Determination and Analysis of Cyanamide Residue in Lettuce Using Electronic Tongue and Principal Component Analysis

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***Abstract*— Adulteration in Lettuce is a common edible plant that is used as a side dish and sometimes used as an add-on to food like burgers, sandwiches and etc. Due to high demand, in order to cultivate lettuce easier, Calcium Cyanamide is often used a fertilizer. Since lettuce is often eaten raw, Calcium Cyanamide in the lettuce may still be present when eaten. Health risks include facial and upper body flushing, accompanied by nausea, vomiting, headache, fatigue, vertigo, dyspnea, rapid breathing and shivering. The present work allows detection of Calcium Cyanamide residue to lettuce using an Electronic Tongue and Principal Component Analysis (PCA). The Electronic Tongue is an array of non-specific, poorly selective sensors that determines compounds in a liquid and generates signals that is sent for data processing. With the help of the Electronic Tongue, we are able to determine if there are traces of Calcium Cyanamide residue.**

Keywords*— Lettuce, Electronic Tongue, Principal Component Analysis, Calcium Cyanamide

I. INTRODUCTION

Lettuce is a common edible plant that is used as a side dish and sometimes used as an add-on to food like burgers, sandwiches and etc. Although lettuce has its good effects on the body, people are forced to cultivate it due to high demand and several methods have been found in order to cultivate it easier. In modern times, they use Cyanamide, a chemical used as a fertilizer to preserve the lettuce and protects the lettuce from unwanted factors. Although there are certain advantages of Cyanamide to lettuce preservation, there is a health risk for the humans who happen to often eat lettuce since they eat the lettuce raw therefore cyanamide could not be fully get rid of. If a human is exposed to Cyanamide, side effects may occur. Side effects of Cyanamide include frequent abdominal symptoms, allergic skin reactions, dizziness, nausea, sleepiness, headache, eye symptoms and a desire to urinate as stated from the Quarterly Journal of Studies on Alcohol by Marconi.

In the Study of Ullmanns Encyklopädie der technischen Chemie by Barbara Elvers that was originally published on 1914, cyanamide can also cause local lesions on the skin and exposed mucosa, Eczema can also develop with the other following skin diseases. Systemic effects of the chemical were found which includes different malfunctions of the body such as anorexia, vomiting, shortness of breath, dyspnea, swelling and many more are some examples stated from Munchener Medizinische Wochenschrift by Gartner. Cyanamide is commonly used in agriculture and has modest toxicity in humans. According to a new study, Determination of cyanamide residue in 21 plant-derived foods by liquid chromatography-tandem mass spectrometry by Cheng Cheng, et al., a new method for detecting cyanamide was derived. The method is simple, sensitive, and widely applicable. It also provides good accuracy and precision. In this method, the samples were homogenized and extracted, it employs clean-up with multi-walled carbon nanotubes (MWCNTs) and derivatization with dansyl chloride. According to the study of Syunataro Hiradate, Tsunashi Kamo, Eri Nakajima, Kenji Kato, and Yoshiharu Fujii titled Direct quantitative determination of cyanamide by stable isotope dilution gas chromatography-mass spectrometry, the method was using gas chromatography-mass spectrometry (GS-MS) equipped with a capillary column for amines was used for quantitative determination of cyanamide.

All the previously mentioned studies use chromatography in detecting cyanamide, but none has ever used electronic tongue in detecting cyanamide residue specifically in a lettuce plant. With the convenient use of electronics, detection is possible with the use of sensors. Lettuce is a common food to eat and has possible residues of cyanamide, therefore Cyanamide detection is possible using an electronic tongue. The electronic tongue uses sensors to compare the chemical content included in the lettuce and then notifies when there is available cyanamide residue in the lettuce itself. It is needed to research more about how the chemical content of Cyanamide that has resided on lettuce so that people could be notified of what they are eating if it is safe or not.

The main objective of the study is to determine and analyze the cyanamide residue in lettuce using Electronic Tongue. Specifically, (1) to develop an improvised electronic tongue that is able to determine cyanamide content in lettuce; (2) to develop a program that is able to detect the cyanamide content of the lettuce using Principal Component Analysis and obtain its output; and (3) to create a statistical analysis involving the content of cyanamide in lettuce from different local supermarkets.

