DETECTION OF FRESHNESS OF FOOD SAMPLE BASED ON COLORIMETRIC SENSOR ARRAY

Vanumu Praneeta

AGRICULTURAL AND FOOD ENGINEERING DEPARTMENT

INDIAN INSTITUTE OF TECHNOLOGY

KHARAGPUR – 721 302

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Project report submitted to

Indian Institute of Technology Kharagpur

for the partial fulfillment of the requirements

of the course curriculum

Bachelor of

Technology in

Agricultural and Food Engineering

by

Vanumu Praneeta

(Roll No.: 20AG10040)

Under the guidance of

Dr. Kanishka Bhunia

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Approved by

Dr. Kanishka Bhunia	*Dr. Madan Kumar Jha*
(Supervisor)	*(Head of the Department)*

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DECLARATION

I certify that

a. The work contained in this report is original and has been done by me under the guidance of my supervisor.

b. The work has not been submitted to any other Institute for any degree or diploma.

c. I have followed the guidelines provided by the Institute in preparing the report.

d. I have conformed to the norms and guidelines given in the Ethical Code of Conduct of the Institute.

e. Whenever I have used materials (data, theoretical analysis, figures, and text) from other sources, I have given due credit to them by citing them in the text of the report and giving their details in the references. Further, I have taken permission from the copyright owners of the sources, whenever necessary.

Signature of the Student

CERTIFICATE

This is to certify that the Dissertation Report entitled, “DETECTION OF THE FRESHNESS OF FOOD SAMPLE BASED ON COLORIMETRIC SENSOR ARRAY” submitted by Ms. “Vanumu Praneeta” to the Indian Institute of Technology, Kharagpur, India, is a record of bonafide Project work carried out by her under my/our supervision and guidance and is worthy of consideration for the award of the degree of Bachelor of Technology in Agricultural and Food Engineering of the Institute.

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Date: Supervisor

ACKNOWLEDGEMENTS

I would like to acknowledge and express my heartfelt gratitude to my ***Dr. Kanishka Bhunia***, who made this work possible. His direction and guidance guided me through all phases of writing my project. I'd also like to thank **Mr. Rahul Kumar** for his cooperation throughout the research tenure.

During all this time for unconditional love, constant support, and guidance, I would like to thank my family ***V. Murali Krishna*** & ***Ch. Bhavani*** for a constant source of inspiration and all my friends who helped me in completing the project successfully, specifically ***K. Suthasri.***

In the end, I would like to thank the ***Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur*** for providing me with the opportunity to work on this Project.

Date: 29/04/2024 Vanumu Praneeta

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ABSTRACT

This report focuses on the detection of the freshness of a food sample by using colorimetric sensor array(CSA) .The report begins with the introduction about the awareness of food quality and how the freshness of perishable foods can be detected. Followed by the Introduction, the report provides an overview of the processes we use to detect the freshness of food sample. A colorimetric sensor array (CSA) was developed to capture the volatile compounds released by the sample. Based on the changes in the CSA, we will get to know whether the food sample is fresh or stale. Overall, this report provides a comprehensive analysis of how we can detect the freshness of food sample by chemical methods and some machine learning methods.

1: Introduction

Introduction to food quality

Accompanied by the dramatic growth in food production and international marketing, food safety, as a dominant global issue, has received considerable attention worldwide. According to statistics, consumption of spoiled food will result in public health risks and even hundreds of thousands of deaths annually. Food safety should be a primary focus for food experts as the world's population expands and consumer demand for food rises. Additionally, the waste of spoilage food is up to 1.3 billion tons every year, which is an extensive resource waste.

