E-nose and E-tongue systems for the Bacteria Detection in milk

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Abstract*– The purpose of this paper is to describe the combination of an E-nose and E-tongue that were evaluated for the E. coli detection at different concentrations, as well as their ability to discriminate this bacterium from others, such as Klebsiella pneumoniae and Salmonella enterica in pasteurized milk. In this study, gold and carbon electrodes were tested in the E-tongue. For data processing, multivariate analysis techniques were used to discriminate the measurements, where the Principal Components Analysis (PCA) and Linear Discriminant Analysis (LDA) methods were applied. Likewise, for the data classification, the Vector Support Machines (SVM) through the linear kernel and Radial Basis Function (RBF) algorithms were used, and the same way as the k-Nearest Neighbor (k-NN) method. When evaluating the capacity of the proposed methodologies to detect and classify E. coli, S. enterica, and K. pneumoniae in pasteurized milk, it was observed that both the E-nose (TGS 826 sensor) and the E-tongue (gold electrode) obtained comparable results with 94.7% and 92.5% success rate respectively. Both devices successfully detected and classified the three bacteria tested, clearly differentiating them from the sterile milk samples. On the other hand, the electronic tongue with a gold electrode achieved a 98.7% success rate in the discrimination of decreasing concentrations of E. coli, from 1x 06 CFU/ml to $1x10^{-2}$ CFU/ml, in pasteurized milk.

Keywords*-- E-nose; E-tongue, bacteria, pasteurized milk, PCA, LDA, SVM, k-NN.

I. INTRODUCTION

Foodborne diseases remain an important cause of morbidity and mortality, therefore are a public health problem worldwide [1] [2]. Consequently, the detection and monitoring of pathogenic bacteria are one of the priorities for the dairy industry, since its food products are consumed by a wide sector of the population, including children [3]. Currently, the main strategy for monitoring hygienic conditions in milk production and its derivatives is the use of indicator microorganisms. Thus, the dairy industry has long since used coliforms for this purpose [4]. Of this group stands out Escherichia coli as this species of coliform bacteria is the best indicator of fecal pollution and the possible presence of pathogens [5]. This bacterium is not only characterized by its use as an indicator microorganism but also includes strains that are pathogenic for humans, and on numerous occasions have been isolated from milk and/or its derivatives as responsible for infective processes resulting from its consumption [2], [6], [7] [8]. Indeed, of the Enterobacteriaceae family, *E. coli* is usually the most frequently isolated species from milk [9]. Additionally, the presence of *E. coli* in milk acquires greater importance at a public health level since strains with resistance properties against antibiotics have been reported [10]. Although pasteurization is considered an effective method for the elimination of pathogenic bacteria from milk [11], in developing countries, there are still reports on the detection of these bacteria in pasteurized milk and in ready-to-eat milk products, which suggests important underlying food security problems [12].

Traditional methods for the bacteria detection in food are based on the growth of bacteria strains in solid culture media, which demands in addition to inputs and a specific infrastructure, time that can vary from 1 to 3 days for obtaining the initial results, and may even be longer to confirm specific pathogens. Therefore, to prevent the spread of infectious diseases, ensure the safety of dairy products, and protect public health, there is an increasing demand for the development and implementation of rapid bacterial detection methods, ideally culture-independent, to have the information in a more immediate way [13]. In recent years, work has been carried out to search for alternative methodologies. At present, this set of methodological strategies can be grouped into three categories: immunological methods, those based on nucleic acids, and those based on chemical sensors or biosensors [14]. Of these categories, the most emerging and promising corresponds to the biosensors that have been used for the construction of artificial systems of smell and taste, called E-nose and E-tongue systems. These systems have demonstrated their ability to detect bacteria in a shorter time, with good sensitivity and selectivity comparable to conventional methods; furthermore, compared to the other categories, and they do not require specialized laboratories with qualified equipment and personnel, or complicated steps for sample preparation [15].

E-noses and E-tongues attempt to mimic the sense of smell and taste, and their communication with the human brain. Besides, E-noses are measuring instruments based on a series of semi-selective gas sensors that interact with volatile molecules that generate physical or chemical reactions that send a signal towards a computational device that uses pattern recognition methods [16]. In the food industry, volatile organic compounds (VOCs) are diverse and can be generated during production, maturation, and storage, causing each product to have a characteristic profile. Similarly, food spoilage will

result in a different but still characteristic profile in the same product [17]. For example, bacterial growth in milk generates VOCs such as ethyl butyrate, acetaldehyde, acetic acid, ethanol, etc., which can be used as markers for the early detection of milk spoilage [18]. In recent years the E-nose has become an instrument with great potential for monitoring, control, and evaluation of food safety, as it helps to quickly and early detect contaminants and/or adulterations in the food production chain [19]–[21], such is the case of meat [4], fish [5], milk and dairy products[22], [23] fruits and vegetables[24], [25], bakery products[26], and drinking water[27], among others[28]. In the dairy sector, the E-noses have been studied for the detection of characteristic volatile and non-volatile compounds related to the growth of bacteria such as *Pseudomonas fragi* or *Escherichia coli* [29]. Moreover, it has been shown that E-noses can have applications in aspects such as: classification of milk by trademark and type, determination of unpleasant taste in UHT milk, prediction of milk shelf life, freshness control and differentiation of microbial species that cause spoilage, and adulterations in skimmed milk, among others [6], [16], [20], [23], [30]–[33].

E-tongues use a variety of non-specific chemical sensors, with high stability, cross-sensitivity, which together with pattern recognition or multivariate analysis tools, allow the classification of samples [34]–[36]. Besides, E-tongues have their most important applications in the food industry, especially in beverages [37]–[41], a sector in which it has been shown that this device has great potential in quality assurance[2] detection of chemical contaminants. It has been reported that, in the dairy industry, E-tongues may have important applications in activities related to spoilage control and quality assurance of dairy products [42], [43]. Its use has been focused mainly on the detection of adulterated milk [5], [44] and they have also been used to evaluate the flavor and freshness of the product [45], and to discriminate between different types of milk[6], [7]. Regarding the fermented products, its use has been described in monitoring the fermentation, post-maturity, and storage processes of yogurt [9].

According to the above, both the E-nose and the E-tongue are very promising tools to assess quality and monitor deterioration in the food industry [19]; nevertheless, few studies have explored the ability of these instruments to detect and discriminate important bacteria in food. This study presents a comparative analysis of the use of an E-nose and an E-tongue to differentiate between three of the most important bacterial species of the *Enterobacteriaceae* family, as well as an evaluation of their ability to discriminate different concentrations of *E. coli* in pasteurized milk.

II. MATERIALS AND METHODS

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A. Bacterial Strains

The following bacterial strains were used in this study: *Escherichia coli* (ATCC® 25922™), *Klebsiella pneumoniae* (ATCC® 27736™), and *Salmonella enterica* (ATCC® 31194™). These strains were supplied and preserved by the microbial culture collection center of the University of Pamplona. To verify the ability of the devices to detect and discriminate between bacteria, a suspension in sterile water was obtained from each strain, at a concentration of 3×10^8 CFU/ml, using the McFarland standard No. 1, as indicated by Isenberg HD 2015. This suspension was diluted 300 times (1/300) to a final volume of 300 ml in pasteurized milk previously sterilized, to achieve a final concentration of 1×10^6 CFU/ml. This procedure was carried out for each of the bacterial strains, preparing three erlenmeyers with milk, each one inoculated with: *E. coli*, *K. pneumoniae* and *S. enterica*.
To evaluate the sensitivity of the devices to detect *E. coli* in milk, the procedure was as described below. From a suspension of *E. coli* in sterile water (at a concentration of 3×10^8 CFU/ml), nine serial decimal dilutions (1/10) were carried out to a final volume of 10 ml in sterile milk, obtaining nine tubes (from 3×10^7 to 3×10^{-1} CFU/ml). Afterward, each of these tubes was diluted 30 times (1/30) to a final volume of 270 ml of sterile milk. Thus, nine flasks were obtained with decreasing concentrations, in an order of magnitude 10, from 1×10^6 to 1×10^{-2} CFU/ml. The above procedure was performed by duplicate. Each of the final concentrations of *E. coli* was verified by a conventional microbiological procedure) figures, plots, drawings and photos for best printing result.

