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Detection of Taste Change of Bovine and Goat Milk in Room Ambient Using Electronic Tongue

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ABSTRACT

An electronic tongue (e-tongue) based on an array of lipid/polymer membranes has been successfully developed for measuring the taste evolution of natural milk. The e-tongue consisted of 16 different lipid/polymer membranes combined with or without a pH sensor. The natural milk of bovine and goat were purchased from the local farming store in Malang-Indonesia. The taste measurement was carried out, from fresh (0 h) to stale (12 h), every two hours under room ambient without any treatment. The responses of the e-tongue were evaluated using a Principal Component Analysis (PCA) and a Linear Discriminant Analysis (LDA). From PCA results, the taste of both milk samples tends to change by time although some groups show a partial overlapping. LDA results show the high precision of the e-tongue in clustering taste evolution. The correctly classified groups after the cross-validation procedure were achieved 95.7 and 87.1% for bovine and goat milk, respectively. The improvement of the classification using LDA was obtained by adding data from a pH sensor of each measurement as 100 and 98.6% for bovine and goat milk, respectively. This work indicates that the lab-made e-tongue may be useful to predict the quality of natural milk for the food industry.

Keywords: *electronic tongue; taste; linear discriminant analysis; principal component analysis*

ABSTRAK

Lidah elektronik berbasis kanal lipid membran telah berhasil dikembangkan, dan digunakan untuk mengukur perkembangan rasa dari susu segar sampai basi. Lidah elektronik yang digunakan dalam penelitian ini terdiri dari 16 membran lipid dan 1 sensor pH. Susu sapi dan susu kambing mentah dibeli dari peternakan lokal di Malang-Indonesia. Pengukuran kualitas rasa susu dilakukan setiap dua jam dalam kondisi suhu kamar tanpa perlakuan khusus dan diukur dari kondisi segar sampai 12 jam. Respon dari lidah elektronik dievaluasi menggunakan metode pengenalan pola analisis komponen utama (PCA) dan analisis diskriminan linier (LDA). Berdasarkan hasil PCA, kedua sampel susu menunjukkan pola perubahan terhadap waktu meskipun terjadi tumpang tindih antara kelompok. Metode LDA menunjukkan ketepatan yang tinggi dalam mengelompokkan perkembangan rasa susu. Berdasarkan LDA, kelompok yang diklasifikasikan secara tepat setelah prosedur cross-validation dicapai 95,7% untuk susu sapi dan 87,1% untuk susu kambing. Perbaikan klasifikasi menggunakan LDA diperoleh dengan menambahkan data sensor pH pada masing-masing pengukuran. Peningkatan ketepatan klasifikasi pada susu sapi ini menjadi 100% dan untuk susu kambing sebesar 98,6%. Hal ini menunjukkan bahwa lidah elektronik ini mampu untuk memprediksi kualitas susu alami.

Kata Kunci: *lidah elektronik; rasa; analisis linear diskriminan; analisis komponen utama*

INTRODUCTION

The body requires energy to optimize daily activities. Energy can be met by consuming food or beverages with enough nutrients for the body. Nutrition is very beneficial for the body because it is a vital necessity for all living beings. Foods and beverages contain nutrients sufficient to meet energy needs. Milk

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is one of the drinks components that it contains protein, lactose, calcium, magnesium, vitamin B and vitamin D [1-2].

Bovine and goat milk can be consumed directly without any treatment, nor heating or additives. This type of milk usually sold in simple packaging, so it was often known as natural milk. The freshness of natural milk was very short because of without preservatives [3,4]. One of the methods to preserve milk was to store it in the freezer. Harmful bacteria could contaminate the natural milk that results in the change of the taste quality rapidly that produce non-consumable sour milk [5-6]

The taste is the primary parameter of milk quality. Measurement of taste for beverage quality assessment usually performed by human tongue [7-9]. For many cases like sour milk, it is impossible to evaluate by human tongue because of harmful. Moreover, the human tongue is usually more subjective if compared to other expensive analytical instruments. When using human tongue, the assessment process of the taste of beverage or food can be influenced by the health condition at this time. The chemical analysis using standard analytical instruments is very expensive, time-consuming for sample preparation, and requires specialized experts although it shows high accuracy. At present, ones seek a method for rapid test of milk taste [10-16].

