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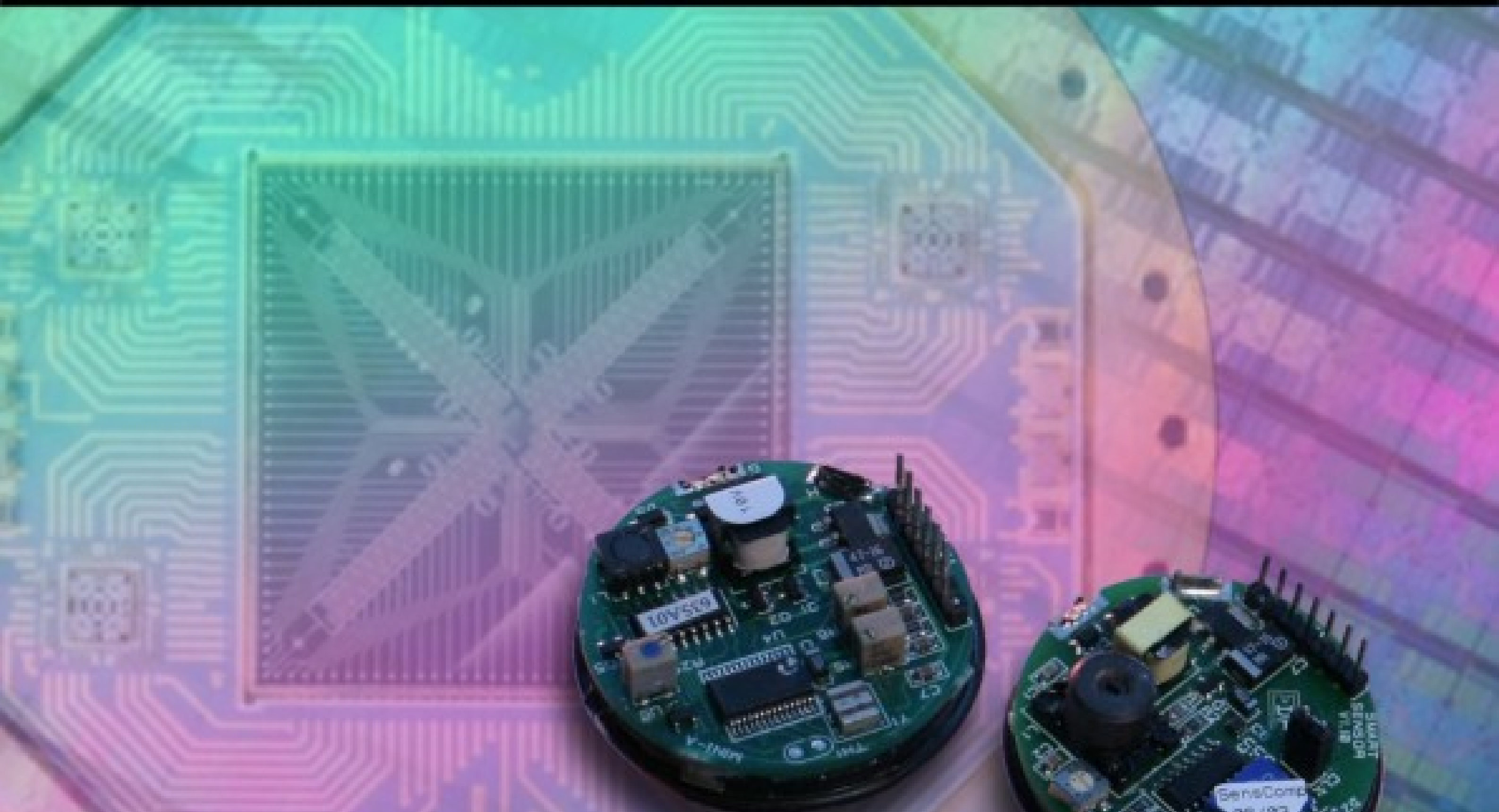

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Conception and Development of a Portable Electronic Nose System for Classification of Raw Milk Using Principal Component Analysis Approach