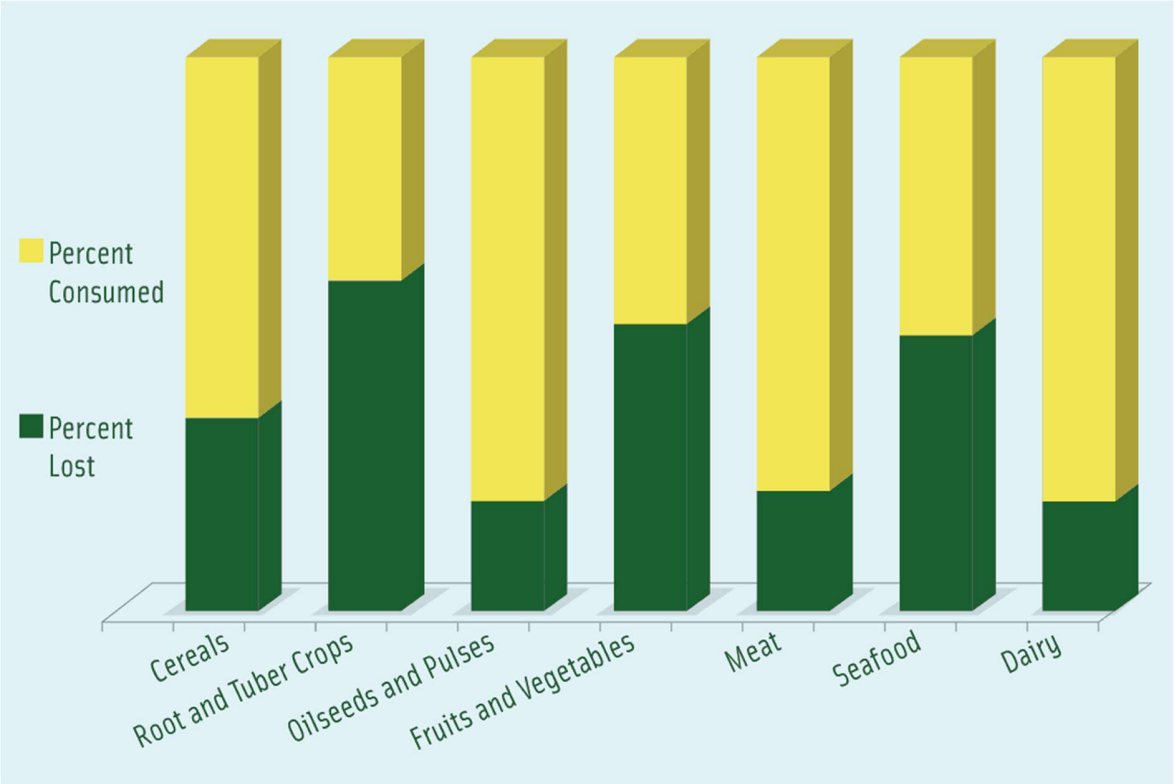

Fig1 : Percentage of waste generated from each industry

(Jia, X., Ma, P., Tarwa, K., & Wang, Q. (2023))

A large number of micro-organisms are responsible for either accidental or deliberate contamination of food, potentially causing food poisoning and foodborne diseases like salmonellosis, listeriosis, botulism, E.coli infection. Some of them can be life-threatening, since

they can produce toxins and release colonization products, leading to nausea or vomiting, and some more serious as abdominal pain, organ failure, abdominal pain, intoxication and even death. Examples of such organisms include Escherichia coli, Clostridium botulinum, Listeria monocytogenes, Salmonella spp., among others.

According to the World Health Organization (WHO), foodborne diseases are those transmitted by water or food that is contaminated with physical, chemical, or biological agents and these are classified as a hazardous. Biological agents are the major causes of ill-health because of their ability to multiply and spread. This category is represented by bacteria, fungi, parasites and viruses. These microorganisms are often present naturally in foods or environments in which they find themselves, but they may be liable to the same hazards resulting from poor storage and handling or even poor hygiene and temperature control.

Some of them are described as below:

Spoilage Organism	Representative Examples	Susceptible Foods	Sensory Changes
Spore formers:			
Thermophilic aciduric bacteria	*Alicyclobacillus*	Pasteurized juices and concentrates, syrups and sugar, soda	Medicinal off-flavors, not necessarily inclusive of visual defects
Acid sensitive bacteria	*Geobacillus*, Clostridia, *Bacillus*, *Paenibacillus*	Bottled tea, fluid milk, juices of moderate acidity	Gas production, turbidity, butyric acid off-flavors
Non-spore forming bacteria	*Erwinia*, *Pseudomonas*, lactic acid bacteria	Fresh fruit and vegetables, meat and poultry	Biogenic amines, polysaccharide production (dextran/slime), breakdown of food matrix
Heat-resistant molds	*Byssochlamys*, *Talaromyces*, *Eupenicillium*, *Neosartorya*, *Aspergillus*	Hot-filled or canned fruit and vegetable products	Visual defect from mycelial growth, occasional gas production
Yeast	*Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Brettanomyces*, *Candida*, *Debaryomyces*	Low a_w foods: salt brined goods, bakery products, jams and jellies, beverages	Gas production, off-flavors, turbidity

