Voltammetric Electronic Tongue for the Qualitative Analysis of Milk Adulterated with Urea combined with multi-way data analysis

Liu-an Li[1,], Yaping Yu[2], Jingjing Yang[1], Renjie Yang[3], Guimei Dong[3], Tianming Jin[1]*

[1]College of Animal Science and Veterinary Medicine, Tianjin Agricultural University, No.22 Jinjing Road, Tianjin 300384, China
[2]School of Electrical Engineering & Automation, Tianjin University, No.92 Weijin Road, Tianjin 300072, China
[3] College of Engineering and Technology, Tianjin Agricultural University, No.22 Jinjing Road, Tianjin 300384, China
[*]E-mail: anliuli2003@163.com

A new approach for qualitative analysis of milk adulterated with urea is proposed by multi-way principal component analysis (MPCA) and multi-way partial least squares discriminant analysis (NPLS-DA) based on voltammetric electronic tongue. 9 pure milk samples and 11 adulterated milk samples with different contents of urea were prepared and the current response values of the voltammetric electronic tongue were measured consequently. MPCA was used to distinguish urea-tainted milk and pure milk. Furthermore, a NPLS-DA model was developed using the first five principal components (PCs) according to the distribution of PCs computed by MPCA. It was shown that the ratios of correct classification were 100% and 88.9% for calibration set and prediction set, respectively. The results indicated that voltametric electronic tongue combined with multivariate methods was efficient for urea-tainted milk discrimination.

1. INTRODUCTION

It is well known that milk has been an important human nutrient source for thoushand years. However, in recent years, in order to get more commercial profit, sometimes natural milk is adulterated with low value or illegal adulterants such as water[1], hydrogen peroxide[2], vegetable oils[3], statch, urea, and melamine[4], etc. Excessive addition of water into milk could increase the

volume of milk, but it would result in the decrease of nutrition substances such as protein and solid content. To avoid supervision, urea and melamine is added to raw milk products to enhance the apparent protein content, since the nitrogen content in urea and melamine is 46.6% and 66% [5],respectively. They improve the nitrogen concentration in milk resulting in a false appearance of a higher level of protein by the Kjeldahl method to mask the milk dillution process[6]. Adulterated milk with urea, above 300mg/L, will cause very serious health related problems such as kidney damage, indigestion, and cancer[7]. Hence, a simple and reliable method for detecting urea level in milk is necessary to control this situation, which has become a global health concern.

Several different methods have been reported for the quantification of milk adulterated with urea, such as spectroscopy [8, 9], scanner digital images [10], impedance sensor [11], electrochemistry [12], and mass spectroscopy [13]. R. J. Yang et al [14] discriminated adulterated milk by using two-dimensional infrared correlation spectroscopy; however, some of these techniques are destructive, time consuming, and require expensive instruments, as well as pre-treatment steps. In addition, most of them are susceptible to environments.

In this work, we report our efforts to develop a rapid, inexpensive, and accurate analysis method based on non-modified electrodes for the qualitative analysis of milk adulterated with urea. We now demonstrate that the fabrication of an array of voltammetric sensors, combined with chemometric tools, is able to distinguish between pure milk samples and the urea-tainted milk samples. This paper proposed a new method by using multi-way variables analysis based voltammetric electronic tongue. The qualitative analysis model is constructed using MPCA, NPLS-DA for the current response values and to study the feasibility of detection the adulterants in milk.

2. EXPERIMENTAL

2.1 Sample collection and preparation

Pure milk samples （Mengniu dairy company, China） was purchased from a local supermarket and spiked at different concentration of urea. The concentration ranges of adulterants were all 0.1-20gL^{-1}，prior to data collection, milk samples adulterated with urea concentration were manually stirred to make them homogeneous.

2.2 Equipment

The electronic tongue consists of three working electrodes made of the metals gold (Au), palladium (Pd) and platinum (Pt), a reference electrode (Ag/Agcl) and a platinum counter electrode. Working electrodes and the counter electrode employed the disk electrodes, which have a diameter of 2mm and a purity of 99.99% with the exception of gold with a purity of 99.999%. Electrochemical experiments were performed by using electrochemical station CHI660E, which is provided by Shanghai Chenhua Company.

