The Pharma Innovation Journal 2021; 10(4): 30-38

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(4): 30-38

www.thepharmajournal.com
Received: 10-02-2021
Accepted: 23-03-2021

Mrinmoy Roy
Department of Planning and Monitoring Cell, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

N Hariharan
Department of Planning and Monitoring Cell, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

Manoj D
Department of Planning and Monitoring Cell, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

Ishita Auddy
Department of Planning and Monitoring Cell, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

S Shanmugasundaram
Department of Planning and Monitoring Cell, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

Corresponding Author:
S Shanmugasundaram
Department of Planning and Monitoring Cell, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

Development of metal oxide semiconductor gas sensor based electronic nose system for adulteration detection in ghee

Mrinmoy Roy, N Hariharan, Manoj D, Ishita Auddy and S Shanmugasundaram

Abstract
The cow ghee is derived from milk and contains high-quality nutrients. The adulteration of cow ghee is very common among traders to earn more profit. The traditional methods of adulteration detection require sample pre-processing and are also time-consuming and costly processes. Therefore, machine olfaction instruments are very common now a day to evaluate the quality of foodstuffs in a fast and precise way. The potential of metal oxide semiconductor (MOS) gas sensor based electronic nose (e-nose) system was developed to detect adulteration in ghee with hydrogenated fat (vanaspati). The data obtained from the e-nose system were analyzed for pattern recognition and classification using multivariate chemometric analysis such as principal component analysis (PCA) and discriminant function analysis (DFA) method for adulteration detection in ghee. The PCA explained 98.10% variance while, DFA explained 99.10% variance in the e-nose dataset. The accuracy in training data and its cross-validation was found to be 98.18% and 97.27%, respectively. The success rate of adulteration identification for test samples using DFA model was 90.90% based on received e-nose signals. The results of PCA and DFA suggest that the developed e-nose system successfully identified pure and adulterated ghee samples based on the e-nose data.

Keywords: Ghee, MOS gas sensor, E-nose, PCA, DFA

1. Introduction
Cow ghee or anhydrous milk fat is extremely utilized in Asian countries, with the highest number of traders and consumers are from India (Anil Kumar *et al*. 2018) [15]. Ghee is rich in vitamin A, polyunsaturated fatty acids, omega-3 fatty acid and conjugated linoleic acid (Karandikar, Bansude, and Angadi 2016) [11]. Ghee can be prepared by traditional/milk-butter method, direct cream method, creamery butter method, pre-stratification method (Anil Kumar *et al*. 2018) [15]. In India, the traditional method of processing ghee is the most common manufacturing method among consumers, involving churning of fermented milk to get butter heated at 115 ℃ until all the water evaporates. Then, this is cooled and strained using a double folded muslin cloth to get the residue-free final product.
Ghee is considered a memory enhancer, grasping booster and controlling senses and strengthening them (Kaushik, Jain, and Rai 2016; Ayari *et al*. 2018) [12, 3]. However, because of the shortage of ghee for consumption and high demand in the lean season, which makes ghee costlier than edible oils. Therefore, ghee manufacturers tend to adulterate it with cheaper edible oils, hydrogenated fat, animal body fat, and starches (Amit Kumar 2008) [14]. Food adulteration is the process by which the authenticity of any high-value food molecule is reduced using cheaper substances of the same nature (Gonzálvez, Armenta, and la Guardia 2010) [9].
Various methods for detection of adulteration in ghee includes physicochemical methods (Gandhi, Kumar, and Lal 2018; Uncu and Uncu 2020) [6, 26], thermal methods (Upadhyay *et al*. 2017) [27], chromatographic methods (Amrutha Kala 2013; Rani *et al*. 2015; Upadhyay *et al*. 2015; Pathania *et al*. 2020) [1, 19, 28] and spectroscopic methods (Antony *et al*. 2018; Saleem 2020) [2, 23]. The drawbacks of these methods are costly, time-consuming, and require sample pre-processing and technical expertise for routine laboratory analysis (Ayari *et al*. 2018) [3].
The above drawbacks bring attention to increasing biological olfactory machines for food quality monitoring. The machine olfaction or an e-nose that approximately mimic human nose could be utilized for odorants detection from any food products in a more rapid, precise, and

cost-effective way with no sample pre-processing and easy handling (Roy and Yadav 2021) [21]. The flavour characteristics to evaluate food quality causes e-noses exceptionally helpful for different industrial applications in the fields of food, pharmaceutics, clinical diagnosis and environment monitoring (Korel and Balaban 2008) [13].

The most widely used sensors in e-nose systems are surface acoustic wave (SAW), conducting polymer (CP) and metal oxide semiconductor (MOS), among which MOS sensors are extensively used due to their high chemical stability, low response to moisture, long life, and reasonable price (Dey 2018; Roy and Yadav 2021) [21]. MOS type gas sensors are extensively used in MQ (Hanwei Electronics Co., Ltd., China) and TGS (Figaro Engineering Inc., Japan) series. A review of recent researches on food adulteration detection using MOS gas sensor based e-nose can be mentioned in the article (Roy and Yadav 2021) [21].

The detection of adulteration in ghee with sunflower oil and cow body fat has been studied by (Ayari *et al*. 2018) [3]. The authors used eight MOS gas sensors among which four sensors are from TGS series which are much costlier than MQ series of sensors. Also, the use of mass flow controller, vacuum pump, three two-way valves, air filter, and data acquisition card makes the e-nose system costly. Considering the above points to reduce economic investments, the present study aimed to develop a low cost e-nose prototype system to detect adulteration of pure cow ghee with hydrogenated fat (vanaspati).

2. Materials and Methods

2.1 Preparation of pure ghee samples: The preparation of pure ghee samples includes the direct cream method described by (Antony *et al*. 2018) [2]. Fresh and pure cow milk was procured from a local farmer of Thanjavur, Tamil Nadu, India. The cream was separated from milk using a mechanical separator in bulk quantities. The cream thus obtained was moderately heated in a pan with continuous stirring until it reaches 115 ± 5 ℃. Thus, the moisture was removed from the product in this process, and a golden yellow colored product appears, filtered using a double folded muslin cloth to get the residue-free final product as ghee.

2.2 Preparation of adulterated ghee samples

The adulterated ghee samples were prepared with a commercial brand of vanaspati that was procured from the local supermarket. The samples were prepared in the molten stage by deliberate adulteration of pure ghee with vanaspati from 0% (pure ghee) to 100% (vanaspati) in 10% steps.