Fig2 : List of some of the microorganisms which spoil the food

(Huang, X., Zhao, J., Shi, J., & Zou, X. (2013))

Organisms that have high spoilage potential are ones that cause obvious sensory changes and typically produce some kind of product-degrading enzyme involved in the organism's metabolic processes like pectinases, proteases, or lipases; are gas producers; or whose growth has visible results, like mycelial development in molds. Janzen's theory on the ecological merit of these sensory changes states that microbes essentially benefit from spoilage that is negatively perceived by vertebrates who may be competing with the microbes for food, so that expression of energetically costly enzymes that render the food unappealing for vertebrates give some microbes a competitive advantage. Tests such as total plate counts that do not take into account variability in spoilage potential are not specific enough, and often not rapid enough, to provide quantifiable data to establish hard cut-offs for raw ingredient quality (Barth et al., 2009). Therefore, detection and quantification of specific organisms or sensory changes are required to move the needle on spoilage prevention.

During the colonization process, bacteria and other organisms have the ability to produce compounds that are volatile and can be released into the environment. These volatile organic compounds (VOC), or sets of them, are unique for each genus or species of microorganisms, producing a chemical fingerprint for each of these microorganisms. As an example, volatile amines (trimethylamine, ammonia and dimethylamine) are responsible for the 'fishy' odor and flavor exhibited by rotting fish suggesting the food has gone past it's use-by date.

2: Review of Literature

The practical use of the human nose as an odor assessment instrument in the food industry is severely limited by the fact that human olfaction is subjective, easily fatigued and disordered. Consequently, there is considerable need for an instrument that could mimic the human olfaction for food industrial applications **(Loutfi, Coradeschi, Mani, Shankar, & Rayappan, 2015)**. Although some successful applications of electronic nose systems have been described in many published studies, they still showed limitations in sensitivity to detect VOCs at lo

concentration, and selectivity to identify different compounds (**Suslick & Neal A. Rakow, 2004**). Furthermore, the selectivity proves especially problematic when interfered by humidity. Recently, a colorimetric sensor array technology was proposed by **Suslick and Neal (2004)** which can be used for "odors and volatile organic compounds (VOCs) visualization".

The development of cheap analytical methods that allows real-time and non-destructive detection to quickly discriminate between different types of contamination avoiding consumption of contaminated food is of great interest to the consumer and retailer alike in order to protect the consumers from foodborne illness and to protect the reputation of retailers respectively. One solution that meets with these requirements is the development of spotted color indicators integrated within the food packaging itself, as a form of smart packaging technology.

The colorimetric sensor array technology has been used to detect and differentiate complex components **(Janzen, Ponder, Bailey, Ingison, & Suslick, 2006)**. This cross-responsive sensor technology aims to mimic the mammalian olfactory system by producing unique composite responses to each odorant. It is the intermolecular interactions between analytes and the active center, often through strong chemical interactions rather than simple physical adsorption, that results in a colorimetric change (i.e., chemo-responsive). With such chemo-responsive colorants, a colorimetric sensor array generates a unique pattern for any odorants or their mixture.

There are four fundamental requirements for the selection of chemo-responsive dyes, (1) the dye should have an interaction center that can interact strongly with analytes, (2) there is a color change after the reaction, and the interaction center of dye must be strongly coupled to an intense chromophore, (3) there are always a group of diverse chemo-responsive dyes that are cross-responsive in a sensor array, and (4) the detection results of the sensor array should be reproducible and reliable **(Askim, Mahmoudi, & Suslick, 2013)**. Normally, the dye classes that could fulfill these requirements were (1) Basic dyes or Bronsted acidic (i.e., pH indicators),

(2) Lewis acid/base dyes (i.e., metal ion containing dyes), and (3) zwitterionic solvatochromic dyes which with large permanent dipoles **(Askim et al., 2013)**. Recent research shows that the mammalian olfactory receptors are mainly metalloproteins and odorant ligation to the metal center that are intrinsic to the mechanism of action **(Rakow & Suslick, 2000)**. The olfactory mechanism highlights the importance of strong interactions between sensor and analyte. Typically, chemo-responsive dyes are porphyrins, metalloporphyrins and pH indicator dyes

The resulting real-time changes in color of the spots in the presence of the VOCs of interest or other by-products responsible for microbial growth will be informative to stakeholders throughout the food chain.