B. Experimental set-up

-E-nose system

For the development of the E-nose, a matrix of 16 TGS-type metal oxide sensors from the manufacturer Figaro sensor (see Table I) was used and conditioned with 4.7 KΩ load resistances and a voltage divider was applied to the acquisition of the sensor signal in resistance value (Rs).

TABLE I
TGS GAS SENSOR

#	Sensor	Target Gas
1	TGS 826	Ammoniate and amine
2	TGS 831	R-22 Monoclorodifluoromethane
3	TGS 821	Hydrogen
4	TGS 826	Ammoniate and amines
5	TGS 842	Methane and natural gas
6	TGS 880	Smoke of the food (Alcohol, odour)
7	TGS 825	Hydrogen sulphide
8	TGS 813	Hydrocarbons in general
9	TGS 800	Air pollutants in general
10	TGS 880	Smoke of the food (Alcohol, odour)
11	TG0S	Alcohol and organic solvents

	822	
12	TGS 821	Hydrogen
13	TGS 832	R-134[a] 1,1,1,2-Tetrafluoroethane
14	TGS 842	Methane and natural gas
15	TGS 831	R-22 Monoclorodifluoromethane
16	TGS 830	R-22 Monoclorodifluoromethane

The measurement chamber was developed in methacrylate material, with a 30 ml volume capacity and two orifices for the entry and exit of volatile compounds. A connector was used for the 5 VDC voltage input and the 16 analog channel output where the sensor signals were acquired by a National Instruments Model 6218 DAQ 16-Bit, 250 kS/s data acquisition card. At the inlet of the measurement chamber, a 1/4-inch 30-cm long hose was placed to connect the volatiles extraction system employing connectors. For heating and subsequent measurement with the sensors, 10 minutes were required. Once the compounds were measured by the E-nose, 5 minutes was set for cleaning the sensor chamber to avoid the memory effect in the next measurement. The time to obtain results with the electronic nose was 20 minutes respectively.

To extract the VOCs from the milk samples for their analysis by the E-nose, 20 ml volume vials were used in which 10 ml of sample were added. Moreover, to generate a headspace sample, a "home-made" volatile extraction system was used, which consists of a heating or resistance unit that allows increasing the temperature from 20°C to 250°C, which allows the extraction of the emitted compounds. by heating the sample. Once the sample was heated to a temperature of 50°C for 10 minutes, the gas was extracted from the headspace of the vial employing a needle and the activation of a 12 VDC electrical pump to draw the compounds towards the sensor chamber. During the warm-up time, the temperature control was carried out with a low-cost data acquisition card "Arduino UNO".

E-tongue system

For the operation of the E-tongue, a two-channel potentiostat (Reference "µStat200") from Dropsens Company was used, which consists of a portable Bi-potentiostat that is used for amperometric and voltammetric measurements. This small device operates with six current levels from 2 nA to 200 µA and with a resolution of 1 pA in the minimum current range.

For the analysis of the samples, two types of screen-printed electrodes from the Dropsens company were used, a carbon electrode (C110) and a gold electrode (220AT). For the acquisition of the signals, the parameters were configured through cyclic voltammetry with the following values: Ebegin: 0, which indicates the scan start potential, Evtx1: -2, scan inversion potential, Evtx2: + 2, voltage with the scan stops, and the number of scans = 1. For the acquisition of measurements in a repetitive way, the potentiostat was set in automatic mode for a time of 1 min, enough to obtain results in 5 minutes.

- **Sample conditioning**

Each of the three bacterial suspensions (at a final concentration of 1×10^6 CFU/ml) described above, was tested. From each flask, 10 ml were taken in a vial to be analyzed by the E-nose, and 50 µl to be analyzed by the E-tongue. The measurements were repeated ten times for each bacteria. Regarding the E-tongue system, the carbon (C110) and gold (220AT) electrodes were also tested.

Furthermore, from each of the nine flasks with different concentrations of *E.coli* (from 1×10^6 to 1×10^{-2} CFU/ml), the same quantities already mentioned were taken to be processed by both the E-nose and the E-tongue. In this test, the performance of the carbon (C110) and gold (220AT) electrodes was also verified and compared. In each case, the analyzes were repeated ten times for each concentration. The microbiological analysis to verify the concentration of the samples contaminated with *E. coli* was carried out according to the procedure defined by the AOAC 17.3.08 (983.25).

In both cases, sterilized pasteurized milk was used as a negative control. Fig. 1 illustrates each of the stages developed in this work, as well as the materials that were used for the development of the tests with the devices based on gas sensors and screen-printed electrodes. The preparation of the samples and the tests with the E-nose and E-tongue were carried out in the microbiology research laboratories of the University of Pamplona (Colombia).

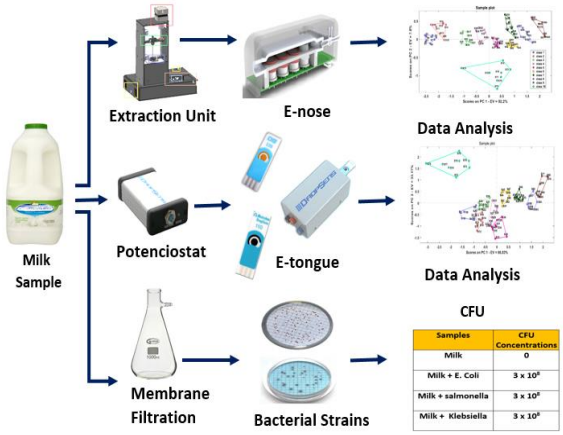

Fig. 1 Overall scheme of the procedure for bacteria detection.

C. Data pre-processing

To obtain the maximum information from the data generated by the devices, a data pre-processing stage was applied to avoid loss of the information or the noise generated during the sample acquisition. In the case of the E-nose, the

static parameter $\Delta R=R_{max}- R_{min}$ was used, in which R_{max} corresponds to the value of the maximum resistance reached by the sensors, and R_{min} to the minimum value of the resistance response of the gas sensors [46].

Likewise, the measurements obtained by the E-tongue by means of the screen-printing electrodes, were pre-processed through the current values (µA), where two parameters were extracted from the data set. One of them was $\Delta U_1=U_{max}- U_{min}$, which extracts the relevant information from the response of the voltammetric signal during the oxidation-reduction process, and on the other hand the parameter $\Delta U_2=U_{final}- U_{initial}$ was acquired in order to extract the behavior of the voltammetric signal during the entire measurement period of the compound.

After extracting the static parameters, two normalization methods were applied in order to reduce the magnitude of the variables, these are normally called "Meancentring" and "Autoscaling", where "Meancentring" extracts the mean value of each of the data, it is used in order to center a subset of vector space to a centroid of the original data set for a better grouping and visualization of the information. On the other hand, "Autoscaling" divides each variable by the standard deviation such as normalization per column, therefore it is a useful method when there are variables with data of different scales. Additionally, "Autoscaling" can be defined as a composition between both methods.

