Original Article

Identification of cow milk in goat milk by nonlinear chemical fingerprint technique

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ABSTRACT

The objective of this paper was to develop a nonlinear chemical fingerprint technique for identifying and detecting adulteration of goat milk with cow milk. In this study, by taking the Belousov-Zhabotinsky oscillatory chemical reaction using acetone and substrates in goat milk or cow milk as main dissipative substances, when the same dosage of goat milk and cow milk was introduced to the "H++Mn2++BrO3-/acetone" oscillating system respectively, nonlinear chemical fingerprints were obtained for goat milk and cow milk from the same origin. The results showed that inductive time value and the content of cow milk in goat milk had a linear relationship in the range of 0–100% and the corresponding regression coefficient was 0.9991. A detection limit of 0.0107 g/g was obtained, and the content of cow milk in mixed milk was calculated. The proposed method in this study was simple, economical and effective. In addition, the method did not need the pretreatment and separation of samples for identifying and evaluating cow milk adulteration in goat milk.

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1. Introduction

Goat milk is a kind of highly nutritious food because it possesses unique properties which distinguish it from cow milk. Recently, goat milk plays an increasingly important role in the human diet, and it suits not only for infants but also for adults, especially for nursing mothers [1]. Furthermore, goat milk has become popular as an infant diet because the fat globules of goat milk are smaller than those of cow milk and goat milk has higher percentage of short-and medium-chain (C6-C14) fatty acids in comparison with cow milk, which is probably main reason for the easy digestion of goat milk [2–4]. However, for higher profit, increasing demand for goat milk might result in the adulteration by cheaper cow milk, which has become a serious quality problem. Due to the similarity of goat milk and cow milk in appearance and composition, it is difficult to...
differentiate goat milk and cow milk [5]. This situation also presents a risk for people afflicted with cow milk allergies, when they consume adulterated goat milk [6,7]. Thus, analytical methods with high selectivity to identify and evaluate adulteration in goat milk are required.

Currently, chemical fingerprint analysis has been applied as an effective method for milk and dairy products quality control. These fingerprinting methods include HPLC [5], capillary electrophoresis [8], isoelectric focusing [9,10], enzyme-linked immunosorbent assay [11], PCR technology [6,12], pulse polarography method [13], liquid chromatography tandem mass spectrometry [14] and so on. Most of these methods exhibit high sensitivity and can meet the requirements for identifying and evaluating adulteration of milk and dairy products. However, these techniques described above are mainly used to identify and distinguish adulteration of milk and dairy products by detecting various chemical compositions of milk, such as the detection of specific protein components by HPLC [15]. Moreover, these methods usually require complex preprocessing, such as separation and purification. So the research on the effective method for directly showing the throng character of chemical components in cow milk and goat milk has become one of the primary tasks which the analysts are facing. In addition, it would be beneficial to develop simple and cost effective methods based on different principles from different aspects for distinguishing and evaluating samples, and the techniques may be complementary with each other.

The discovery of Belousov–Zhabotinsky (B–Z) oscillatory reaction (Belousov and Zhabotinsky were the names of the two Russian scientists, who were the first ones to study the reaction) began the times to investigate nonlinear chemical reaction systems [16], and the research on the application of nonlinear chemical reaction had aroused increasing attention. Chemical oscillation based on Belousov–Zhabotinsky (B–Z) reaction was a common phenomenon in nonlinear chemistry. The phenomena of the reaction are complex, involving chemical oscillation, chemical turbulence, chemical patterns and chemical waves [16]. The reaction mechanism and applications of chemical oscillation in single component detection had been investigated extensively and thoroughly by domestic and foreign scholars [16–19]. While the research to apply it in fingerprint analysis for characterizing throng characteristic of the components in foods was rare abroad, and also started rather late in China. Such as authenticity identification and quality evaluation of soya sauce had been reported by nonlinear electrochemical fingerprint and system similarity [20]. According to the literatures [21,22], because of the differences in the types and the content of their components, different samples had different influences on an identical nonlinear chemical reaction, which caused that the shapes of the relevant potential-time (E–t) curves were different from each other. The E–t curve is very helpful for the rapid identification and evaluation of cow milk in goat milk because it contains abundant qualitative and quantitative information. In this work, we introduced goat milk with or without artificially added cow milk to “H+ + Mn2+ + BrO3− + acetone” oscillating system, and cow milk adulteration in goat milk was identified and evaluated by a nonlinear chemical fingerprint technique.