Odors are recognized by pattern combinations captured by olfactory cells; these patterns are carried to the brain where olfactory information is decoded. Recognition systems (receptor/transducer) have been mimicked using synthetic materials integrated with sensors and have been reported in the literature as electronic nose technologies. Likewise, there are other systems that mimic the taste, referred to as electronic tongues8 as well as vision (electronic eye). These electronic devices work the same way as human analogues, capturing information by patterns, using the concept of global selectivity, a system that does not identify particular substances but extracts the information into patterns that can be decoded by the brain.

The brain, in the case of synthetic devices, is an unsupervised pattern recognition tool, which is able to transform these sets of signals into useful information for recognition. In contrast to a single colorimetric spot, or colorimetric sensor, an array of colorimetric sensors using a paper device, with pattern recognition, one can easily discriminate between more than a single compound or the total volatile basic nitrogen (TVB-N) species. These devices can qualitatively discriminate the compound released discerning the types of microorganisms found based on the volatile chemical fingerprint produced.

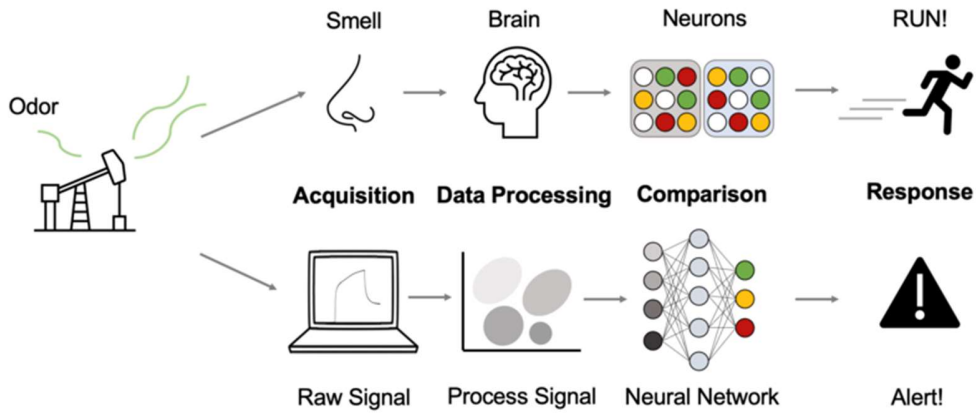

Fig 3: Comparison of CSA to the olfactory nerves

(Protoplasmix, 2012; Huang Xiaowei, 2013; Huang et al., 2014; Jie-wen, Jian, & Xiaobo, 2008)

3: Materials and Methods

The colorimetric sensor array was prepared using a PVDF membrane as the flexible substrate, and the colorimetric dyes were first immobilized by sol–gels and then drop-coated on each sensing unit. The use of sol–gels in building a colorimetric array was attempted for the first time. The sensor array was used for the quantification of volatiles in the sample. Data acquired from the sensor array were processed with hierarchical cluster analysis (HCA) and principal component analysis (PCA). Here the volatiles like acetone and acetaldehyde are measured as a traditional freshness indicator was also measured and compared with the sensing results. Finally, the fabricated array was employed to differentiate food sample freshness at different storage intervals.

3.1 Preparation of the sample and array

The colorimetric sensor array was prepared using a PVDF membrane as the flexible substrate, and the colorimetric dyes were first immobilized by sol-gels and then drop-coated on each sensing. A colorimetric sensor array of 5x5 dye is used, and a beaker or a tumbler with a small mouth is taken. The CSA was put on the beaker's mouth and left for a few minutes.

Pure acetone and acetaldehyde of different concentrations like 10ppm, 20ppm, 100ppm, and 500ppm were taken and for each concentration, 3 different samples were taken, i.e., a total of 27 samples were taken including the blank samples. Each sample was taken in a beaker and CSA was put on the beaker. The CSA will capture the volatile compounds released by the sample and generate unique scent fingerprints and due to this, the dye color gets changed.