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Abstract: The analysis of the aroma of milk is an especially complex problem due to the heterogeneous nature of milk. In the present study, a portable electronic nose has been fabricated and characterized using an oxide semiconductor gas sensor array. The portable electronic nose system, based on Taguchi Gas Sensors (TGS), consists of a microcontroller PIC16F877 as CPU, an LCD for displaying gas conductance and a LabVIEW© PC interface for data acquisition, etc. To check its separation capability a pattern recognition method namely Principal Component Analysis (PCA) has been performed. The PCA method permits a good classification between three types of raw milks from different dairy farms. On the other hand, the data coming from the response of the sensors have been elaborated by PCA and Support Vectors Machines (SVMs) in order to obtain a classification of the data clusters related to different milk ageing days and so track the dynamic evolution of milk rancidity. It was found that the portable electronic nose system together with a pattern recognition technique, PCA or SVMs is able to show a characteristic development of the milk quality, when it is stored at 4 °C, dependent on storage time. The last section draws the evaluation of the hygienic quality of raw milk, the following microbiological counts were determined: yeast and mould counts, total coliform count and total aerobic mesophilic flora count. *Copyright © 2009 IFSA.*

Keywords: Electronic nose, Principal component analysis, Support vector machines, Milk quality, Raw cows' milk

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1. Introduction

One of the most important technological steps in the production of dairy products is the quality control of starting milk. The chemical analysis of flavours in dairy products is complicated by the heterogeneous nature of milk. The control of milk quality and freshness is of increasing interest for both the consumer and the beverages industries. The profile of the volatile components of milk and dairy products is performed today by gas chromatography-mass spectrometry (GC-MS) [1, 2] coupled with highly efficient systems of extraction, such as dynamic or static headspace. These Classical analytical techniques are, however, time-consuming, expensive, and laborious, which can hardly be done on-site or on-line [3].

It has been shown that a new system, named “electronic nose” can be used as a powerful method to control the quality of food in some specific applications [4-10]. This innovative system consists of a multi-sensors array capable to monitor the interaction with specific molecules as a consequence of adsorption/desorption processes occurring on the surface of the sensors. A suitable electronics and software allow to perform quantitative and qualitative analysis of a mixture of gases using headspace analysis method. Static headspace analysis involves sampling air, equilibrated above a milk sample and injecting into a gas analytical instrument (into sensor’s chamber of the electronic nose system) for classification and identification. Dynamic headspace analysis requires a carrier gas in the sampling of volatiles above a milk sample.

In the work reported here, a portable electronic nose system has been fabricated and characterized using an oxide semiconductor gas sensor array. The employed sensor array consists of six different available Taguchi gas sensors (TGS). The data coming from the response of the sensors have been elaborated by principal component analysis (PCA) and support vectors machines (SVMs). The aim of this work was to explore the capabilities of a simple electronic nose device to discriminate among raw milks of three different dairy farms. And also to perform a characterization of one raw milk in different ageing days, simple PCA algorithm was used to process the data and to follow the evolution of the data clusters related to different ageing days. SVMs have been used for the recognition of different storage days of raw milk.

2. Experimental

2.1. Milk Samples

The raw cows’ milks used in this study were collected from three different dairy farms around the city of Meknes (Morocco) in the cool season (April 2007). Fresh samples were, immediately after receiving, placed in plastic bottles (1.5 L/bottle) and introduced in a refrigerator kept at a constant temperature of 4 °C to be analyzed at 1, 2, 3, and 4 days of storage.

2.2. Description of the Developed Portable Electronic Nose

The electronic nose used has been home-fabricated and home-developed for milk aroma purposes. The developed system is mainly composed of three parts: E-nose case, sampling vessel, and laptop PC equipped with a software acquisition of data. The E-nose case includes sensors cell, sensors array and a programmable microcontroller.

2.2.1. Sensors Array

The sensors of the electronic nose are assembled in an array. The array is normally a small electronic unit that integrates the different sensors into a practical circuit card that is easy to insert into the electronic nose instrument. The resistive gas sensors we used are of MOS (Metal-Oxide Semiconductor) such as tin dioxide. The sensors array comprised six TGS 8XX (with XX= 15, 21, 22, 24, 25 and 42), a relative humidity sensor (HIH4000-01) and a temperature sensor (LM335Z) from National Semiconductor are used to monitor the conditions of the experiment [9, 10]. The inside arrangement of components of the sensing module is shown in Fig. 1.

Fig. 1. Photo of TGS sensors array.

2.2.2. Sampling Vessel and Measurement Rig

The sampling system consists of a dynamic headspace sampling. For the measurement a volume of 100 mL of milk in a 200 mL vial kept at the temperature of 35 °C, was stripped by means of the 1000 sccm nitrogen flow for 10 mn. In this way, the volatile compounds were directly transferred by the carrier gas into the sensors chamber. The vial had two small holes in their covers to allow the headspace to be analyzed with the electronic nose equipment. Each time that a new set of milk was analyzed, new glass vials were used.

