Detection of the adulteration in pure cow ghee by electronic nose method (case study: sunflower oil and cow body fat)

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ABSTRACT
Cow ghee is very used in some regions of Iran, such as Kermanshah province. Cow ghee is a natural source that contains high-quality nutrients which are needed for the human body. Adulteration in dairy products is not only a serious threat to human health but also it causes economic losses. Diagnosis of foodstuff cheating and its estimation is one of the key concerns in recent years. The aim of this study was the detection of the adulteration in cow ghee by olfactory machine system. Therefore, an electronic nose system was used for the different levels of sunflower oil and cow body fat mixed with pure cow ghee (10\%, 20\%, 30\%, 40\%, and 50\%). The principal components analysis (PCA) and artificial neural networks (ANNs) methods were used to achieve this goal. Based on the results, the accuracy of the principal components analysis of sunflower oil and cow body fat were 96\% and 97\% of the data variance, respectively. According to the results, artificial neural networks identified the adulteration with sunflower oil and cow body fat with an accuracy of 91.3\% and 82.5\%, respectively.

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Introduction
Cow ghee or clarified butter is very used in some regions of Iran, such as Kermanshah province.\textsuperscript{[1]} Cow ghee is prepared by both traditional and industrial methods. In Iran, the traditional method is more common in villages. In the traditional way, firstly milk is converted to yogurt. Butter and yogurt drink are produced from the yogurt. After separating the butter from the yogurt drink, it is processed to produce ghee.\textsuperscript{[2]} Ghee is an effective material enhancing memory power, grasping power, and power to control senses and to make these stronger.\textsuperscript{[3,4,5]} However, because of the shortage of ghee, and comparatively more demand, it is very expensive (costing 7 to 10 times more than edible vegetable oils). Therefore, ghee is prone to adulteration by the traders in the market. The commonly used adulterants are vegetable oils, animal body fats, mineral oils, starchy material, and so on.\textsuperscript{[3]}

Adulteration is defined as the process by which the quality or the nature of a given substance is reduced through the addition of a foreign or an inferior substance and the removal of vital elements.\textsuperscript{[6]} The adulteration is a very serious problem. In recent years, more attention is being paid for developing biological olfactory machines which use biosensors for detection and discrimination of specific odorants.\textsuperscript{[7]} Utilizing sensitive elements, biosensors are able to detect odorants as well as providing a new platform to investigate the performance of the olfactory system. Excellent properties of smell and taste receptors are generally recognized in the development of biomimetic smell receptors-based biosensors.\textsuperscript{[8,9]}

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Electronic noses (e-noses) are instruments, which mimic the sense of smell. These devices are typically used an array of sensors to detect and distinguish odors precisely in complex samples and at low cost. These features make e-noses very useful for diverse applications in the food, cosmetic and pharmaceutical industry as well as in environmental control or clinical diagnostics. In the recent years, different research have been done about using of e-nose in the field of food control such as meat, fish, milk, oils, Saffron, Chinese pecan, honey, rapeseed and orange juice and soil.

Several methods have been developed for the detection of the adulteration in cow ghee. These methods were mostly based on chemical parameters like the fatty acid composition and the physicochemical analysis. According to a survey of the literature, a special machine or system for the detection of pure cow ghee has never been studied. So, in this research, an electronic nose system was considered for detecting pure cow ghee from the adulterated samples. To achieve this goal, an olfactory machine was evaluated by different samples of pure and faked samples (including sunflower oil and cow body fat).

**Materials and methods**

All experiments were done in the department of mechanical engineering of Biosystems, Razi University, Kermanshah, Iran.

**Structure of the e-nose**

In order to detect the amount of adulteration in cow ghee, an olfactory machine was used (Figure 1). This system mainly includes sampling unit, detection unit, and a control unit. The sampling unit includes a cylindrical container with a capacity of 100 g, a mass flow controller, three two-way valves, a micro-vacuum pump, and power supply. The detection unit includes a sensor array, air pump, air filter, and a data acquisition card (DAC, USB), which is used to convert odor information to electrical signal. The control unit consists of a computer equipped with software that was designed specifically for the e-nose system in Lab VIEW 2012 environment.

The system is equipped with a 12-V micro-vacuum pump to transfer the sample smell (headspace) to the sensor chamber. In the present research, the sensor array was composed of eight different gas sensors. MOS gas sensors, as a typical commercial sensor, are extensively used in e-nose. These sensors are widely used in olfactory machines due to their high chemical stability, long life, low response to moisture, and reasonable prices. These eight sensors are circulated on an electronic screen. By entering the gas around the sample into the sensor chamber, the change in the output voltage of each sensor is proportional to the type of sensor and its sensitivity. Specification of the selected gas sensors has been listed in Table 1. The odor stimulus patterns from known odors are

![Figure 1. The used e-nose system.](image-url)
then utilized to generate a database that is subjected to multivariate analysis so that unknown odors can, therefore, be identified and classified.\textsuperscript{[26,27]}

**Preparation of adulterated ghee samples**

In this study, the quality of the used pure cow ghee samples was guaranteed since they were obtained directly from the producers. Sunflower oil and fat (from cow body) were used as a common cheating in cow ghee. For the preparation of adulterated ghee samples, sunflower oil and cow body fat were heated to 40–50°C for 10 min before mixing. To prepare specimens with various adulteration levels, cow ghee was mixed with sunflower oil and cow body fat to prepare five concentration levels in proportions of 10%, 20%, 30%, 40%, and 50% (w/w). Also, 15 replications were considered for each adulteration level.