A sensor-array based electronic tongue (e-tongue), fortunately, has been reported as potential, versatile and advantageous electrochemical tools within food analysis. It is because e-tongue is able to qualitatively estimate characteristic properties of food samples, to distinguish among several types of foods and to recognize taste attributes [17].

An e-tongue has been reported as a qualitative and rapid analysis of foods and dairy products. It composes of an array of taste sensors, a data acquisition system, and a pattern recognition system. The taste sensor is made from an active material of lipid/polymer and has a low selectivity. Originally, it was used to evaluate and recognize the five basic tastes (sweet, salty, sour, bitter, and umami), but recently, it is employed in many applications. If the taste sensor is utilized in an array, there would be the cross sensitivity of each sensor [8,18-19]. Some applications of e-tongue include differentiating some brand of milk and yogurt [9,20].

Because of resulting a particular pattern, an e-tongue should also be equipped with a pattern recognition system. A multivariate analysis of Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) are a pattern recognition system which used for the purpose of classification or discrimination [21-22]. PCA is a non-supervised pattern recognition system [23-25], while LDA is a supervised pattern recognition system [17,26-28].

The objective of this study is to investigate the ability of self-developed e-tongue in detecting the taste evolution of natural milk without preservation at room ambient. The taste of natural milk changes rapidly with time because of the absence of the preservation. For this purpose, natural milk of bovine and goat were used as the sample of the investigation. PCA was applied to a pattern recognition system, starting from fresh milk until different taste conditions for up to 12 h. Moreover, LDA method was applied as the comparison of a pattern recognition system. The last analysis would result in the best pattern recognition multivariate model.

EXPERIMENTAL SECTION

Sensor Array

The sensor array of the working electrodes consisted of 16 types of lipid/polymer membranes coupled with a pH sensor. The membrane composed of polyvinyl chloride (PVC) as a matrix, 2-nitrophenyl octyl ether (2-NPOE) as a plasticizer, and many kinds of lipid as an active material. All the above materials were then dissolved in tetrahydrofuran (THF) for producing the membranes. Table 1 shows the composition of 16 kinds of the membrane. Finally, each solution was attached to the gold layer of the end of electrode [29,30].

Data Acquisition System

Fig. 1 shows a diagram of an e-tongue system used in this study. The sensor array consisted of a pH electrode and 16 kinds of membrane connected to silver electrodes. From the definition, a Data Acquisition Systems (DAQ) is a system consists of sensors or transducers, signal conditioning, and processing, an analog to digital converter and a computer. The roles of DAQ includes measuring physical quantities, recording data (as a data logger) and also controlling the measurement system [30]. In the case of e-tongue, the recorded data were in the form of patterns. A pattern recognition system was then applied to cluster or classify the data.

Measurement

Two types of milk, bovine milk and goat milk, purchased from the local farming in Indonesia were used as samples. For measurement, a milk sample was put into a beaker glass and stirred using a magnetic stirrer at a speed of 450 rpm and temperature of (27 ± 2) °C. The measurement was carried out starting from fresh to the next 12 h. Table 2 is a number of groups related to the measurement time,

Table 1. The composition of membranes (S1 to S16), all materials are in PVC Matrix (32%)