2.2.3. Microcontroller Circuit

The developed portable electronic nose system consists of a microcontroller PIC16F877 as CPU, an LCD. Fig. 2 gives the electronics circuit for the microcontroller used. For each measurement the response of sensor arrays was converted with a 10-bits ADC implemented in the microcontroller. An LCD is used for displaying gas conductance, relative humidity and temperature.

2.2.4. Data Acquisition

Data acquisition and device control are accomplished using a PIC16F877 microcontroller with a software programme running on the laptop PC. The Laptop communicates via RS232 with the microcontroller electronics. Fig. 3 depicts a schematic view of the portable electronic nose measurements. There is a software programme running on the laptop, which controls the electronics and acquires data from the sensor operations (Fig. 4). This software was developed using LabVIEW© software (National Instruments Inc.).

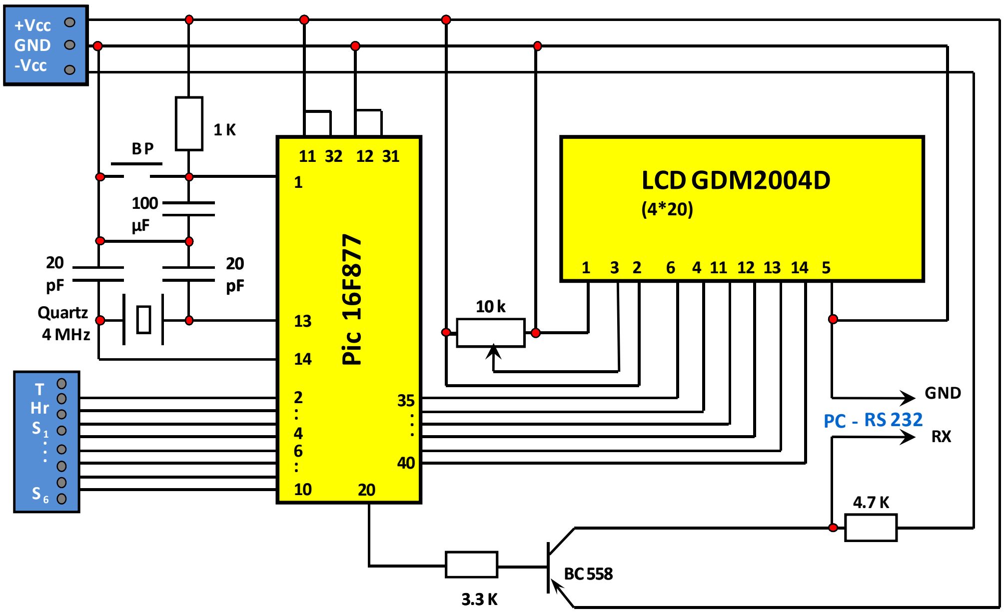

Fig. 2. Circuital schematics of the microcontroller-LCD.

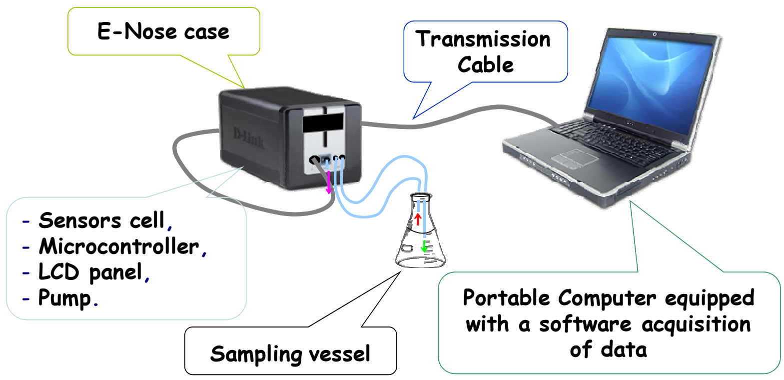

Fig. 3. Schematic representation of the portable electronic nose system.

