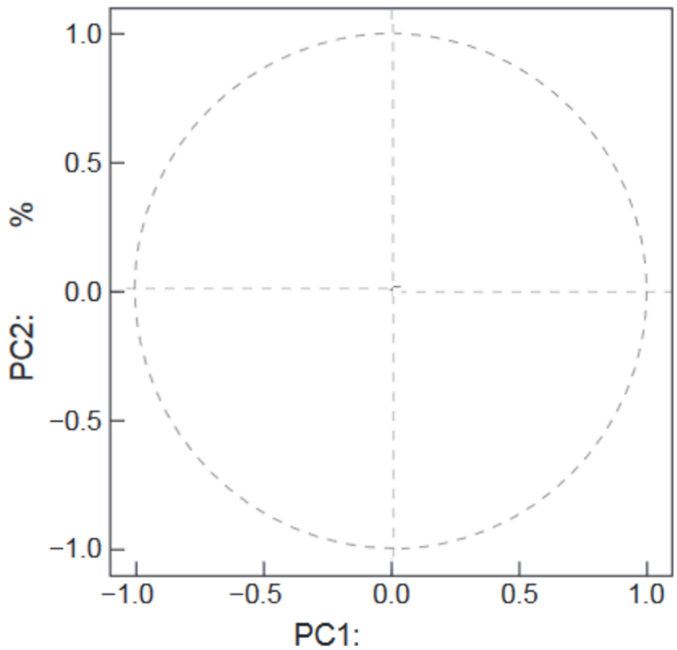

Figure 3.20c Principal Component Analysis for Paraoxon

Table 3.8 Malaoxon (C10H14NO6P)

Test Sample, n = 25 broccoli

Broccoli sample ID	**pH**	**Concentration in mg/kg**	**% Composition found in broccoli sample**	**Maximum residue level mg/kg**
1		mg/kg	%	mg/kg
2		mg/kg	%	mg/kg
...		mg/kg	%	mg/kg
n		mg/kg	%	mg/kg