Sensor	Active Material Lipid (3%)	Plasticizer (65%)
S1	Octadecylamine	2-NPOE
S2	Oleyl alcohol	2-NPOE
S3	Methyltrioctylammonium chloride	2-NPOE
S4	Oleic acid	2-NPOE
S5	Octadecylamine	Bis(2-ethylhexyl) sebacate
S6	Oleyl alcohol	Bis(2-ethylhexyl) sebacate
S7	Methyltrioctylammonium chloride	Bis(2-ethylhexyl) sebacate
S8	Oleic acid	Bis(2-ethylhexyl) sebacate
S9	Octadecylamine	Bis(2-ethylhexyl) phosphate
S10	Oleyl alcohol	Bis(2-ethylhexyl) phosphate
S11	Methyltrioctylammonium chloride	Bis(2-ethylhexyl) phosphate
S12	Oleic acid	Bis(2-ethylhexyl) phosphate
S13	Octadecylamine	Bis(1-butylpentyl) adipate
S14	Oleyl alcohol	Bis(1-butylpentyl) adipate
S15	Methyltrioctylammonium chloride	Bis(1-butylpentyl) adipate
S16	Oleic acid	Bis(1-butylpentyl) adipate

Table 2. List of groups

Groups	Description of measurement time (h)
1	0 (fresh)
2	2
3	4
4	6
5	8
6	10
7	12 (stale)

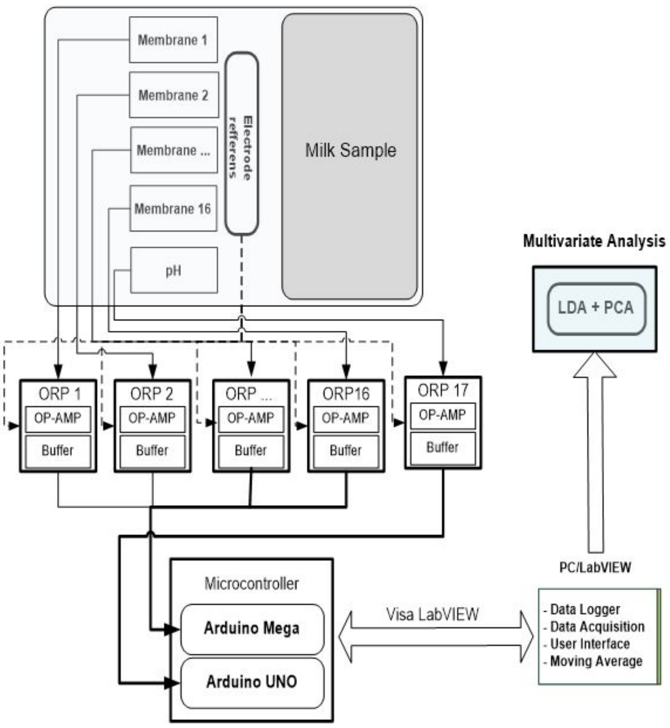

Fig 1. Schematic diagram of e-tongue and the measurement process

at 0 h (fresh condition) to 12 h (stale condition). Measured data of bovine milk were saved as Dataset01, while that of goat milk were saved as Dataset02.

Statistical Analysis

PCA and LDA are statistical methods that use an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values which are linearly uncorrelated. Both unsupervised (PCA) and supervised (LDA) statistical techniques can be used to analyze the ability of the different techniques to discriminate qualitatively between samples.

In this case, PCA and LDA were applied for reducing of 16 or 17 sensors to a smaller number of new variables as principal components. Those methods were used for visualization and classification of groups of milk data. Using PCA and LDA, the ability of the e-tongue for recognizing milk taste was analyzed. For this purpose, the effect of a pH sensor addition to the sensor array was also investigated related to the performance of the e-tongue.

RESULT AND DISCUSSION

Basically, an e-tongue is an array of potentiometric sensors that give a non-selective and non-Nernstian response to various analytes as a representative of various taste. The response mainly comes from the interaction between the analyte with the active compound in the membrane, so that a gradient concentration is generated in the membrane and at the interface between the membrane and aqueous solution. As a result, a potential will be generated in the electrodes and measures as the response of the sensor. As each sensor contains a different combination of active compound and membrane solvent (plasticizer), then each sensor will give different response pattern to different taste. The response pattern of the 16 sensors to a particular analyte then can be used as a marker for that taste, that can be analyzed further using PCA or LDA.