The significance of the research is it will give us information to determine cyanamide residue left on lettuce which is commonly eaten especially by most vegetarians and health conscious individuals today. Since it is known that cyanamide could cause adhesive effects to the human body at a certain amount, it is then the significance of this study to make sure that the people that are consuming lettuce are not

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able to intake a high amount of cyanamide residue left on the lettuce that might cause adhesive effects to the human body. Also, the study focuses on how to approach this agricultural problem with the use of electronics which is widely used in the industry today therefore it integrates the idea of finding cyanamide residue in a modern-day fashion.

The study is limited only to determining of cyanamide residue on lettuce. Using only electronic tongue and Principal Component Analysis. The study does not cover the cultivation of lettuce that is to be used in the said study. The type of lettuce to be used is Iceberg lettuce that can be bought in local markets here in the Philippines, specifically local markets or supermarkets in Manila. The electronic tongue to be used is improvised and produced by the group working in this study. The study does not cover the research on the adhesive effects of cyanamide to a human body and is only limited to detecting cyanamide residue in lettuce.

II. METHODODLOGY

A. *System Flowchart*

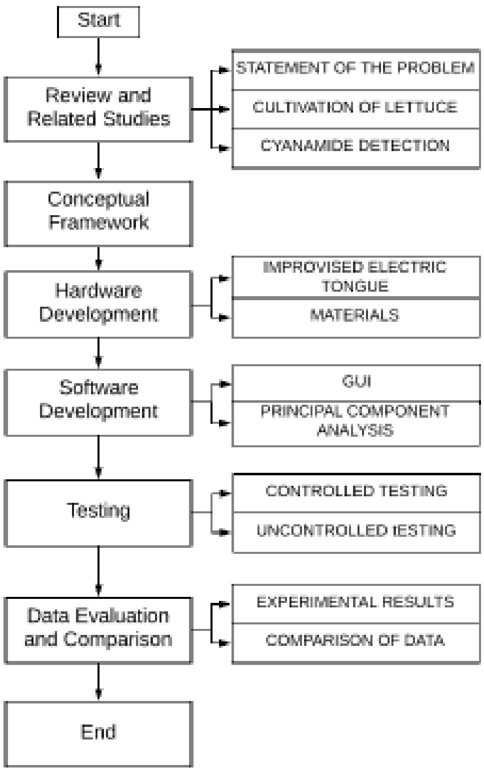

Fig. 1. Process of Methodology

In Figure 1, the process of methodology shows a series of steps to be followed with subcategories to be considered. The process of Methodology includes the Review of Related Studies, Conceptual Framework and the phases where the objectives are to be followed with subcategories to be considered.

B. *Conceptual Framework*

The conceptual framework of the study (Figure 2) shows the process of the hardware and the software of the how would the operation be able to detect Cyanamide residue in lettuce as its output. As Cyanamide is a compound that is soluble with water, water could be used as a reagent for the sensors to be able to read the chemical compounds, the data obtained from the solution is transferred first to a high impedance buffer before processed. The measured data from the sensors will then be converted to a digital signal and a GUI will be used in order to compare the values obtained from the solution. The output should show signs of Cyanamide residue that is present or not in lettuce.

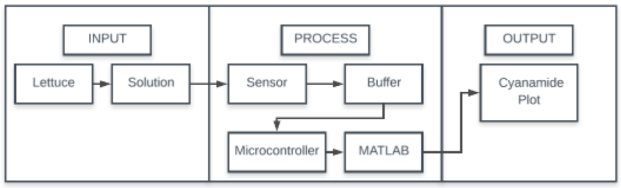

Fig. 2. Conceptual Framework

C. *Hardware Development*

1) *Improvised Electronic Tongue*

An electronic tongue is a device that identifies the specific components of a liquid substance and compares the given data to the data recorded in the electronic tongue itself. The hardware mechanism consists of an array of sensors much like the e-nose, but it uses an ISE (Ion-Selective Electrode) as its sensor.

