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Pure Milk Brands Classification by Means of a Voltammetric Electronic Tongue and Multivariate Analysis

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This paper deals with the application of a voltammetric electronic tongue towards discrimination of various brands of pure milk samples. The electronic tongue consists of three working electrodes made of gold, platinum and silver, and the applied waveform is staircase voltammetry. Both the principle component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were applied to evaluate the data of the current responses of the working electrode array. The results indicate PCA can not completely identify three pure milk brands whatever the current responses are from individual working electrode or all the working electrodes, but the algorithm of partial least square –discriminant analysis has better predict effect for various pure milk brands. Data fusion from all the working electrodes was found to be more beneficial and improvement of discrimination rate of various brands of pure milk samples has been demonstrated by PLS-DA. The rate of correct classification from data of the silver and three working electrodes was 83.33% and 100%, respectively. The analysis showed the voltammetric electronic tongue combined with PLS-DA method has good discrimination effect for various pure milk brands.

Keywords: Pure Milk; Electronic Tongue; Staircase Voltammetry; PCA; PLS-DA

1. INTRODUCTION

Dairy products have high nutritional value and are consumed by people all around the world. There are various pure milk brands in the markets. Quality, safety, and uniformity of products are important problems in the dairy industry. In milk samples analysis, a wide range of traditional methodologies are used to determine or detect compounds characteristics of them. These methodologies show good precision, accuracy and reliability, but they are destructive, time-consuming, and require expensive equipments. To overcome above drawbacks, electronic tongues have emerged as rapid and ease-to-use tools very promising for evaluation of food quality [1]. Regarding the sensor array used in the design of electronic tongues, such sensors have been widely employed as potentiometric, voltammetric, amperometric, impedimetric, conductimetric sensors, etc. Voltammetric electronic tongue has already proven valuable in many applications [2]. The measuring technique collects a lot of information variables with low, partially overlapping, non-specificity on each variable. Even though the specificity of each variable is low, considerable information can be extracted through the combination of several selectivity working electrodes. The information in the vast data material is processed with help of multivariate data analysis. Studies have shown that this electronic tongue could be used to identify milk from different sources in dairy industry [3], monitor the freshness of milk [4], and for discrimination of urea and melamine adulterated skimmed milk power [5]. It also devoted to the identification of different types of samples or quantification of components present in those, such as tea [6-7], water [8-9], edible oils [10], beers [11] and monofloral honey [12]. The idea of the recognition of milk type with the use of an electronic tongue relies on the fact that the variety of technologies applied in the production of the milky product result in differences in the milk composition [13]. This paper is focused on the recognition of milk samples produced by various producers. α-ASTREE electronic tongue, potentiometric sensors, was used to identify various pure milk brands and gained good effect [14-15]. However, the price of the potentiometric electronic tongue is expensive. This paper adopted voltammetric electronic tongue coupled with multivariate data analysis in order to evaluate the discriminating ability of the electronic tongue to differentiate milk from different producers.

2. EXPERIMENTAL

2.1 Equipment

The electronic tongue consists of three electrodes made of the metals gold, platinum, and silver, a reference electrode (Saturated Calomel Electrode, SCE) and a platinum counter electrode. The disk electrodes that serve as working electrodes have a diameter of 2mm and a purity of 99.999%. Electrochemical experiments were performed by using electrochemical station CHI660E, which is provided by Shanghai Chenhua Company.

2.2 Sample

Three various brands of pure milk samples from three separate producers (Mengniu dairy company, Xiajin dairy company and Sanyuan dairy company, China), are investigated and their nutrition information (per 100 g) from the packages that customers are provided with the pure milk by the producers is as shown in Table 1. The pure milk samples of various brands were purchased from a local supermarket in Tianjin City.

Table 1. The content of different brands pure milk (per 100 g)

Content \ Brands	Mengniu	Xiajin	Sanyuan
Energy (kJ)	270	260	261
Protein(g)	3.0	3.0	3.0
Fat (g)	3.7	3.5	3.6
Carbohydrate (g)	4.8	4.7	4.5
Na (mg)	62	55	60

2.3 Principles of the Voltammetric Method

In voltammetric measurement, when the electrodes are inserted into the tested solution, a double layer charged is formed between the counter electrode and the working electrode. A current is measured between the working electrode and the counter electrode when a voltage is applied between the working electrode and the reference electrode. If there are substances in the solution that are electrochemically active at the applied potential, a Faradic current is created in addition to the capacitive current. Different metals can extract information to different extent [2].