Taste components are sourness, saltiness, umami, bitterness, and sweetness. Each taste can be represented chemically such as acid for sourness, NaCl as saltiness, sodium glutamate for umami, coffee for bitterness, sucrose for sweetness. The active compound in a series of 16 sensors will respond unselectively to the various compounds representing various flavors in the sample. The response of each sensor will be a cumulative potential for each taste-representative compounds to that membrane sensor.

Membrane component consists of PVC as the membrane matrix, membrane solvent (plasticizer) and sensing-active compound. As the membrane solvent, four types of compounds were used, namely 2-NPOE, bis(2-ethylhexyl) sebacate, bis(2-ethylhexyl) phosphate, and bis(1-butylpentyl) adipate. These four plasticizers have different lipophilicity (log $P_{o/w}$) and dielectric constant (ε) that will affect the sensitivity and speed of the electrochemical response of each membrane. Eventually, the difference in membrane solvent will give different response patterns.

The active compounds that used for the preparation of the 16-sensor in this work are expected to have an interaction with a group of taste-representative compounds. These compounds can be grouped into (1) amines (octadecylamine); (2) ammonium salts (methyltrioctylammonium chloride); (3) acid (oleic acid); and (4) alcohol (oleyl alcohol). The amine compound will respond to the acidic taste component; while the ammonium salt will respond to chloride ions as a saltiness representation. The active acid compound will respond to the base compound as a bitter taste representation, while the alcoholic compound will interact with the nonionic polar compounds, such as sugars. By combining four active compounds and four membrane solvent, then a series of 16 sensors can be constructed, that each sensor will give a different response to each taste to give a response pattern.

Fig. 2 are typical responses of a sensor array in the e-tongue when detecting the same sample. Each curve represents the corresponding potential (mV) of a sensor against time (s). Significant changes took place in 0-275 s for sensor S1, while the remain sensors achieved a dynamic balance very fast. The last 50 signal values of the e-tongue sensors were chosen for average treatment. From the sensor array, there are 17 (with pH sensor) original variables in total for each sample (bovine or goat milk), that is, a 17 × 70 (10 replications × 7 groups) data matrix.

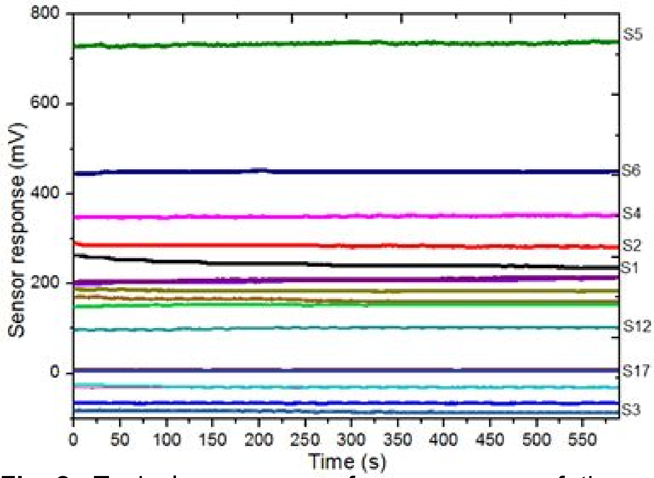

Fig 2. Typical responses of sensor array of the e-tongue

Characteristic Response of the Sensors

Sixteen sensors used in the e-tongue here were a combination of four types of lipid and four types of plasticizer. The lipids used were octadecyl amine, oleyl alcohol, methyltrioctylammonium chloride and oleic acid, while the plasticizer was 2-NPOE, bis(2-ethylhexyl) sebacate, bis(2-ethylhexyl) phosphate and bis(1-butylpentyl) adipate. The analyte used were KCl, MSG, NaCl and $MgCl_2$ as a representative for four types of taste, i.e., sour, umami, salty, and bitter, respectively. Octadecylamine, as a base, interacts with acidic components (H_3O^+) or with a cation (ion-dipole interactions), whereas oleyl alcohol interacts weakly with organic molecules such as MSG through hydrogen bonding. Methyltrioctylammonium chloride, on the other hand, as cationic organic molecules, interacts with the anion (negative slope) while oleic acid interacts with bases or cationic depending on the pH. The interactions strength can be determined by the magnitude of the slope of the plot of potentiometric response curve vs. log concentration of the analyte. An ideal response will follow a Nernstian pattern that the slope of 59.16 mV/decade for singly charged ions. The sensors used in the e-tongue usually have a small slope which shows that the interactions between the lipid with the analyte is not too strong nor selective. The effects of plasticizer on the response of the sensor are usually not as significant as that of lipid used. Data of the slopes for each type of lipid that is the average of the four types of plasticizer against four types of analytes are shown in Table 3.