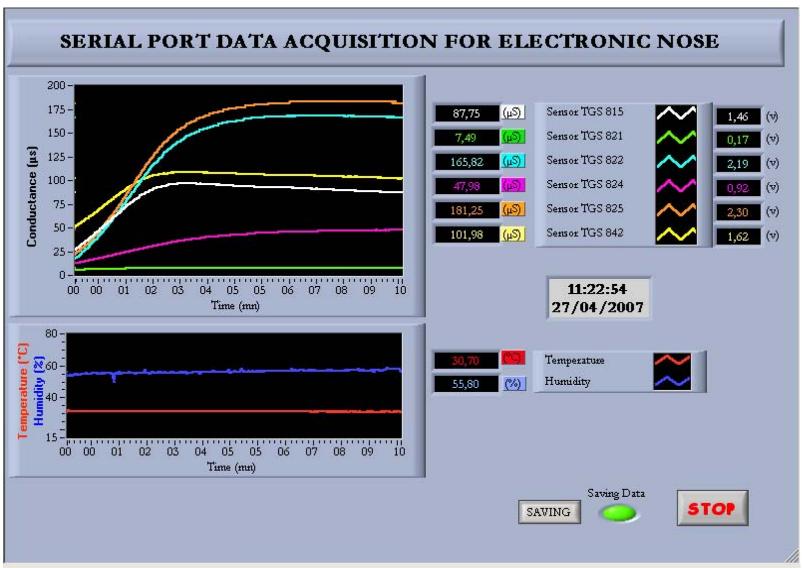

Fig. 4. Typical LabVIEW© PC interface for the electronic nose system.

2.2.5. Data Analysis

The use of PCA to assess clustering within the datasets is discussed bellow. The objective of using these methods was to establish classes according to the states of rancidity of milk. PCA is a powerful, linear, and 'unsupervised' pattern recognition technique that has been shown to be effective for discriminating between the responses of an electronic nose to simple and complex odours [11]. PCA decomposes the primary data matrix by projecting the multidimensional data onto a new coordinate base formed by the orthogonal directions with data maximum variance. The eigenvectors of the data matrix are called Principal Components (PC) and they are uncorrelated among them. The principal components are ordered so that the first one displays the greatest amount of variance, followed by the next greatest and so on.

Support vector machines approach was also used, as a supervised learning technique, for discriminate analysis. SVMs have demonstrated to be a powerful learning method [12] and its use in the electronic nose field is getting more importance [13]. SVMs were originally designed for binary classification. Currently there are two types of approaches for multi-class SVM. One is by constructing and combining several binary classifiers "one-against-one or one-against-all methods", while the other is by directly considering all data in one optimization formulation [14]. The direct approach consists to construct a classifier recognizing the set of the classes: the determination of the hyper plane between these different classes permits to choose a class among the k when a new input is presented.

In our case, we have applied the one-against-all strategy to recognize the milk storage day; the "leave-one-out" validation procedure has been adopted for all the SVMs analysis. All the evaluations have been performed in Matlab 5.2.

3. Results and Discussion

3.1. Electronic Nose Responses and Signal Analysis

Fig. 5 shows the evolution of the signals generated by the 822 TGS sensor during the measurement of raw milk number 2 (dairy farm number 2) for all storage days. We show the same behaviour for all the other sensors. Each line represents the average signal variation of all milk samples measured in the same day respectively for each sensor of the array.

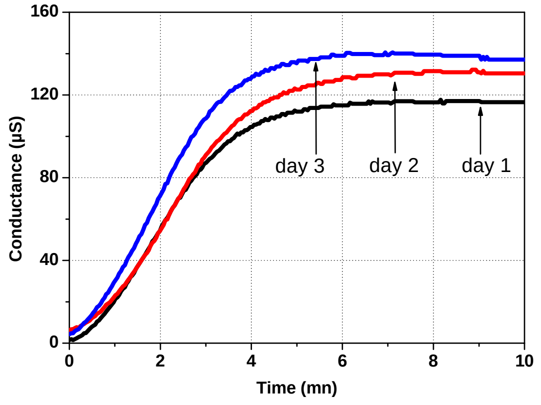

Fig. 5. Time conductance evolution of the TGS 822 sensor during storage days.

The evolution in sensor conductance can be directly attributed to the increasing number of storage days of milk (day 1, day 2 and day 3) and the effect of sensor drift can be ignored. This behaviour can be justified by an increase in the concentration of volatile gases given out by milks as a function of storage time. It can be shown that the sensor signals reach a plateau at the longest time. The measurement procedure was as follows: data acquisition started 30 sec before the transferring the volatile compounds by the carrier gas into the sensors chamber. This allowed for the baseline conductance of the sensors to be acquired. Acquisition ended after 10 mn and the sensor chamber was flushed with nitrogen air. The features extracted from the response of each sensor in the array are:

• G_0: the initial conductance of a sensor calculated as the average value of its conductance during the first 30 second of a measurement.

• G_s: the steady-state conductance calculated as the average value of its conductance during the last 2 min of a measurement.

These two features were extracted from the response of each sensor. Since there were 6 sensors within the array, each measurement was described by 12 features.