2.3 Measurement procedure

In voltammetric measurement, when a voltage is applied between the working electrode and the reference electrode, a response current is measured between the working electrode and the counter electrode. When the electrodes are inserted into the tested solution, a double layer charged is formed between the counter electrode and the working electrode [15]. If there are substances in the solution that are electrochemically active at the applied potential (being oxidized or reduced), a Faradic current is created in addition to the capacitive current.

In this paper, the cyclic voltammetric waveform was adopted[16], for the Au electrode, with its maximal value at 1.6V and minimal value at -0.5V; for the Pd and Pt electrode, with its maximal value at 1.6V and minimal value at 0V; scan rate 0.05V/s, sample interval 0.01V, and sample quiet time was 2s.

After each measurement, all electrodes were rinsed with ultrapure water (milliQ, 18.2MΩ)，and the surfaces of disk working electrodes were mechanically polished for 15 seconds with aluminum oxide of 1 and 0.3μm consecutively[17]. Thereafter, working electrodes and counter electrode were sonicated (Ultrasonic cleaner KQ5200DE) in ethanol: water (1:1) for 5 minutes. Reference electrode was placed in a saturated KCl aqueous solution. Finally, all electrodes were thoroughly rinsed with ultrapure water and used in the next measurement [18].

In cyclic voltammetry, the potential difference between the working electrode and the reference electrode is varied linearly with time, while the reference electrode maintains a constant potential. We have used a electrochemical station CHI660E (Chenhua instrument inc. Shanghai, China), where the working electrodes are gold, palladium and platinum(Aida instrument inc. Tianjin, China) and the reference electrode (Ag/Agcl , Chenhua instrument inc. Shanghai, China) and the counter electrode is of platinum (Aida instrument inc. Tianjin, China). When the potential is applied between the working electrodes and the reference electrode, the current is measured between the working electrodes and the counter electrode. In the present work, the output response current values of the applied potential were considered for the analysis.
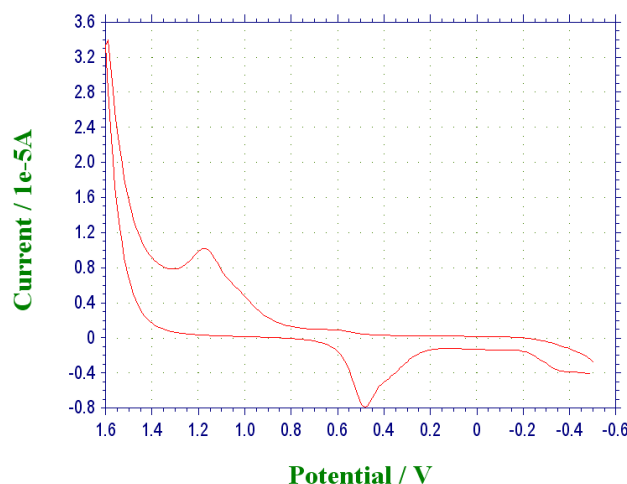
(a)The current response of Au electrode 20g/L milk adulterated with urea
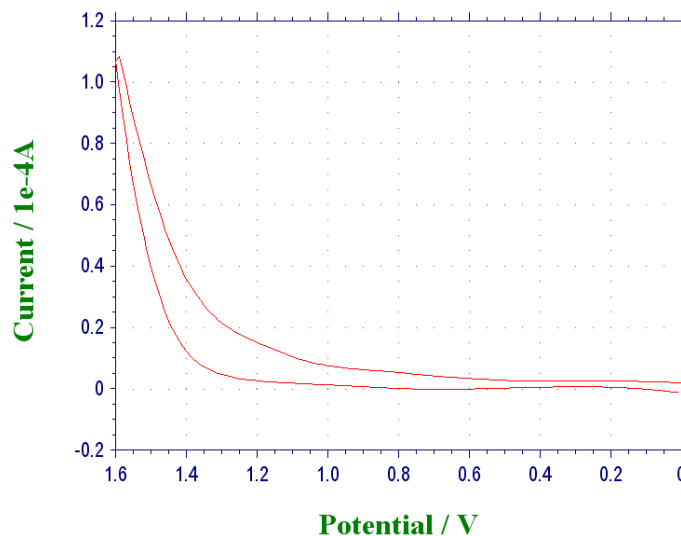
(b)The current response of Pd electrode 20g/L milk adulterated with urea
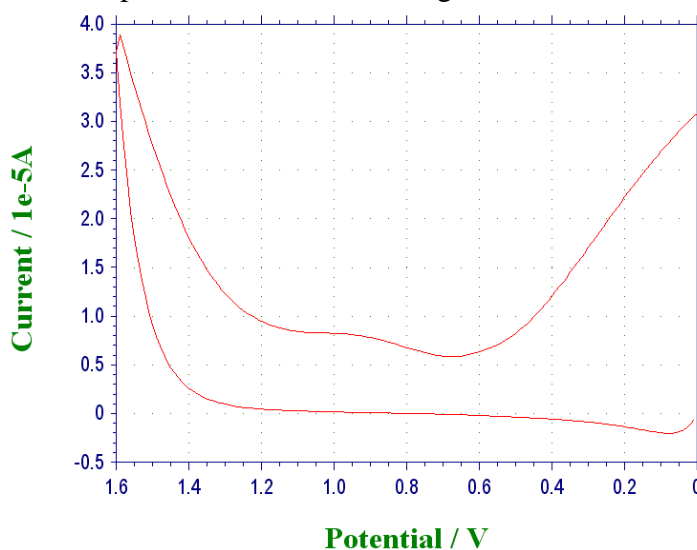
(c)The current response of Pt electrode 20g/L milk adulterated with urea