2.3 Screening of sensors

Initial sensor screening was made from a set of commercial MOS gas sensors (Hanwei Electronics Co., Ltd., China). Table 1 describes the primarily identified nine specific sensors with different selectivity on the basis of capability of sensing volatile chemical compounds. However, the initial sensor combination is not optimal because of overlapping selectivity among sensors. For instance, the selectivity to detect alcohol is the same for MQ 2, MQ 3, MQ 4, MQ 8 and MQ 135. Hence, the screening of these sensors was performed to select the best combination of e-nose sensor array to reduce the redundant features (Ayari *et al*. 2018) [3].

Table 1: Various MOS gas sensors used for screening

Sensor number	Sensor type	Applications	Detection ranges (ppm)
1	MQ 2	LPG, iso-butane, propane, methane, alcohol, hydrogen, smoke	LPG and propane 200-5000, butane 300-5000, methane 5000-20000, H_2 300-5000, alcohol 100-2000
2	MQ 3	Alcohol	0.5-10
3	MQ 4	CH_4, natural gas, alcohol	200-10000
4	MQ 5	LPG, natural gas, iso-butane, propane, smoke	200-10000
5	MQ 6	Propane, butane and LPG, natural gas, methane	300-10000
6	MQ 7	Carbon monoxide	20-2000
7	MQ 8	Hydrogen, alcohol, LPG	100-10000
8	MQ 9	CO, combustible gas, methane, propane	CO 10-1000, combustible gas 100-10000
9	MQ 135	NH_3,NOx, alcohol, Benzene, smoke,CO2	NH_3 10ppm-300, benzene 10-1000, alcohol 10-300

The sensitivities of each of these sensors (Table 1) were checked and recorded individually by exposing 8 g of pure ghee and adulterated ghee samples to each of the sensors separately in a glass container of 40 ml volume. The air-tight glass container was used with having provision to hold the sensor on its lid. Then, the sensors were attached to the provision on lid and sealed airtight with the help of a rubber gasket to avoid the leakage of volatiles. Before gas sensor measurements, the samples were heated continuously in a water bath at 60 ℃ for 300 s in air-tight mode to release the volatiles from the sample towards the container's headspace. The analog values of these gas sensors were measured for 120 s (at every 100 milliseconds) after the sample heating was over. The pictorial representation of the setup was depicted in Figure 1. The analog values were recorded in a computer with the help of an Arduino Uno microcontroller and Universal serial bus (USB) to Transistor–transistor logic (TTL). The USB to TTL allows connecting the computer to a TTL serial port via USB.

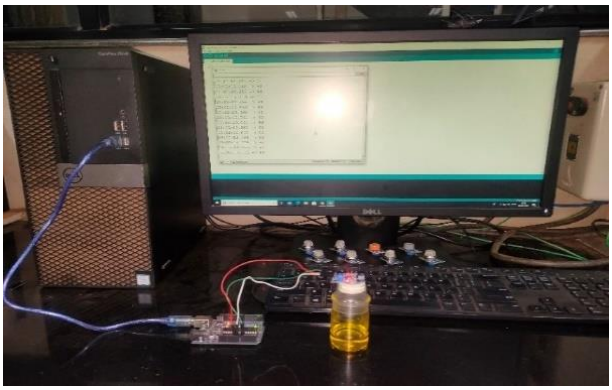

Fig 1: Screening of sensors

2.4 Development of metal oxide semiconductor based e-nose system

2.4.1 Development of sensor array

The array is the series of sensors attached together in a board.

For the development of sensor array, the provision was made for attachment of screened sensors over the acrylic lid. The acrylic lid was chosen because it is non-toxic, corrosive resistant, non-adhesive, heat stable, has good insulation properties, good strength, high durability, lightweight and low cost (Pawar 2016; Babo *et al*. 2020) [18, 4]. The screened sensors (MQ2, MQ3, MQ6, MQ7, MQ8) were attached to the provided space on acrylic lid. The pins of the sensors were connected to Arduino uno microcontroller via a breadboard. The developed sensor array was depicted in Figure 2.

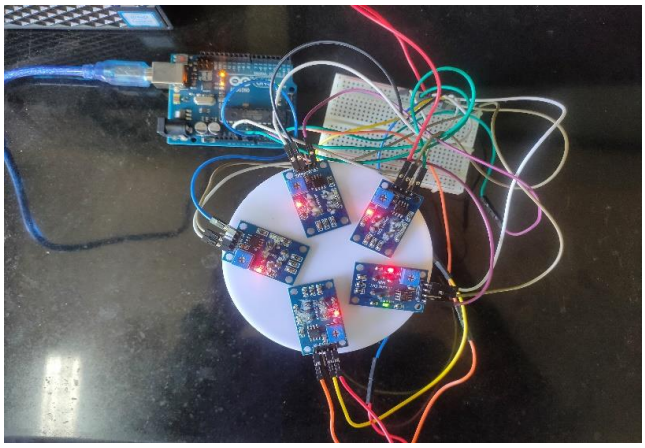

Fig 2: Developed sensor array

2.4.2 Development of e-nose prototype system

The developed e-nose prototype system mainly consists of a sampling unit, a detection unit and a control unit (Figure 3AB). A sample holder of volume 250 ml was fabricated as sampling unit for this study. The detection unit comprises of developed sensor array. In order to detect the adulteration in pure ghee, the screened sensor array was used to build an olfactory machine (e-nose) prototype system. The sensor array board was placed over the sample holder to facilitate airtight environment. The microcontroller was further connected to a computer equipped with software (control unit) via USB to TTL to get the output analog value of the prototype e-nose system.