3.2. Characteristic Parameter Evolution

Fig. 6 shows the evolution of maximum conductance change as a characteristic parameter. We notice that the 822 and 825 TGS sensors showed the highest responses of the sensors of all the samples. However, the response of the sensors changes with storage days. It is shown that this characteristic parameter increases with storage days.

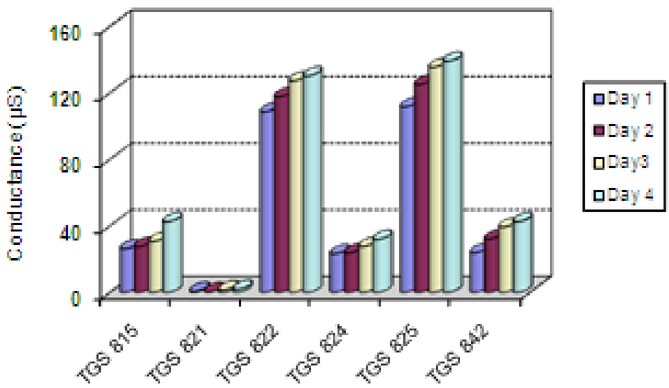

Fig. 6. Time variation of the maximum conductance change.

3.3. Classification of Raw Milks Using PCA Analysis

This experiment was devoted to study a dynamic evolution of the clusters related to different degrees of rancidity during a fixed time. In order to see whether the portable electronic nose was able to distinguish between different spoilage states, a PCA analysis was applied to the database [15, 16]. The analysis was done using all the responses that were obtained in the different measurements for milk number 2.

A mean centring pre-processing technique was applied to the response matrix. With PCA, the three first principal components allow us to clearly represent 99.31 % of the information in the database. Fig. 7 report, in three dimensional plot (PC1-PC2-PC3), the data coming from the measurements collected during a period of 4 days for raw milk no. 2. From the figure, it is quite evident as the 3D

PCA plot allows to well distinguishing the different days in which the milk has different rancidity values. It is reflecting how PCA analysis allows us to identify a preferential direction according to different of rancidity, giving an indication on the dynamic evolution of the system.

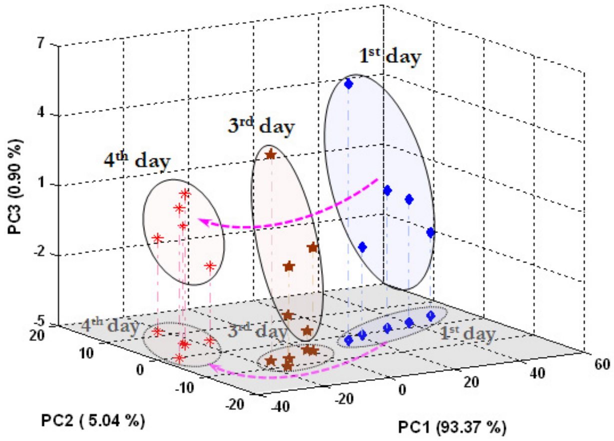

Fig. 7. PCA plot of raw milk no. 2 in different days.

3.4. Milk Ageing Analysis

In this study, we examine the second day of storage and we perform the same PCA analysis as described in Section 3.3. The first two components, PC1 and PC2, can be used to represent the 97.93 % of the data variance.

Fig. 8 shows the projections of the experimental results on a two-dimensional (2D) plane PC1-PC2. Samples can be grouped together in three different clusters. Each of these groups corresponds to 1st-2nd day, 3rd day and 4th day of storage, respectively. The 1st and 2nd day are not so distinctly separated, so they have similar properties and they are combined in one group. However, the 3rd and 4th days are sufficiently far from the other days so we can conclude that the DLC (date limit for consumption) of this raw milk, stored at 4 °C, is 2 days.

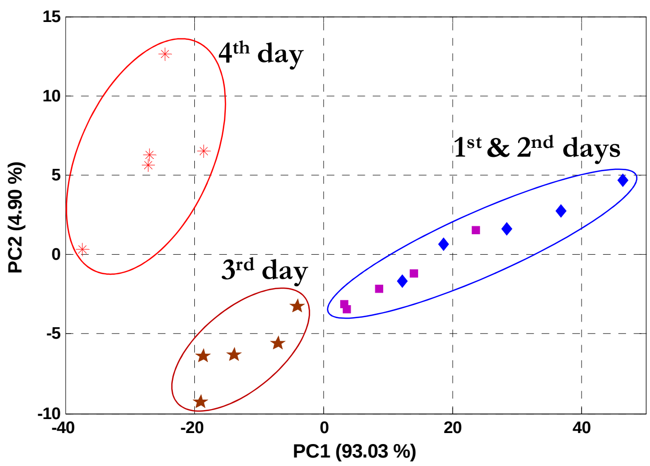

Fig. 8. PCA results in the PC1-PC2 plane for the analysis of milk at different storage days.