D. Data processing

For processing and data analysis, different Pattern Recognition (PARC) methods and classification techniques were used, such as Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), Support Vector Machine (SVM) by using linear kernel and Radial Basis Function (RBF); finally, the k-Nearest Neighbor (k-NN) algorithm was used as classification method.

PCA creates a dimensional graph in 2D or 3D using the previously normalized data matrix, thus reducing the number of initial variables (sensors) and obtaining a set of principal components, which are the new variables built from the originals ones [47]. It is the most widely used method in E-noses and E-tongues since it performs discrimination employing a linear transformation that converts a number of observations of correlated variables into uncorrelated variables that correspond to the principal components [48]. Therefore, most of the information of the original variables is distributed in the first two components (PC1 and PC2) [45] [49]. In the analysis of the milk samples, both for the bacteria discrimination and the concentrations of *E.coli*, the matrix was reduced to 2 variables from the original 16, maintaining the information of the data set.

Additionally, through the LDA multivariate analysis method the first two factors are used, and unlike PCA, it is more used in data classification, where the main objective is to obtain information from the two factors and project the set of measures in a two-dimensional space through adequate separation between classes and good repeatability between the same class [49]. LDA is a supervised method that calculates directions through the use of linear discriminant functions that represent the axes or coordinates that maximize the separation in different classes depending of the variance [50]. This means that with a single discriminant factor it could be determined whether the set of measures can be classified [51].

SVM allows in most cases to solve different classification problems since the algorithm offers an excellent reliable predictor and with the characteristic of having a good performance because it performs minimum overfitting to the data set [52]. SVM algorithm is optimized by the polynomial, linear and radial basis function (RBF) Kernel function. In this study, several tests were carried out with the milk samples, both linear and RBF Kernels to find the best percentage of success rate in the classification of milk measurements [53], [54].

Another classification method widely used in solving classification problems is the k-NN algorithm owing to its simplicity and low error rate. This method is also known as k-nearest neighbors, which works using an input vector with the determined number of closest training samples in the space to which each category belongs. k-NN also requires training to define the neighbors as a function of the distance from the test sample to another sample, and thus determine the class to which the test sample belongs [55]. Due to the ease of implementation and because it is a non-parametric classifier method, its error probability is limited by the Bayesian error [56]. For the classification of bacteria and milk samples with concentrations, the "Fine" k-NN classifier was used, which makes detailed distinctions between classes with the number of neighbors set to one [57].

III. RESULTS AND DISCUSSION

A. Bacteria discrimination in milk

This analysis was carried out by means of a controlled contamination of sterilized pasteurized milk with each of the following bacteria *E. coli*, *K. pneumoniae*, and *S. enterica*, at a final concentration of 1×10^6 CFU/ml. These are three of the species of the Enterobacteriaceae family with the greatest relevance in milk and dairy products owing to their prevalence, pathogenicity, and their involvement in foodborne outbreaks [58].

B. E-nose

Fig.2 shows the result obtained through the PCA analysis, in which a total variance between the first two components reached 86.69%. In the PC1 a greater representation of the samples was obtained, where the clusters of *S. enterica* and *E. coli* are discriminated very close to each other, coming to infer

similarities between the two classes; however, these categories are clearly distinguished. The proximity between these two clusters has biological bases since these are species are known to be evolutionarily closely related based on a high level of similarity between their housekeeping genes, and the two species are considered to have diverged from a common ancestor [59].

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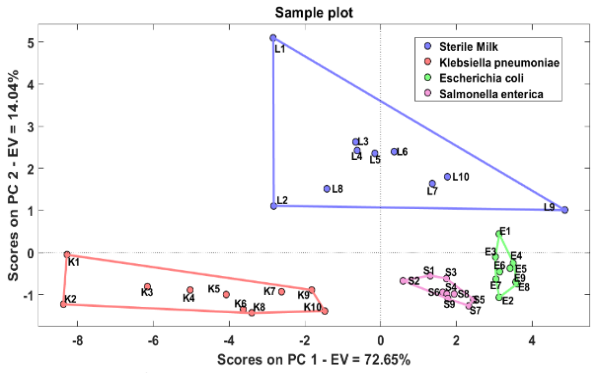

Fig. 2 PCA Plot for the bacteria discrimination by using the E-nose

Besides, it is observed that the samples contaminated with *K. penumoniae* have a greater dispersion than the rest of the categories. This may be because the bacteria had a memory effect on the sensors and the recovery was very slow. Furthermore, it is important to clarify that two outliers were discarded from the group of 40 measurements due to problems in the acquisition process.

Fig. 3 illustrates the behavior of the classes for the variable (V1) or sensor 1 (TGS 826) applying the Boxplot graph, which presented a better performance, and clearly shows the differences between the contaminated samples with bacteria and the sterile milk. The classes are represented as: 1) Sterile water, 2) *K. pneumoniae*, 3) *E. coli*, and 4) *S. enterica*. additionally, for the classification of the measures, the SVM classifier with the Gaussian mean function was used, which obtained a success rate of 94.7% in the classification of the measures and the cross-validation method was applied with a k-fold = 5 (see Figure 4). Although a very good classification was obtained, there were two errors in categories 1 and 4, since some measurements of class 3 (*E. coli*) generated overlaps between sterile water and *S. enterica*.

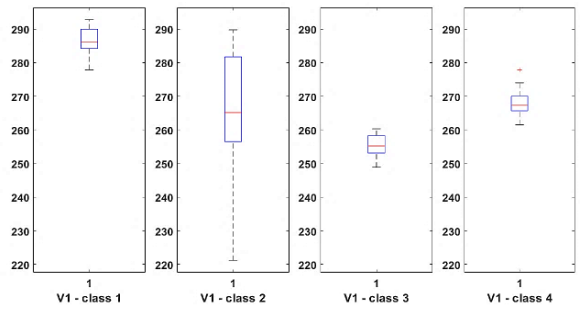

Fig. 3 Boxplot for samples analysis by using TGS 826 sensor. 1) Sterile water, 2) *K. pneumoniae*, 3) *E. coli*, and 4) *S. enterica.*

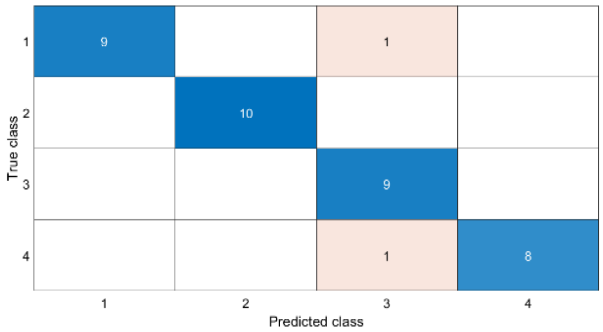

Fig. 4 Confusion matrix obtained from SVM classifier for bacteria classification through the E-nose.

In general terms, the E-nose showed a high discrimination capacity between the bacteria tested; not only because of the clear separation between them and with regards to sterile milk but also because the results of the PCA and SVM allow to show a closer relationship between the strains of *E. coli* and *S. enterica* than with regards to *K. pneumoniae*. This result is very interesting because the data generated by the E-nose are consistent with the results obtained by phylogenetic inference based on nucleic acid sequences (16S ribosomal RNA and housekeeping genes), which have been carried out in the Enterobacteriaceae family. Analysis by DNA markers has clearly shown an evolutionary closeness between the genera *Escherichia* and *Salmonella*, while the genus *Klebsiella* has a greater genetic distance.

