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# Identification of Adulterated Milk Using Electronic Nose

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Over the past few years, electronic nose (E-nose) technology has enhanced the possibility of exploiting information on aroma to assess food. The objective of this study is to monitor the adulteration of milk with water or reconstituted milk powder using an E-nose (PEN 2) containing 10 different metal oxide sensors. Whole fluid milk, reconstituted milk powder and whole fluid milk adulterated with different proportions of water or reconstituted milk powder were examined in experiments over 7 days of storage. The results were obtained by principal-component analysis (PCA) and linear-discriminant analysis (LDA). In the LDA plot, for the samples of 1st day, two samples of 100% skim milk and 100% reconstituted milk could be clearly discriminated, and four other samples with different proportions of skim milk and reconstituted milk were examined. In the LDA plot, the results for the six samples from the 1st day to the 3rd day were well separated. However, from the 5th day to the 7th day, the LDA plots of the samples overlapped completely. The E-nose has the capacity to discriminate the purity of milk when skim milk is adulterated with different volumes of water, and results for the three samples of skim milk, skim milk adulterated with reconstituted milk, and skim milk adulterated with water were distributed regularly in the LDA and PCA plots. By LDA and PCA, the E-nose could also discriminate between milk samples that had been aged for different numbers of days, and the 1st–4th day results were well separated.

## 1. Introduction

Aroma is an important food-quality attribute. The aroma of a food is detected when its volatiles enter the nasal passages at the back of the throat and are perceived by the receptors of the olfactory system.[1] The exploitation of information contained in the headspace above food has been recently studied using conventional analytical chemistry

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equipment, and the correlation between the quality and the aroma has also been expressed in both quantitative and qualitative terms.

A strategy for determining quality consists of sensing the aromatic volatiles emitted from food using electronic olfactory systems.[2] In the last decade, electronic nose (E-nose) technology has enabled the possibility of exploiting, from a practical point of view, the information contained in the headspace above food for many different application fields. The E-nose offers a fast and nondestructive alternative method of analyzing aromas, and, hence, may be advantageously used to develop quality indices for food.

Commercially available E-noses use an array of sensors combined with pattern recognition software. There have been several reports on electronic sensing in environmental control, medical diagnostics and in the food industry.[3–6] E-noses have been developed for classification and recognition in the dairy industry to analyze milk ageing, for shelf-life prediction, the correct classification of UHT and pasteurized milk,[7–8] off-flavours in milk,[9] dairy products by geographical origin,[10] and bacteria cultures in milk,[11] the screening of aroma-producing lactic acid bacteria,[12] quality assessment,[13,14] and the recognition of different types of milk.[15] Although the E-nose has been used as a new tool to control the quality of dairy produce, there has been no application to identify adulterated milk.

Furthermore, little detailed information is available on whether the E-nose is able to discriminate between milk adulterated by water or reconstituted milk powder. The objectives of this study are: (1) to evaluate the capacity of the PEN 2 E-nose for monitoring milk adulterated by water or reconstituted milk powder, (2) to compare two types of statistical data (PCA and LDA), and (3) to identify milk storage time.

## 2. Materials and Methods

### 2.1 *Milk samples*

The skim milk (fluid milk) and skim milk powder were bought from the supermarket; they were the same brand named "Yili" and were made from the same milk. The protein content in the skim milk was about 2.9 g per 1000 ml and that in the skim milk powder was 22 g per 100 g. Skim milk powder (13.5 g) was dissolved in 1000 ml distilled water, then the mixture (referred to as reconstituted milk) was homogenized by ultrasonic waves. The reconstituted milk powder was formed so that its protein content was near to that of the skim milk.

Adulterated milk samples were made by adulterating fluid milk with different proportions of reconstituted milk. There were six samples with different percentages of reconstituted milk powder added to fluid milk: 100% skim milk, 80% skim milk adulterated with 20% reconstituted milk, 60% skim milk adulterated with 40% reconstituted milk, 40% skim milk adulterated with 60% reconstituted milk, 20% skim milk adulterated with 80% reconstituted milk, and 100% reconstituted milk. Six replicas of each sample were made.

The skim milk was adulterated with different volumes of water. There were 5 samples with different percentages of distilled water added to the fluid milk: 40 ml skim milk, 35 ml skim milk adulterated with 5 ml water, 30 ml skim milk adulterated with 10

ml water, 25 ml skim milk adulterated with 15 ml water, and 20 ml skim milk adulterated with 20 ml water. Six replicas of each sample were made.