The tests were done after filling the cylindrical container by an oil sample and tying the container cap. The interval time between each of the two tests was about 30 min for mixing the air inside the compartment with the volatiles from the sample and filling the space above the sample. In each test, the electronic nose dealt with baseline correction, injecting the sample smell and clearing the sensors chamber. After filling the sample container, in the baseline correction step (200 s) the clean air was passed through sensors to reach the steady state. At the injection stage (180 s), the sample smell as a gas is entered in the sensor chamber and causes to change the output voltage of each sensor that is proportional to the type of sample, sensor and its sensitivity. At the stage of clearing the sensors and the container, clean air was entered into the sensor chamber to reach the steady state. At this stage, the pump also removed the odor remaining inside the sample container. This stage also lasted for 120 s. Sensor voltage response was collected in 500 s by the data acquisition system.

**Data analysis**

The signals obtained from the sensors were first recorded as raw data. In the next step, sensor signals were pre-processed consisted of three stages of baseline correction, compression, and normalization of data. The purpose of the correction of the baseline is to compensate the drift and increase the quality of the response of the sensors. In this study, the deficit method was used to correct the baseline by Equation 1. This method is widely used in semiconducting metal oxide sensors\textsuperscript{[18,28]}:

\[
Y_s(t) = \frac{X_s(t) - X_s(0)}{X_s(0)}
\]

where \(X_s(0)\) is the sensor response before the test, \(X_s(t)\) is the sensor response at time \(t\) and \(Y_s(t)\) is the normalized response of the sensor.

In this research, data were analyzed using principal component analysis (PCA) and artificial neural networks (ANN) methods. PCA is a projection method that allows an easy visualization of all the information contained in a data set.\textsuperscript{[28]} This method helps to understand how a sample varies
from other samples (score plot) and which variables mostly contribute the distinction (loading plot). Artifical neural networks (ANN) can be defined as a set of very simple calculation units (nodes) that start out from a data set and transform it into a set of response values. An ANN is a technique with great potential for the treatment of the signals generated by electronic noses based on sensors that afford non-linear responses. For classification purposes, the network builds a model based on a set of input objects (the training set) with known outputs, adjusting the weights associated with each connection so that output values as similar as possible to the real values are generated. Pre-processed data were used as input matrix to the analytical method. All calculations and analysis were performed using the Unscrambler x10.4 and MATLAB R2013a software.

Results and discussion

Sensor selection

The sensor’s response was normalized using the fractional method and, their radar graph was drawn. Figure 2, shows the radar graph corresponding to the sensors’ responses for pure cow ghee (a), pure sunflower oil (b) and pure cow body fat (c).

The radar graph shows that the odor of pure cow ghee, pure sunflower oil and pure cow body fat has the most and least effect on the MQ136 and MQ135 sensors, respectively. The MQ136 sensor is sensitive to sulfur dioxide odor, and MQ135 sensor is sensitive to ammonia, benzene and sulfide vapor. According to the results obtained from the radar graph, it can be concluded that the MQ136 sensor has the most role in classification among other sensors.

PCA results for the combination of cow ghee and sunflower oil

PCA was used to reduce the dimension and for primary evaluation of the similarity of classes. Based on the results of the PCA analysis, the two main components, PC1 and PC2, showed 70% and 26% variance between the pure and adulterated cow ghee samples, respectively. To understand the effect of each sensor in pattern identification analyzes, the sensors were depicted in a plot called a loading plot with special value coefficients (Figure 3(a)).

Loading plot demonstrates the sensors role in systems. Therefore, we can select the correct sensors to detect particular components in cow ghee and sensors that produce similar results can be removed to reduce the cost of making the olfactory machine. Based on Figure 3(a), it was observed that sensors No. 1 and 4 and sensors No. 2 and 8 have a similar loading coefficient. So we can select or remove one of them in the next step (construction of the apparatus).

The score plot, which describes the first and second components (PC1 and PC2) expresses the variance between the data obtained from the tests. Score plot is used to determine the existence of separate data clusters for pattern recognition. In Figure 3 (b), the score plot diagram is shown for the two main components. This plot is used to detect the distinction between the sample groups for the pattern recognition process. As shown in the figure, there is a difference between pure cow ghee samples and adulterated ones.

In a research, the capability of e-nose machine for detecting formalin, hydrogen peroxide and sodium hypochlorite in raw milk was investigated. According to the results, principal component analysis (PCA) showed that PC1 and PC2 have 97%, 87% and 83% of variance within data for formalin, hydrogen peroxide, and sodium hypochlorite, respectively.