The staircase waveform is used as a tool for fundamental and diagnostic studies [7]. In this paper, the staircase waveform was adopted as the shape of applied potential between the working electrode and the reference electrode, with its maximal value at 2V and minimal value at 0 V; the amplitude of the step 0.05 V, the pulse length 300 ms, sample interval 0.001 s, sample quiet time 2 s. All voltages mentioned are versus the SCE electrode.

2.4 Measurement Procedure

All pure milk were sealed and placed in the laboratory in order to have the same temperature as the room temperature. Each of three brands of pure milk was prepared twelve times in random order. All samples were un-pretreated before measurement. Each of those 36 pure milk samples was measured three times and then averaged into one point. After each measurement, all electrodes were rinsed with distilled water (milliQ, 18.2MΩ)，and the planar working electrodes were mechanically polished for 15 seconds with aluminum oxide of 1 and 0.3μm consecutively [16]. Thereafter, working electrodes and counter wire electrode were sonicated (Ultrasonic cleaner KQ5200DE) in ethanol: water (1:1) for 5 minutes. Reference electrode (SCE) was stabilized in a saturated KCl aqueous solution. Finally, all electrodes were thoroughly rinsed with distilled water and used in the next measurement [5].

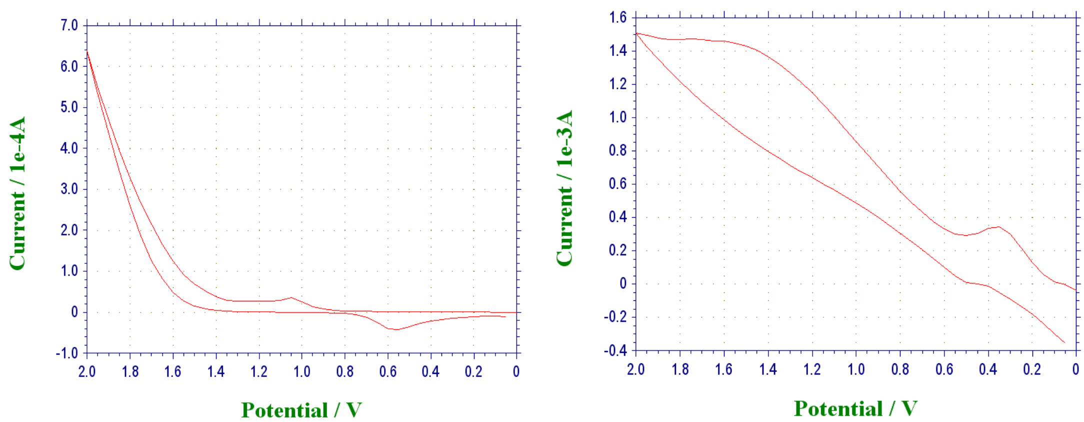
2.5 Data Analysis

Multivariate analysis was used to treat all current responses collected from the working electrodes, specifically principal component analysis (PCA) [17] and partial least square (PLS) [18]. The raw data obtained from working electrodes were exported in Excel format from the instrument software to MATLAB. The rows represented samples; the columns represented the current responses created by the applied potential. The PCA produces a score plot that is visualizing differences between samples, which can be used for classifications of different samples [19]. Although PCA itself can only be used as an unsupervised pattern recognition method, the behavior can indicate the data trend in a visualizing dimension. The aim of PLS-DA is to predict Y from X, by simultaneous decomposition of those matrixes or vector in a group of components which explain as much as possible the covariance of X and Y. Prediction models are built by using the calibration set, which is the part of the collected data from the working electrodes. The other part of the collected data is tested to obtain prediction accuracy of the built model. All data management was carried out with Matlab (Version R2007 b, Math Works).