The samples were held in 500 ml beakers and sealed with polyethylene film. A luer lock needle (20 g) connected to Teflon tubing (3 mm) was used to perforate the seal (polyethylene film) of the beaker and to absorb the air inside it during the measurements. The beakers were sealed by a new polyethylene film after the air was absorbed. The data were collected daily during 7 days of storage at 20±1°C.

### 2.2 *E-nose data acquisition and analysis*

An E-nose device, PEN 2, provided by WMA Airsense Analysentechnik GmbH, Schwerin, Germany, was used. The portable electronic nose PEN 2 contains an array of 10 different metal oxide sensors (listed in Table 1) positioned in a small chamber (V=1.8 ml). Table 1 lists all the sensors used and their main applications. The table also contains currently known or specified reactions.

Table 1
Sensors used and their main application in PEN 2.

| Number in array | Sensor name | General description | Reference |
|---|---|---|---|
| 1 | WIC | Very sensitive to aromatic compounds. | Toluene, 10 ppm |
| 2 | W5S | Very sensitive, broad range of sensitivity, reacts with nitrogen oxides, very sensitive with negative signal. | $NO_2$, 1 ppm |
| 3 | W3C | Sensitive to ammonia. Used as sensor for aromatic compounds. | Benzene 10 ppm |
| 4 | W6S | Selective sensitivity to mainly hydrogen (gases in breath). | $H_2$, 100 ppb |
| 5 | W5C | Sensitive to alkanes, aromatic compounds, less polar compounds. | Propane 1 ppm |
| 6 | W1S | Sensitive to methane (environment) *ca*. 10 ppm. Broad range, similar to No. 8. | $CH_4$, 100 ppm |
| 7 | W1W | Reacts with sulfur compounds, $H_2S$ 0.1 ppm. Otherwise sensitive to many terpenes and sulfur organic compounds, which are important for smell, such as limonene and pyrazine. | $H_2S$, 1 ppm |
| 8 | W2S | Detects alcohols and partially aromatic compounds, broad range. | CO, 100 ppm |
| 9 | W2W | Detects aromatic compounds, sulfur organic compounds. | $H_2S$, 1 ppm |
| 10 | W3S | Reacts with methane at high concentrations >100 ppm, sometimes very selective. | $CH_4$, 10 $CH_4$, 100 ppm |

During the measurements, the headspace gas was pumped over the sensors in the electronic-nose with a flow of 100 ml/min; during the measurements process, three different phases can be distinguished: concentration, measurement, and standby. Electrovalves, controlled by a computer program, guide the air through different circuits depending on the measurement phase. For each phase, the airflow was kept constant through the measurement chamber. During the measurement phase, the bomb pushes the volatiles through a closed loop that includes the measurement and concentration chambers. No air enters, or exits the loop. The measurement phase lasts 100 s, sufficient time for the sensors to reach a stable value. The collected data interval was 1 s. When the measurement is completed, the standby phase was activated (100 s). Its main purpose is to clean the circuit and return the sensors to their baseline. Clean air enters the circuit, crosses the measurement chamber followed by the empty concentration chamber, and pushes the remaining volatiles out of the circuit.

The E-nose experiments were conducted at the temperature of 20±1°C and 50–60% relative humidity (RH) during all experiments. When the sensors are exposed to volatiles during the measurement phase, a computer records the resistance changes that the sensors undergo. When the measurement is completed, the acquired data is stored for later use.

The set of signals of all sensors during the measurement of a sample constitute a pattern. Patterns of multiple measurements dealing with the same type of sample are stored in a pattern file and act as the training set. The pattern data are recorded, checked visually, and analyzed using WinMuster (version 1.5.2.4 Jun. 2003, copyright 1996-2002 WMA Airsense Analysentechnik GmbH 2003).

### 2.3 *Principal-component analysis and linear-discriminant analysis*

The sensor response signal obtained by the measurement was subjected to different pattern recognition techniques such as principal-component analysis (PCA) and linear-discriminant analysis (LDA).

PCA is a chemometric linear, and unsupervised pattern recognition technique used for analyzing, classifying, and reducing the dimensionality of numerical datasets in a multivariate problem. This method enables the extraction of useful information from the data, exploration of the data structure, the relationship between objects, the relationship between objects and variables, and the global correlation of the variables. The main features of PCA are that the coordinates of the new data in the database (score plot) and the contribution to each component of the sensors (load plot) can be shown. The score plot is usually used for studying the classification of data clusters, while the load plot can provide information on the relative importance of the array sensors in analyzing each principal component and their mutual correlation.