Thus, the objective of this study was to evaluate the feasibility to apply nonlinear chemical fingerprint technique to detect and identify cow milk adulteration in goat milk. The content of cow milk in mixed milk was calculated by the least square method, and the mechanism of the method was introduced using H+, Mn2+, BrO3−, acetone and glucose as the reaction substrates. The method developed in this study has the advantages of low operational cost and no pretreatment for identifying and evaluating cow milk adulteration in goat milk.

2. Materials and methods

2.1. Materials

All chemicals used were of analytical grade. Sulfuric acid (1.0 mol L−1), acetone (1.0 mol L−1), sodium bromate (0.8 mol L−1) and manganese sulfate (0.08 mol L−1) were used. Solutions were kept at a constant temperature (50.0 °C) until used. Double distilled water was used throughout the experiments. In addition, acetic acid (5%), dichloromethane (200 μL) and formic acid (0.2%) were used.

2.2. Milk samples

Raw goat milk and cow milk were collected from local farms. Then, milk samples were freeze dried milk powder. A series of adulteration samples were made of raw cow milk and raw goat milk in volume ratios 0, 5%, 10%, 20%, 40%, 60%, 80%, and 100% v/v.

2.3. Main apparatus

A nonlinear chemical fingerprint instrument (Model MZ-1B) developed by Central South University and Xiangtan Ltd. (Hunan, China) was used. A Type 217 calomel electrode was used as reference electrode and a Type 213 platinum electrode was used as working electrode (both were purchased from Shanghai Precision & Scientific Instrument Co., China). All studies were conducted using the experimental set-up which was illustrated in the literature [21]. In addition, an ion trap mass spectrometer (Thermo Finnigan, San Jose, USA) was used.

2.4. Procedure

The following procedure was used in all experiments. The nonlinear chemical reaction mixture was prepared by mixing 25.00 mL of sulfuric acid, 10.00 mL of acetone, 12.00 mL of manganese sulfate, 10.00 mL of double distilled water and appropriate dosage of cow milk or goat milk sample. All components of reaction mixture were added into the reactor. The reactor cover with two injection holes, the electrodes and a thermometer was closed. The instrument was then turned on, with temperature and stirring rate adjusting to 50.0 °C and 800 r/min, respectively. After stirring for 5.0 min, 5.00 mL of sodium bromate solution was injected into the reactor. Electric potential-time (E–t) curve was immediately obtained and finished as soon as the potential oscillation disappeared.
3. Results and discussion

3.1. Reaction condition and basic process of nonlinear chemical fingerprint

Kinetic and thermodynamic conditions on nonlinear chemical fingerprint had been discussed, and the entropy change laws and expression suitable for describing the entropy change rates of any thermodynamic system had been interpreted on the basis of the relevant literature [22]. It was demonstrated that an open system without complementariness of dissipative substances and a close system far from the equilibrium were suitable for studying nonlinear chemical fingerprint since the chemical reaction was able to be accomplished in a properly short period of time in these systems.

Process of a nonlinear chemical reaction is very complicated and involves oxidation—reduction reaction, precipitation reaction, neutralization reaction and free radical reaction [23]. In this study, the mechanism of B—Z oscillatory reaction to use Mn$^{3+}$, BrO$_3^-$, H$_2$O, acetone and glucose as the reaction substrates was discussed. Although B—Z oscillatory reaction includes many kinetic steps like dozens of elementary reactions [24, 25], its mechanism can be summed into two main processes, namely, inductive process and oscillatory process [25], which are explained by Process I and Process II, respectively.