3.1 Segmentation and analysis of datasets

For analysis of each sample, was employed to capture the images of the array before and after analyte exposure. The images were then processed with color measurement software, which is based on the same principle as the usual Photoshop and ImageJ, which can read out red, green, and blue (RGB) values of each colorimetric unit, and then calculate changes in the RGB color values of each element of the array by subtracting the averaged color intensity of each element before and after exposure to the sample as shown in the below equations.

$$\Delta R = | R_{after} - R_{before} |$$

$$\Delta G = | G_{after} - G_{before} |$$

$$\Delta B = | B_{after} - B_{before} |$$

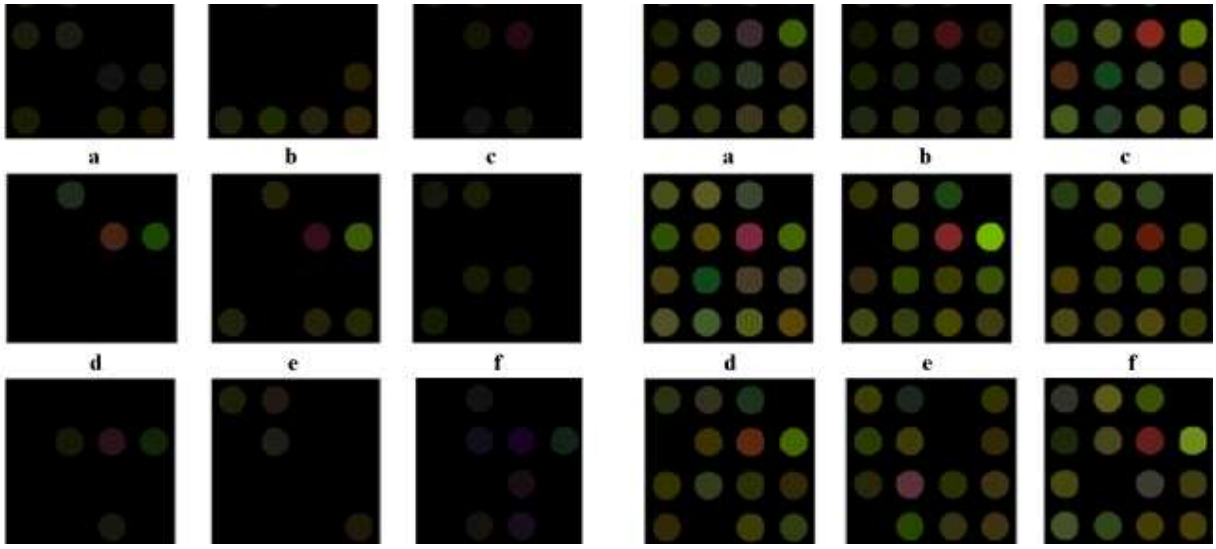

Fig 4: CSA of 25 dyes before and after exposure to the samples

(Xu, W., He, Y., Li, J., Zhou, J., Xu, E., Wang, W., & Liu, D. (2023))

3.1 PCA and KNN analysis

To evaluate the performance of the sensor array in discriminating the amines, the collected sensing data were processed by PCA, which is an unsupervised exploratory data analysis. PCA is a data dimensionality reduction technique that can reveal the scale of the chemical active space detected by the array, among data points and cluster them in multivariate vector space.

To avoid overfitting and make computations easier, feature extraction reduces the number of dimensions in the feature space while keeping as much of the original variance as possible. Methods like Principal Component Analysis (PCA) are often used to do this. PCA changes the original variables into a new set of variables called principal components. By keeping as much of the original variation as possible, PCA makes it possible to show the data in a more concise way, which makes modelling more efficient and effective.

First, we have done correlation between all the datapoints to know how all the values are co-related and the dependency between each datapoint.