LDA calculates the discriminant functions and is similar to the PCA in that it offers a 2-dimensional display of the training set data. The difference between PCA and LDA is that PCA does not consider the relation of data points to the specified classes, while the LDA calculation uses the class information that was given during training. LDA considers the distribution within classes and the distances between them. Therefore, LDA is able to collect information from all sensors to improve the resolution of classes.

## 3. Results and Discussion

### 3.1 *Discrimination among skim milk, reconstituted milk, and their mixture*

On the last day when the milk was fresh, the six samples (100% skim milk, 80% skim milk adulterated with 20% reconstituted milk, 60% skim milk adulterated with 40% reconstituted milk, 40% skim milk adulterated with 60% reconstituted milk, 20% skim milk adulterated with 80% reconstituted milk, and 100% reconstituted milk) were clearly discriminated using the E-nose by LDA and PCA based on the response signals of the E-nose sensors, as shown in Fig. 1. The results for skim milk and reconstituted milk were well separated and distributed on the top right and top left, respectively, in Fig. 1(a). There may be an obvious difference between the volatiles of skim milk and skim milk powder, leading to the longest distance between the two samples of 100% skim milk and 100% reconstructed milk. In Fig. 1(a), the six samples are distributed in order of the proportion of reconstituted milk in the sample. The lower the proportion of skim milk, the longer the distance from the sample of skim milk, and the higher the proportion of reconstituted milk, the shorter the distance from the sample of reconstituted milk.

The data points of each sample obtained by LCA were more concentrated than those in the PCA plot in Fig. 1(b). The 3 samples that were adulterated with lower proportions of reconstituted milk (100% skim milk, adulterated with 20% reconstituted milk, and adulterated with 40% reconstituted milk) were at the bottom right of the plot. The distances among the 3 samples were relatively small. The other 3 samples that were adulterated with higher proportions of reconstituted milk (adulterated with 60% reconstituted milk, adulterated with 80% reconstituted milk, and 100% reconstituted

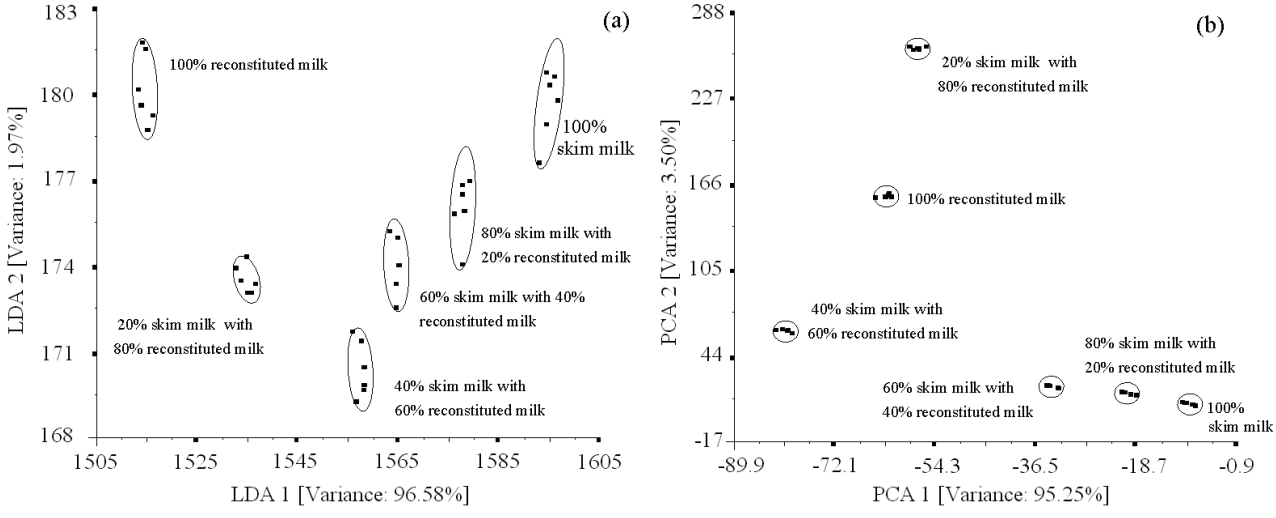


Fig. 1. Plot of the six samples on 1st day: (a) LDA result and (b) PCA result.

milk) were on the left side of the plot, and the distances among the 3 samples were longer than those among the other 3 samples.