Figure 1. The current response of cyclic voltammetry from (a) the Au electrode, (b) the Pd electrode and (c) the Pt electrode.

All measurements were carried out in a 50 mL beaker by immersing the three-electrode system directly into 50 mL milk samples at room temperature (20℃) without any pre-treatment. All voltammograms were obtained with a scan rate of 50mV/s, a step potential of 10mV. Three cyclic voltammograms of different working electrodes were recorded sequentially using the CHI660E software, which is shown in Figure 1a-c.

3. DATA ANALYSIS

In this paper, the output current values obtained from all cyclic voltammograms have been considered for data analysis, which were used as input data for the chemometric analyses. In order to

retain more characteristic information of measured objects from different working electrodes, multi-way analysis methods were adopted.

3.1 Multi-way Principal Component Analysis (MPCA)

Principal component analysis (PCA) is widely used in statistics to reduce the number of the variables of an experimental data matrix by several principal components. The PCA produces a score plot that is visualizing different between samples, which can be used for classifications of different samples. Multi-way principal component analysis (MPCA) [19], which is an extension of the PCA algorithm, was initially proposed by Nomikos and MacGregor. Multi-way methods are particularly useful for analytical data where a response is being measured as a function of 2 or more parameters. In this study, current response values of different working electrodes can be summarized in the three dimensional (I samples ×J working electrodes ×K response current variables) array X. MPCA decomposition is the same as the principles of PCA. The matrix X is decomposed into a series of principal components (PCs) consisting of score vectors (t) and loading matrices (P) plus a residual array E that is minimized in a least squares sense.

$$X = \sum_{r=1}^{R} t_r \otimes P_r + E \qquad (1)$$

Here $\otimes$ is the outer product operator.

3.2 N-partial least square discriminant analysis (NPLS-DA)

NPLS-DA is an extension of the PLS-DA algorithm to be applied in multi-way data. NPLS-DA can be applied to classification of objects measured [14]. NPLS-DA is a linear regression method that has been employed successfully for prediction modeling in various biological and biochemical applications [20, 21].

For X(I×J×K) and Y(I×L×M), the mathematical notation of N-PLS calibration model can be written as:

The X is decomposed as $X = T(W^K \otimes W^J)^T + E_X$ \qquad (2)

Where T is scores matrices, W^K and W^J is weight of vector of K and J, respectively. E_X is residual matrices.

The Y is decomposed as $Y = U(Q^M \otimes Q^L)^T + E_Y$ \qquad (3)

Where U is scores matrices, Q^M and Q^L is weight of vector of M and L, respectively. E_Y is residual matrices.

The core of N-PLS is that the maximum covariance is gained between T and U by looking for one group of appropriate loading matrices W^K, W^J and Q^M, Q^L. The regression model of scores matrices is expressed as follows:

$$U = TB + E_U \qquad (4)$$

$$B = (T^T T)^{-1} T^T U \qquad (5)$$

where, B is the regression coefficient.

3.3 Data processing

MPCA was performed using "PLS_Toolbox 2.1" (for use with Matlab, Eigenvector Research, Inc., Manson, WA). Then the nway310 Toolbox (available at http://www.models.kvl.dk) was used to construct NPLS-DA based on current response value of different working electrodes of voltammetric electronic tongue in adulterated milk. NPLS-DA algorithm was performed in MATLAB(The Mathworks Inc.).