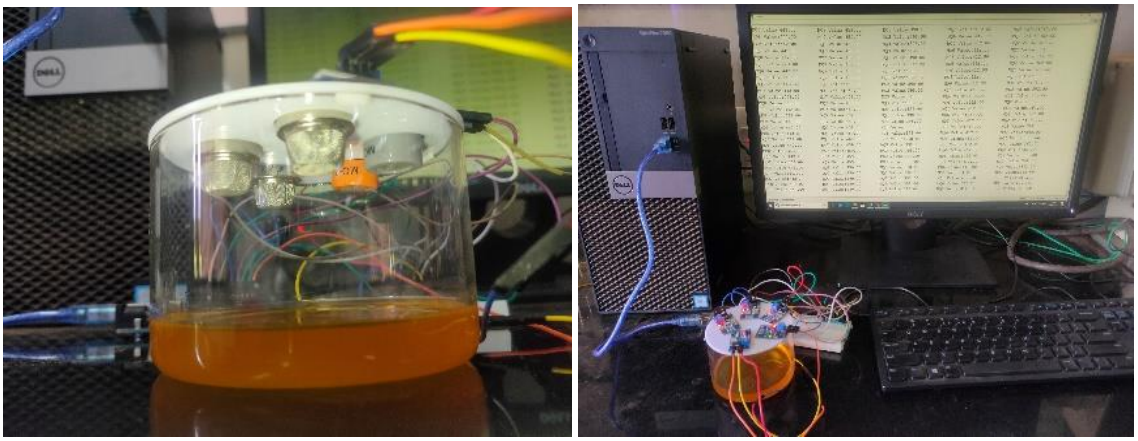

Fig 3: The developed e-nose system prototype. (A) Sensor array with sample holder, (B) prototype e-nose system

2.6 E-nose experimental methodology

The sample holder containing 50 g ghee samples was placed on a water bath and maintained a constant temperature of 60 ℃ to release the volatiles from the samples for 300 s. Subsequently, the sample gas (headspace) concentration was sensed by the detection unit, thus varying the output responses of each sensor for a certain period of time. The output analog value was collected for 120 s displayed on computer. The raw datasets were further utilized for data pre-processing to remove outliers produced by environmental noise or by experimental errors. A time interval of 600 s was maintained between two experiments and the array was exposed to clean air in order to eliminate the gases attached to sensor and reach its baseline (initial) stage.

The e-nose output analog values were first recorded as raw dataset. Then, the outliers were eliminated from the raw dataset using the following steps described by (Sainis, Srivastava, and Singh 2018) [22]:

$$\text{Inter quartile range, IQR} = Q_3 - Q_1 \quad \text{... (1)}$$

Where IQR is the range of values from Q_1 to Q_3

Q_1 is the lower quarter of the dataset

Q_3 is the upper quarter of the dataset

$$\text{Lower range limit} = Q_1 - (1.5 * \text{IQR}) \quad \text{..... (2)}$$

$$\text{Higher range limit} = Q_3 + (1.5 * \text{IQR}) \quad \text{..... (3)}$$

The output data below and above this lower and higher range limit was considered an outlier.

2.7 Pattern recognition, classification and performance evaluation of the developed prototype e-nose system

For pattern recognition, classification and performance evaluation of the developed prototype e-nose system, chemometric analyses were performed (Yu *et al*. 2009; Yin and Zhao 2019; Okur *et al*. 2021) [30, 29, 16]. The chemometric analyses included in this work were principal component

analysis (PCA) and discriminant function analysis (DFA) that are usually applied to discriminate the samples based on e-nose dataset and to identify adulterations. Both PCA and DFA helps to identify the patterns of various samples with classification accuracy. The performance of the developed prototype e-nose system was analysed based on the DFA method where, the unknown adulterations can be identified based on the known adulterations. OriginPro 9.5 (OriginLab Corporation) statistical software was used to perform PCA and DFA analysis. Also, ten replications were considered for each sample group.

2.7.1 Principal component analysis

The principal component analysis (PCA) is an unsupervised pattern recognition and dimensionality reduction technique that reduces large data sets into smaller ones by retaining most of the original information in the new data set in reduced form and identifying the correlations and patterns (Śliwińska *et al*. 2016) [24]. This statistical method identifies the patterns in the data and expresses the data in such a way, which highlights their similarities and differences by making clusters and illustrating the variances with classification accuracy (Roy and Yadav 2021; Ghasemi-Varnamkhasti *et al*. 2012) [21, 8].

The clusters can be identified with a score plot of PCA model. The PCA models create new variables as linear combinations, which are called principal components (PCs). The PCs are calculated for each variable, indicating the distance of any variable from the calculated axis with a calculated Eigenvalue corresponding to the amount of variation explained by that axis (Karami, Rasekh, and Mirzaee-Ghaleh 2020) [10]. The PCs possess most of the important data information that was scattered in the raw dataset. Each PC represents the variance direction in the data, with the largest variances are the most important. The X axis of PCA represents the first PC (PC1) with the most variance explained while the Y axis represents the second PC (PC2) with the second most variance explained. A loading plot in combination with the PCA score plot describes how strongly each characteristic influences a PC (Ghasemi-Varnamkhasti *et al*. 2011) [7].

2.7.2 Discriminant function analysis

The DFA is also called canonical discriminant analysis (CDA) or linear discriminant analysis (LDA), or quadratic discriminant analysis (QDA), is a supervised multivariate analysis used to differentiate different sets of samples and assign new samples to the previously defined groups. The classification algorithm is evaluated by a technique called confusion matrix for summarizing the quality of prediction (Okur *et al*. 2021) [16]. The confusion matrix is represented by a table of predicted (identified) value versus true (actual) value. The two important goals of DFA models are discrimination and classification. The model constructs a classifier to differentiate the samples from a known population. It also distributes the unknown population into the known groups with the classifier. The emphasis is on developing a classifier that can be utilized to sort new populations into the previous groups (Tohidi *et al*. 2018) [25]. The variable features are utilized to differentiate the unknown food samples by maximizing the variance between groups and minimizing the variance within groups to improve the goal between clusters. This method utilizes the discriminant functions (DFs) to determine which group or cluster from the original dataset it would assign each sample in the final dataset. It ensures this via a cross-validation method, where the DFs are determined while eliminating one variable from the dataset. The DFs are then used to classify the variables into their individual groups. Hence, building the DFA model is based on the trained dataset and the grouping rules obtained in the first step (Śliwińska *et al*. 2016) [24]. The higher the correct classification of particular groups, the easier the sample group differentiation and vice-versa (Śliwińska *et al*. 2016) [24].

3. Results and Discussions

3.1 Sensor screening

The responses of each sensor were collected as the raw data. Sensor responses were recorded from 0 to 1023 as analog values to maximize the difference in output response. The output responses of each sensor were shown in Figure 4 for pure ghee and vanaspati.