### 3.2 *Identification of milk stored for different number of days*

On the 2nd day, for the milk analyzed by LDA and PCA, similar results were obtained from the LDA and PCA plots.

On the 4th day, some samples of milk became dense and cheeselike. In the LDA plot, by comparing Fig. 2(a) with Fig. 1(a), it can be seen that the distribution of samples changed. Three of the samples (adulterated with 20% reconstituted milk, adulterated with 40% reconstituted milk, and adulterated with 60% reconstituted milk) had results that overlapped partly. However, in the PCA plot, the 6 samples could still be discriminated. It was found that PCA was better than LDA for identifying the skim milk adulterated with reconstituted milk. On the 4th day (Fig. 2), although some samples had become rancid, the 6 samples could still be discriminated by PCA.

On the 5th day (Fig. 3), when the deterioration of the milk was evident, the data points had a disperse distribution, and the results for the 6 samples overlapped completely and were not distributed in order of proportion of reconstituted milk. When the rancidity occurred, maybe the odor of the skim milk was similar to that of skim milk powder. Thus, from the 5th day, the E-nose could not discriminate between the 6 samples.

### 3.3 *Identification of skim milk adulterated with water*

The data acquired for skim milk adulterated with water were analyzed by PCA and LDA, and the results are shown in Fig. 4. In Fig. 4(a), the plot for 40 ml skim milk was at the bottom left. The 5 samples were distributed regularly in order of the volume

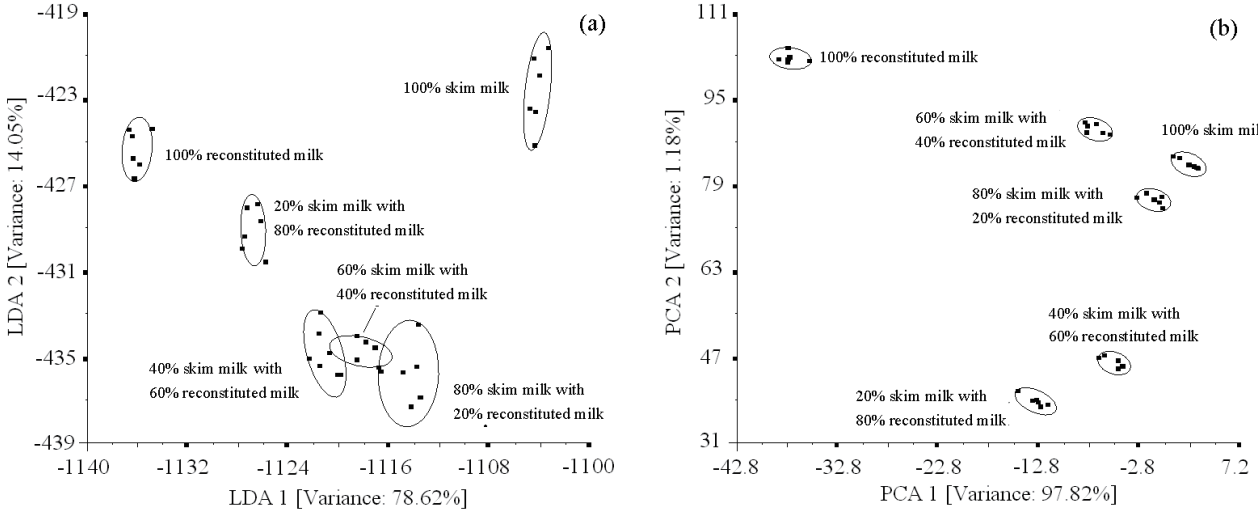


Fig. 2. Plot of the 6 samples on 4th day: (a) LDA result and (b) PCA result.