Process I:

\[3\text{BrO}_3^- + 6H^+ + 6\text{Mn}^{2+} + 2\text{CH}_2\text{COCH}_3 \rightarrow \]

\[3\text{Br}^- + 6\text{Mn}^{3+} + 3\text{H}_2\text{O} + 2\text{HCOOH} + 2\text{CH}_3\text{COOH} \]  (2)

It can be seen from process I that bromine ion is generated in the inductive reaction.

Process II:

\[2\text{C}_6\text{H}_5\text{O}_6 + \text{HCOOH} + 2\text{CH}_2\text{COCH}_3 + 2\text{Br}^- + 2\text{BrO}_3^- + 4\text{H}^+ \rightarrow \]  (3)

\[\text{Br}_2 + \text{CO}_2 + 2\text{BrCH}_2\text{COCH}_3 + 2\text{C}_6\text{H}_5\text{O}_7 + 4\text{H}_2\text{O} \]  (4)

Process II is the total reaction of the oscillating reaction in the reaction system. It involves three processes, namely, process A, process B and process C.

Process A:

\[2\text{H}^+ + \text{BrO}_3^- + \text{Br}^- \rightarrow \text{HOBr} + \text{HBrO}_2 \]  (5)

\[\text{Br}^- + \text{H}^+ + \text{HBrO}_2 \rightarrow 2\text{HOBr} \]  (6)

\[\text{Br}^- + \text{H}^+ + \text{HOBr} \rightarrow \text{Br}_2 + \text{H}_2\text{O} \]  (7)

Process B:

\[2\text{HBrO}_2 \rightarrow \text{HOBr} + \text{H}^+ + \text{BrO}_3^- \]  (8)

\[\text{BrO}_3^- + \text{H}^+ + \text{HBrO}_2 \rightarrow 2\text{BrO}_2^+ + \text{H}_2\text{O} \]  (9)

\[\text{BrO}_2^+ + \text{H}^+ + \text{Mn}^{2+} \rightarrow \text{HBrO}_2 + \text{Mn}^{3+} \]  (10)

Process C:

\[\text{CH}_3\text{COCH}_3 + \text{Br}_2 \rightarrow \text{Br}^- + \text{H}^+ + \text{BrCH}_2\text{COCH}_3 \]  (11)

\[\text{C}_6\text{H}_5\text{O}_6 + 2\text{Mn}^{3+} + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_5\text{O}_7 + 2\text{Mn}^{2+} + 2\text{H}^+ \]  (12)

\[\text{HCOOH} + \text{HOBr} \rightarrow \text{H}^+ + \text{Br}^- + \text{CO}_2 + \text{H}_2\text{O} \]  (13)

B—Z oscillating reaction is initiated by Br$^-$ when the concentration of Br$^-$ in the reaction system is higher than $[\text{Br}^-]_{\text{crit}}$, namely, the critical concentration [24, 25]. In process A, Br$^-$ is consumed and Br$_2$ is accumulated. With the reactions prolonging, the concentration of Br$^-$ gradually decreases in system [26]. When the concentration of Br$^-$ is lower than $[\text{Br}^-]_{\text{crit}}$, the whole oscillating reaction is dominated by process B, during which Mn$^{3+}$ and HOBr are accumulated. HBrO$_2$ is an important intermediate that operates the switch from process B to process C. Accumulation of Br$_2$, Mn$^{3+}$ and HOBr initiates process C that regenerates Br$^-$. Then the next new cycle will start as the concentration of Br$^-$ being accumulated. In this way, process A, B and C move in cycles and form oscillating reaction.

According to the above described, it can be observed that nonlinear chemical reaction can not take place in the reaction system without reducing agent such as glucose can reduce Mn$^{3+}$ into Mn$^{2+}$. In fact, there are many reducing substances in different samples, such as vitamin C in milk, and these reducing substances may reduce Mn$^{3+}$ into Mn$^{2+}$. In other words, the condition of taking place nonlinear chemical reaction is that reducing agent is required in the reaction system. The differences of the reducing substances in different samples result in the differences of reaction mechanisms for inductive process and oscillatory process. Thus, the shape of nonlinear chemical fingerprint is changed with reaction condition, reactants, products and coexisting substances in the reaction system.