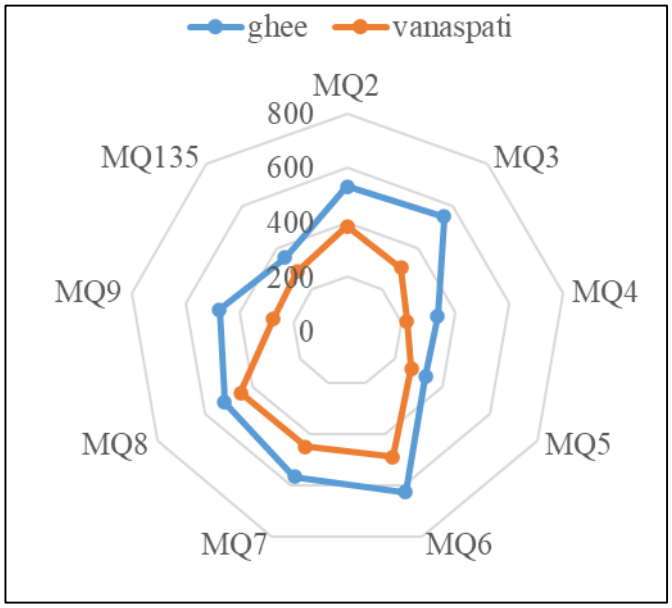

Fig 4: Radar graph showing responses of each sensor on pure ghee and vanaspati.

As can be seen from Figure 4, pure ghee and vanaspati have the maximum effect on MQ 6 sensor. The gas applications of MQ 6 is propane, butane, LPG, natural gas and methane (Table 1). Since MQ 2, MQ 3, MQ 6, MQ 7, MQ 8 sensors have the maximum sensitivity towards both the samples and their target gas applications match MQ 4, MQ 5, MQ 9, MQ 135. Hence, MQ 4, MQ 5, MQ 9 and MQ 135 were removed to avoid redundant features of the final e-nose prototype machine system construction.

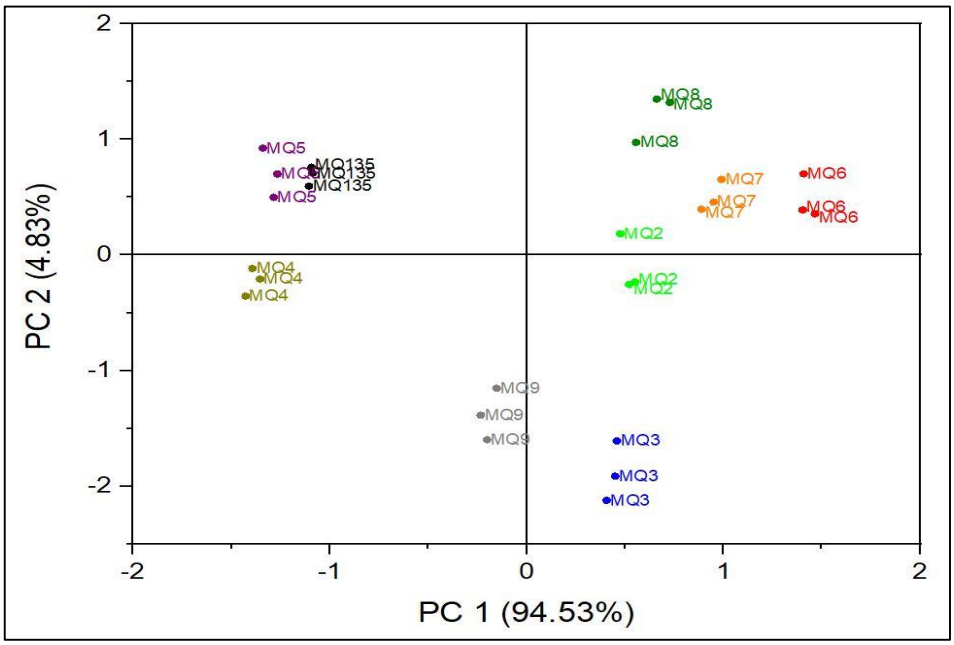

Fig 5: PCA score plot for sreening of sensors

The PCA was also performed to confirm the optimal sensor classification towards the output responses. The PCA score plot (Figure 5) also revealed that the sensors with higher sensitivity are located on the positive side of PC1 with positive loadings. On the other hand, those with lower sensitivity are located on the negative side of PC1. The negative variation from PCA analysis suggests less sensitivity of target gases towards MQ 4, MQ 5, MQ 9 and MQ 135 sensors. These results revealed that MQ 2, MQ 3, MQ 6, MQ 7, MQ 8 sensors have the most roles in the classification of ghee and vanaspati samples.

3.2 Prototype e-nose response to pure and adulterated ghee samples

The final array of the developed prototype e-nose consists of optimized five MOS sensors MQ 2, MQ 3, MQ 6, MQ 7 and MQ 8. The e-nose mean responses for pure ghee, vanaspati and adulterated ghee samples are presented in Figure 6. The e-nose array responses toward pure ghee is more than vanaspati. From Figure 6, it is clear that the MQ 6 sensor has the highest sensitivity towards all the samples.