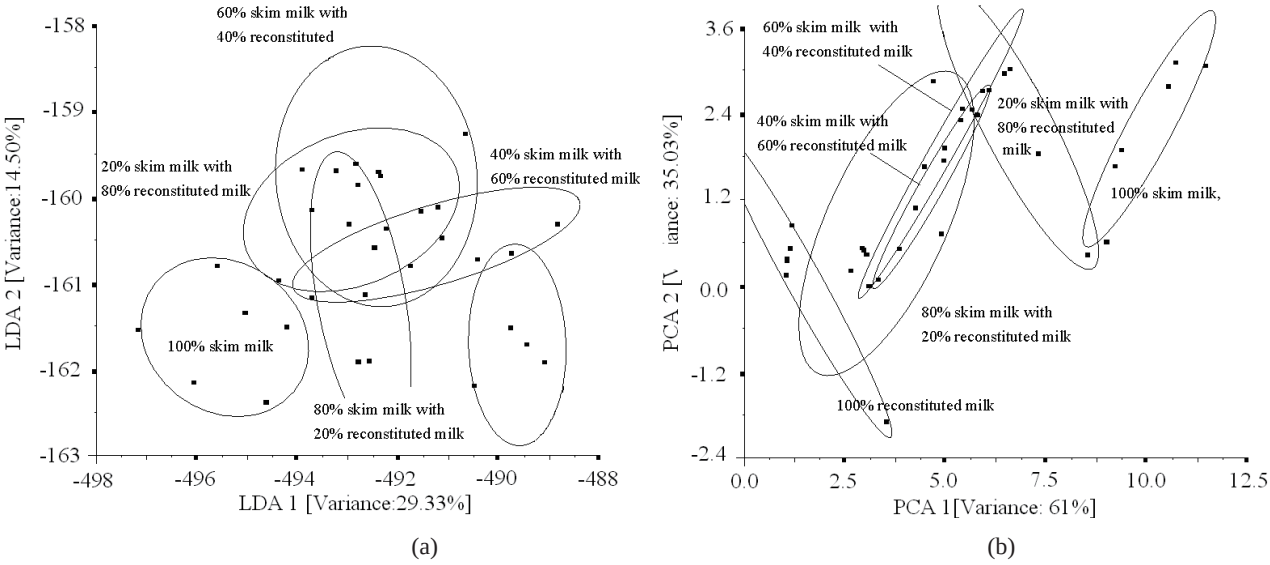


Fig. 3. Plot of the 6 samples on 5th day: (a) LDA result and (b) PCA result.

of water added. The result for milk adulterated with 10 ml water was at the highest point. It was shown that the LDA had the capacity to identify the adulterated milk and monitor its purity. Figure 4(b) shows that the results of PCA for the 3 samples that were adulterated with lower proportions of water (40 ml skim milk, adulterated with 5 ml water, and adulterated with 10 ml water) were at the bottom left. However, the other two samples, which were adulterated with a higher proportion of water, were at the top right and could clearly be discriminated.

### 3.4 *Discrimination among skim milk adulterated with reconstituted milk and water*

To test the capacity of the E-nose for identifying pure skim milk, skim milk adulterated with reconstituted milk, and skim milk adulterated with water, 3 samples were used (40 ml skim milk, 35 ml skim milk adulterated with 5 ml water, and 80% skim milk adulterated with 20% reconstituted milk). The acquired data was analyzed by PCA and LDA. In LDA plot shown in Fig. 5(a), the 3 samples were distributed triangularly. The result for 40 ml skim milk was at the bottom left, the result for 35 ml skim milk adulterated with 5 ml water was at the highest point, and the result for the 80% skim milk adulterated with 20% reconstituted milk was at the middle right. The 3 samples were well separated. In the PCA plot shown in Fig. 5(b), the result for 40 ml skim milk was close to that for 35 ml skim milk adulterated with 5 ml water. Maybe the aroma of the skim milk was the only detectable odor of the 2 samples. The only difference between the two samples was whether the skim milk was adulterated with water or not. The sample of 80% skim milk adulterated with 20% reconstituted milk emanated at least 2