3.2. Optimization of detecting dosage of milk

Nonlinear chemical fingerprint is very sensitive to the change of determining condition, therefore, it must be determined under the constant condition. In the experiment, the universal optimum conditions recommended in the literature [22] were utilized, and the optimization experiment of the determining dosage of milk was carried out. On selecting the determining dosage in order to determine the fingerprint of milk, both of the characteristic of the fingerprint and the determining time must be considered simultaneously. Under the optimal experimental conditions, namely, 25 mL of 1.0 mol L$^{-1}$ sulfuric acid, 15 mL of 1.0 mol L$^{-1}$ acetone, 5 mL of 0.8 mol L$^{-1}$ sodium bromate, 12 mL of 0.08 mol L$^{-1}$ manganese sulfate, 10 mL of double distilled water and temperature of 50.0 °C in the reactor, the detecting dosage of milk could be evaluated by employing oscillatory end time as the measured parameter. Therefore, the applied dosage of adulterated milk samples to the experiment was 1.00 g, and the detecting dosage was 0.90 g for the reproducibility of milk samples. Under the condition of the determining dosage, not only the characteristic differences of the fingerprints were conspicuous, but also the determining time was not too long.
3.3. Essential information in nonlinear chemical fingerprint of milk

Nonlinear chemical fingerprint contained abundant quantitative information and intuitionistic information due to its dynamic property. In this study, nonlinear chemical fingerprint was obtained by adding 0.90 g of goat milk into the reactor. The fingerprint was shown in Fig. 1.

It was obvious from Fig. 1 that the essential characteristic information of nonlinear chemical fingerprint mainly included inductive time (t\text{ind}), undulatory period (T\text{und}), undulatory life (L\text{und}), canyon potential (E\text{can}), canyon time (t\text{can}), peak top potential (E\text{top}), peak top time (t\text{top}), oscillatory start potential (E\text{start}), oscillatory end potential (E\text{end}), oscillatory end time (t\text{end}), maximum amplitude (AE\text{max}), which were defined as quantitative information. All of them were described in detail in the literature \cite{22,23}. The researchers \cite{23} pointed out that oscillation wave shape of fingerprint could reflect the characteristics of complex samples, and inductive time, oscillatory life, oscillatory period and oscillatory curve were holistic quantitative information on chemistry components of samples. Furthermore, the fingerprint information was very important and helpful for distinguishing and evaluating of goat milk and cow milk. Thus, inductive curve, oscillatory curve, oscillatory-end curve and a part of equilibrium curve constituted the whole nonlinear chemical fingerprint \cite{23,25}.

3.4. Reproducibility and precision of nonlinear chemical fingerprint of milk

Goat milk and cow milk were analyzed to evaluate the repeatability of the method. Under the same determination conditions, according to “Procedure”, 0.90 g of goat milk or cow milk with the same origin was added into the reactor, and their nonlinear chemical fingerprints were obtained. The experiments were carried out in quadruplicate. The reproducibilities of nonlinear chemical fingerprints of goat milk and cow milk were shown in Fig. 2.

The precision was assessed by determining the relative standard deviation (RSD). It can be seen from Table 1 that the RSDs were less than or equal to 1.93%. Thus, these values confirmed that nonlinear chemical fingerprint had very good reproducibility and precision.

3.5. Identification for goat milk and cow milk

In this study, by taking B–Z nonlinear chemical reaction to use acetone and substrates in goat milk or cow milk as main dissipative substances, when the same dosage of goat milk and cow milk was introduced to the “H⁺ + Mn²⁺ + BrO₃⁻ + acetone” oscillating system respectively, the fingerprints of goat milk and cow milk were obtained. As shown in Fig. 3.

The profiles of inductive curves and oscillating curves had obvious characteristic differences between the fingerprint of goat milk and cow milk. Inductive curve of goat milk was smoother. On the contrary, inductive curve of cow milk had slight fluctuation. Furthermore, compared with cow milk, the time taken to reach the maximum potential was longer for goat milk. In addition, it can be seen from Fig. 3 that inductive time of cow milk and goat milk was 1720.56 s and 2865.03 s, respectively. This indicated that goat milk and cow milk could be distinguished by comparison of the fingerprints.