4. RESULTS AND DISCUSSION

4.1 Classification of Milk Adulterated with Urea by MPCA

Voltammograms of working electrodes Pd and Pt were recorded between 0 and 1.6V, and that of working electrode Au was recorded between -0.5 and 1.6V, with data less than 0V not included for data analysis. The MPCA algorithm was applied to the three-dimensional matrix X, with dimensions 20×3×320, where I=20 is the number of samples and J=3 is the number of working electrodes, K=320 is the number of current response values[9]. PCA can reduce the immense data set by several principal components. The percent of variance of each principal component represent original information content. The several principal components can represent almost all information of the original experimental data. Figure 2 shows the percent of variance of each principal component of matrix X.
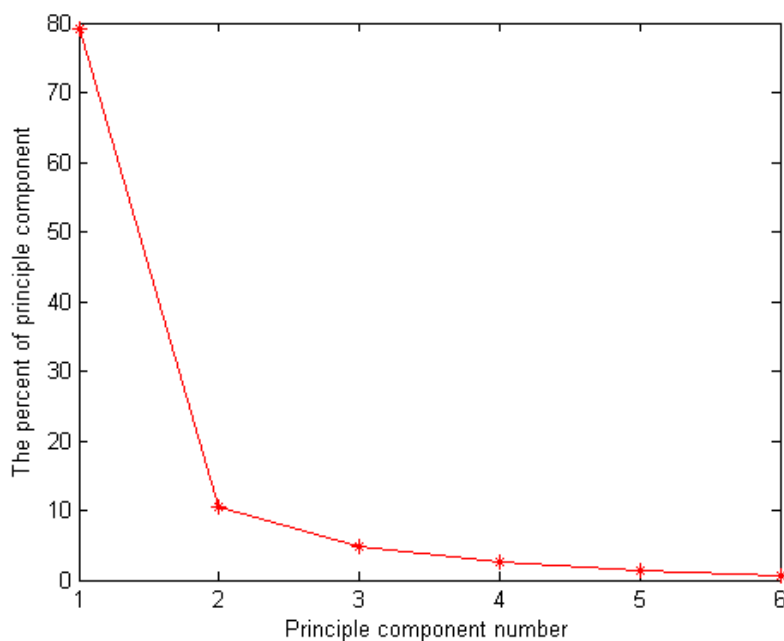

Figure 2. The percent of variance carried out by each principal component of the first six principal components

The first three most important principal components (PCs), which accounted for the most current response variation 94.42% (79.11%, 10.49%, and 4.82% for PC1, PC2, and PC3, respectively). They were used to differentiate pure milk samples and adulterated milk samples. Analyzing the distribution of data plotted on the multi-way principal components analysis, it was evident that twenty samples from pure milk samples and adulterated milk samples with urea were distinguished into two concentrated areas, as shown in Figure 3.
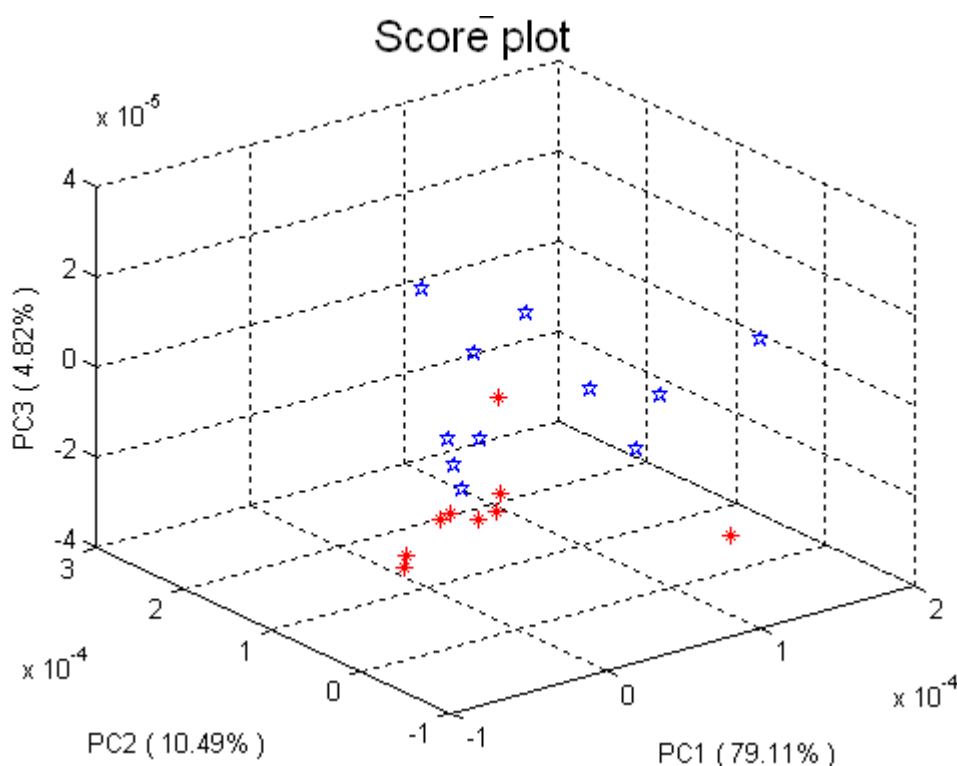