3.5. Evolution of Milk Quality with Time

The fact that samples appear ordered along the first principal component (PC1) according to the number of storage days is a significant result. The first principal component explains the main variance in the data (i.e., in the response of the sensors), and Fig. 8 shows that this variance is well correlated with the number of storage days. In other words, the sensor array employed seems appropriate to envisage an application where the main goal would be to predict the number of days of cold storage undergone by raw milk. A linear fit of the scores, whose equation is shown in Fig. 9, suggests that the value of the scores on the first PC could be used to predict the period of storage and, therefore, the decay in raw milk. These scores show a monotonic decrease during the period of storage.

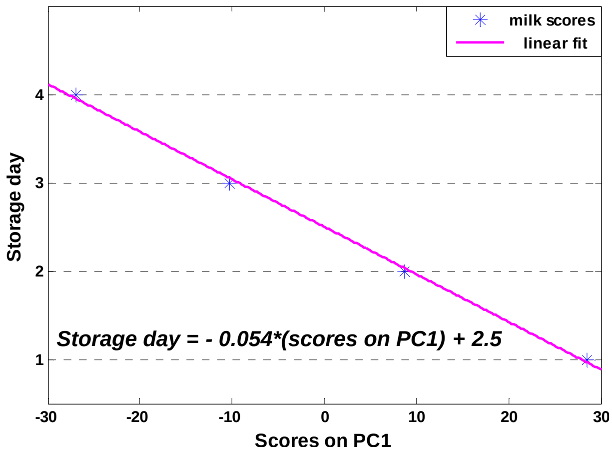

Fig. 9. Evolution of the scores on the first principal component with the period of storage and linear fitting.

3.6. Support Vector Machines Analysis

A SVMs one-against-all classification method was applied to develop the classifier model for our portable electronic nose. As in the PCA, 12 response features from the sensor array are used as inputs to the SVMs. Second-order of radial basis function (RBF) kernel are used to project the training data to a space that maximizes the margin hyper plane. The optimal regularization parameter of the SVM was set to C = Inf.

The performance of the SVM model is evaluated using a leave-one-out cross-validation method. In the first, autoscale pre-processing technique was applied to SVM dataset. Four binary classification models were performed (i.e., four groups). Then the output of SVM classifier was calculated with the largest voting.

Table 1. SVM classification results.

Predicted	**Actual**			
	Class 1	**Class 2**	**Class 3**	**Class 4**
Class 1	5	1	0	0
Class 2	0	4	0	0
Class 3	0	0	5	0
Class 4	0	0	0	5

The SVM reached a 95 % success rate in the recognition the four days of milk storage. Table 1 shows the confusion matrix of the SVM classifier. Columns indicate true values and rows, predicted categories. As it can be noticed in this table, one mistake is signaled: one measurement belonging to class 2 (i.e., second storage days) was misclassified as belonging to class 1 (i.e., first storage days). The SVM method gives a good generation.

3.7. Discrimination Among Three Types of Raw Milk Using PCA

In this case, PCA of the data was performed to determine the capability of the prototype system to discriminate between three types of raw milk from three different dairy farms. The dataset here was pre-processed using a standard mean centring approach. A good separation was obtained between the three clusters indicating three separate regions in the 3D space for the three types of raw milk. Fig. 10 shows the projections of the experimental results on a three-dimensional plot (3D).

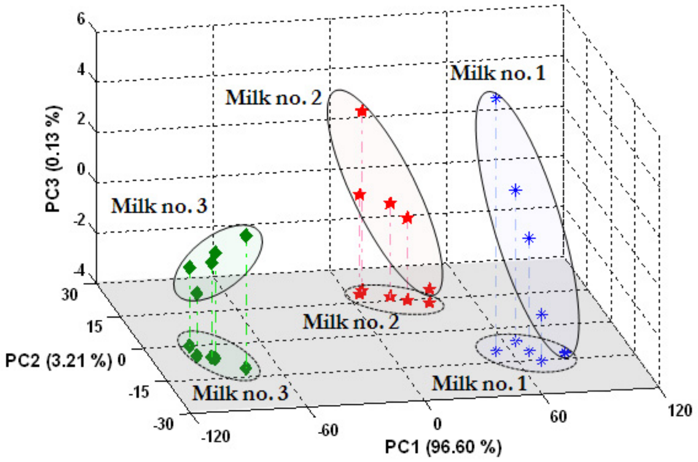

Fig. 10. PCA plot of different milks measured in the second day.