At present, chromatogram and spectrum fingerprints are the most widely used methods in milk analysis. However, characteristic differences of the chromatogram and spectrum fingerprints are not obvious and intuitive. Moreover, their features must be interpreted by chemometric methods or mathematics \cite{27}. In contrast, the shapes of nonlinear chemical fingerprints of different types of milk are obviously different. At the same time, compared with other methods such as chromatogram and spectrum, the proposed method avoids the laborious process of separation and purification of samples. Since a large number of samples contained different chemical components and contents, which leaded to be different for quantifiable parameters and intuitionistic shapes in nonlinear chemical fingerprints of goat milk and cow milk. Moreover, the holistic content of chemical constituents was higher, while it is the content of active components was also higher in terms of the same dosage of goat milk or cow milk. Thus, based on the above explanation, it was important to estimate the quality of milk by the throng character of chemical components in cow milk or goat milk. However, the shapes and quantifiable parameters of nonlinear chemical fingerprint of milk were decided by the types of milk and the content of chemical components in milk. Firstly, with regard to the content of chemical components in goat milk and cow milk, some researchers had reported that cow milk had lower percentage of short-and medium-chain (C₆-C₁₄) fatty acids in comparison with goat milk \cite{28}. Secondly, goat milk was richer in monounsaturated (MUFA), polyunsaturated fatty acids (PUFA) and medium chain triglycerides (MCT) \cite{7,29}. In addition, goat milk contained relatively higher levels of beta-casein and alpha-s2 casein, whereas alpha-s1 casein was the most abundant in cow milk \cite{2,30,31}. The factors described above were probably one of the reasons for influencing on the shape of E–t curves between goat milk and cow milk. At the same time, season, climatic condition \cite{32}, feeding schemes \cite{33} and so on were also mainly factors. This indicated that
experiments with the same dosage could provide different fingerprints for different types of milk.

3.6. Feasibility of nonlinear chemical fingerprint for adulterated milk samples

In order to evaluate feasibility of the method for detecting and evaluating adulterated milk samples, raw goat milk was spiked with different volume percentages of raw cow milk (0, 5%, 10%, 20%, 40%, 60%, 80%, and 100% v/v), which were also used to determine the detection limit for milk adulteration. Then adulterated samples were analyzed by nonlinear chemical fingerprint. The results were shown in Fig. 4. In order to find a strategy to evaluate the percentage of cow milk present in adulterated goat milk, the attention was focused on inductive time. It could be seen that inductive time was decreasing with increasing of the volume percentage of cow milk added in goat milk. Moreover, when adding different proportion of cow milk into goat milk, the shapes of nonlinear chemical fingerprint had obvious changes. From the fingerprints in Fig. 4(a), there were obvious characteristic differences between the fingerprints of mixed milk. The proposed method was validated using high-performance liquid chromatography with electrospray ionization mass spectrometry method (HPLC/ESI/MS) [5]. Thus, the method of this study was feasible for identifying and evaluating cow milk adulteration in goat milk.

The content of cow milk in mixed milk had greatly influenced on the quantitative information of nonlinear chemical fingerprint. As shown in Fig. 4(b). The results showed that inductive time value and the content of cow milk in goat milk had a linear relationship in the range of 0–100%, and the regression equation was $t_{ind} = -1249.9C + 2550.2$ with a linear correlation coefficient ($R^2$) of 0.9991, which provided a theoretical basis for identification of samples and determination of the content of adulterated milk. In this study, the least square method was adopted as a quantitative assessment model for identification and evaluation of cow milk adulteration in goat milk.