C. E-tongue

The processing with the E-nose was carried out taking into account two characteristics of the voltammetric signal obtained by the screen-printing electrodes of both carbon (C110) and gold (220AT). The characteristics correspond to the maximum and minimum value extracted from the signal together with the final and initial value of the voltagram. The values of the two characteristics were acquired to obtain relevant information from the data set.

- **Gold sensor**

The score graph of PCA analysis for this sensor (Fig. 5) shows how the sterile milk and the samples contaminated with *E. coli* and *S. enterica* were grouped and discriminated appropriately, with very good repeatability and selectivity among categories. 100% of the total variance in the two PCs was obtained. Similar to what was observed with the carbon sensor, the category corresponding to *K. pneumoniae* showed dispersion among the samples. These observations are supported by the data of the distribution of the classes shown in the Boxplot graph (Fig. 6). This graph shows how class 2 (*K. penumoniae*) presents higher outliers, which explains the dispersion of the samples.

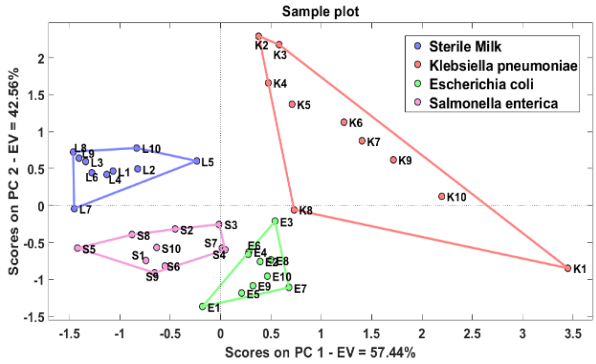

Fig. 5 PCA analysis using the gold electrode.

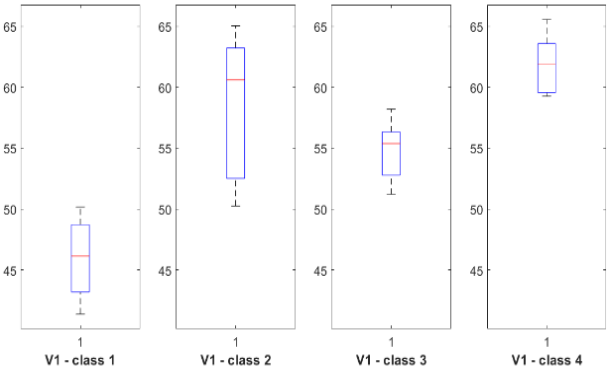

Fig. 6 Boxplot for the samples analysis through the gold electrode.

For the classification of the measures, the LDA classifier was used, which obtained a success rate of 92.5% with the cross-validation method and a k-fold = 5. It should be noted that the classification carried out in this case obtained 3 errors that can be seen in Fig. 7, and it can be compared to the carbon electrode that obtained 5 samples misclassified (see Fig. 10).

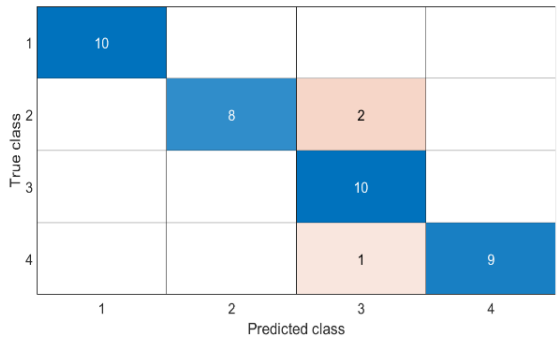

Fig. 7 Confusion matrix obtained from LDA algorithm through the gold sensor (220AT) for the bacteria classification in milk.

Carbon sensor

Fig. 8 shows the discrimination of the different categories of bacteria and the sterile milk samples. With 99.66% of the total variance in PC1, *E. coli* and *S. enterica* bacteria can be discriminated with good repeatability between samples and selectivity between categories. However, the category corresponding to the bacterium *K. pneumoniae* showed a significant dispersion among the samples, even some of them were located close to the cluster of samples with *E. coli*.

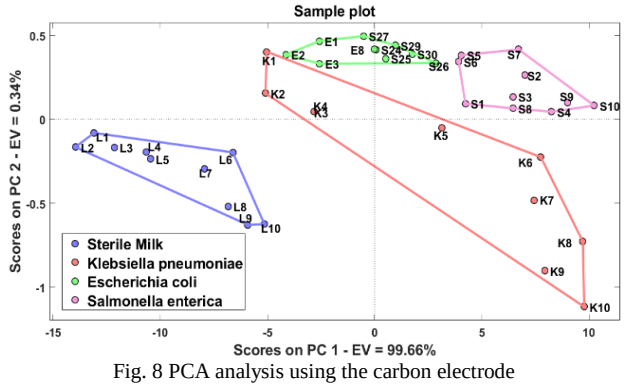

Fig. 8 PCA analysis using the carbon electrode

Fig. 9 illustrates the Boxplot graph that represents the class distribution using the carbon electrode (C110). The graph shows the differences between the classes of bacteria and sterile milk. In class 2 (*K. pneumoniae*) higher atypical values are observed, which leads to the dispersion of the samples.

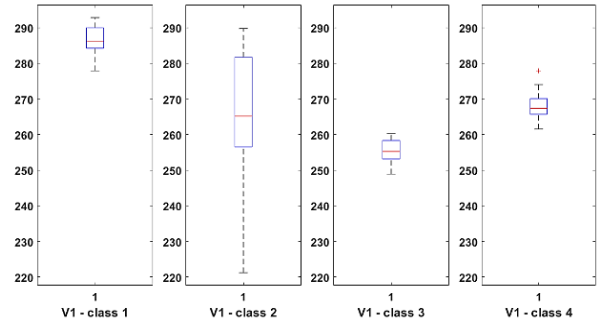

Fig. 9 Boxplot for the samples analysis through the carbon electrode.

The confusion matrix (Fig. 10) presents the result of the classification of the measurements using the LDA and K-NN classifiers with fine adjustment, which obtained success rates of 87.5% with the cross-validation method with k-fold = 5 and 10. It should be noted that the classification obtained for this case had 5 errors compared to the E-nose where only 2 unclassified samples were obtained. In this case, the *E.coli* bacteria also overlapped with the other two bacteria.

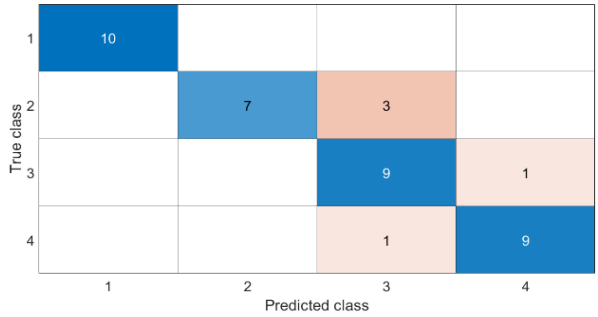

Fig. 10 Confusion matrix obtained from LDA and k-NN algorithms by using the C110 electrode for the bacteria classification in milk.

Finally, we can mention that the use of the E-tongue produced satisfactory results, with both the carbon and gold sensors despite the dispersion observed in the samples contaminated with *K. pneumoniae*; if we compare the two sensors, the use of the gold sensor produced slightly more consistent results and with a distribution more in line with what was observed when using the E-nose.

D. Discrimination of the E.coli concentration in milk

Taking into account the use of *E. coli* as an indicator microorganism of food quality, the ability of E-nose and E-tongue to discriminate different levels of *E. coli* contamination in pasteurized milk was verified. The results obtained from the measurements acquired and the different data processing methods that were applied for the discrimination and classification of contaminated samples and sterilized milk, are described below.