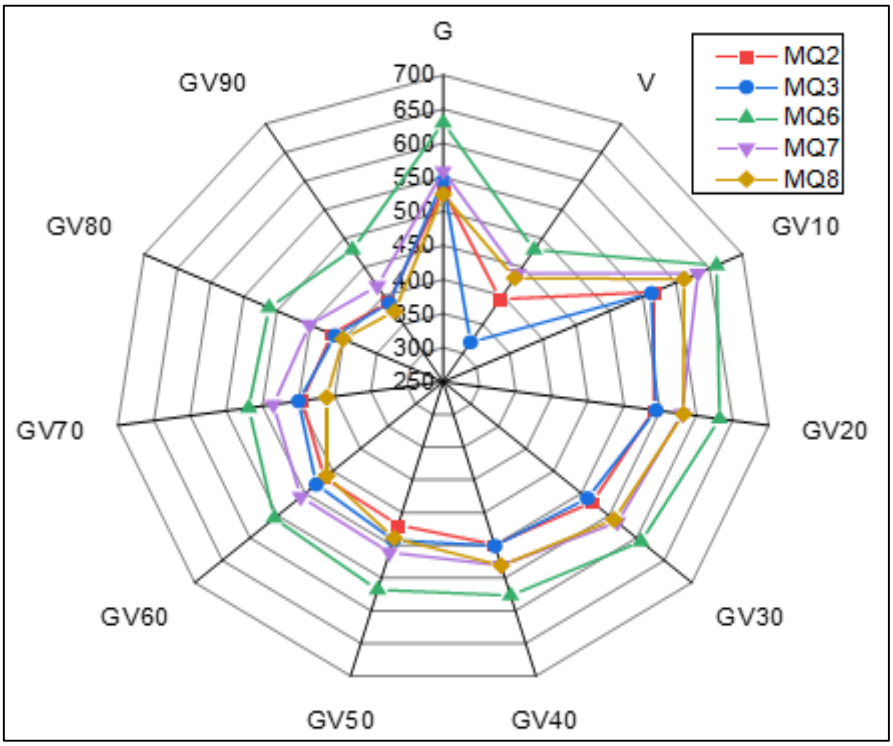

Fig 6: Radar graph response of developed prototype e-nose system for pure ghee, vanaspati and adulterated ghee samples. G: ghee, V: vanaspati, GV10 to GV90: ghee adulterated with vanaspati from 10% to 90% adulteration concentration levels in 10% step.

The e-nose response was increasing for low adulteration samples (GV10 and GV20). This might be due to the addition of vanaspati in lower concentrations since vanaspati also contains an adequate ghee-like aroma. Then, it decreases for

remaining adulterated ghee samples and thus moving towards vanaspati, when the level of vanaspati adulteration in ghee was maximum. It can be conferred from this result that the sensor array responds to the aroma produced by all the samples during the process of adulteration and the MQ 6 sensor has the most role in the classification of samples. The outliers from e-nose measurements were eliminated from the raw dataset which were less than five on an average for each e-nose measurement. The processed e-nose mean values were further used as the input data for PCA and DFA algorithm to identify the pure ghee from counterfeit specimens.

3.3 PCA results for pure and adulterated ghee samples

The PCA is the most widely used dimensionality reduction process to evaluate the similarities and differences between samples. The score plot is presented in Figure 7(A) to identify the patterns and classification of e-nose dataset. In addition, a loading plot was presented in Figure 7(B) to detect the relative role of the sensor array in the e-nose measurements (Ghasemi-Varnamkhasti *et al.* 2011) [7]. As can be seen from the PCA score plot, the two PCs called PC1 and PC2 explains a variance of 92.92% and 5.18% (overall 98.10%) of the total data in the PCA method to identify the adulteration among pure and adulterated ghee samples, respectively.

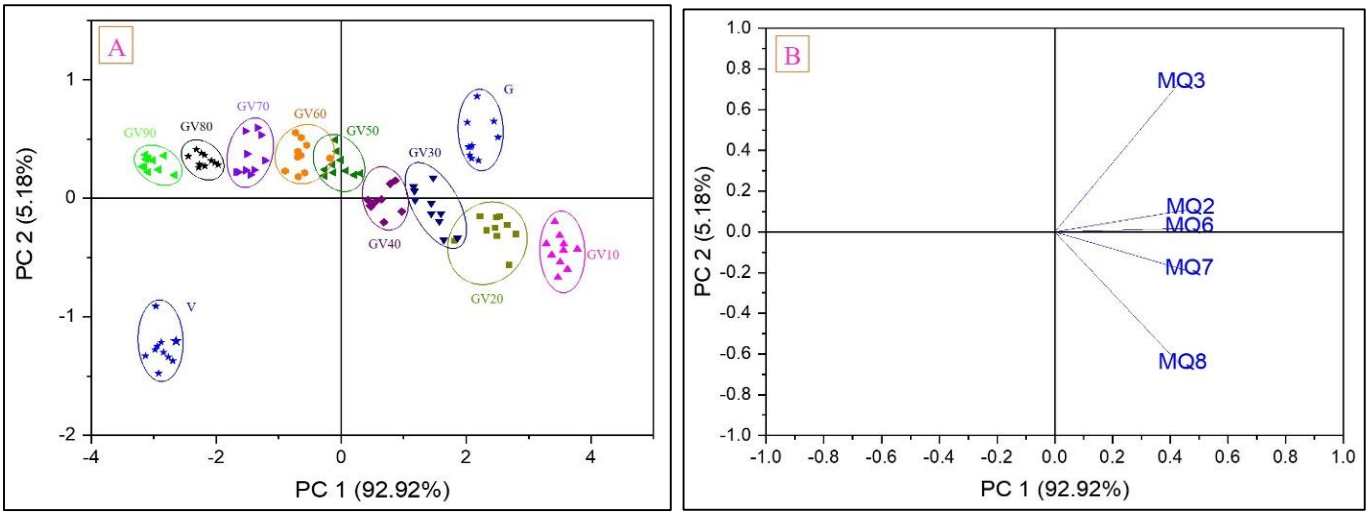

Fig 7: PCA algorithm model obtained from e-nose data analysis (A) score plot, (B) loading plot. G: ghee, V: vanaspati, GV10 to GV90: ghee adulterated with vanaspati from 10% to 90% adulteration concentration levels in 10% step.

There is a clear distinction between all the samples of pure and adulterated ghee. The potential reason for the location of all the samples on the score plot and variables on the loading plot could be distinguishable based on e-nose measurement characteristics. The pure ghee samples are located in the positive loadings of PC1, while the vanaspati samples are far away from the axis situated in a unique location, indicating a negative correlation with pure ghee samples. Pure ghee has rich aroma characteristics, which vanaspati has less compared to pure ghee. The loading plot is used to understand the relationships between variables. The variables placed right at the loading plot of PC1 are positively correlated. The MQ 6 sensor close to the X-axis of PC1 has the highest role in classification of all the samples. While MQ 3 and MQ 8 are far away from the axis and thus they have the lowest role in classification. Similar results were found by Ayari *et al*. 2018 [3] in discriminating pure ghee samples mixed with soybean oil and cow body fat.

3.4 DFA results for pure and adulterated ghee samples

A total of 110 samples were analysed for training of discriminant functions using LDA method. The cross-validation was done based on leave-one-out method to test the DFA algorithm (Ren *et al.* 2019) [20]. This methodology suggests a computation for every measurement (110 times). Each time, training is completed with 109 measurements, and the prediction is made on the remaining one observation. Hence, all the measurements finally joined in training as well as the testing (cross-validation of trained data) process. The training dataset was utilized to obtain the boundaries while earlier trained boundaries transformed the test dataset in their earlier groups.