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The principle of the method was that inductive time ($t_{ind}$) and the content of cow milk (C) in mixed milk had a good linear relation, namely, $t_{ind} = KC + B$. For the convenience of calculation, all inductive time ($t_{ind}$) was denoted by Y, namely

$$Y = KC + B$$

(14)

To calculate the content of cow milk in mixed milk by using quantitative information of nonlinear chemical fingerprint, K and B must be obtained firstly. Let $C_i$ and $Y_i$ be the content of cow milk of the $i$th standard sample in mixed milk and the

| Name of milk | $E_{uni}$ (V) | $t_{ind}$ (s) | $E_{uni}$ (V) | $\Delta E_{max}$ (V) | $t_{ind}$ (s) | $E_{pet}$ (V) |
|--------------|----------------|---------------|----------------|----------------------|---------------|---------------|
| Cow milk     | 1.128          | 1720.56       | 8251.03        | 0.9174               | 0.1750        | 43.4304       |
| RSD (%)      | 0.31           | 0.43          | 1.13           | 1.45                 | 1.93          | 0.68          |
| Goat milk    | 1.130          | 2865.03       | 9159.78        | 0.9151               | 0.1325        | 46.835        |
| RSD (%)      | 0.96           | 0.19          | 0.38           | 0.80                 | 0.62          | 1.72          |

Fig. 2 – Reproducibility of nonlinear chemical fingerprint of goat milk (a) and cow milk (b) (Nonlinear chemical fingerprints of each type of milk were obtained in quadruplicate).

Fig. 3 – Nonlinear chemical fingerprint of goat milk (1) and cow milk (2).
corresponding inductive time of nonlinear chemical fingerprint, respectively. Substituting known data of \( C_i \) and \( Y_i \) into Equation (14), a linear calibration model was obtained, namely

\[
Y = kC + b
\]

(15)

where \( k \) and \( b \) were the estimated values of \( K \) and \( B \), \( Y \) was the estimated value of \( Y \). The essence of the least square method was that the parameter estimation value was optimal when the sum of square of deviations for the real value \( Y_i \) and the estimated value \( bY_i \) was minimal.

Denote

\[
Q(k, b) = \sum_{i=1}^{n} (Y_i - \bar{Y})^2 = \sum_{i=1}^{n} (Y_i - kC_i - b)^2 \rightarrow \text{min}
\]

Namely

\[
\frac{\partial Q}{\partial b} = -2 \sum_{i=1}^{n} (Y_i - kC_i - b) = 0
\]

(16)

\[
\frac{\partial Q}{\partial k} = -2 \sum_{i=1}^{n} (Y_i - kC_i - b)C_i = 0
\]

(17)

After some manipulations, we obtained

\[
\begin{align*}
nb + \sum_{i=1}^{n} kC_i &= \sum_{i=1}^{n} Y_i \\
\sum_{i=1}^{n} bC_i + \sum_{i=1}^{n} kC_i^2 &= \sum_{i=1}^{n} C_i \cdot Y_i
\end{align*}
\]

(18)

where \( k \) and \( b \) were calculated by using \( n \) known data of \( C_i \) and \( Y_i \) of \( n \) standard samples. Then, the values of \( k \) and \( b \) were obtained by Equation (18). When the content of cow milk in unknown mixed milk sample was predicted under the same condition of the correction model, inductive time of unknown mixed milk sample was determined. According to Equation (14), we obtained,

\[
C = k^{-1}(Y - b)
\]

(19)

The content of cow milk in unknown mixed milk could be calculated in terms of Equation (19). Thus, Equation (19) may be as the general model of mixed milk of goat milk and other milk for quantitative identification and evaluation.

According to the general model, the content of cow milk in mixed milk was obtained, and determination results, precision and accuracy were shown in Table 2. It can be seen from Table 2 that the RSDs of determination results of the content of cow milk were less than or equal to 1.87%, and the recovery rates were between 95.09 and 106.12%. These experimental data interpreted that nonlinear chemical fingerprint method could be used to estimate the amount of adulterated cow milk. In addition, the limit of detection (LOD) at signal-to-noise ratio of 3.0(S/N = 3.0) was 0.0107 g/g. The proposed method could meet the requirements for the maximum amount of cow milk added in goat milk. Therefore, it suggests that the proposed method has good precision and accuracy for calculation of the content of cow milk in goat milk by nonlinear chemical fingerprint and the least square method.