Figure 3. Three-dimensional scores plots of multi-way principal component analysis results for twenty samples by principal component PC1, PC2, and PC3, urea-tainted milk (blue ☆) and unadulterated milk (red ✳).

The result shows that a good discrimination trend can be observed between pure milk samples and adulterated milk samples; in PC3 direction, the upper samples are almost urea-tainted milk. Because the difference between pure milk samples and urea-tainted milk samples is very small and the first three PCs mainly extract their common characteristic information，the classification edge between pure milk samples and urea-tainted milk samples appears unclear. Therefore, in order to achieve an improved separation, more PCs are needed to obtain different characteristic information of measured samples. For this purpose, according to the result of Figure 2, the PC6 is only 0.66%，the first five PCs were used for qualitative analysis of samples with accumulative contributing rate of 98.4%， which can represent the original information content. N-PLS discrimination model was employed for an improved separation rate.

4.2 Discrimination of the NPLS-DA model

NPLS-DA was developed between the arrays containing the current response values of different working electrodes *X* and 'dummy matrix' *Y* containing information about samples groups [14]. In present work, the calibration set (11×3×320) and prediction set (9×3×320) were applied to build NPLS-DA model. The calibration set, which was used to construct the model, had 11 samples including 5 pure milk samples and 6 urea-tainted milk samples. The prediction set was formed by the remaining 9 samples including 4 milk samples and 5 urea-tainted milk samples. The model was used for qualitative analysis of milk adulterated with urea.

Choosing the optimal factors is a crucial step for building a stable N-PLS model with high accuracy. In this work, we selected 5 factors according to the contribution of PCs. In the NPLS-DA models used in this study, the threshold value was set to 0.5. Below 0.5, the sample was estimated as 0 and was assigned to pure milk samples. Above 0.5, the sample was estimated as 1 and was assigned to urea-tainted milk samples.

In calibration set, samples 1 to 5, which represent the pure milk samples, are belonging to class 1. Samples 6 to 11, which represent the urea-tainted milk samples, are belonging to class 2. The rate of correct classification was 100%, and the predicted result in calibration set was shown in Figure 4.
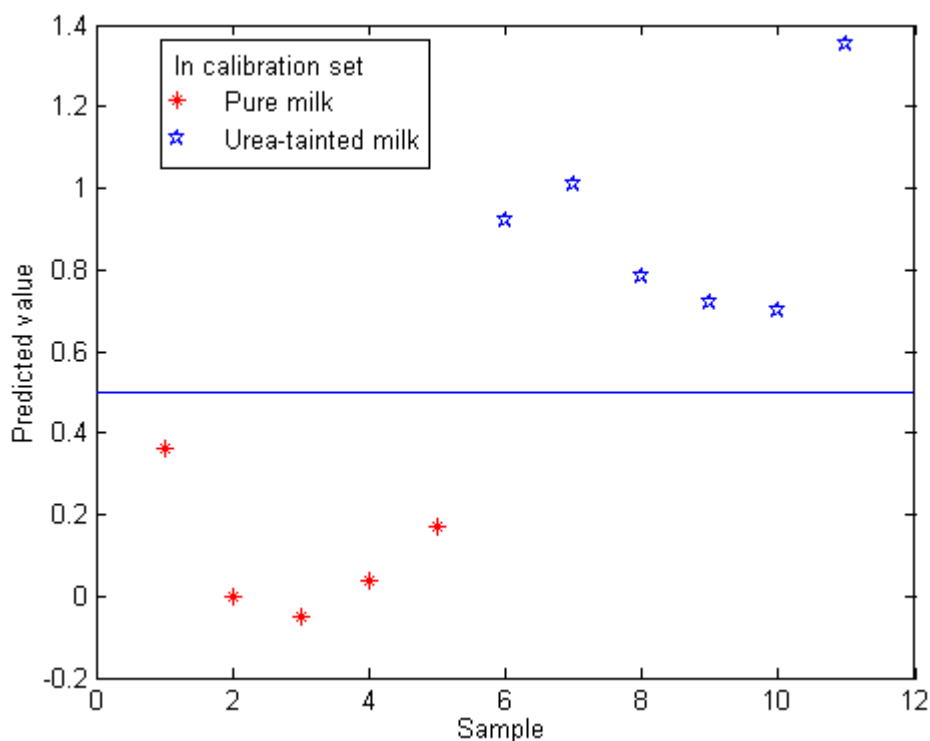