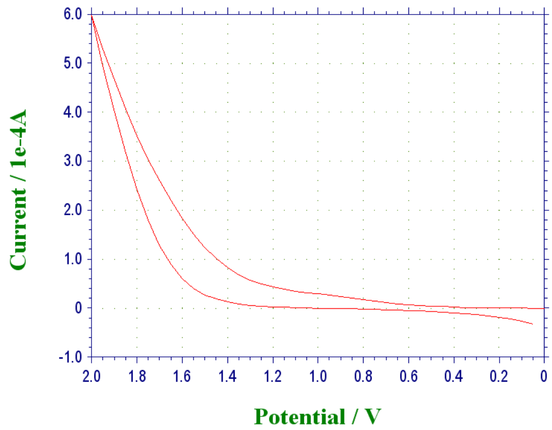
3. RESULTS AND DISSCUSION

3.1 The Current Responses of Different Working Electrodes

The current responses for different working electrodes are shown in Figure1. The current responses are the ones from Mengniu pure milk, because current response curves of different pure milk are similar for the same working electrode. As can be seen in Figure 1, it is shown that different current responses were formed from different working electrodes. For the gold working electrode, the voltammogram showed one anodic peak at around +1.05V and one cathodic peak at around +0.55V. While, the voltammogram of the sliver working electrode only showed one anodic peak at around +0.35V. Besides, there was no obvious peak on the voltammogram of the platinum working electrode.



(a) (b)



(c)

Figure 1. The current responses of staircase voltammetry from (a) the gold electrode, (b) the sliver electrode and (c) the platinum electrode.

After a double layer charged is formed between the counter electrode and the working electrode, once the potentials were applied, on the one hand, the ions Na^+, Ca^{2+} in milk formed a charging current; on the other hand, the fat in pure milk was oxidized or reduced on the electrode surface to form the redox current. Different substance content in different brands of pure milk resulted in different current value in response.

3.2 Classification of the Pure Milk Brands by PCA

Preprocessing stage is very useful as it contributes to increased accuracy of prediction. The difference between the measurements of the same pure milk is caused by drift in the instrument, difference between samples, and most importantly, adsorption to the electrodes. It is possible to reduce this drift by cleaning the surface of the electrodes between each measurement and by mathematical methods [20]. Mean center with respect to the electrode is given below:

$$x_u^{'} = x_{ik} - \bar{x}_k \qquad (1)$$

Where x_{ik} denotes k^{th} measured value of i^{th} sample, $\bar{x}_k$ denotes the mean value of measured current values under the same potential.

The centered data are used in PCA; the score plots are compared containing three kinds of pure milk brands here. The obtained PCA score plots from the individual working electrodes respectively and from all three working electrodes are shown in Figure 2. It can be seen that the score plot can not entirely differentiate three pure milk brands. With the two first principle components (PCs), both accumulated variance were more than 96%. Despite these large values, not enough clear discrimination between the different pure milk brands was achieved. The reason is the first principle component represents the similar information of the tested pure milk. The difference of the tested pure milk of different brands is very small, we can not discriminate them by the first principle component. So we analyze the relationship between other principle components [21].