### 4. Conclusion

Compared with other analytical instruments, nonlinear chemical fingerprint method was rather economic in price. Nonlinear chemical fingerprint was a kinetic fingerprint based on potential change with time, which was determined by all
components in sample. Therefore, the reproducibility of the proposed method was very well, and it not only had the advantages of simple operation and no pretreatment, but also contained abundant quantitative information and intuitive information. Meanwhile, the method may be easy received by routine analysis, and it can be used to calculate the content of cow milk adulteration in goat milk. The adulteration can be quantified in a range from 0 to 100%, with a detecting limit of 0.0107 g/g. When an assay was being considered for use in large scale analysis of milk and dairy products, nonlinear chemical fingerprint method was very helpful for the identification and evaluation of milk. More importantly, the proposed method can reflect the throng characteristics of chemical substances by adding a sample into the reactor, so it was more suitable for discriminating complicated adulterated milk samples. Accordingly, as a nonlinear chemical analysis of throng components, nonlinear chemical fingerprint technique could be a promising method for routine dairy product inspection in future. In addition, the proposed method in this study may also identify and evaluate other foodstuffs unable to be treated directly by chromatography, such as honey, vinegar, jam, syrup and so on.

Conflicts of interest
The authors declare that they have no conflicts of interest.

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References
[1] Baldo BA. Milk allergies. Aust J Dairy Technol 1984;39:120–8.
[2] Fevrier C, Mourtou J, Jaquin Y, Mounier A, Lebreton Y. Comparative digestive utilization of UHT goat’s and cow’s milks: nutritional effects of gelation-use of a swine model. Lait 1993;73:581–92.
[3] Haenlein GFW. Nutritional value of dairy products of ewe and goat milk. In: Proceedings of the IDF/Greek national committee of IDF/CIRVAL seminar; 1996. p. 159–78.
[4] Lopez-Aliga I, Diaz-Castro J, Alfredo MM, Barrionuevo M, Campos MS. A review of the nutritional and health aspects of goat milk in cases of intestinal resection. Dairy Sci Technol 2010;90:611–22.
[5] Chen RK, Chang LW, Chung YY, Lee MH, Ling YC. Quantification of cow milk adulteration in goat milk using high-performance liquid chromatography with electro spray ionization mass spectrometry. Rapid Commun Mass Spec 2004;18:1167–71.
[6] Cheng YH, Chen SD, Weng CF. Investigation of goats’ milk adulteration with cows’ milk by PCR. Asian Aust J Anim Sci 2006;19:1503–7.
[7] Hauenlein GFW. Goat milk in human nutrition. Small Rumin Res 2004;51:155–63.
[8] Molina E, Jesus Martin-Alvarez P, Ramos M. Analysis of cows’, ewes’ and goats’ milk mixtures by capillary electrophoresis: quantification by multivariate regression analysis. Int Dairy J 1999;9:99–105.
[9] Amigo L, Ramos M, Martin-Alvarez PJ, Barbosa M. Effect of technological parameters on electrophoretic detection of cow’s milk in Ewe’s milk cheeses. J Dairy Sci 1991;74:1482–90.
[10] Kim HHY, Jimenez-Flores R. Two-dimensional analysis of skim milk proteins using preparative isoelectric focusing followed by polyacrylamide gel electrophoresis. J Food Biochem 1992;16:307–21.
[11] Song SX, Xue HY, Han Y. Detection of cow’s milk in Shaanxi goat’s milk with an ELISA assay. Food Control 2011;22:883–7.
[12] Bania J, Ugorski M, Polanowski A, Adamczyk E. Application of polymerase chain reaction for detection of goats’ milk adulteration by milk of cow. J Dairy Res 2001;68:333–6.
[13] Yilmaz UT, Ergun F, Yilmaz H. Determination of the food dye carmine in milk and candy products by differential pulse polarography. J Food Drug Anal 2014;22:329–35.
[14] Ozdemir N, Kahraman T. Rapid confirmatory analysis of avermectin residues in milk by liquid chromatography tandem mass spectrometry. J Food Drug Anal 2016;24:90–4.
[15] Luikx DMAM, Cordewener JHG, Ferranti P, Frankhuizen R, Bremer MEG, Hoeijirink H, et al. Identification of plant proteins in adulterated skimmed milk powder by high-performance liquid chromatography-mass spectrometry. J Chromatogr A 2007;1164:189–97.
[16] Field RJ, Schneider FW. Oscillating chemical reactions and nonlinear dynamics. J Chem Educ 1989;66:195.
[17] Field RT, Koros E, Noyes RM. Oscillations in chemical systems. II. Thorough analysis of temporal oscillation in the bromate-cerium-malic acid system. J Am Chem Soc 1972;94:8649–64.
[18] Wang J, Yang ST, Cai RX, Lin ZX, Liu ZH. A new method for determination of uric acid by the lactic acid-acetone-BrO3-Mn2+-H2SO4 oscillating reaction using the anlyte pulse perturbation technique. Talanta 2005;65:799–805.
[19] Gan NQ, Cai RX, Lin ZX. Determination of ascorbic acid based on a peroxidase oscillator reaction. Anal Chim Acta 2002;466:257–60.
[20] Zhang J, Qiao JX, Zhang TM, Zhao Z, Xiang FQ, Fang XQ, et al. Nonlinear electrochemical fingerprint and system similarity as well as their applications in authenticity identification and quality evaluation of soya sauce. J Food Technol 2014;105:189–200.
[21] Zhang TM, Liang YZ, Yuan B, Ding F, Zhang YP, Wei MQ, et al. Determining method and conditional factors of electrochemical fingerprint of Chinese traditional medicine. Chin Sci Bull 2007;52:2190–202.
[22] Zhang TM, Zhao Z, Fang XQ, Qiao JX, Xiang FQ, Zhu R, et al. Determining method, conditional factors, traits and applications of nonlinear chemical fingerprint by using dissipative components in samples. Sci China Chem 2012;55:285–303.
[23] Zhou JF, Fang XQ, Zhang TM, Zhao Z, Zhu R, Xiang FQ, et al. Quantitative similarity assessment of non-linear chemical fingerprint of traditional Chinese medicine by similarity system theory. J Central South Univ Technol 2011;18:343–52.
[24] Gao JZ, Yang H, Liu XH, Ren J, Lu XQ, Hou JG, et al. Kinetic determination of ascorbic acid by the BZ oscillating chemical system. Talanta 2001;55:99–107.
[25] Zhang TM, Zhao Z, Fang XQ, Qiao JX, Xiang FQ, Zhu R, et al. Principle of nonlinear chemical fingerprint by using
dissipative components in samples as well as calculation and evaluation of similarity. Sci China Chem 2012;55:304–22.