Table 3. Average slope of sensors based on octadecyl amine (S1, S5, S9, S13), oleyl alcohol (S2, S6, S10, S14), methyltrioctylammonium chloride (S3, S7, S11, S15) and oleic acid (S4, S8, S12, S16)

Sensors	Average slope (mV/decade)			
	KCl	MSG	NaCl	$MgCl_2$
S1, S5, S9, S13	22.2	23.1	-15.7	41.7
S2, S6, S10, S14	15.8	16.7	14.9	12.5
S3, S7, S11, S15	-10.3	-6.6	-10.9	-9.9
S4, S8, S12, S16	3.1	5.7	-10.3	3.2

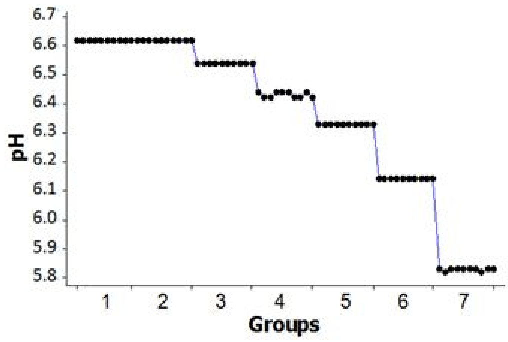

Fig 3. Typically of signal pH sensor on each group

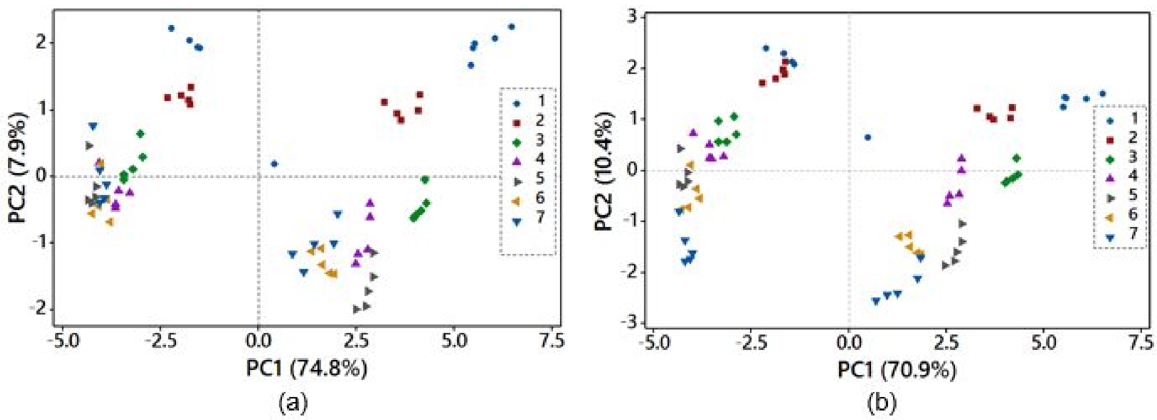

Fig 4. Score plot of PCA for natural bovine milk, (a) without and (b) with involving a pH sensor in which seven groups refer to Table 2