-E-nose

The measurement discrimination using the PCA analysis and the pre-processing method "autoscaling" with the data obtained by the E-nose, are shown in Fig. 11. Each of the concentrations was labeled with a letter from A to I. The letter A indicates the lowest concentration (1x10-2 CFU/ml) and the letter I the highest ($1x10^6$ CFU/ml). The graph shows that all the concentration clusters are correctly separable by the algorithm, obtaining a variance in PC1 of 88.22%.

The results clearly show that all concentrations were recognized and separated without overlaps, they follow a sequential order depending on the contamination level. For instance, cluster I with a concentration of $1x10^6$ CFU/ml is at the top of the PCA graph; likewise, cluster J, which corresponds to sterile milk, is projected towards the right side of the graph, which makes it easily identifiable (Fig. 11). The organization of the graph makes it easier to discriminate between the concentrations of *E. coli* and sterile milk. It should be clarified that an "outlier" was detected in the H concentration ($1x10^5$ CFU/ml), therefore this measurement was eliminated from this group of measurements since during the sample collection process there were failures in the data acquisition.

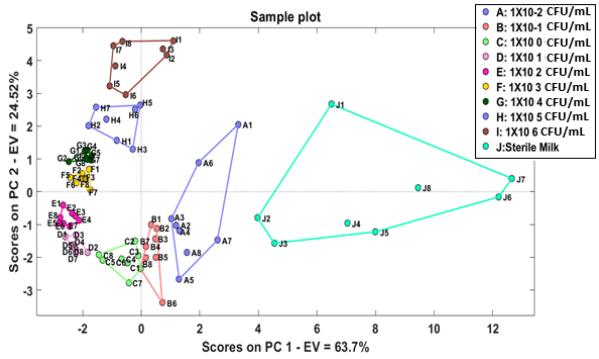

Fig. 11 Scores plot of PCA analysis for *E. coli* concentrations and sterile milk by using the E-nose.

Analyzing the distances projected in PC1, it can be observed that from cluster D to J there are greater differences when compared with the distances projected in PC2. In clusters E, F, G, H, I the behavior is inverse; however, the repeatability was better than that shown by the samples with low concentrations.

Once the original variables were analyzed with the PCA, it was observed that the TGS 831 sensor obtained the best information from the measurements and was able to differentiate all the groups of measurements. The boxplot graph (Fig. 12) shows the behavior of sensor 15 (TGS 831) in detecting samples with different concentrations of *E. coli* and sterile milk. It is observed that the group of samples J (Sterile milk) labeled as class 10 has a significant difference for the rest of the milk samples contaminated with *E. coli* since the distribution of the data is more atypical than the rest.

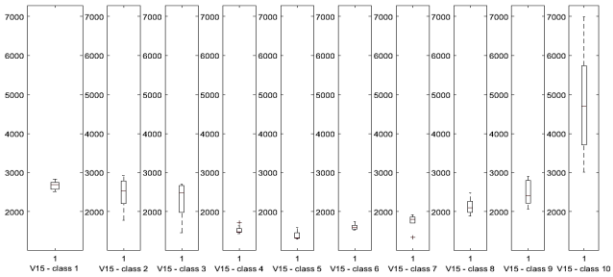

Fig. 12 Boxplot of contaminated milk concentrations with *E. coli* and sterile milk.

Fig. 13 shows the confusion matrix obtained with the SVM classifier with the Gaussian mean function, with which a percentage of success of 82.3% was obtained in the classification of the data set based on k-fold cross-validation (k = 5), which was applied to determine the accuracy of the classification method. The PCA scores were used as input to the algorithm, observing that the confusion matrix identified 14 measures that were not well classified. In the analysis of the confusion matrix, it is seen that four measures of class 6 ($1x10^3$ CFU/ml) were categorized as measures of class 7 ($1x10^4$ CFU/ml). This error in the classification may be owing to the similarity between the data of some samples and the variations in the spatial distribution of the bacteria in the suspension at the time of homogenizing and taking the volume to be analyzed.

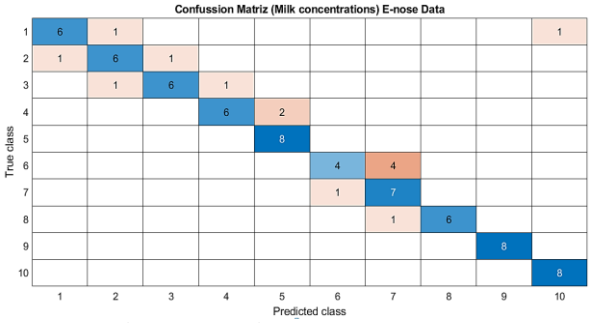

Fig. 13 Confusion matrix of contaminated milk concentrations with *E.coli* and sterile milk by using SMV classifier.

E-tongue

Gold sensor

Fig. 14 shows the projection of the measurements in the PCA graph in which the different concentrations were discriminated through the response of the gold electrode used for the detection of the samples and analysis with the device. Once the voltammetric (cyclic) signals were acquired through the potentiostat, they were normalized through the "Autoscaling" function, and later they were discriminated by the PCA method, obtaining a variance in PC1 of 92.2%. Therefore, between the first two main components, they added a total of 100% of the variance captured by the first two "scores", reaching a clear separation of the concentrations projected in PC1. Furthermore, it is observed that the concentrations do not overlap and follow a sequential order depending on the level of contamination. For instance, cluster I that represents the highest concentration was located at the opposite end of cluster A, which in turn corresponds to the lowest concentration. Likewise, cluster J that corresponds to sterilized milk is projected in the lower part of the PCA graph, which is easily identifiable. In this way, all the categories tested were clearly and correctly discriminated.

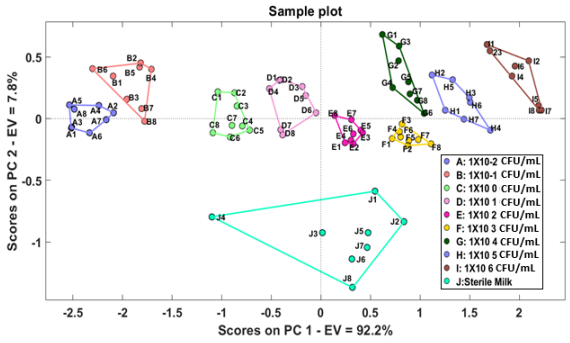

Fig. 14 Scores plot of PCA analysis for *E. coli* concentrations and sterile milk by using E-tongue (gold electrode).

The corresponding confusion matrix was performed to verify the accuracy of the k-NN algorithm since it obtained the best classification of 98.7% of the data set. For the validation of the responses of the k-NN classifier, the cross-validation with k-fold (k = 5) with fine adjustment was used, which was applied to determine the response of the classification method. The PCA scores were used as input to the algorithm and as a result, the confusion matrix identified only 2 misclassified measurements (J1 and J4) which were projected very close to categories C and E respectively (Fig. 15).

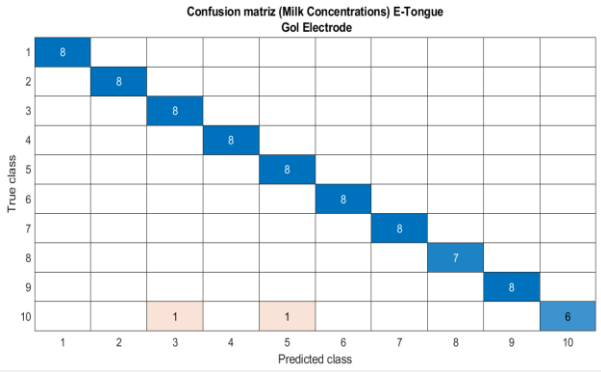

Fig. 15 Confusion matrix of contaminated milk concentrations with *E. coli* and sterile milk by using k-NN classifier. E-tongue (gold electrode).