[26] Gao JZ, Wei XX, Yang W, Lv DY, Qu J, Chen H, et al. Determination of 1-naphthylamine by using oscillating chemical reaction. J Hazard Mater 2007;144:67–72.

[27] Fang XQ, Zhang TM, Zhao Z, Xiang FQ, Liang YZ, Wang M, et al. Application of nonlinear chemical fingerprinting to identification, evaluation and clinical use of Glycyrrhiza. Chin Sci Bull 2010;55:2937–44.

[28] Kondyli E, Katsiari MC, Voutsinas LP. Variations of vitamin and mineral contents in raw goat milk of the indigenous Greek breed during lactation. Food Chem 2007;100:226–30.

[29] Posati LP, Orr ML. Composition of foods, dairy and egg products, agriculture handbook. USDA-ARS.

[30] Hurley IP, Coleman RC, Ireland HE, Williams JHH. Use of sandwich IgG ELISA for the detection and quantification of adulteration of milk and soft cheese. Int Dairy J 2006;16:805–12.

[31] Trujillo AJ, Guarnisi B, Carretero C. The major protein of goat milk. Alimentaria 1997;19:19–28.

[32] Bruhn JC, Franke AA. Regional differences in nitrogen fractions in California herd milks. J Dairy Sci 1979;62:1326–8.

[33] Baker LD, Ferguson JD, Chalupa W. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. J Dairy Sci 1995;78:2424–34.