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Monitoring of quality and storage time of unsealed pasteurized milk by voltammetric electronic tongue

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ABSTRACT

A voltammetric electronic tongue (VE-tongue) was self-developed and applied to monitor the quality and storage time of unsealed pasteurized milk. The VE-tongue comprised four working electrodes: gold, silver, platinum, and palladium electrode. Two potential waveforms: Multi-frequency rectangle pulse voltammetry (MRPV) and multi-frequency staircase pulse voltammetry (MSPV) were applied to working electrodes in the study, and both of MRPV and MSPV consisted of three frequency segments: 1 Hz, 10 Hz, and 100 Hz. The total areas under the corresponding curves obtained by VE-tongue in the three frequencies were applied as characteristic data, which were evaluated by the principal component analysis (PCA) and cluster analysis (CA). The results of PCA and CA indicate that the milk samples of different storage time could be successfully classified by the VE-tongue based on MRPV and MSPV, respectively. Combining the areas obtained by the VE-tongue based on MRPV and MSPV, the classification results of PCA and CA were improved evidently. The total bacterial count, acidity and viscosity of the milk samples were also measured during the storage, and those physicochemical characteristics showed regular configuration in PCA and CA plots. Furthermore, the total bacterial count and viscosity properties were predicted by partial least squares regression (PLSR) and least squares-support vector machines (LS-SVM), and the combination of the areas obtained by the VE-tongue based on the MRPV and MSPV were applied as the input data of PLSR and LS-SVM. Both the prediction techniques performed well in predicting viscosity and total bacterial count, and the prediction results of LS-SVM were better than that of PLSR. Those results demonstrate that the VE-tongue could be applied to monitor the quality storage time of unsealed pasteurized milk.

1. Introduction

Milk is one of the most common balanced and nutritious foods. It contains significant nutrients including lactose, amino acids, vitamins, nucleotides, inorganic salts, and trace elements that make milk an ideal growth medium for a wide range of pathogenic and spoilage microorganisms [1]. Additionally, the high water activity (0.98) and neutral pH (6.6) provide very conductive conditions for rapid growth of those microorganisms [2]. The Grade 'A' Pasteurized Milk Ordinance (PMO) establishes that raw milk cannot be stored in a single container for more than 72 h [3]. High-temperature short-time thermal pasteurization (HTST) has been used effectively for decades as a method to extend the shelf life of fluid milk and to eradicate pathogenic bacteria from it [4].

With the pace of life gradually accelerated, the large packing of pasteurized milk ($\geq$1 L) which could be consumed in a day or two become the first choice to people. The unsealed milks were easy be contaminated by the bacteria in the air during subsequent refrigerated storage. Furthermore, HTST cannot destroy heat resistant proteolytic and lipolytic enzymes produced by psychrotrophic bacteria, which may recover during the storage. The action of bacteria could induce chemical, physicochemical or microbiological changes in the milk product. In the past study, chemical, microbial, and sensory testing were applied to detect the storage time of pasteurized milk [5–7]. However, these techniques require certain expertise and complex pre-treatment process. Some modern analysis techniques, such as gas chromatography/mass spectroscopy [8], high performance liquid chromatography [9], and capillary zone electrophoresis [10] were also employed to detect the odor active and nonvolatile flavor compounds in milk products during the storage. However, these analytical methods are time-consuming, and the sample preparations required for them are rather complex and expensive. Therefore, the device for the fast detection, quantification and evaluation of quality and storage time of unsealed pasteurized milk product is urgently needed.

Electronic tongue (ET), which is based on an array of non-specific, low selective chemical sensors with partial specificity (cross-sensitivity) to different components in solution

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accompanied by an appropriate method of pattern recognition and/or multivariate calibration for the data-processing [11], was developed and tested as faster, low cost and reliable alternatives to these expensive and time-consuming determinations [12]. The features of ET sensors are different from those traditional chemical sensors: instead of high selectivity for the detecting substances, ET sensors have the overall selectivity that could obtain global information about the solution. The global information, which can be applied as digital fingerprint of gustatory compounds, is analyzed with appropriate pattern recognition tools. ETs were widely declared for food analysis, such as quality control [13,14], process monitoring [15] and shelf-life investigation [16]; environment monitoring, such as pesticide residue detection [17], and water quality monitoring [18]; medical and pharmaceutical applications, such as disease diagnosis [19,20], and development of liquid dosage forms [21]. ETs of several types (potentiometric, impedimetric and voltametric) may provide such alternatives [22–24].