Carbon sensor

The result of the PCA analysis obtained a variance between the two scores of 100%. In the PC1 score, a better representation of the different concentrations was obtained and it can be observed how cluster J (sterile milk) is projected from PC2, providing a greater difference with the rest of the categories (Fig. 16). However, it is observed that at low concentrations (clusters A, B, and C) the ability of the sensor to discriminate is not as good as the gold sensor.

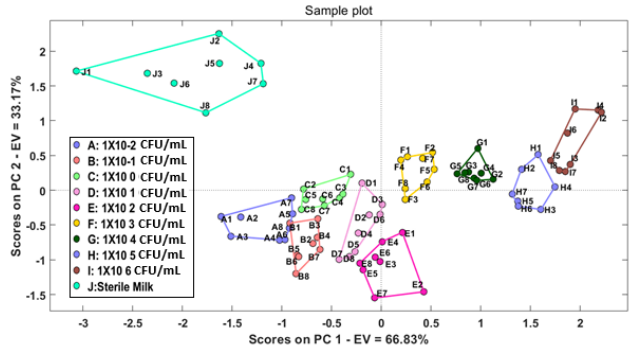

Fig. 16 Scores plot of PCA analysis for *E.coli* concentrations and sterile milk by using E-tongue (carbon electrode).

To verify the above analysis, a confusion matrix was made in which the k-NN algorithm was applied with fine adjustment and a percentage of the success rate of 89.9% was obtained in the classification of the measurements. For the validation of the response of the k-NN classifier, the cross-validation with k-fold (k = 5) was used, which was applied to determine the response of the classification method. The PCA scores were used as inputs to the algorithm and the confusion matrix identified 8 misclassified measurements, 5 of which correspond to the lowest concentrations (Fig. 17).

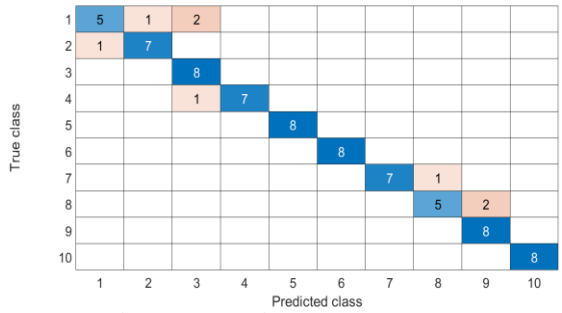

Fig. 17 Confusion matrix of contaminated milk concentrations with *E.coli* and sterile milk by using k-NN classifier and PCA scores.

When comparing the results obtained with both electrodes, it can be observed that the gold electrode offered a better response for the discrimination between the different concentrations of *E.coli* in pasteurized milk. This electrode showed an even better capacity than that observed for the E-nose since it allowed to classify and separate clearly and effectively all the categories tested. These results make it possible to project the use of these devices, not only for the detection of bacteria in milk but also for their quantification.

IV. Conclusions

Of the differentiation tests between bacteria of the Enterobacteriaceae family, promising results were obtained with the E-nose and the behavior of the TGS 826 sensor, since it was able to achieve 94.7% success in classifying the data set using the SVM algorithm with Gaussian mean. On the other hand, the electronic tongue with the gold sensor achieved a similar result, reaching a 92.5% success rate in the classification of bacteria. Although the carbon sensor did not present the best response, its application can be postulated in this type of analysis since the result of 87.5% of data classification promises that it can be used for other applications.

The best results to classify and discriminate milk samples contaminated with different concentrations of *E. coli* were achieved with the gold electrode of the E-tongue. The data processing carried out on the sample signals allowed classification of 98.7% of success through the k-NN algorithm with fine adjustment. These results show the great potential of this device for use in the detection and quantification of *E. coli* in pasteurized milk. Notwithstanding the above, the E-nose and the E-tongue with carbon electrode also produced good results, with 82.3% and 89.9% success respectively, for the classification of the different concentrations of *E. coli*.

Taking into account the usual limits required by the regulations on the presence of *E. coli* in milk, and despite the differences that may present between them, both the E-nose and the E-tongue showed high sensitivity to detect this bacteria in pasteurized milk. Both methodologies were able to classify and separate from the sterile milk, those samples whose concentrations were even lower than 0 CFU/ml. These results support the possibility of using this type of device for microbiological quality control since added to its sensitivity, they are quick response methods that would allow real-time monitoring of the product.

These promising results allow us to consider its possible use for the detection and quantification of *E. coli* in pasteurized milk. The performance shown by the tested devices was high, according to the requirements of the microbiological quality regulations of dairy products. The results obtained showed the potential that both multisensory systems have to be used as microbiological control tools in the dairy industry and with the potential to quantify the presence of different kinds of bacteria such as *E.coli*.

On the other hand, the next steps and future work would be to create a product to be applied to the market.

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References

[1] M. L. Rodríguez Méndez, Electronic Noses and Tongues in Food Science. Elsevier Inc., 2016.
[2] P. Ciosek, “Milk and Dairy Products Analysis by Means of an Electronic Tongue,” in Electronic Noses and Tongues in Food Science, Elsevier Inc., 2016, pp. 209–223.
[3] S. Raithore, J. Bai, A. Plotto, J. Manthey, M. Irey, and E. Baldwin, “Electronic Tongue Response to Chemicals in Orange Juice that Change Concentration in Relation to Harvest Maturity and Citrus Greening or