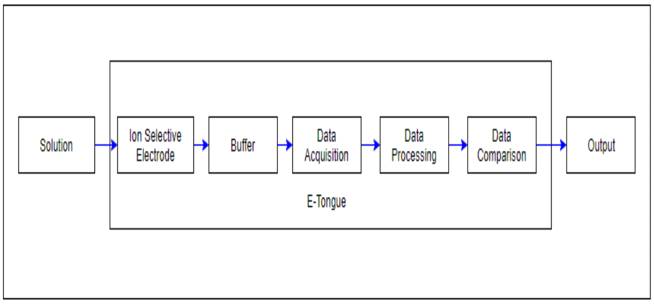

Fig. 3. Block Diagram of the Improved Electronic Tongue

Figure 3 shows the process of how the Electronic Tongue works. The use of Ion sensors (ISE) is essential as it is the main component of the Improvised Electronic Tongue. Electrodes are dipped into the solution in order to obtain the measurable voltage level. A certain reaction occurs from the sensors when the corresponding ions from the element are met 1by the sensor and a potential voltage is produced given there is a reference electrode to complete the flow in the circuit.

2) *Controlled Testing*

Table 1 shows the data from the sensors; it has total of 100 samples of getting the data to ensure the graph of PCA to be accurate. As we can see the nitrogen samples shows a high amount, since we put 1/4 tablespoon of Calcium Nitrate, a type of fertilizer that has the main component of nitrogen which is the component that is abundant, and which is made of by cyanamide.

TABLE I. SAMPLE DATA OF FIRST CONTROLLED SAMPLE WITH ¼ TABLESPOON OF CYANAMIDE

Nitrogen (mV)	Hydrogen (pH)	Temperature (°C)
644.53	10	28.12
751.95	9.79	28.12
634.77	10.82	28.12
551.76	9.95	28.12
527.34	9.78	28.12

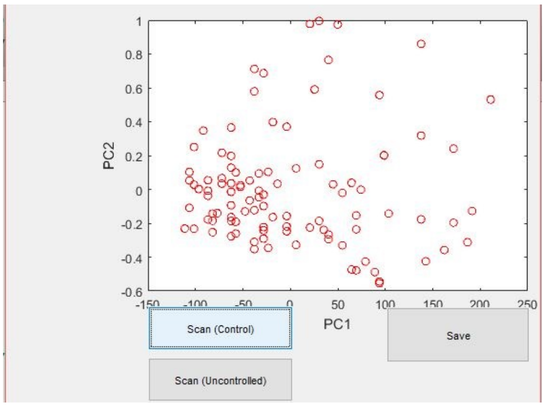

Fig. 4. —¼ Tablespoon - PCA Graph of the First Controlled Sample

This graph shows the PCA graph of the data in Table 1 above, if you notice the concentration of the cyanamide at the left side of the graph. It indicated the strong traces of cyanamide. And the equation of this PCA plot is

$$y = -6.4x10^{-16}X - 1.3x10^{-19} \tag{1}$$

3) Uncontrolled Testing

1st Market Uncontrolled Samples

TABLE II. DATA OF FOURTH UNCONTROLLED SAMPLE FROM MARKET 1.

Nitrogen (mV)	Hydrogen (pH)	Temperature (°C)
230.30	7.35	29.09
217.33	6.80	29.09
202.14	7.25	29.09
305.88	7.58	29.09
249.55	6.83	29.09

Table 2 holds the data from the fourth sample of uncontrolled testing. Just like the previous ones it also has minimum amount of nitrogen compared to its controlled counterpart and can be considered to no cyanamide content at all, since it is uncontrolled, and we did not put any cyanamide/fertilizer in it.