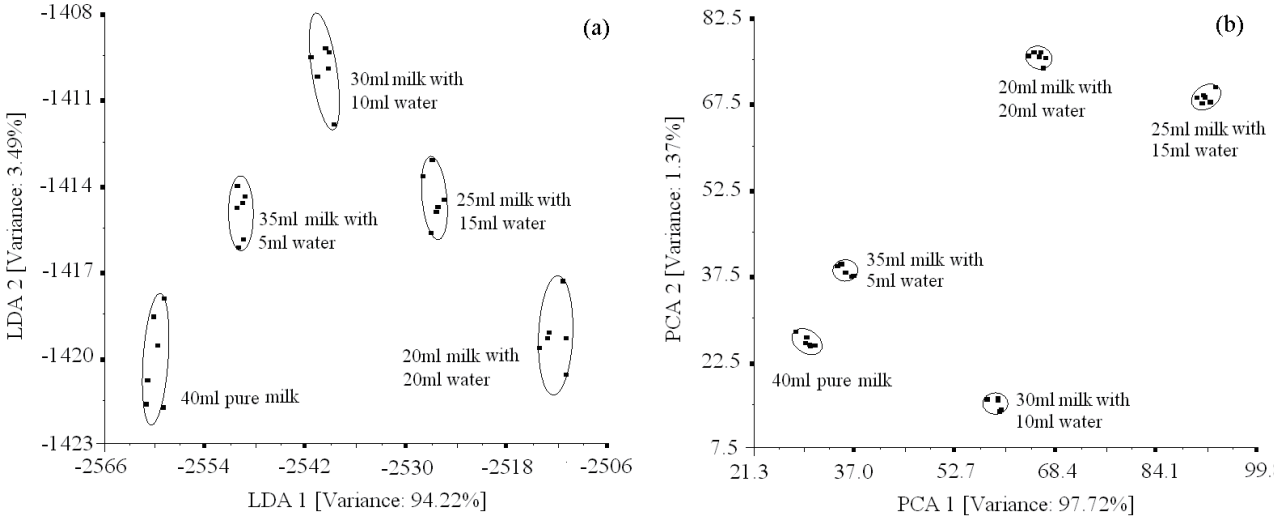


Fig. 4. Plot of milk adulterated with different volumes of water: (a) LDA result and (b) PCA result.

kinds of aroma, namely, the aromas of skim milk and reconstituted milk. Thus, the result for this sample was far from the others in the PCA plot.

### 3.5 *Milk ageing analysis*

To identify the freshness of skim milk, the data collected from skim milk from the 1st day to the 7th day were analyzed by PCA and LDA. In the LDA plot (Fig. 6(a)), the results for the 1st–4th days were well separated; on the 4th day, data had a disperse distribution; but on the 5th–7th days, results overlapped. The freshness evidently deteriorated day by day from the 1st day to the 7th day. Thus, the odors of the milk on the 1st–4th days could be discriminated by the E-nose. By the 5th day, most of the samples had become rancid, and the E-nose could not discriminate among them. The same result was obtained by PCA. These results were similar to those obtained by Capone *et al.*[7] The data of the other 5 samples (adulterated with 20% reconstituted milk, adulterated with 40% reconstituted milk, adulterated with 60% reconstituted milk, adulterated with 80% reconstituted milk, and 100% reconstituted milk) were analyzed by PCA and LDA from the 1st day to the 7th day. The results of 2 samples (adulterated with 60% reconstituted milk, and 100% reconstituted milk) are shown in Figs. 7 and 8, respectively. The results for not only skim milk, but also skim milk adulterated with reconstituted milk underwent a similar trend during the process of rancidity. Namely, in the PCA and LDA plots, the 1st–4th day results were well separated, but 5th–7th day results overlapped. The E-nose could identify the change in the odor of milk day by day, and the freshness level can be identified in the PCA and LDA plot.