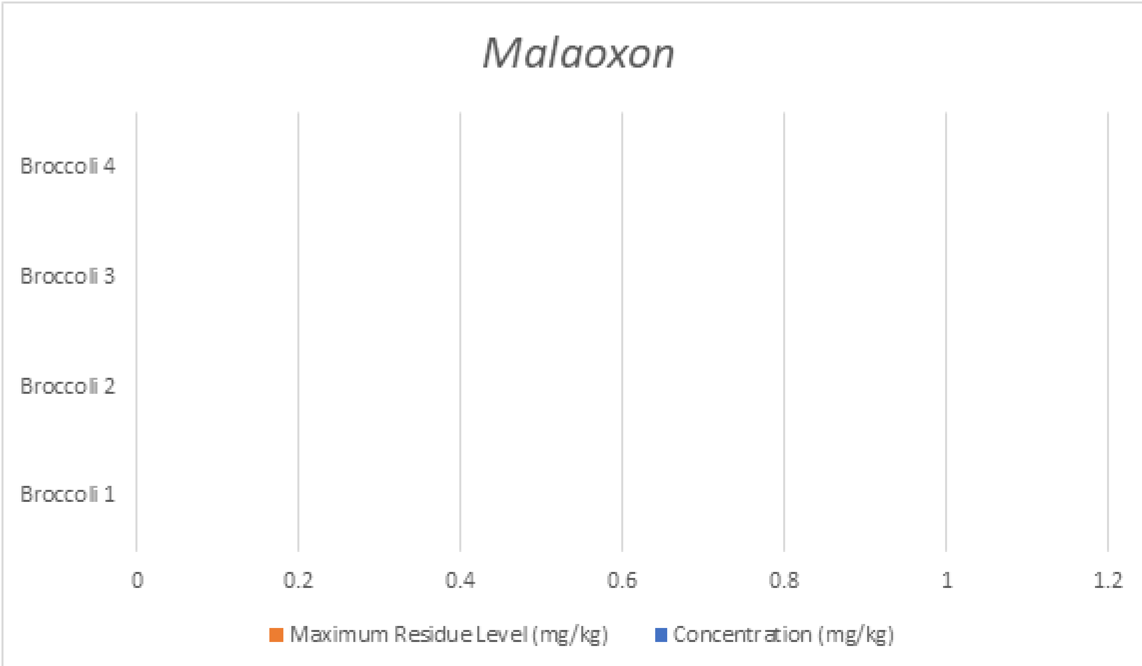

Figure 3.21a Malaoxon Maximum Residue Levels and Concentrations

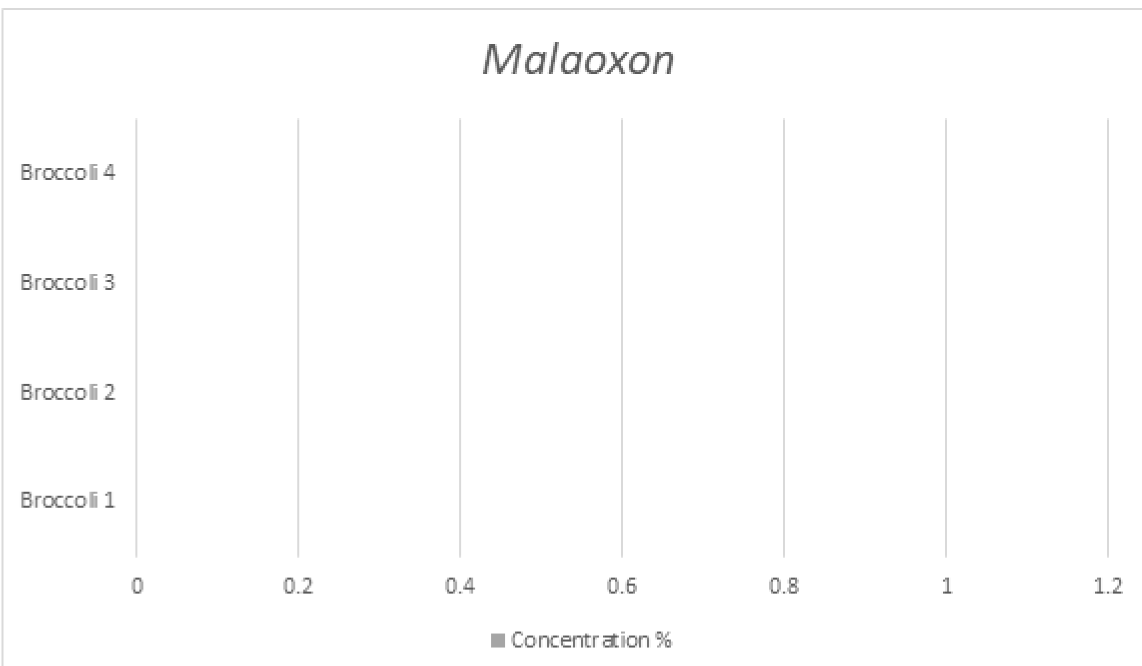

Figure 3.21b Malaoxon Percent Concentration

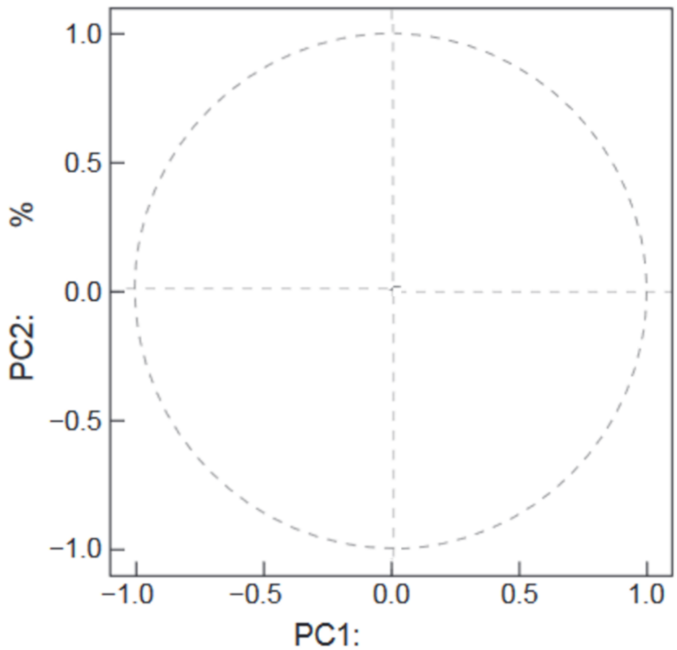

Figure 3.21c Principal Component Analysis for Malaoxon

Output Conclusion Table

Table 3.9 Conclusion Table

Sample size: 50 Broccoli

Pesticide Compound	**Standard Maximum Residue Limit in mg/kg**	**Number of Broccoli with Chemical Pesticide Compound**	**Number of Broccoli Exceeding the Maximum Residue Limit**
Chlorpyrifos C9H11Cl3NO3PS	0.05 mg/kg		
Paraoxon C10H14NO6P	0.01 mg/kg		