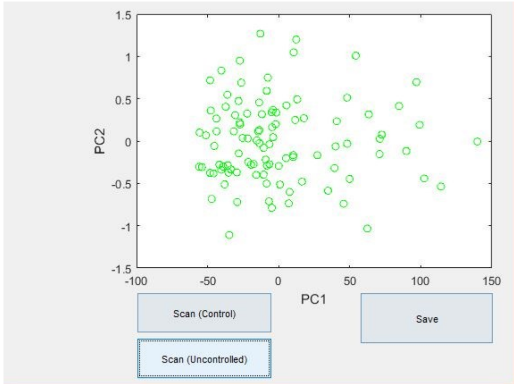

Fig. 5. — PCA Graph of the Sample from Table 2

Figure 5 shows that the points are concentrated on the left center of the graph, which indicates that it has races of cyanamide. And the equation of the PCA plot is

$$y = -6x10^{-15}X - 7.7x10^{-19} \tag{2}$$

III. RESULTS AND DISCUSSION

TABLE III. SUMMARY OF SAMPLES IN SUPERMARKET 1

Supermarket 1		
Sample	Remarks	
	Cyanamide	Cyanamide
1	Affirmative	-
2	Affirmative	-
3	-	Affirmative
4	-	Affirmative
5	Affirmative	-

Table 3 shows that 3 samples of lettuce have cyanamide, but two samples do not have cyanamide.

A. Statistical Analysis

T-Test is used in order to determine if there was a significant difference from the results of the randomly selected uncontrolled samples and their corresponding actual laboratory results. Each sample has 100 trials and thus, the degrees of freedom will be 99. The acceptance level is set to 5% (0.05). For all t-Test done, the Null Hypothesis and Alternative Hypothesis wil be:

$$H_0: \mu_{\text{experimental}} = \mu_{\text{actual}}$$
$$H_1: \mu_{\text{experimental}} \neq \mu_{\text{actual}}$$

All the t-Tests between the actual and experimental results for EC and H of the uncontrolled samples lead to t-Stat values less than the Critical two-tail t-value. Thus, the null hypothesis for all EC and H T-Tests will be accepted, which states that there is no significant difference between the means of the experimental and actual results. The average percentage error for EC are the following: 7.43% for sample from Market 1, 9.21% for sample from Supermarket 1, 7.81% for sample from Wet Market 1, 8.92% for sample from Public Market 1, and 8.57% for sample from Market 2, which indicates that the prototype has an average of 8.39% error for every trial run on uncontrolled samples.

The average percentage error for H are the following: 3.69% for sample from Market 1, 2.82% for sample from Supermarket 1, 2.82% for sample from Wet Market 1, 4.71% for sample from Public Market 1, and 3.69% for sample from Market 2, which indicates that the prototype has an average of 3.95% error for every trial run on uncontrolled samples.

V. Conclusion

The research has three objectives. First, (a) to develop an improveised electronic tongue that can determine cyanamide content in lettuce. The researchers were able to make an improvised electronic tongue using three sensor arrays, EC sensor, pH sensor and temperature sensors were used. These sensor arrays were able to detect the components of cyanamide, the nitrogen content, pH, and temperature from the solution containing the sample. Second, (b) to develop a program that can detect the cyanamide content of the lettuce using Principal Component Analysis and obtain its output. The researchers were able to develop a program that provided a GUI to solve for the PCA of each sample. Out of 25 samples from 5 different markets, 23 of the samples tested positive of cyanamide. Third, (c) to create a statistical analysis involving the content of cyanamide in lettuce from different local supermarkets. All the t-Tests between the actual and experimental results for EC and H of the uncontrolled samples lead to t-Stat values less than the Critical two-tail t-value. Thus, the null hypothesis for all EC and H T-Tests will be accepted, which states that there is no significant difference between the means of the experimental and actual results.

VI. Recommendations

The electronic tongue was able to detect the cyanamide by getting its unique components using each of the sensors, but cyanamide a chemical being composed of more than three components or elements, the sensor was not able to detect all the components, and compounds of cyanamide. It is recommended to use better and more sensor arrays to detect cyanamide. PCA was able to classify the samples that have cyanamide, but another machine learning algorithm might even find the amount of cyanamide there is in a sample. It is recommended to use more than machine learning algorithm or a proper algorithm to determine the amount of cyanamide in a sample. The researchers conducted the experiment with fully grown lettuce, but in every stages of the lettuce they are fertilized in different amount, so it is possible to make a research of how much cyanamide is absorbed by the plant in its lifetime until being sold off to local markets.

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