Figure 4. The discrimination results of urea-tainted milk and pure milk in calibration set by PLS-DA model

In prediction set, samples 1 to 4, which represent the pure milk samples, are belonging to class 1. Samples 5 to 9, which represent the urea-tainted milk samples, are belonging to class 2. The rate of

correct classification of urea-tainted milk samples was 100%, but there was 1 sample that was not correctly recognized in the pure milk samples. Predicted result in prediction set was shown in Figure 5.
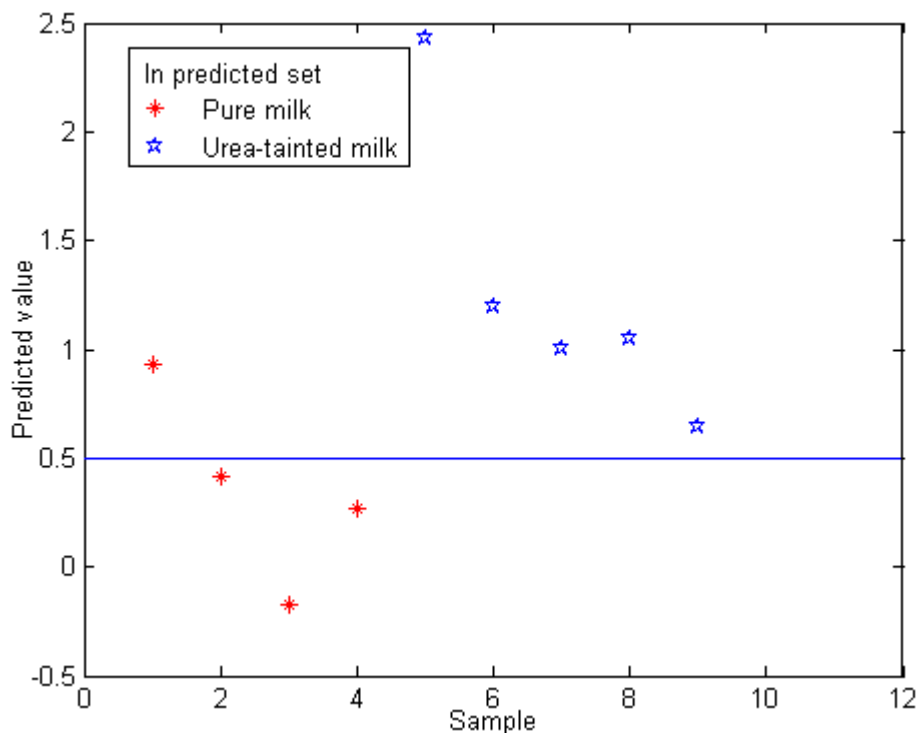

Figure 5. The discrimination results of urea-tainted milk and pure milk in predicted set by PLS-DA model

Performance results for NPLS-DA models to classify adulterated milk were listed in Table 1.It has been proved that NPLS-DA can achieve good effect for discrimination of urea-tainted milk and pure milk.