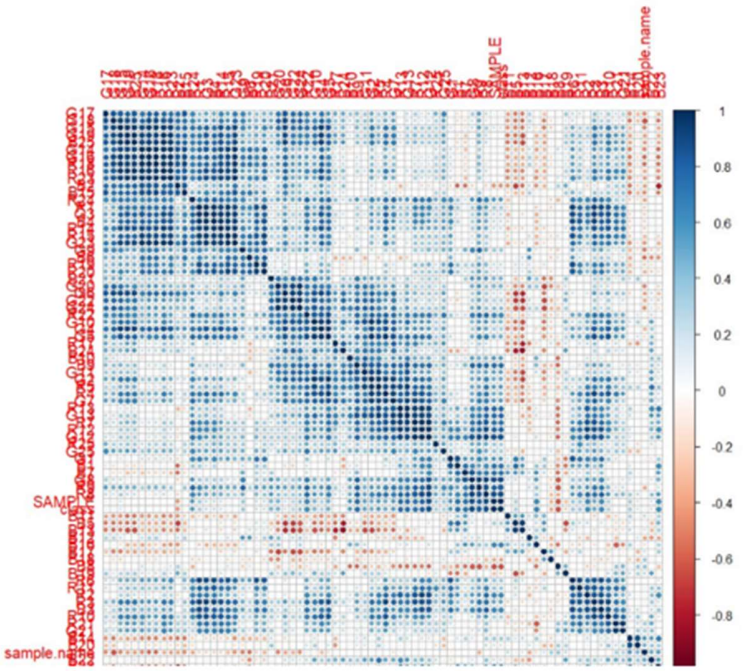

Fig 5: Correlation between the datapoints

In PCA, first we converted the categorical data into numerical one by using label encoding. Then we plot the graph between PC1 and PC2 which is shown below. After that we plotted the PC for the dataset. At 7 rate of change decreases, so we took 7 principal components and we plot PCA.