PCA results for the combination of cow ghee and cow body fat

Loading plot was used to determine the role of sensors in separating groups. Loading plot for the detection of pure cow ghee from animal fat is shown in Figure 4a. As seen, sensors that have the
Figure 2. Radar graph response of the sensors for pure cow ghee (a), pure sunflower oil (b) and pure cow body fat (c).
highest values for the main component are sensors 2 (TGS822), 3 (MQ136), 4 (MQ9) and 8 (TGS2620). The score plot which describes the first and second components (PC1 and PC2) expresses the variance between the data obtained from the tests. As shown in Figure 4(b), sensors 6 (MQ135) and 7 (TGS2602) among the other sensors, have the least role in classification. Also, it was observed that sensors No. 1 and 4 and sensors No. 2, 5 and 8 have a loading coefficient. So we can select or remove one of them in the next step.

Based on the results of PCA analysis, for the combination of pure cow ghee with animal fat, two main components of PC1 and PC2 were 81% and 16%, respectively, and the variance between samples was 97% of the total data. In Figure 4(b), a score plot diagram is shown for the two main components. As shown in the figure, there is a distinct distinction between pure cow ghee samples and adulterated samples (combination cow ghee with cow body fat). In similar research, an electronic nose was applied to the detection of adulteration of virgin coconut oil. Principal component analysis (PCA) was used to differentiate between pure and adulterated samples. Based on the results, the PCA provided a good differentiation of samples. Also, in another research, PCA analysis was used for detection pig body fat in pure ghee by total reflectance Fourier transform infrared spectroscopy (ATR–FTIR) method, and for detection of goat body fat adulteration in pure ghee using ATR-FTIR spectroscopy coupled with chemometric strategy.

**ANN results**

In this research, an artificial neural network with multilayer perceptron structure was used for better performance than other types of artificial neural network with error propagation learning algorithm.
In the structure of the artificial neural network, three layers are also used, since, according to Kolmogorov, three layers are suitable for the separation of any type of space. The structure of artificial neural network consists of three different layers, including the input layer, the middle layers (hidden) and the output layer. Within each layer, there are a number of neurons that are related to the weighted connections that the number of these neurons depends on the number of input and output variables of the model, but the selection of the number of neurons in the middle layer is determined by trial and error. The last layer or output layer contains the predicted values by the network and introduces the output of the model and the middle layers formed by the processor nodes.

In this study, the feed forward neural network with the Lewenberg-Markowart post-propagation training function was used. The performance evaluation of the designed networks was evaluated using the mean squared error (MSE) and correlation coefficient (r). The transfer function of the network was the hyperbolic tangent sigmoid function. In this study, 20% of the data was randomly for testing, 20% for cross-validation and 60% of the data was used for the network training. The number of neurons of the hidden layer was changed to reach the minimum mean squared error and maximum correlation coefficient. ANN has eight inputs (eight data achieved from each sensor's signal) and the output layer has 7 neurons for classifying cow ghee and adulterated oils.

Based on the results, the network with the final structure of 8–15-7 (8 neurons in the input layer, 15 neurons in the hidden layer, and 7 neurons in the output layer) was the best structure with the

Figure 4. Loading plot (a) and score plot (b) of PCA analysis in the diagnosis of cow ghee mixed with cow body fat.
correct classification rate of 91.3% (Tables 2 and 3). Also, the mean squared error (MSE) and correlation coefficient (r) were 0.086222 and 0.87747, respectively, for the best structure.

According to the results for classification of different groups of adulterated cow ghee (with cow body fat), the network with the final structure of 8–17-7, was the best model with the correct classification rate of 82.5% (Table 4). Also, the mean squared error (MSE) and correlation coefficient (r) were obtained by the best network as 0.094352 and 0.81238, respectively (Table 5).

Similar research was conducted by Oliveros et al.[34] They concluded that ANN can difference between adulterations of virgin olive oil. Also, an electronic nose was used for the detection of maize oil adulteration in camellia seed oil and sesame oil. The artificial neural network (ANN) model was
used to detect the percentage of adulteration in camellia seed oil and sesame oil. The results showed that, based on ANN as its pattern recognition technique, the electronic nose cannot predict the percentage of adulteration in camellia seed oil, but can be used in the quantitative determination of adulteration in sesame oil.\[28\]

**Conclusion**

The e-nose machines are designed to detect and distinguish various odors. In this research, an electronic nose based on 8 metal oxide semiconductor sensors was used for detection of the pure cow ghee from adulterated ones (mixed with sunflower oil and cow body fat). Principal component analysis (PCA) and artificial neural network (ANN) methods were used to classify different amounts of adulteration in cow ghee. Based on the results, the used system successfully detected pure cow ghee and the adulterated one by pattern recognition method. To classify cow ghee with different percentages of sunflower oil and cow body fat using the olfactory machine, the PCA results indicated the 96% and 97% variance of total data for sunflower oil and cow body fat, respectively. Also, the correct classification rate by ANN method was 91.3, 82.5% for sunflower oil and cow body fat, respectively.

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