The electronic tongue based on potentiometric working electrodes (those working electrodes composed of several kinds of lipid/polymer membranes) was first presented by Toko and coworkers [25]. Each lipid/polymer membrane was fitted on the part of a plastic tube, which has a hole, such that the inner part of the cylinder is isolated from the outside. The end of the cylinder was sealed with a stopper that holds an Ag/AgCl reference electrode. The potentiometric electronic tongue is widely declared now to monitor storage time for foods and beverage, such as pork [26], fish [27] and red wine [28]. The process of making potentiometric sensors, which requires some expertise, is complex. Otherwise, potentiometric sensors operate in terms of the charging of a membrane, limiting their use to the detection of charged ionic species [29]. The electronic tongue based on impedance spectroscopy was first described by Riul et al. [24]. The sensors were constructed by pure and composite nanostructured films of conducting polymers. The impedance measurement is often considered as a slow and unnecessarily accurate method for repeated and rapid electrical measurements, and is more often used for preliminary characterization of complex circuits [30]. The concept of voltammetric electronic tongue (VE-tongue) has been advanced recent years [31]. The VE-tongue comprised different metallic working electrodes positioned in three-electrode configuration, and transient current values were collected from working electrodes. The working electrodes are of particular interest due to their cross-sensitivity: each metallic electrode has different sensitivity to samples, and the signals obtained by different electrodes presented different information regarding the samples. Two kinds of three-electrode configuration were applied in the previous studies. The first one, the metallic working electrodes were inserted in a circular ceramic disc around a reference (Ag/AgCl) electrode, and the disc was mounted in a stainless steel tube, which was also used as an auxiliary electrode. The large amplitude pulse voltammetry (LAPV) was always applied to the working electrodes as the potential waveform of the VE-tongue, and the characteristic data were collected at a fix frequency, such as 200 Hz [32], 900 Hz [33], 2000 Hz [34]. The second one, the metallic working electrodes were embedded in a polytetrafluoroethylene tube placed around the platinum auxiliary electrode, and the reference (Ag/AgCl) electrode wes attached to the polytetrafluoroethylene tube. The multi-frequency large amplitude pulse voltammetry (MLAPV) was applied as the potential waveform, and 4 characteristic data (the maximum value, the minimum value, and two inflection values) were exacted from the sequence by each electrode for analysis [35]. Due to its high sensitivity, versatility, simplicity and robustness, the VE-tongue has already proven to be valuable in some applications [36,37].

The VE-tongues have been used for recognizing the quality of milk due to microbial growth [38], classification of different types of fermented milk [39], recognition of milk adulteration [40] and monitoring of off-flavors in the incoming milk [41], and LAPV and cyclic voltammetric (CV) was used to the working electrode as the potential waveform. However, the working electrodes based on MLAPV could obtain more information from samples than that based on other potential waveforms (the charging current of the double layer capacitance did not decay to zero and the electroactive compounds next to the electrode surface was not oxidized or reduced completely in higher frequency segments of MLAPV, and more information could be obtained from those frequency segments) [35]. Meanwhile, thousands of responses were collected by VE-tongue in the course of the experiments, and lots of them were redundant for pattern recognition analysis. In this work, a VE-tongue was self-developed to detect the unsealed pasteurized milk samples during storage at 4 °C. Four noble metallic electrodes (gold, silver, platinum, and palladium electrode) were integrated together as a new working electrode array of VE-tongue. Two types of MLAPV, multi-frequency rectangle pulse voltammetry (MRPV) and multi-frequency staircase pulse voltammetry (MSPV), were first applied to the working electrodes as the potential waveform (rectangle and staircase pulse were applied to working electrode as potential waveforms, and both of them worked good in classifying samples [42,43]. MRPV and MSPV comprised of many frequency segments of rectangle and staircase pulse, respectively, and working electrodes could obtain more classification information from samples based on MRPV and MSPV). A new method, which could reduce those redundant data, was employed to obtain the characteristic data: the sum of the areas under the corresponding curves obtained by VE-tongue in three frequencies was analyzed by pattern recognition techniques. The physicochemical and microbiological changes in unsealing pasteurized milk were also tested during the storage, and those changes were analyzed by VE-tongue based on some pattern recognition techniques.

The main purposes of this study were as follows: (1) to investigate whether the unsealing pasteurized milk of different storage time could be classified by VE-tongue with principal component analysis (PCA) and cluster analysis (CA), and to compare the classification results based on MRPV and MSPV; (2) to investigate whether the total bacterial count and viscosity of the milk samples could be predicted accurately by VE-tongue with partial least squares regression (PLSR) and least-squares support vector machine (LS-SVM).

2. Materials and methods

2.1. Samples

The 1 L-packaging pasteurized milk samples were manufactured by GuangMing Company (ShangHai, China, 12 a.m., October 2, 2011), all the samples were stored at 4 °C before experiment. 26 1 L-packaging pasteurized milks were unsealed at the same time. For the measurements, 100 mL milk were sampled from each unsealed package at 7 different times spread over 72 h (milk samples were detected at hour 0, 12, 24, 36, 48, 60, and 72 after unsealing, respectively; the testing at hour 0 was carried out on milk samples immediately after unsealing.). The changes in total bacterial count, acidity (pH) and viscosity of milk samples were also measured after VE-tongue experiment. Each sample was measured 3 replicates successively, and the average values of the 3 replicate measurements were used in the data evaluation.

2.2. Voltammetric electronic tongue

The VE-tongue self-developed and comprised four working electrodes (gold, silver, platinum, palladium, all purity 99.9%, length 5 mm, diameter 2 mm), a reference electrode (Ag/AgCl