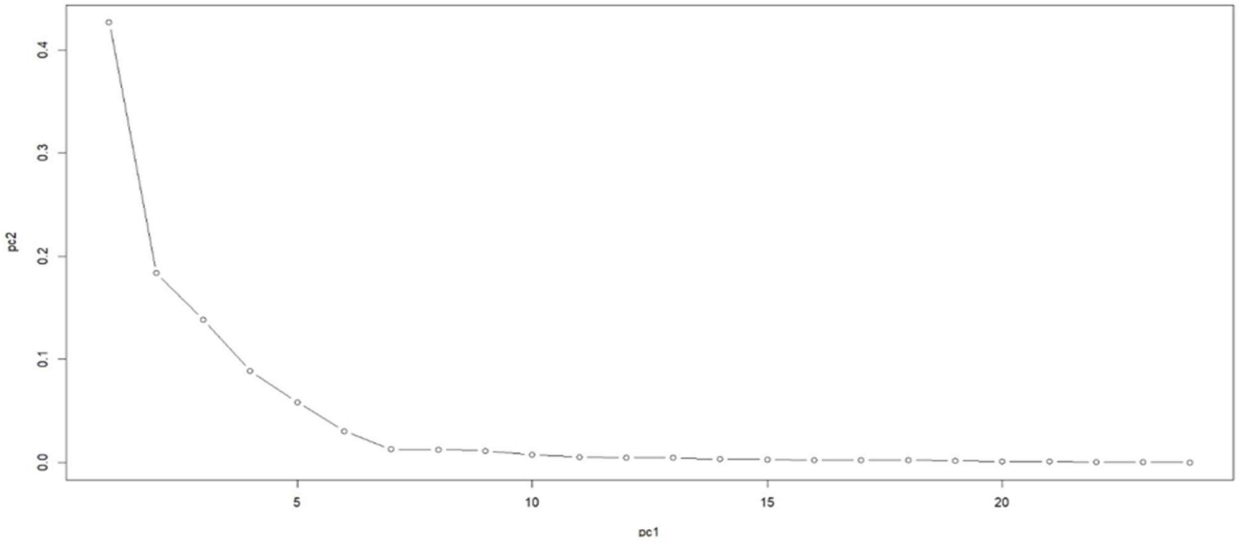

Fig 6: Graph between PC1 and PC2 components

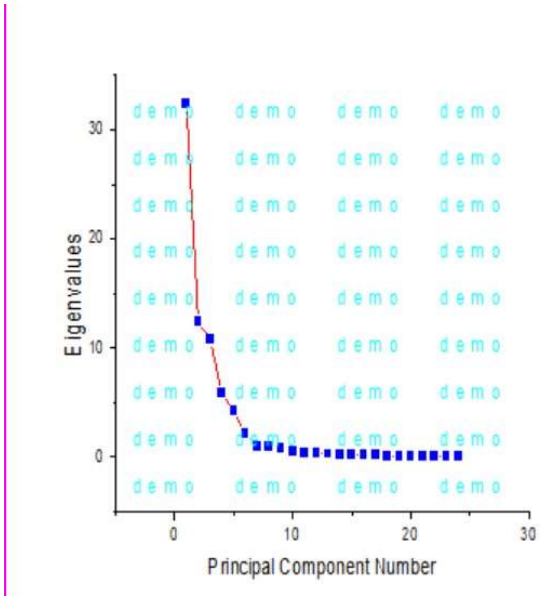

Fig 7: Plot to know the number of principal component

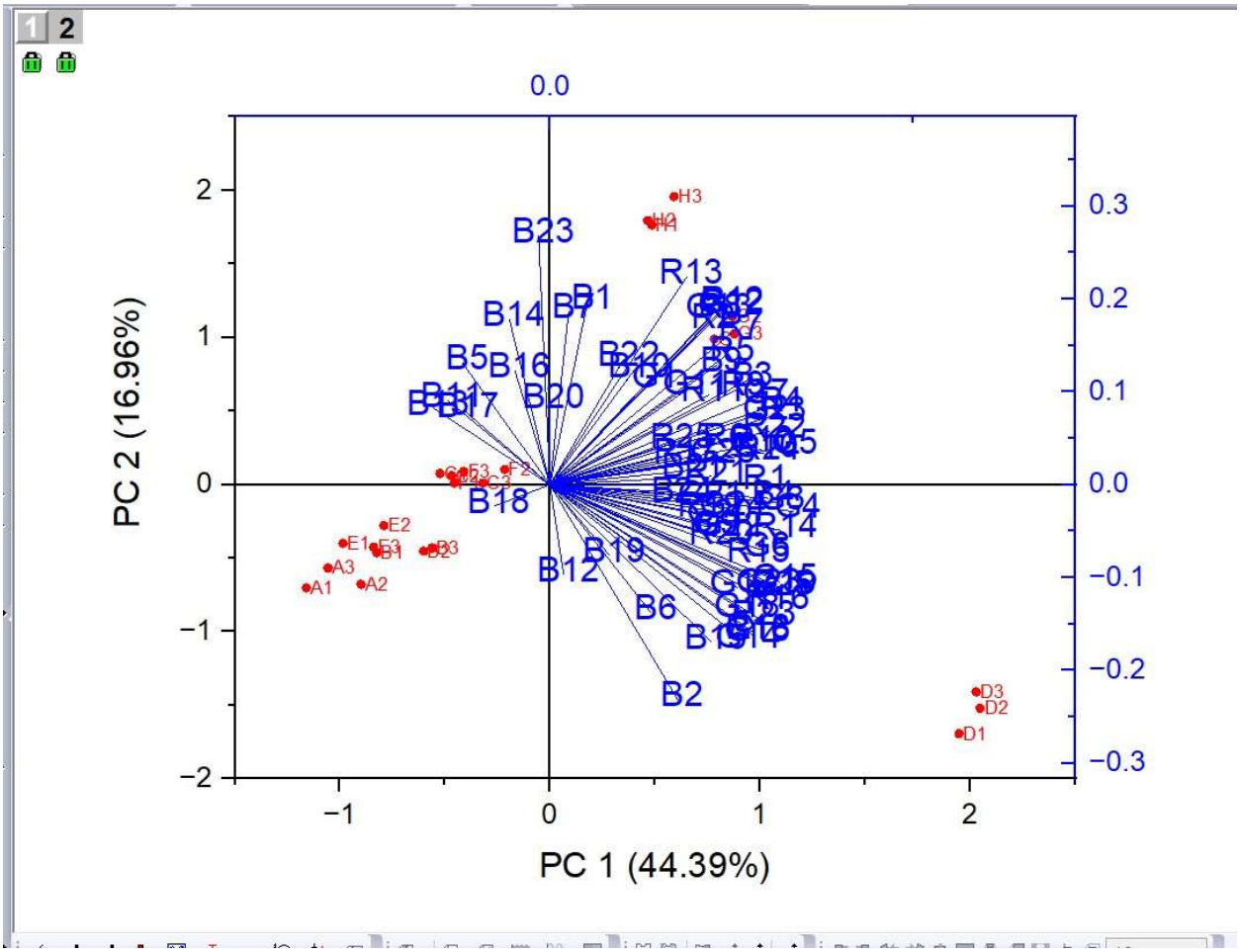

Fig 8: PCA plot analysis of the datapoints(Biplot)

K-Nearest Neighbors (KNN) is a simple yet effective supervised machine learning algorithm used for classification and regression tasks. It works based on the principle of similarity, where the prediction for a new data point is determined by the majority class or average value of its nearest neighbors in the feature space.