Malaoxon C10H14NO6P	0.02 mg/kg		

Device Output Graph

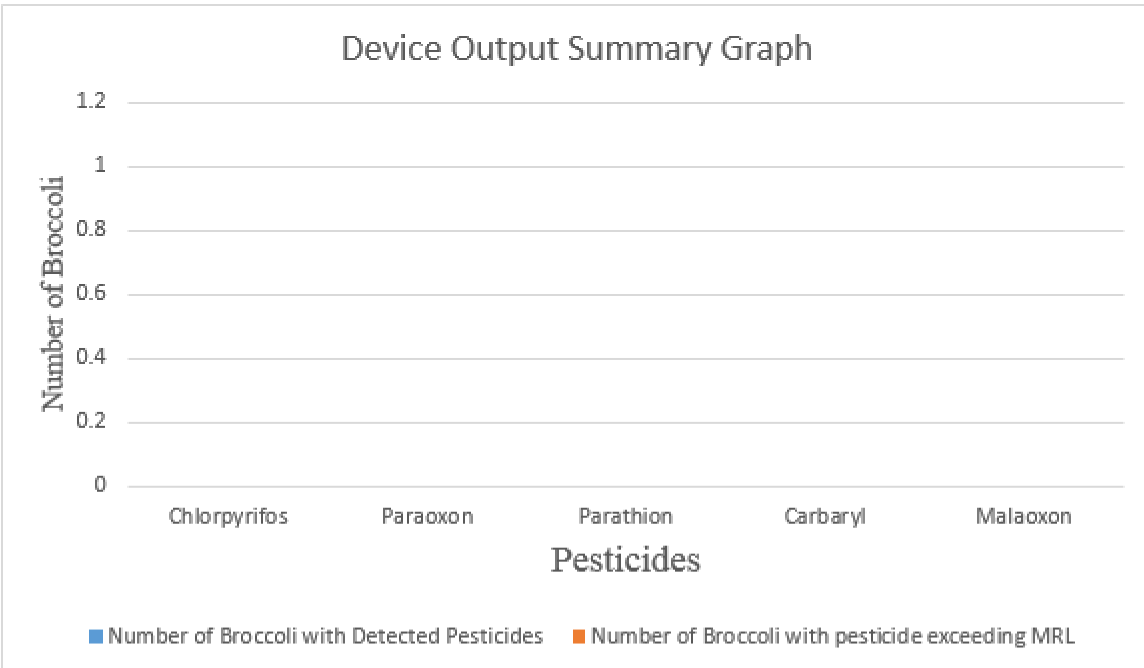

Fig 3.22 Expected output bar graph

3.5 Statistical Analysis

Table 3.10 T-Test Table

Broccoli Datasets	Standard Deviation	Mean	Total Number of Values
Laboratory Testing			
E-Tongue Based Sensor			
T-test Results			

Table 3.11 Statistical Table

Number of tests n = 20			
Parameters	Standard MRL	Statistical Tests	
		Laboratory Test	E-Tongue Test
Chlorpyrifos $C9H11Cl3NO3PS$			
Paraoxon $C10H14NO6P$			
Malaoxon $C10H14NO6P$			

$$\boldsymbol{t} = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{(s_1)^2}{n_1} + \frac{(s_2)^2}{n_2}}}$$ *Equation 3.7 T-Testing*

$\overline{x_1}$ = Mean of first set of values

$\overline{x_2}$ = Mean of second set of values

s_1 = Standard deviation of first set values

s_2 = Standard deviation of second set values

n_1 = Total number of values in the first set

n_2 = Total number of values in

$$S = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$$ *Equation 3.8 Standard Deviation*

S = Standard Deviation

$\bar{x}$ = Mean Value

n = Total number of values

In the statistical testing, T-test will be utilized for comparing two different set of values. T-test will be applied to a normal distribution for which contains small set of value samples. Set of data and results from the conventional standard laboratory testing that will focus on GC-MS will be compared to the data acquired from the E-tongue sensors with application of PCA Equation 3.7 Equation 3.8, shows the formula for the mean and standard deviation of the two data samples to be compared. T-test compares mean of two specimen samples.

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