The three first principal components allow us to well represent 99.94 % of the information in the database. The data used in this study corresponding to the second storage day. So the 3D PCA plot allows us to well distinguish the three raw milks.

Winquist et al. [17] report also a correct separation between milks from different sources, and thus, also having different quality properties with a voltammetric electronic tongue. This system has been further developed for industrial applications, and due to the ruggedness and simplicity, this concept has already proven valuable in many applications. Studies have shown that this electronic tongue could be used to monitor quality changes of milk [18], recognize different microbial species [19], and for supervision of rinses in a washing machine [20].

Fig. 11 shows the validation results obtained with the PCA model. This stringent test of the ability of the array and e-nose to identify samples was made by using the data from the milk experiment (dataset 1) as a training set and the second data (dataset 2) as the “unknown” for identification. The new samples appear correctly distributed according to their dairy farms. These results prove that the electronic nose system leads to reproducible results and that it can be used as a screening tool to differentiate between the three types of raw milk.

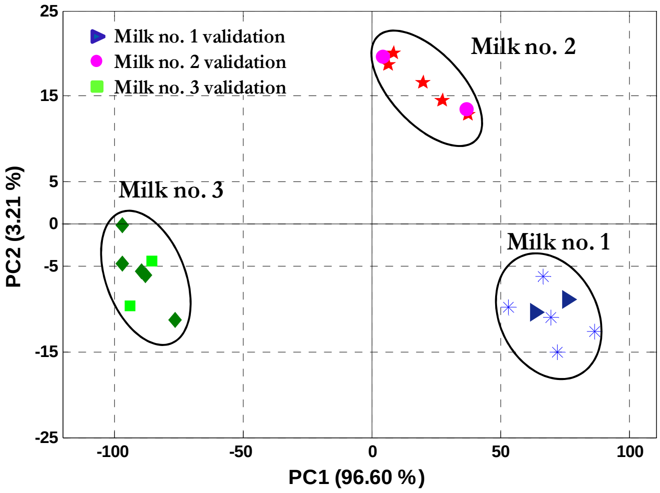

Fig. 11. Projection of the second set of measurements onto the PCA model built using the first set of measurements.

3.8. Microbiological Analysis

One of the most common tests is the viable cell count, the enumeration of viable microorganisms. It gives information on food quality, food spoilage and food safety. Even if they cannot be considered to be a "rapid method", they will be used due to their importance for routine food testing. Originally, these methods were introduced to perform total viable counts: yeast and mould, total coliform and total aerobic mesophilic flora counts. The objectives of the present study were to determine the prevalence of potentially pathogenic microorganisms that indicate the hygienic and sanitary conditions of raw milk samples [21-23].

Yeast and mould counts: The yeast and mould count method proposed by Andrews (1992) was used to enumerate the yeast and moulds of the samples. An agar medium was employed, in which organisms other than yeast and mould were inhibited by using acidified media. After incubation at 25 °C for 3-5 days, the colonies were counted [24].

Total coliform count: The coliform count method proposed by Collins et al. (1995) was used. Make serial tenfold dilution and do plate counts with violet red bile lactone agar. Incubate at 30 °C for 24 hours. Counts only red colonies that are 0.5 mm in diameter or larger [24].

Total aerobic mesophilic flora count: The method of aerobic plate count proposed by Andrews (1992) was followed to determine the total colony count of the samples. A series of dilutions of the samples (10^{-1} and 10^{-2}) was made and 1 mL dilution mixed with aerobic plate agar in Petri dishes. After incubation at 37 °C for 48 hours, the colonies were counted [24].

The bacterial growth on the raw milk no. 2 stored at 4 °C is presented in Fig. 12. This figure shows the evolution of log10 (cfu mL^{-1}) of total coliform, total aerobic mesophilic flora, and yeast and mould counts developed in raw milk as a function of the days of cold storage. The result of this work showed a big difference in microbial counts between the 2 first days and the 3rd - 4th days (Fig. 12). This is in agreement with the results found by using PCA analysis (section 3.4).