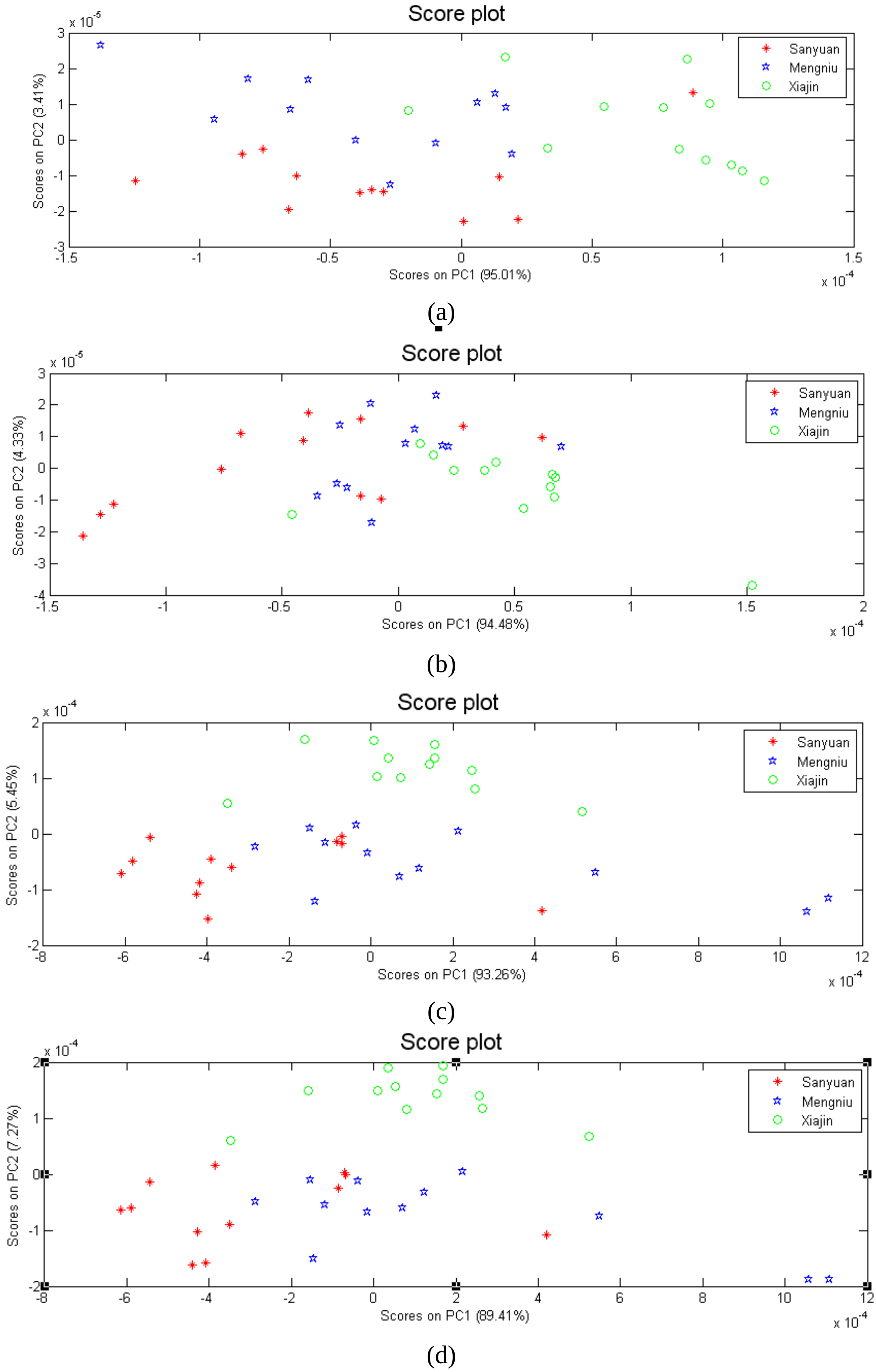


Figure 2. The score plots obtained from the current responses of (a) the gold working electrode, (b) the platinum working electrode, (c) the sliver working electrode and (d) all working electrode

The score plot of the silver electrode between PC2 and PC3 is showing the best separation ability in all scores plot, as shown in Figure 3. Despite these PCs are small values, it can discriminate more clearly between the different pure milk brands.

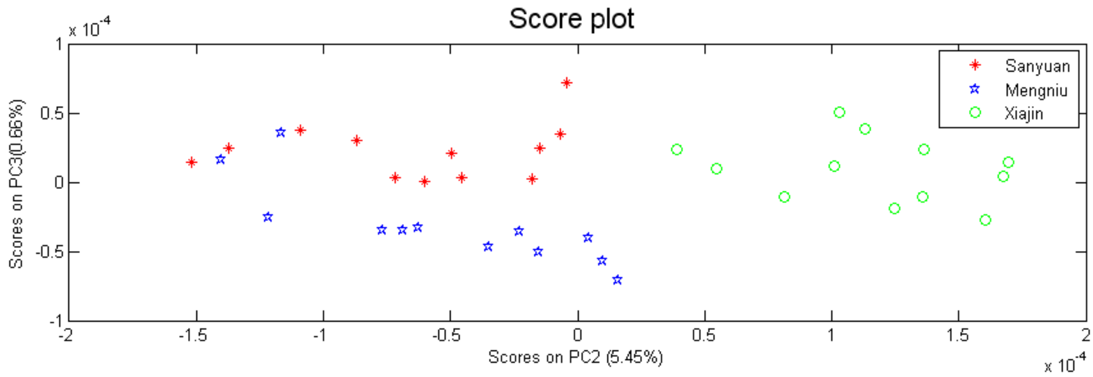


Figure 3. The score plot obtained from the current responses of the sliver working electrode (PC2 and PC3)

The reason is the second and the third principle component extracted the dissimilar information in the tested pure milk. However, there are 5 samples, 3 Sanyuan pure milk sample and 2 Mengniu pure milk samples, can not be distinguished clearly. Silver electrode gave the best results when used to discriminate different pure milk brands in comparison to the other working electrodes, therefore, this work is focused on the sliver working electrode and fusion of three working electrodes, respectively. From above results, classification of three kinds of pure milk based on PCA score plots only gives the cluster trend in the 2-dimension space but can not be used as a classification tool. The ultimate aim of this work is to classify three pure milk brands by electronic tongue and multivariable data processing method. So we selected raw data to build the PLS-DA model.