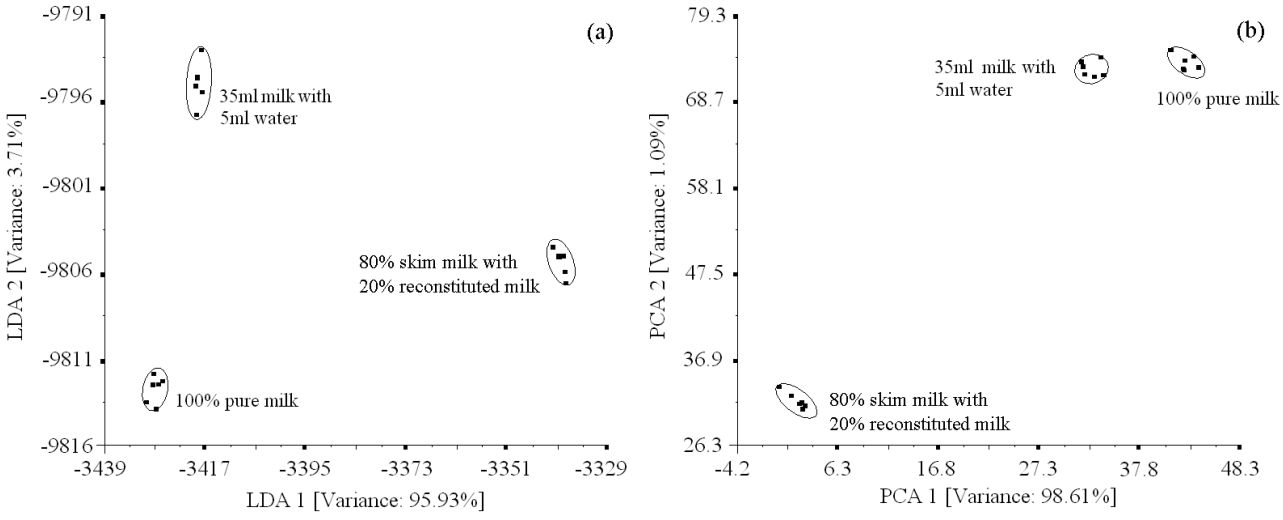


Fig. 5. Plot of milk adulterated with reconstituted milk or water: (a) LDA result and (b) PCA result.

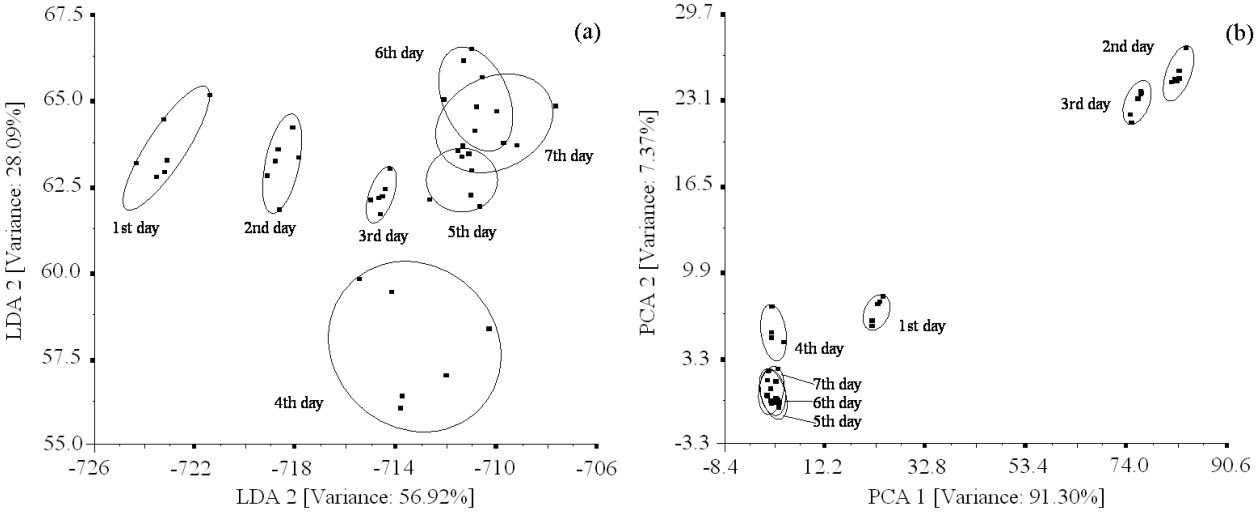


Fig. 6. Plot of 100% skim milk on different days: (a) LDA result and (b) PCA result.

## 4. Conclusions

(1) The E-nose could discriminate between skim milk and reconstituted milk by their aroma. When skim milk was adulterated with different proportions of reconstituted milk, the samples could be identified by the E-nose through their aroma. From the 1st day to the 4th day, in the LDA plot, the results for 6 samples were distributed regularly in order