Based on the data in Table 3, it was observed that the sensor based on octadecyl amine exhibited the high responses to KCl, MSG, and $MgCl_2$. The essential character of the amine as a strong electron pair donor could explain the relatively strong interaction between the lipid and cation. However, the basic character of the amine depends on the pH of the solution. In an acidic media, the amine group will bind a proton and become $–NH_3^+$, which will respond to the anion. Therefore, the strength of the sensor response based on amine compound will depend on the pH of the solution. As expected, oleyl alcohol showed a weaker response compared to that of octadecyl amine. Methyltrioctylammonium chloride showed a negative slope that in accordance with the theory, the sensor based on methyltrioctylammonium chloride responds to the anionic analyte. As for oleic acid, that could exist in two types of species, as molecular acid and as oleate anionic. The proportion of the two species will depend on the pH of the solution. In acidic solution, the molecular acid will be dominant, while in more basic solution the anionic species will be dominant. As the molecular acid, the lipid may interact with anionic species, while as anionic species the sensor will generate a response to cationic species. As a result, there will be a competition of cationic and anionic responses that depend on the pH of the solution. The result in Table 3 for sensor based on oleic acid exhibited a positive slope that means the cationic response is more dominant than that of the anionic response.

The process of being sour in fresh milk is the activity of *Lactobacillus* in fresh milk converting lactose into lactic acid, so that the milk becomes sour (decrease in pH) [31]. In this study, an unconventional approach is attempted by adding a selective sensor to the acidic taste (H^+) that is pH electrode. By the addition of one pH probe to the series of 16-unselective membrane sensors, it strengthened the confirmation of the taste change during the process of milk decay. Our experiment showed that there was a significant change in the pH of the milk from 6.6 to 5.8, slightly acidic. An example of pH sensor signal in each group is shown in Fig. 3. The change in the pH of the solution will affect the sensitivity of the sensor based on octadecyl amine compound and oleic acid. In lower pH, the sensitivity of the sensor based on amine compound will decrease its cationic response character; while for the sensor based on oleic acid will be affected oppositely.

The sensors based on oleyl alcohol and methyltrioctylammonium chloride will not be affected significantly by the change in the pH of the milk because of the souring process. The change in the

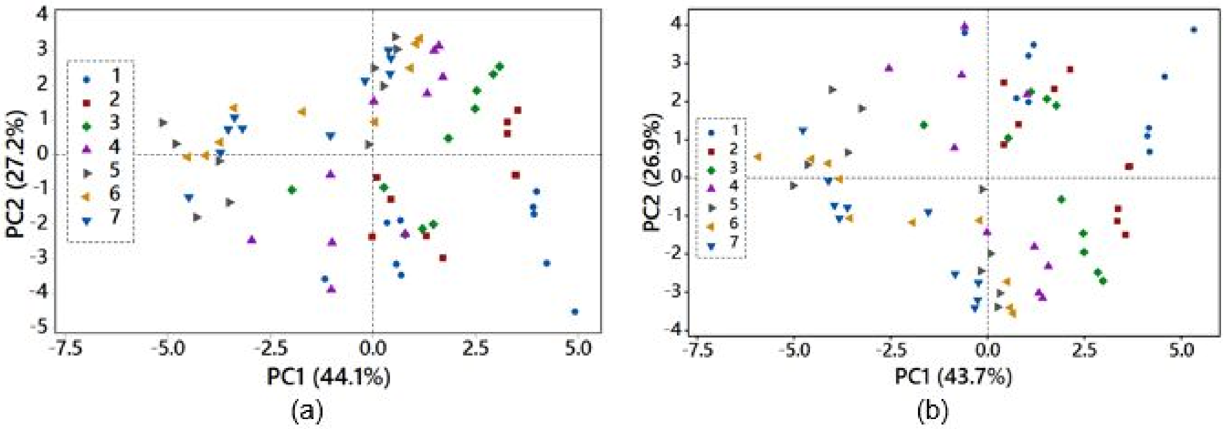

Fig 5. Score plot of PCA for natural goat milk, (a) without and (b) with involving pH sensor in which seven groups refer to Table 2