The trained groups were utilized to create the linear discriminant functions, while the test group was utilized to assess the consistency of the network. Five test samples from each group (totalizing 55 test samples) were randomly taken to evaluate the performance of the e-nose system based on DFA model. The results of the DFA score plot for training data and test data was presented in Figure 8. As can be seen from the DFA score plot that two discriminant functions DF1 and DF2 explained a total variance of 99.10% in e-nose dataset. In the training data, the pure ghee, vanaspati and all the adulterated ghee samples were well classified as individual groups except one observation level each from GV20 and GV60 overlapped with GV30 and GV50, respectively. The training results were cross-validated using leave-one-out coss-validation and it was observed that one more observation level from GV30 was mis-classified in GV20 group. For testing sample, five samples were mis-classified out of 55 test samples. Two samples each from GV20 and GV30 was mis-classified into GV10 and GV40, respectively. Also, one sample from GV60 group was mis-classified into GV70 group. This results could further be confirmed with DFA classification summary plot (Figure 9) for training samples and test samples The location of pure ghee samples were different from vanaspati samples. This may be explained that the volatile compositions in ghee was different from vanaspati.

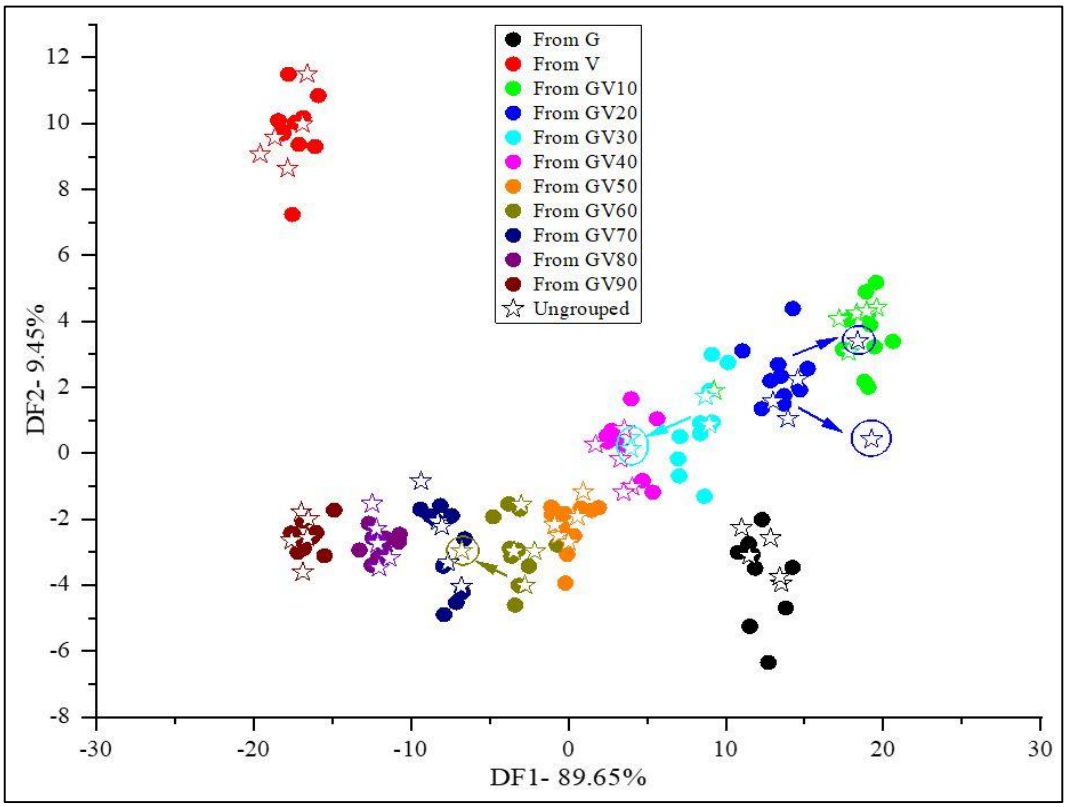

Fig 8: DFA model of training samples and test samples obtained from e-nose data analysis