Huanglongbing (HLB) Disease," Sensors, vol. 15, no. 12, pp. 30062–30075, Dec. 2015, doi: 10.3390/s151229787.
[4] Z. Wei, J. Wang, and X. Zhang, "Monitoring of quality and storage time of unsealed pasteurized milk by voltammetric electronic tongue," Electrochimica Acta, vol. 88, pp. 231–239, Jan. 2013, doi: 10.1016/j.electacta.2012.10.042.
[5] L. A. Dias, A. M. Peres, A. C. A. Veloso, F. S. Reis, M. Vilas-Boas, and A. A. S. C. Machado, "An electronic tongue taste evaluation: Identification of goat milk adulteration with bovine milk," Sensors and Actuators, B: Chemical, vol. 136, no. 1, pp. 209–217, 2009, doi: 10.1016/j.snb.2008.09.025.
[6] I. Tazi, K. Triyana, D. Siswanta, A. C. A. Veloso, A. M. Peres, and L. G. Dias, "Dairy products discrimination according to the milk type using an electrochemical multisensor device coupled with chemometric tools," Journal of Food Measurement and Characterization, vol. 12, no. 4, pp. 2385–2393, Dec. 2018, doi: 10.1007/s11694-018-9855-8.
[7] P. Ciosek and W. Wróblewski, "Miniaturized electronic tongue with an integrated reference microelectrode for the recognition of milk samples," Talanta, vol. 76, no. 3, pp. 548–556, Jul. 2008, doi: 10.1016/j.talanta.2008.03.051.
[8] I. Tazi, K. Triyana, D. Siswanta, A. C. A. Veloso, A. M. Peres, and L. G. Dias, "Dairy products discrimination according to the milk type using an electrochemical multisensor device coupled with chemometric tools," Journal of Food Measurement and Characterization, vol. 12, no. 4, pp. 2385–2393, 2018, doi: 10.1007/s11694-018-9855-8.
[9] Z. Wei, W. Zhang, Y. Wang, and J. Wang, "Monitoring the fermentation, post-ripeness and storage processes of set yogurt using voltammetric electronic tongue," Journal of Food Engineering, vol. 203, pp. 41–52, Jun. 2017, doi: 10.1016/j.jfoodeng.2017.01.022.
[10] M. Ghasemi-Varnamkhasti, C. Apetrei, J. Lozano, and A. Anyogu, "Potential use of electronic noses, electronic tongues and biosensors as multisensor systems for spoilage examination in foods," Trends in Food Science and Technology, vol. 80. Elsevier Ltd, pp. 71–92, Oct. 01, 2018, doi: 10.1016/j.tifs.2018.07.018.
[11] M. Anderson, P. Hinds, S. Hurditt, P. Miller, D. McGrowder, and R. Alexander-Lindo, "The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country," Asian Pacific Journal of Tropical Biomedicine, vol. 1, no. 3, pp. 205–211, 2011, doi: 10.1016/S2221-1691(11)60028-2.
[12] S. P. Oliver, B. M. Jayarao, and R. A. Almeida, "Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications," Foodborne Pathogens and Disease, vol. 2, no. 2. Mary Ann Liebert, Inc. 2 Madison Avenue Larchmont, NY 10538 USA , pp. 115–129, Jun. 01, 2005, doi: 10.1089/fpd.2005.2.115.
[13] J. Hu et al., "Rapid analysis of Escherichia coli O157:H7 using isothermal recombinase polymerase amplification combined with triple-labeled nucleotide probes," Molecular and Cellular Probes, vol. 50, p. 101501, Apr. 2020, doi: 10.1016/j.mcp.2019.101501.
[14] X. Zhao, C. W. Lin, J. Wang, and D. H. Oh, "Advances in rapid detection methods for foodborne pathogens," Journal of Microbiology and Biotechnology, vol. 24, no. 3. Korean Society for Microbiology and Biotechnology, pp. 297–312, Mar. 28, 2014, doi: 10.4014/jmb.1310.10013.
[15] C. Adley, "Past, Present and Future of Sensors in Food Production," Foods, vol. 3, no. 3, pp. 491–510, Aug. 2014, doi: 10.3390/foods3030491.
[16] E. A. Baldwin, J. Bai, A. Plotto, and S. Dea, "Electronic Noses and Tongues: Applications for the Food and Pharmaceutical Industries," Sensors, vol. 11, no. 5, pp. 4744–4766, May 2011, doi: 10.3390/s110504744.
[17] M. E. Fleming-Jones and R. E. Smith, "Volatile Organic Compounds in Foods: A Five Year Study," Journal of Agricultural and Food Chemistry, vol. 51, no. 27, pp. 8120–8127, Dec. 2003, doi: 10.1021/jf0303159.
[18] A. Poghossian, H. Geissler, and M. J. Schöning, "Rapid methods and sensors for milk quality monitoring and spoilage detection," Biosensors and Bioelectronics, vol. 140. Elsevier Ltd, p. 111272, Sep. 01, 2019, doi: 10.1016/j.bios.2019.04.040.
[19] M. Ghasemi-Varnamkhasti, C. Apetrei, J. Lozano, and A. Anyogu, "Potential use of electronic noses, electronic tongues and biosensors as multisensor systems for spoilage examination in foods," Trends in Food Science and Technology, vol. 80. Elsevier Ltd, pp. 71–92, Oct. 01, 2018, doi: 10.1016/j.tifs.2018.07.018.
[20] I. Casalinuovo, D. di Pierro, M. Coletta, and P. di Francesco, "Application of Electronic Noses for Disease Diagnosis and Food Spoilage Detection," Sensors, vol. 6, no. 11, pp. 1428–1439, Nov. 2006, doi: 10.3390/s6111428.
[21] M. Peris and L. Escuder-Gilabert, "Electronic noses and tongues to assess food authenticity and adulteration," Trends in Food Science and Technology, vol. 58, pp. 40–54, 2016, doi: 10.1016/j.tifs.2016.10.014.
[22] M. Peris and L. Escuder-Gilabert, "Electronic noses and tongues to assess food authenticity and adulteration," Trends in Food Science and Technology, vol. 58. Elsevier Ltd, pp. 40–54, Dec. 01, 2016, doi: 10.1016/j.tifs.2016.10.014.
[23] M. T. Kalit, K. Marković, S. Kalit, N. Vahčić, and J. Havranek, "Primjena elektronskog nosa i elektronskog jezika u mljekarskoj industriji," Mljekarstvo, vol. 64, no. 4, pp. 228–244, 2014, doi: 10.15567/mljekarstvo.2014.0402.
[24] H. Shi, M. Zhang, and B. Adhikari, "Advances of electronic nose and its application in fresh foods: A review," Critical Reviews in Food Science and Nutrition, vol. 58, no. 16. Taylor and Francis Inc., pp. 2700–2710, Nov. 02, 2018, doi: 10.1080/10408398.2017.1327419.
[25] R. Banerjee (Roy), B. Tudu, R. Bandyopadhyay, and N. Bhattacharyya, Application of Electronic Nose and Tongue for Beverage Quality Evaluation. Elsevier Inc., 2019.
[26] R. Needham, J. Williams, N. Beales, P. Voysey, and N. Magan, "Early detection and differentiation of spoilage of bakery products," in Sensors and Actuators, B: Chemical, Apr. 2005, vol. 106, no. 1 SPEC. ISS., pp. 20–23, doi: 10.1016/j.snb.2004.05.032.
[27] J. Carrillo-Gómez, C. Durán-Acevedo, and R. García-Rico, "Concentration Detection of the *E. coli* Bacteria in Drinking Water Treatment Plants through an E-Nose and a Volatiles Extraction System (VES)," Water, vol. 11, no. 4, p. 774, Apr. 2019, doi: 10.3390/w11040774.
[28] A. Sanaeifar, H. ZakiDizaji, A. Jafari, and M. de la Guardia, "Early detection of contamination and defect in foodstuffs by electronic nose: A review," TrAC - Trends in Analytical Chemistry, vol. 97, pp. 257–271, 2017, doi: 10.1016/j.trac.2017.09.014.
[29] Z. Ali, W. T. O'Hare, and B. J. Theaker, "Detection of bacterial contaminated milk by means of a quartz crystal microbalance based electronic nose," Journal of Thermal Analysis and Calorimetry, vol. 71, no. 1, pp. 155–161, 2003, doi: 10.1023/A:1022274419166.
[30] H. Yu, J. Wang, and Y. Xu, "Identification of Adulterated Milk Using Electronic Nose," 2007. Accessed: Jul. 09, 2020. [Online].
[31] N. Magan, A. Pavlou, and I. Chrysanthakis, "Milk-sense: A volatile sensing system recognizes spoilage bacteria and yeasts in milk," Sensors and Actuators, B: Chemical, vol. 72, no. 1, pp. 28–34, Jan. 2001, doi: 10.1016/S0925-4005(00)00621-3.
[32] F. Korel and M. O. Balaban, "Microbial and Sensory Assessment of Milk with an Electronic Nose," Journal of Food Science, vol. 67, no. 2, pp. 758–764, Mar. 2002, doi: 10.1111/j.1365-2621.2002.tb10672.x.
[33] M. Tohidi, M. Ghasemi-Varnamkhasti, V. Ghafarinia, M. Bonyadian, and S. S. Mohtasebi, "Development of a metal oxide semiconductor-based artificial nose as a fast, reliable and non-expensive analytical technique for aroma profiling of milk adulteration," International Dairy Journal, vol. 77, pp. 38–46, Feb. 2018, doi: 10.1016/j.idairyj.2017.09.003.
[34] Y. Vlasov, A. Legin, A. Rudnitskaya, C. di Natale, and A. D'Amico, "Nonspecific sensor arrays ('electronic tongue') for chemical analysis of liquids: (IUPAC technical report)," in Pure and Applied Chemistry, Nov. 2005, vol. 77, no. 11, pp. 1965–1983, doi: 10.1351/pac200577111965.
[35] E. A. Baldwin, J. Bai, A. Plotto, and S. Dea, "Electronic noses and tongues: Applications for the food and pharmaceutical industries," Sensors, vol. 11, no. 5. Multidisciplinary Digital Publishing Institute (MDPI), pp. 4744–4766, May 2011, doi: 10.3390/s110504744.