3.3 PLS-DA Model

PLS is a linear transformation procedure, which models both the electronic tongue response (x) and target (y) matrices simultaneously to find the latent variables in x that will best predict the latent variables in y. Choosing appropriate principle component number is important to build a good PLS-DA model with high classification accuracy and stability [22-23]. In present work, All 36 pure milk samples were divided into two subsets randomly. The calibration set, which was used to construct the model, had 24 samples including 8 Sanyuan pure milk samples, 8 Mengniu pure milk samples, and 8 Xiajin pure milk samples. The prediction set was formed by the remaining 12 samples including 4 Sanyuan pure milk samples, 4 Mengniu pure milk samples, and 4 Xiajin pure milk samples. The model was used to predict results in prediction set of different pure milk brands. The traditional root mean square error of cross validation (RMSECV) was used to determine the optimal number of principal components. The optimal number of principal components was selected according to the lowest RMSECV. We analyzed the current responses of the sliver electrode and all the working electrodes, respectively. In this case, 10 principal components were chosen to build PLS-DA model. The relationship between RMSECV and principal component number was shown in Figure 4.

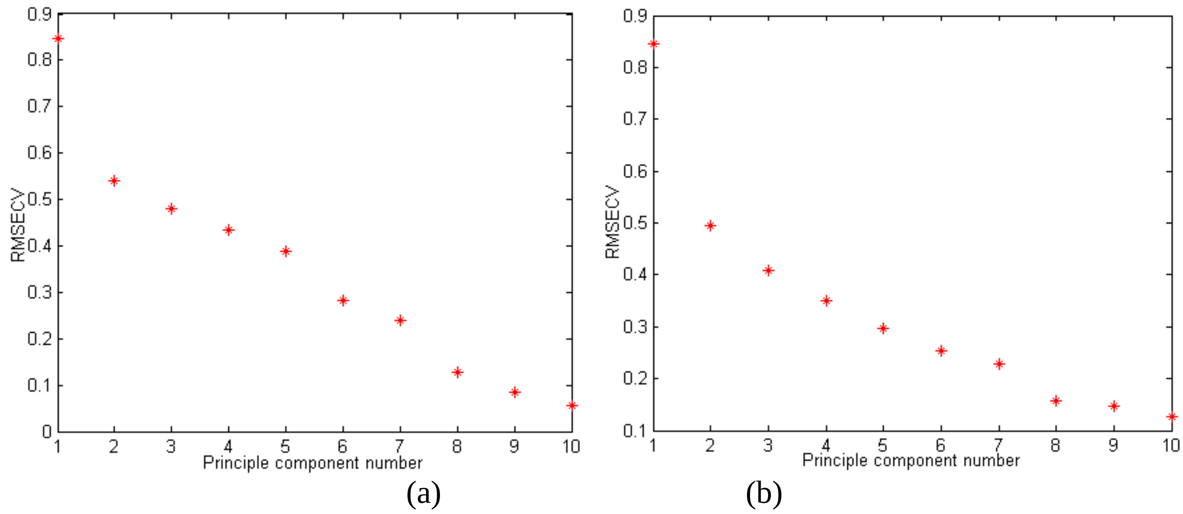
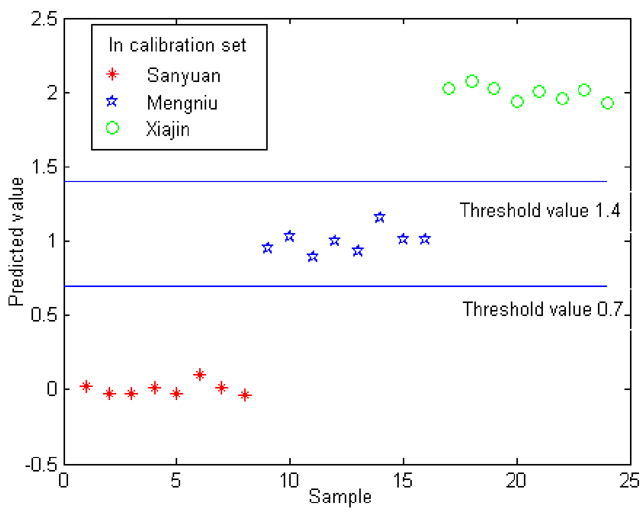
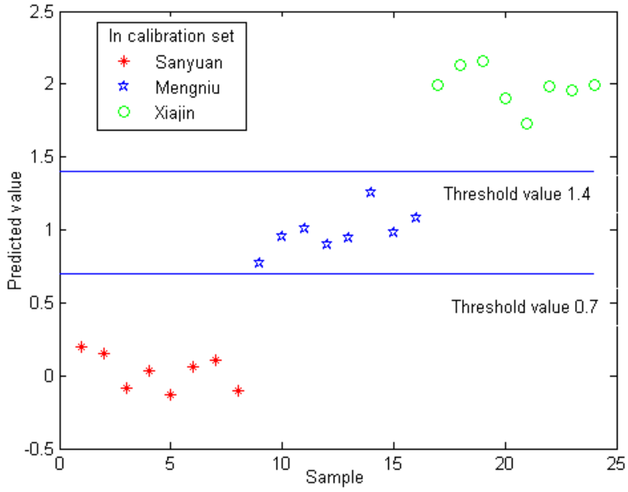