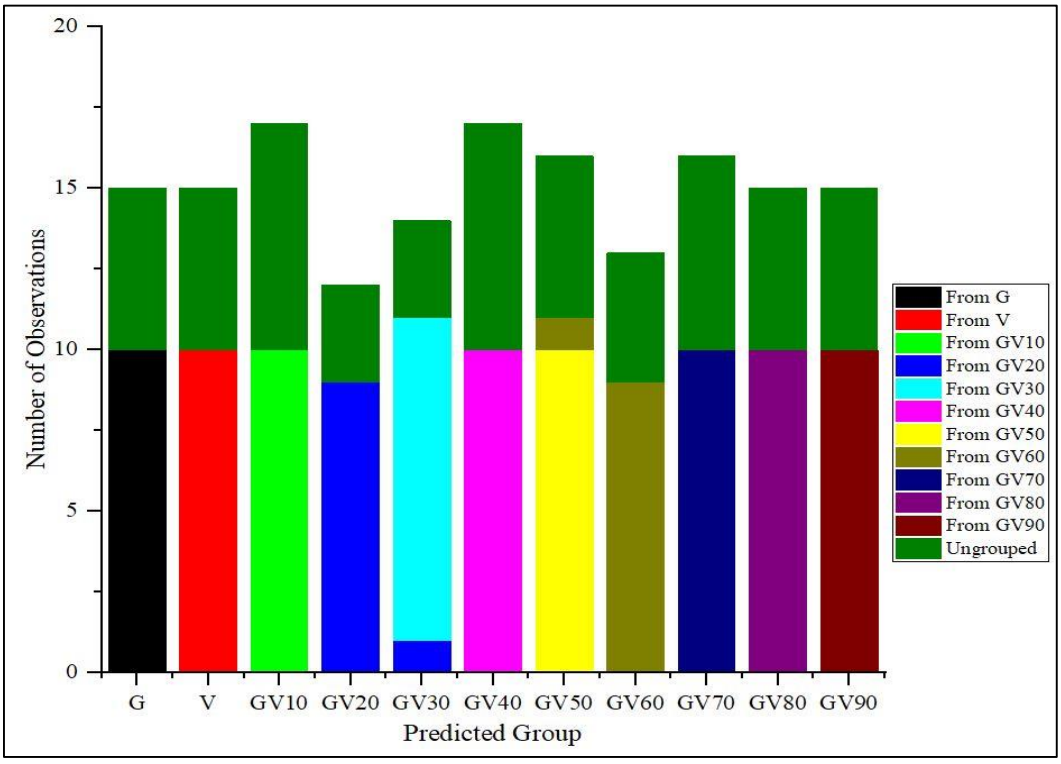

Fig 9: DFA classification summary plot for training samples and test samples

The fisher discriminant function eigenvalues were shown in Table 2, where the five discriminant functions were used to classify selected features with 100% cumulative. The results of confusion matrices of training data and cross-validated training data were presented in Table 3 and Table 4, respectively. Diagonal values in bold corresponds to the correct classification of samples. The DFA method shows a correct classification of 98.18% for the selected feature of training data. The cross-validated leave-one-out classification for the training data achieved a correct classification of 97.27%.

Table 2: Fisher discriminant function eigenvalues

Function	Eigenvalue	Variance %	Cumulative %	Canonical Correlation
1	158.00211	89.65%	89.65%	0.99685
2	16.65193	9.45%	99.10%	0.97126
3	1.39838	0.79%	99.90%	0.76358
4	0.15332	0.09%	99.98%	0.36461
5	0.03026	0.02%	100.00%	0.17138

Table 3: Confusion matrix obtained from DFA training samples

	Predicted Group										
	G	**V**	**GV10**	**GV20**	**GV30**	**GV40**	**GV50**	**GV60**	**GV70**	**GV80**	**GV90**
G	10	0	0	0	0	0	0	0	0	0	0
V	0	10	0	0	0	0	0	0	0	0	0
GV10	0	0	10	0	0	0	0	0	0	0	0
GV20	0	0	0	9	1[a]	0	0	0	0	0	0
GV30	0	0	0	0	10	0	0	0	0	0	0
GV40	0	0	0	0	0	10	0	0	0	0	0
GV50	0	0	0	0	0	0	10	0	0	0	0
GV60	0	0	0	0	0	0	1[a]	9	0	0	0
GV70	0	0	0	0	0	0	0	0	10	0	0
GV80	0	0	0	0	0	0	0	0	0	10	0
GV90	0	0	0	0	0	0	0	0	0	0	10

Error rate for classification of training data is 1.82%

[a] Mis-classified test samples

Table 4: Confusion matrix obtained from leave-one-out cross-validated DFA training samples

	Predicted Group										
	G	**V**	**GV10**	**GV20**	**GV30**	**GV40**	**GV50**	**GV60**	**GV70**	**GV80**	**GV90**
G	10	0	0	0	0	0	0	0	0	0	0
V	0	10	0	0	0	0	0	0	0	0	0
GV10	0	0	10	0	0	0	0	0	0	0	0
GV20	0	0	0	9	1[a]	0	0	0	0	0	0
GV30	0	0	0	1[a]	9	0	0	0	0	0	0
GV40	0	0	0	0	0	10	0	0	0	0	0
GV50	0	0	0	0	0	0	10	0	0	0	0
GV60	0	0	0	0	0	0	1[a]	9	0	0	0
GV70	0	0	0	0	0	0	0	0	10	0	0
GV80	0	0	0	0	0	0	0	0	0	10	0
GV90	0	0	0	0	0	0	0	0	0	0	10

Error rate for cross-validation of training data is 2.73%

[a] Mis-classified test samples

Since, only five samples were mis-classified by DFA method for e-nose random test samples, therefore the classification accuracy of the developed prototype e-nose system is 90.90%. The results of DFA analysis confirms that discriminant functions in the test were steady and could be utilized to distinguish the adulterated and pure ghee samples.

4. Conclusions

The MOS gas sensor based e-nose system was developed to detect adulteration in ghee. The array of e-nose system consisted of five MOS gas sensors. Post-processing chemometric analysis such as PCA and DFA discriminated eleven groups of known samples with excellent accuracy based on e-nose signals. The PCA explained a total variance of 98.10% accuracy in the e-nose dataset of ghee and its adulteration with vanaspati while, DFA explained 99.10%. The classification accuracy of the developed e-nose system was found to be 90.90% for testing of pure and adulterated ghee samples. The DFA algorithm model revealed that the proposed feature selection method was effective in identifying adulteration in pure ghee with vanaspati. Based on the results, it is concluded that the developed e-nose system successfully identified the pure ghee samples and adulterated ghee samples with good accuracy. Furthermore, the developed e-nose system and the proposed methodology may universally be applied with other adulterants mixed with pure cow ghee.

5. References

1. Amrutha Kala AL. Detection of Possible Adulteration in Commercial Ghee Samples Using Low-Resolution Gas Chromatography Triglyceride Profiles. International Journal of Dairy Technology 2013;66(3):346-51.
2. Antony, Bency, Bhavbhuti M Mehta, Saurabh Sharma, Ratnam K, Aparnathi KD. "Comparative Appraisal of Ghee and Common Vegetable Oils for Spectral Characteristics in FT-MIR Reflectance Spectroscopy." Journal of Food Science and Technology 2018;55(9):3632-39.
3. Ayari, Fardin, Esmaeil Mirzaee-Ghaleh, Hekmat Rabbani, Kobra Heidarbeigi. "Detection of the Adulteration in Pure Cow Ghee by Electronic Nose Method (Case Study: Sunflower Oil and Cow Body Fat). International Journal of Food Properties 2018;21(1):1670-79.
4. Babo, Sara, Joana Lia Ferreira, Ana Maria Ramos, Anna Micheluz, Marisa Pamplona, Maria Helena Casimiro, *et*