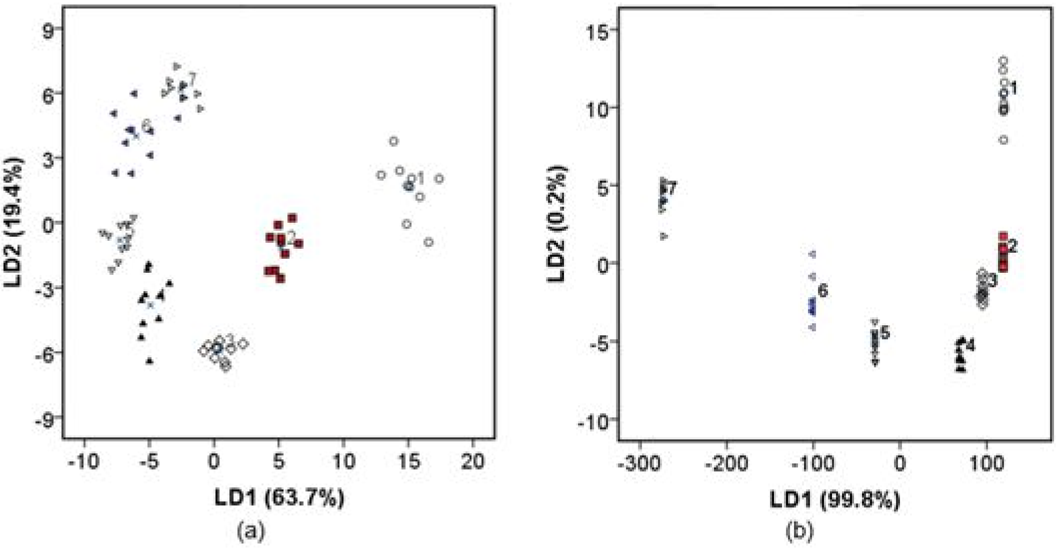

Fig 6. LDA results of seven groups of bovine milk measured using the e-tongue: (a) without and (b) with employing a pH sensor, in which seven groups refer to Table 2

response pattern of the sensors during the souring process could be used to track the souring process in the fresh milk using a chemometric method as described in the next section.

Identification Results Based on PCA

The discrimination amongst different groups of the samples was analyzed. Firstly, PCA was conducted on the recorded data. Fig. 4(a) shows the two-dimensional score plot of PCA of bovine milk (based on Dataset01) where separation amongst groups is not complete. The variances explained by the first and the second principal components were 74.8 and 7.9%, respectively. Similarly, Fig. 4(b) also shows that some of the groups are overlapped. The variances explained first and the second principal components are 70.9 and 10.4%, respectively. It indicates that the addition of using pH sensor did not change the separation amongst the groups completely. From these two plots, there is a certain area of overlapping in which no clear differentiation could be made between bovine milk samples at the condition of fresh (0 h) to stale (12 h).

Fig. 5(a) shows the score plot of PCA (PC1 and PC2) of goat milk (based on Dataset02) in which separation amongst groups is also not clear. Here, the total variances explained by PC1 and PC2 are only 44.1 and 27.2%, respectively. Based on Fig. 5(b), the total variances contributed by PC1 and PC2 are 43.7 and 26.9%, respectively. Similar to the bovine milk, the use of a pH sensor also did not improve the separation amongst the groups. As shown previously, PCA is mainly used for feature extraction by finding the features with the highest variation in a large dataset.