Figure 4. The relationship between RMSECV and principal component number for (a) data of the sliver working electrode and (b) data fusion of three working electrodes.

In the PLS-DA models used in this study, two threshold values were set to 0.7 and 1.4. Below 0.7, the sample was estimated as 0 and was assigned to Sanyuan pure milk. Above 0.7 and less than 1.4, the sample was estimated as 1 and was assigned to Mengniu pure milk. Above 1.4 the sample was estimated as 2 and was assigned to Xiajin pure milk. Here, samples 1 to 8, which represent the Sanyuan milk sample, are belonging to class 1. Samples 9 to 16, which represent the Mengniu pure milk samples, are belonging to class 2. Samples 17 to 24, which represent the Xiajin pure milk samples, are belonging to class 3.



(a)

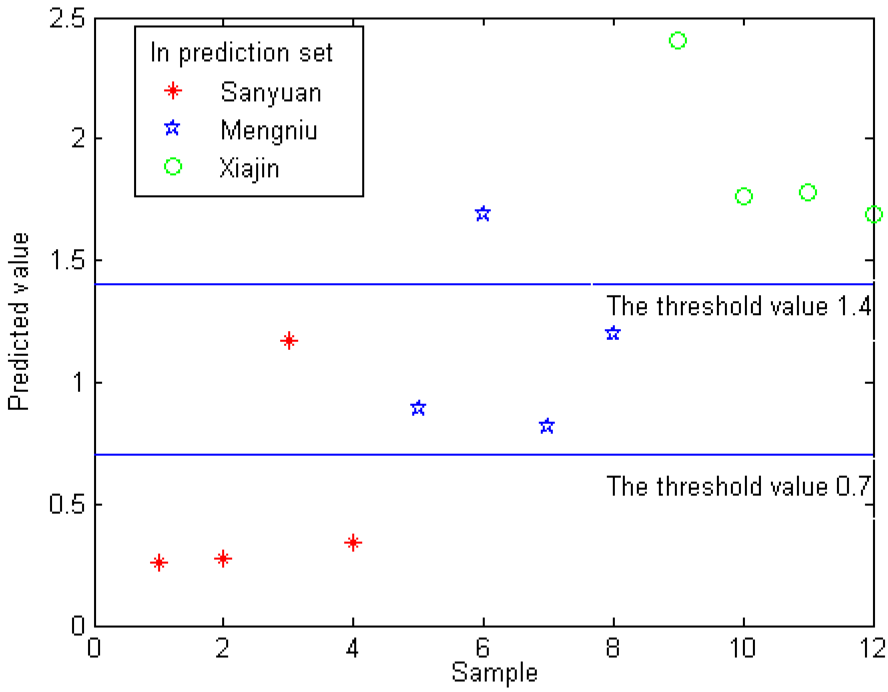


(b)