al. "Characterization and Long-Term Stability of Historical PMMA: Impact of Additives and Acrylic Sheet Industrial Production Processes." *Polymers* 2020;12(10):2198.
5. Dey Ananya. "Semiconductor Metal Oxide Gas Sensors: A Review." Materials Science and Engineering: 2018;B229:206-17.
6. Gandhi, Kamal, Anil Kumar, Darshan Lal. "Solvent Fractionation Technique Paired with Apparent Solidification Time (AST) Test as a Method to Detect Palm Olein and Sheep Body Fat in Ghee (Clarified Milk Fat)." *Indian J Dairy Sci* 2018;71(3):246-51.
7. Ghasemi-Varnamkhasti, Mahdi, Seyed Saeid Mohtasebi, Maryam Siadat, Jesus Lozano *et al*. "Aging Fingerprint Characterization of Beer Using Electronic Nose. Sensors and Actuators B: Chemical 2011;159(1):51-59.
8. Ghasemi-Varnamkhasti, Mahdi, Maria Luz Rodriguez-Méndez, Seyed Saeid Mohtasebi, Constantin Apetrei, *et al*. Monitoring the Aging of Beers Using a Bioelectronic Tongue. Food Control 2012;25(1):216-24.
9. Gonzálvez A, Armenta S, la Guardia M. Adulteration Detection of Argan Oil by Inductively Coupled Plasma Optical Emission Spectrometry. Food Chemistry 2010;121(3):878-86.
10. Karami, Hamed, Mansour Rasekh, Esmaeil Mirzaee-Ghaleh. "Application of the E-Nose Machine System to Detect Adulterations in Mixed Edible Oils Using Chemometrics Methods." Journal of Food Processing and Preservation 2020;44(9):e14696.
11. Karandikar, Yogita Surendra, Akshata Sanjay Bansude, Eesha Ajit Angadi. "Comparison between the Effect of Cow Ghee and Butter on Memory and Lipid Profile of Wistar Rats." Journal of Clinical and Diagnostic Research: JCDR 2016;10(9):FF11.
12. Kaushik R, Jain J, Rai P. "Therapeutic Potentials of Cow Derived Products-a Review." International Journal of Pharmaceutical Sciences and Research 2016;7(4):1383.
13. Korel, Figen, Murat Ö Balaban. "16Electronic Nose Technology in Food Analysis." Food Analysis Instruments 2008.
14. Kumar, Amit. "Detection of Adulterants in Ghee." NDRI, Karnal 2008.
15. Kumar, Anil, Shreya Tripathi, Nidhi Hans, Pattnaik HSN, Satya Narayan Naik. "Ghee: Its Properties, Importance and Health Benefits." *Lipid Universe* 2018;6:6-14.
16. Okur, Salih, Mohammed Sarheed, Robert Huber, Zejun Zhang, Lars Heinke *et al*. "Identification of Mint Scents Using a QCM Based E-Nose." Chemosensors 2021;9(2):31.
17. Pathania, Parul, Vivek Sharma, Sumit Arora, Priyanka Singh Rao. "A Novel Approach to Detect Highly Manipulated Fat Adulterant as Reichert--Meissl Value-Adjuster in Ghee (Clarified Butter) through Signature Peaks by Gas Chromatography of Triglycerides." Journal of Food Science and Technology 2020;57(1):191-99.
18. Pawar, Eshwar. "A Review Article on Acrylic PMMA." *IOSR J. Mech. Civ. Eng* 2016;13(2):1-4.
19. Rani, Anupama, Vivek Sharma, Sumit Arora, Darshan Lal, Anil Kumar. "A Rapid Reversed-Phase Thin Layer Chromatographic Protocol for Detection of Adulteration in Ghee (Clarified Milk Fat) with Vegetable Oils." Journal of Food Science and Technology 2015;52(4):2434-39.
20. Ren, Ruibo, Kechao Han, Pinhui Zhao, Jingtao Shi, Lei Zhao *et al*. "Identification of Asphalt Fingerprints Based on ATR-FTIR Spectroscopy and Principal Component-Linear Discriminant Analysis. Construction and Building Materials 2019;198:662-68.
21. Roy, Mrinmoy, Yadav BK. Electronic Nose for Detection of Food Adulteration: A Review. Journal of Food Science and Technology, 2021, 1-13.
22. Sainis, Nachiket, Durgesh Srivastava, Rajeshwar Singh. Feature Classification and Outlier Detection to Increased Accuracy in Intrusion Detection System. International Journal of Applied Engineering Research 2018;13(10):7249-55.
23. Saleem M. "Fluorescence Spectroscopy Based Detection of Adulteration in Desi Ghee." Journal of Fluorescence 2020;30(1):181-91.
24. Śliwińska, Magdalena, Paulina Wiśniewska, Tomasz Dymerski, Waldemar Wardencki, Jacek Namieśnik. Application of Electronic Nose Based on Fast GC for Authenticity Assessment of Polish Homemade Liqueurs Called Nalewka. Food Analytical Methods 2016;9(9):2670-81.
25. Tohidi, Mojtaba, Mahdi Ghasemi-Varnamkhasti, Vahid Ghafarinia, Seyed Saeid Mohtasebi, Mojtaba Bonyadian. "Identification of Trace Amounts of Detergent Powder in Raw Milk Using a Customized Low-Cost Artificial Olfactory System: A Novel Method." *Measurement* 2018;124:120-29.
26. Uncu, Ayse Ozgur, Ali Tevfik Uncu. "A Barcode-DNA Analysis Method for the Identification of Plant Oil Adulteration in Milk and Dairy Products." *Food Chemistry* 2020;326:126986.
27. Upadhyay, Neelam, Ankit Goyal, Anil Kumar, Darshan Lal. "Detection of Adulteration by Caprine Body Fat and Mixtures of Caprine Body Fat and Groundnut Oil in Bovine and Buffalo Ghee Using Differential Scanning Calorimetry." International Journal of Dairy Technology 2017;70(2):297-303.
28. Upadhyay, Neelam, Anil Kumar, Gopal Rathod, Ankit Goyal, Darshan Lal. "Development of a Method Employing Reversed-Phase Thin-Layer Chromatography for Establishing Milk Fat Purity with Respect to Adulteration with Vegetable Oils." International Journal of Dairy Technology 2015;68(2):207-17.
29. Yin, Yong, Yuzhen Zhao. "A Feature Selection Strategy of E-Nose Data Based on PCA Coupled with Wilks $Λ$-Statistic for Discrimination of Vinegar Samples." Journal of Food Measurement and Characterization 2019;13(3):2406-16.
30. Yu, Huichun, Jun Wang, Hong Xiao, Miao Liu. "Quality Grade Identification of Green Tea Using the Eigenvalues of PCA Based on the E-Nose Signals." Sensors and Actuators B: Chemical 2009;140(2):378-82.