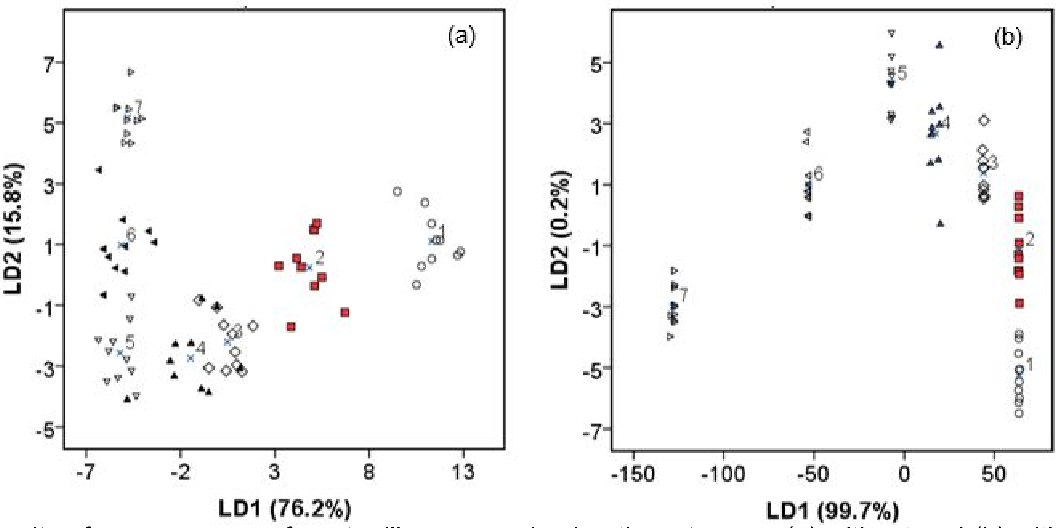

Fig 7. LDA results of seven groups of goat milk measured using the e-tongue: (a) without and (b) with employing a pH sensor, in which seven groups refer to Table 2

From both datasets, the features explain that the taste of milk samples tend to change by time.

Identification Results Based on LDA

Different from PCA, LDA is mainly used for classification. Here, a stepwise LDA procedure was then employed to visualize the distribution of groups. For this purpose, the leave-one-out cross-validation was applied to avoid overoptimistic data modulation, while the Wilks' Lambda test was carried out to verify which canonical discriminant functions were significant.

Fig. 6(a) shows LDA plot of the 16 e-tongue sensors (without pH sensor) for the first two discriminant functions (LD1 and LD2). When using Dataset01 (for bovine milk), the stepwise LDA resulted in better discrimination with a complete separation amongst the groups. These two functions explained 83.1% of the total variance of the e-tongue data to the bovine milk (the first explaining 63.7% and the second 19.4%). All samples (100%) are correctly classified for the original groups, but only 95.7% are correctly classified for the cross-validation procedure. The improvement of classification based on Dataset01 obtained by employing a pH sensor in the sensor array is shown in Fig. 6(b). From this figure, the 100% groups are perfectly separated from each other. Therefore, from LDA, all samples (100%) are correctly classified both for the original groups and for the cross-validation procedure.

Fig. 7(a) shows LDA plot of seven groups of Dataset02 using 16 e-tongue sensors (without pH sensor) for LD1 and LD2. These two functions explained 83.1% (LD1 = 63.7% and LD2 = 19.4%) of the total variance of the e-tongue data to the goat milk. From the validation procedure, 97.1% of Dataset02 are correctly classified for the original groups, while 87.1% of cross-validated grouped cases correctly classified. The improvement of the classification based on Dataset02 was also obtained by employing a pH sensor in the sensor array, as shown in Fig. 7(b), in which all groups are perfectly separated from each other. These two functions explained 99.9% of the total variance of the e-tongue data to the goat milk (the first explaining 99.7% and the second 0.2%). From LDA results, all samples (100%) are correctly classified for the original groups while 98.6% are correctly classified for the cross-validation procedure.

CONCLUSION

It has been shown the potential application of an e-tongue which comprised an array of 16 different lipid/polymer membranes combined with a pH sensor. The performance of the e-tongue was evaluated for detecting the taste change of raw milk samples in room ambient during 12 h. The results showed that using the potentiometric fingerprints gathered from the sensor array it was possible to differentiate the change of taste every two hours. It was also demonstrated that the classification using LDA was obtained to be 100% and 98.6% for bovine and goat milk, respectively by adding data from a pH sensor for each measurement. Thus, it could be concluded that the e-tongue represented a feasible and accurate rapid detection tool that could be used to identify the freshness of raw milk (bovine and goad milk) used in quality control of the production of dairy products.

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