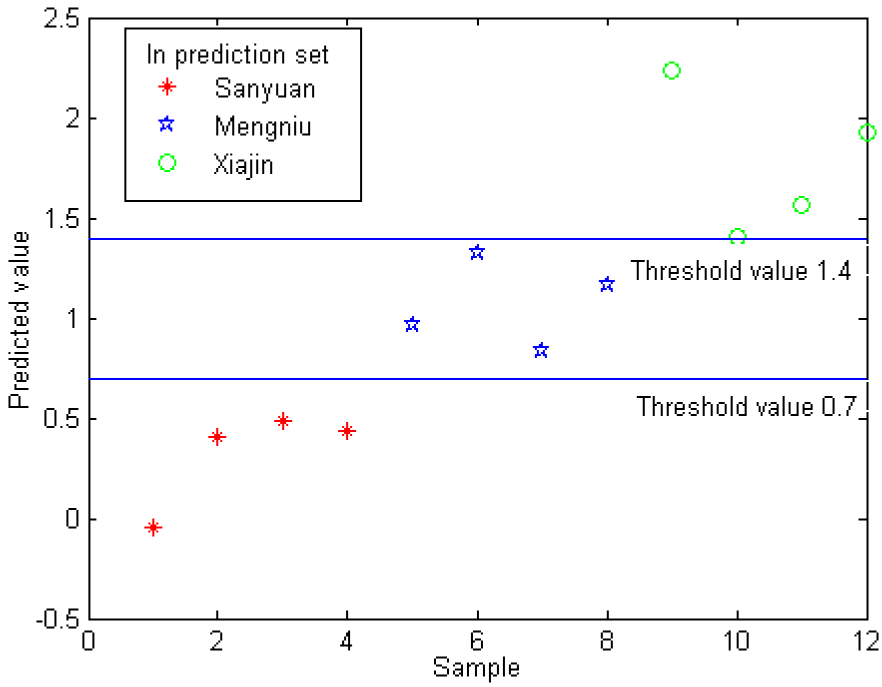
Figure 5. Predicted results in calibration set by PLS-DA model of (a) data of the sliver electrode and (b) data fusion of three working electrodes.

The result was from the data of the sliver electrode. Correlation Coefficient for calibration (R^2c) is 0.9951 in the X block, and R^2c is 0.9273 in the Y block. Predicted result in calibration set was shown in Figure 5(a), the rate of correct classification was 100%. For the result which was derived from the data fusion of the three electrodes. R^2c is 0.9762 in the X block, and R^2c is 0.8749 in the Y block, the rate of correct classification also was 100%, predicted result in calibration set was shown in Figure 5(b).

The predicted results of samples in prediction set were shown in Figure 6.



(a)



(b)

Figure 6. Predicted results in prediction set by PLS-DA model of (a) data of the sliver electrode and (b) data fusion of three working electrode

As can be seen in Figure 6(a), the result was from the data of the sliver electrode. The prediction value of sample No.3 was between 0.7 and 1.4, and the prediction value of sample No.6 was above 1.4, which indicated these samples were incorrectly classified. There were 2 samples that were not correctly recognized in the 12 samples, so the rate of correct classification was 83.33 %.

As can be seen in Figure 6(b), the result was from the data fusion of all working electrodes. the prediction values of all Sanyuan pure milk were below 0.7; all Mengniu pure milk were between 0.7 and 1.4; all Xiajin pure milk were above 1.4. Obviously, all pure milk samples were correctly recognized. So the rate of correct classification was 100% in the whole data set.

Performance results for PLS-DA models to classify pure milk brands were listed in Table 2, using the sliver electrode, and the three working electrode respectively. It can be seen from the table that the PLS-DA model using the three working electrode have better predictive ability than the only sliver electrode. The reason may be that the three working electrode can provide more useful information than only one working electrode.

Table 2. Discrimination results of pure milk brands

Sample brands	Number		The rate of correct classification					
			Only the Sliver electrode			the three working electrode		
	Training set	Testing set	Training set	Testing set	Total	Training set	Testing set	Total
Sanyuan	8	4	100%	75%		100%	100%	
Mengniu	8	4	100%	75%	83.33%	100%	100%	100%
Xiajin	8	4	100%	100%		100%	100%	

4. CONCLUSION

A Voltammetric electronic tongue based on three kinds of metals has been designed. It is shown that each electrode separates the pure milk differently and not equally well, and the silver electrode was proved had the best sensitivity in individual electrode. It is clear that the metal material of the working electrodes is important according to the separating capability. Certainly, a suitable algorithm is also essential for increasing accuracy. The better results were achieved by PLS-DA. The total discrimination rate from the silver working electrode responses is 83.33%, while the total discrimination rate from the fusion of three working electrode responses is 100%. The results indicate the voltammetric electronic tongue is capable of distinguishing pure milk brands coupled with PLS-DA.

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