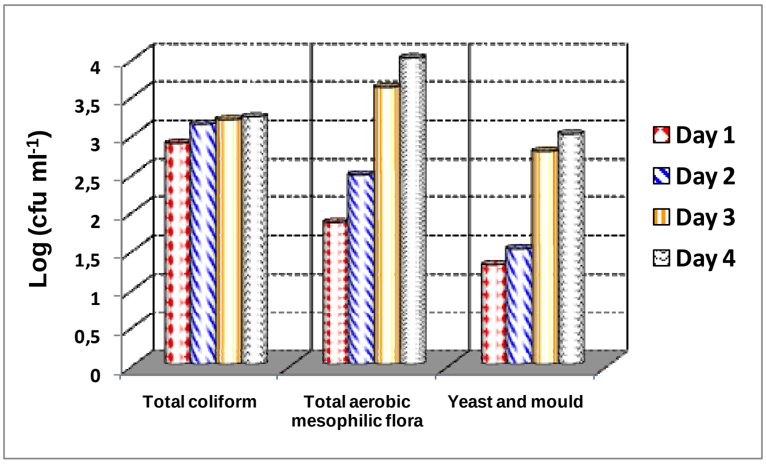

Fig. 12. Evolution of milk contamination (Log10 of total coliform, total aerobic mesophilic flora, and yeast/moulds counts) during storage days.

4. Conclusion

We have shown the feasibility of our prototype portable electronic nose based on an array of semiconductor gas sensors to detect off-odours from milk. It can be used to discriminate among milks from three different dairy farms. The sensor array coupled with features extraction and pattern recognition methods was found applicable to obtain selective discrimination of milk of different rancidity levels.

The proposed approach based on PCA application is of very good generalization with a success rate in discrimination of 99.94 %. The multi-class classifier model based on SVMs and the one-against-all, achieves very good accuracy in the classification. The results obtained with the developed approach and equipment are highly promising. PCA is able to show a characteristic development of the milk quality dependent on storage time; it gave a good estimation of the number of storage days. In conclusion, the system described may be of great use in the dairy industry. However the field of applications may further be widened and thus, it might become a challenging promise for food and beverage industry.

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Guide for Contributors

Aims and Scope

Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because it is an open access, peer review international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per annual by International Frequency Association (IFSA). In additional, some special sponsored and conference issues published annually.

Topics Covered

Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

- Physical, chemical and biosensors;
- Digital, frequency, period, duty-cycle, time interval, PWM, pulse number output sensors and transducers;
- Theory, principles, effects, design, standardization and modeling;
- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
- Sensors interfaces, buses and networks;
- Signal processing;
- Frequency (period, duty-cycle)-to-digital converters, ADC;
- Technologies and materials;
- Nanosensors;
- Microsystems;
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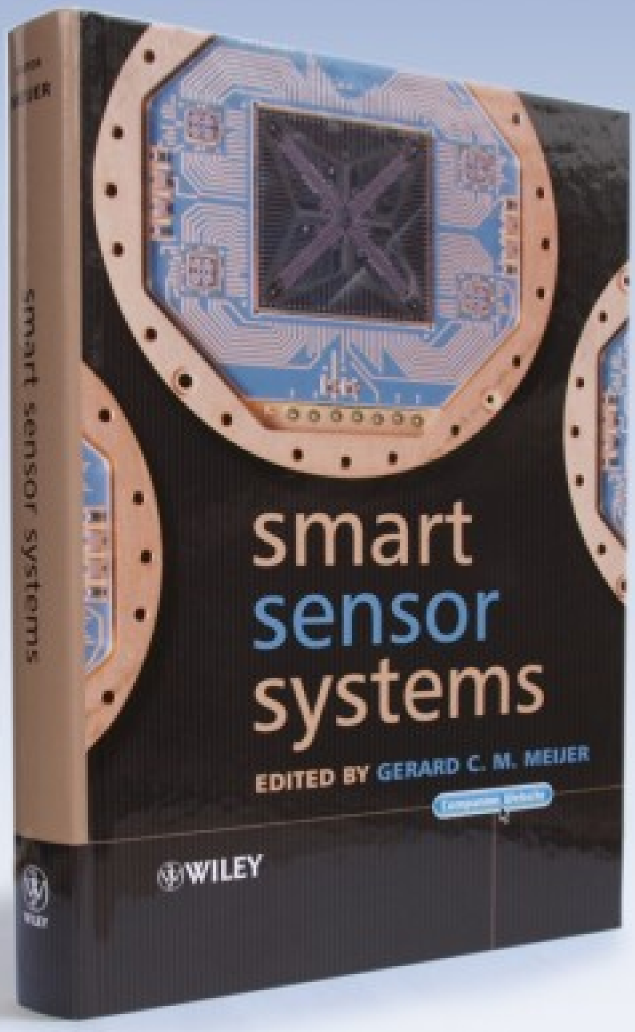
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