Table 1. Discrimination results of urea-tainted milk and pure milk by NPLS-DA

Sample	Number		The rate of correct classification		
	Training set	Testing set	Training set	Testing set	Total
Pure milk	5	4	100%	75%	88.9%
Urea-tainted milk	6	5	100%	100%	

Electrochemistry analysis of adulterated milk usually adopted 2-dimentional data analysis method [4]. Before multi-way data are processed by 2-dimensional data analysis methods, they must be straightened into a vector, so that some of information will be lost, which reduce the accuracy of the model. While the multi-way linear analysis methods decomposed multi-way experimental data into a

number of linear model to make full use of all data information. In this paper, multi-way data analysis was adopted for qualitative analysis of urea-tainted milk and achieved good effect.

5. CONCLUSION

A new approach based on voltametric electronic tongue in combination with MPCA and NPLS-DA model was developed for discrimination between milk adulterated with urea and pure milk. The correct classification rates were 100% for calibration set, and 88.9% for prediction set using NPLS-DA. The results show that the proposed method is feasible. This method in which multi-way data analysis algorithms coupled with voltametric electronic tongue technique can have a large applicability not only in dairy analysis, but also other food safety detection areas.

References

1. A .A .H. Adam. *Pakistan Journal of Nutrition*, 8(2009) 439-440.
2. T.R.L.C. Paixao, M. Bertotti. *Sensors and Actuators B: Chemical*, 137(2009) 266-273.
3. M.P. Ntakatsane, X.M.Liu, P.Zhou. *Journal of dairy science*, 96(2013) 2130-2136.
4. L.Bueno, W.R. de Araujo, M.O. Salles, M.Y. Kussuda and T.R.L.C. Paixão. *Chemosensors*, 2(2014) 251-266.
5. W. R. de Araujo, T.R.L.C. Paixão. *Electrochimica Acta*, 117(2014) 379-384.
6. J.C. Moore, J.W. DeVries, M.Lipp, J.C. Griffiths and D.R.Abernethy. *Food Science and Food Safety,* 9(2010) 330-357.
7. U. B Trivedi, D. Lakshminarayana, I.L. Kothari, N.G. Patel, H.N. Kapse, K.K. Makhija, P.B. Patel, C.J. Panchal. *Sensors and Actuators B: Chemical,* 140(2009) 260-266.
8. P.M Santos, E.R. Pereira-Filho, L. E. Rodriguez-Saona. *Food chemistry,* 138(2013) 19-24.
9. R. Yang, R. Liu, K.Xu, Y.Yang. *Acta Photonica Sinica,* 42(2013) 580-585.
10. P.M. Santos, P.D. Wentzell, E.R. Pereira-Filho. *Food Analytical Methods,* 5(2012) 89-95.
11. S. Das, M. Sivaramakrishna, K.Biswas, et al. *Sensors and Actuators A: Physical,* 167(2011) 273-278.
12. R. Ramesh, P. Puhazhendi, J. Kumar, M.K. Gowthaman, S.F. D'Souza and N.R. Kamini. *Materials Science and Engineering*: C, 49(2015)786-792.
13. X. Dai, X. Fang, F. Su, M. Yang, H. Li, J. Zhou and R.Xu. *Journal of Chromatography B,* 878(2010) 1634-1638.
14. R. Yang, Y. Yang, G. Dong and W.Zhang, Y. Yu. *Analytical Methods,* 6(2014) 3436-3441.
15. F. Winquist. *Microchimica Acta,* 163(2008) 3-10.
16. K. Tiwari, B. Tudu, R. Bandyopadhyay and A. Chatterjee. *Journal of Food Engineering,* 117(2013) 205-210.
17. S. Pilehvar, F. Dardenne, R. Blust and K. De Wael. *Int. J. Electrochem. Sci,* 7(2012) 5000-5011.

18. A. Hilding-Ohlsson, J.A. Fauerbach, N.J. Sacco, M.C. Bonetto and E. Cortó. *Sensors,* 12(2012) 12220-12234.
19. K. Villez, M. Ruiz, G. Sin, J.Colomer, C.Rosén and P.A. Vanrolleghem. *Water Science and Technology,* 57(2008) 1659-1666.
20. P.C. Damiani, I. Durán-Merás, A. García-Reiriz, A. Jiménez-Girón and A.M. de la. Peña. *Analytical chemistry,* 79(2007) 6949-6958.
21. B. Li, P. W. Ryan, M. Shanahan, K. J. Leister and A.G. Ryder. *Applied spectroscopy,* 65(2011) 1240-1249.