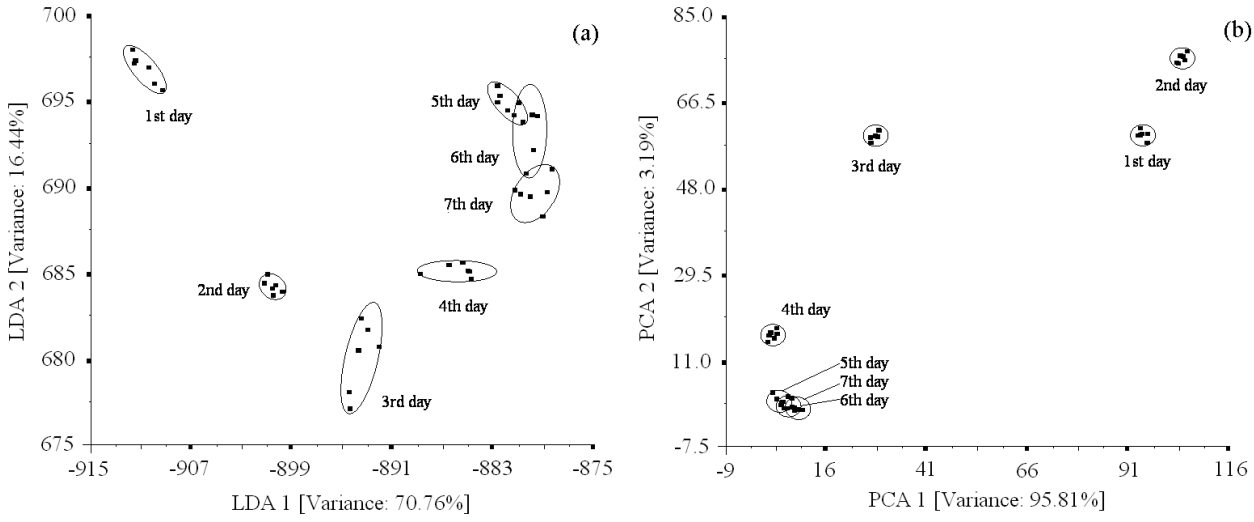


Fig. 7. Plot of milk adulterated with 60% reconstituted milk on different days: (a) LDA result and (b) PCA result.

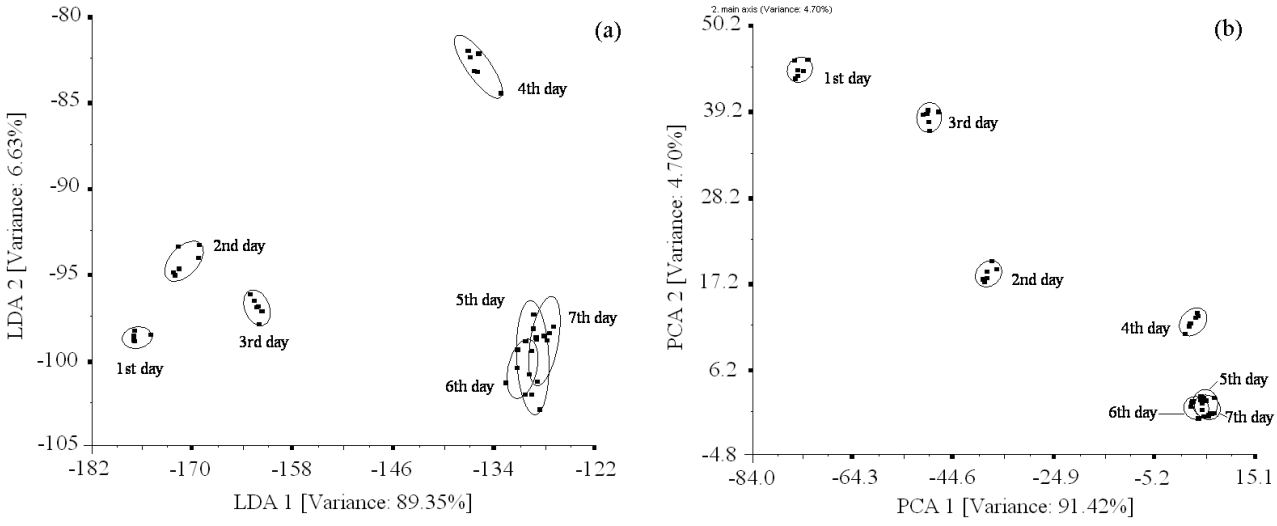


Fig. 8. Plot of 100% reconstituted milk on different days: (a) LDA result and (b) PCA result.

of the proportion of adulterated reconstituted milk.

(2) The E-nose could discriminate the samples of skim milk adulterated with different volumes of water. Results for the 5 samples were distributed regularly in LDA plot, and the samples adulterated with lower proportions of water and those adulterated with higher proportions of water could be discriminated between.

(3) The E-nose could discriminate among milk stored for 1–4 days for skim milk, reconstituted milk, and skim milk adulterated with different proportions of reconstituted milk, but could not discriminate between milk stored for 5–7 days. The E-nose could identify the change in the odor of milk day by day. The freshness of the milk could be obtained from the PCA and LDA plots.

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