[36] R. S. Latha and P. K. Lakshmi, “Electronic tongue: An analytical gustatory tool,” Journal of Advanced Pharmaceutical Technology and Research, vol. 3, no. 1. Wolters Kluwer -- Medknow Publications, pp. 3–8, Jan. 2012, doi: 10.4103/2231-4040.93556.
[37] M. Yu. Vagin, M. Eriksson, and F. Winquist, “Drinking Water Analysis Using Electronic Tongues,” Electronic Noses and Tongues in Food Science, pp. 255–264, Jan. 2016, doi: 10.1016/B978-0-12-800243-8.00025-1.
[38] A. Wadehra and P. S. Patil, “Application of electronic tongues in food processing,” Analytical Methods, vol. 8, no. 3. Royal Society of Chemistry, pp. 474–480, Jan. 21, 2016, doi: 10.1039/c5ay02724a.
[39] H. Jiang, M. Zhang, B. Bhandari, and B. Adhikari, “Application of electronic tongue for fresh foods quality evaluation: A review,” Food Reviews International, vol. 34, no. 8. Taylor and Francis Inc., pp. 746–769, Nov. 17, 2018, doi: 10.1080/87559129.2018.1424184.
[40] W. Wang and Y. Liu, “Electronic tongue for food sensory evaluation,” in Evaluation Technologies for Food Quality, Elsevier, 2019, pp. 23–36.
[41] T. Titova and V. Nachev, “‘Electronic tongue’ in the Food Industry,” Food Science and Applied Biotechnology, vol. 3, no. 1, p. 71, 2020, doi: 10.30721/fsab2020.v3.i1.74.
[42] Z. Wei, J. Wang, and X. Zhang, “Monitoring of quality and storage time of unsealed pasteurized milk by voltammetric electronic tongue,” Electrochimica Acta, vol. 88, pp. 231–239, Jan. 2013, doi: 10.1016/j.electacta.2012.10.042.
[43] A. Poghossian, H. Geissler, and M. J. Schöning, “Rapid methods and sensors for milk quality monitoring and spoilage detection,” Biosensors and Bioelectronics, vol. 140. Elsevier Ltd, p. 111272, Sep. 01, 2019, doi: 10.1016/j.bios.2019.04.040.
[44] L. Bueno, W. de Araujo, M. Salles, M. Kussuda, and T. Paixão, “Voltammetric Electronic Tongue for Discrimination of Milk Adulterated with Urea, Formaldehyde and Melamine,” Chemosensors, vol. 2, no. 4, pp. 251–266, Nov. 2014, doi: 10.3390/chemosensors2040251.
[45] H. Singh et al., “Metal oxide SAW E-nose employing PCA and ANN for the identification of binary mixture of DMMP and methanol,” Sensors and Actuators, B: Chemical, vol. 200, pp. 147–156, Sep. 2014, doi: 10.1016/j.snb.2014.04.065.
[46] A. D. Wilson, “Advances in electronic-nose technologies for the detection of volatile biomarker metabolites in the human breath,” Metabolites, vol. 5, no. 1. MDPI AG, pp. 140–163, Mar. 02, 2015, doi: 10.3390/metabo5010140.
[47] M. R. Mendez, “Electronic Noses and Tongues in Food Science - 1st Edition,” 2016. https://www.elsevier.com/books/electronic-noses-and-tongues-in-food-science/rodriguez-mendez/978-0-12-800243-8 (accessed Jul. 23, 2020).
[48] I. Jolliffe, “Principal Component Analysis,” in International Encyclopedia of Statistical Science, Berlin, Heidelberg: Springer Berlin Heidelberg, 2011, pp. 1094–1096.
[49] L. Xu, X. Yu, L. Liu, and R. Zhang, “A novel method for qualitative analysis of edible oil oxidation using an electronic nose,” Food Chemistry, vol. 202, pp. 229–235, Jul. 2016, doi: 10.1016/j.foodchem.2016.01.144.
[50] K. Mahmodi, M. Mostafaei, and E. Mirzaee-Ghaleh, “Detection and classification of diesel-biodiesel blends by LDA, QDA and SVM approaches using an electronic nose,” Fuel, vol. 258, p. 116114, Dec. 2019, doi: 10.1016/j.fuel.2019.116114.
[51] S. Zhang, C. Xie, C. Fan, Q. Zhang, and Q. Zhan, “An alternate method of hierarchical classification for E-nose: Combined Fisher discriminant analysis and modified Sammon mapping,” Sensors and Actuators, B: Chemical, vol. 127, no. 2, pp. 399–405, Nov. 2007, doi: 10.1016/j.snb.2007.04.045.
[52] N. Cristianini and J. Shawe-Taylor, An Introduction to Support Vector Machines and Other Kernel-based Learning Methods. Cambridge University Press, 2000.
[53] K. Brudzewski, S. Osowki, and T. Markiewicz, “Classification of milk by means of an electronic nose and SVM neural network,” Sensors and Actuators, B: Chemical, vol. 98, no. 2–3, pp. 291–298, Mar. 2004, doi: 10.1016/j.snb.2003.10.028.
[54] O. S. Papadopoulou, C. C. Tassou, L. Schiavo, G.-J. E. Nychas, and E. Z. Panagou, “Rapid Assessment of Meat Quality by Means of an Electronic Nose and Support Vector Machines,” Procedia Food Science, vol. 1, pp. 2003–2006, Jan. 2011, doi: 10.1016/j.profoo.2011.09.295.
[55] S. Sriramprakash, V. D. Prasanna, and O. V. R. Murthy, “Stress Detection in Working People,” in Procedia Computer Science, Jan. 2017, vol. 115, pp. 359–366, doi: 10.1016/j.procs.2017.09.090.
[56] J. Lozano, A. García, C. J. García, M. Aleixandre, J. P. Santos, and M. C. Horrillo, “RECONOCIMIENTO DE PATRONES BASADO EN REDES NEURONALES Y ANÁLISIS DE COMPONENTES INDEPENDIENTES PARA NARICES ELECTRÓNICAS.” Accessed: Jan. 13, 2021. [Online].
[57] J. Vitola, F. Pozo, D. Tibaduiza, and M. Anaya, “A Sensor Data Fusion System Based on k-Nearest Neighbor Pattern Classification for Structural Health Monitoring Applications,” Sensors, vol. 17, no. 2, p. 417, Feb. 2017, doi: 10.3390/s17020417.
[58] K. Anand and M. W. Griffiths, “Pathogens in Milk: Enterobacteriaceae,” in Encyclopedia of Dairy Sciences: Second Edition, Elsevier Inc., 2011, pp. 67–71.
[59] B. Hu et al., “Structural and genetic evidence for the close relationship between Escherichia coli O71 and Salmonella enterica O28 O-antigens,” FEMS Immunology & Medical Microbiology, vol. 59, no. 2, pp. 161–169, Jul. 2010, doi: 10.1111/j.1574-695X.2010.00676.x.