3.2 Logistic Regression and SVM analysis

Regression methods predict continuous outcomes from input data. These methods clarify variable relationships and simplify model interpretation. They use control variables to

improve predictions and evaluate assumptions to ensure validity. Predicting future outcomes, analysing variables and making data-driven decisions which require regression.

In this project, we are given RGB values of the CSA and the class of the sample. We have to train the model with the RGB values of the dataset and the model has to predict what type of class it comes under. As we have multi-classes to predict, we use Logistic Regression to train the data and predict the values.

We want to see whether the data is getting perfectly separated or not. For that we have plotted between the RGB values and class of the sample.

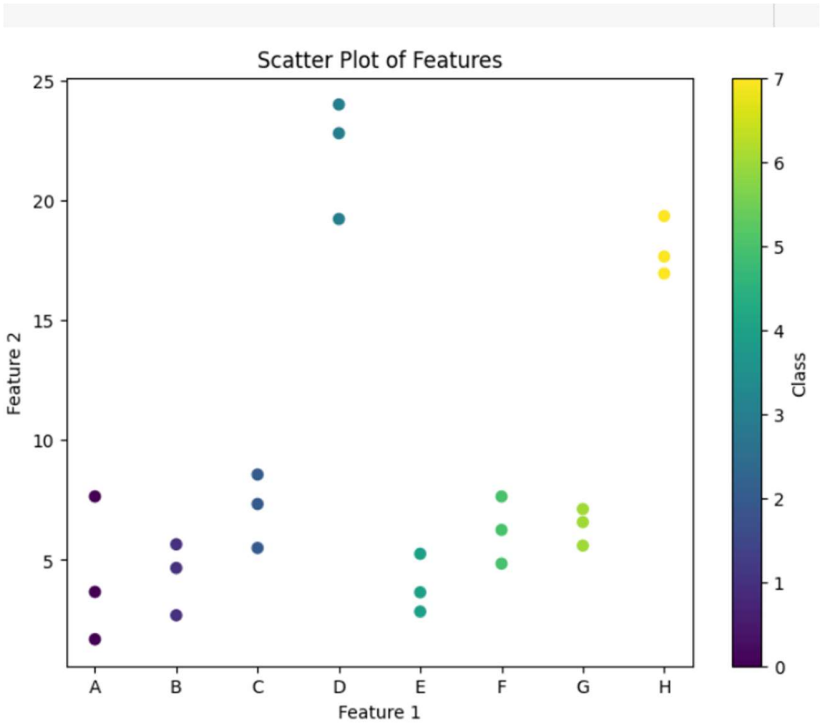

Fig 9: Datapoints separation and classification

From the above plot, we can say that the data is getting perfectly separated. As we have to predict the class of the sample which is a categorical variable, we encode the variables and then split the data into training and testing, and applied the Logistic Regression model to it and calculated the metrics for the data

4: Results and discussions

From the above figures, we can say that in PCA analysis, the clusters have been done and how much variance is there from each value to the PC1 and PC2. In the KNN algorithm, we have divided the dataset into the training and testing dataset and we trained the model with the training dataset and after that we tested the model with the validation dataset to get to know whether we are getting the accurate results or not.

To know which model is giving better accuracy, we trained the dataset with various models like Logistic Regression, SVM, kNN etc….

We also calculated the accuracy and I got the accuracy as 68.7%. I also plotted the graph between the error value and k value and I got that the error is least at k = 1. While training the dataset with Logistic Regression, we got accuracy 74.76%.

5: Conclusion

In conclusion, a novel, effective and non-destructive colorimetric sensor array has been used for the detection of the freshness of a food sample. The sensor array resembles the mammalian olfactory system, which can sense volatiles released from rotting samples and form scent fingerprints. By extracting unique features, the sensor array can quickly identify the volatiles with detection limits down to the low ppm level.

These results indicate that the colorimetric sensor array has great potential for monitoring food freshness and is a promising candidate for use in food transport, storage, and consumption. It also serves as a useful alternative to other methods of food safety inspection.

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