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Analysis of milk adulteration by means of a potentiometric electronic tongue

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ABSTRACT

Milk adulteration presents substantial challenges in the food industry, prompting the need for efficient detection methods. This study introduces a potentiometric electronic tongue for rapid and accurate detection of milk adulteration. Using polymeric membranes with various integrated additives, the electronic tongue distinguished between different milk types and detected common adulterants. Experimental results demonstrated its effectiveness in discriminating raw, pasteurized, and medicated cow milk, as well as goat milk. Moreover, it successfully identified adulterants, such as water and cow milk, in goat milk samples. Chemometric analyses, including principal component analysis and partial least squares regression, correlated sensor responses with traditional milk parameters such as fat, protein, and lactose content with an R^2 of up to 0.97 on the validation step. Strong correlations validated the electronic tongue's potential for rapid milk quality assessment. This innovative approach offers a cost-effective, reliable solution for detecting milk adulteration in contrast to current techniques that require numerous time-consuming experiments.
Key words: potentiometric electronic tongue, cow milk, water-adulterated milk, goat milk

INTRODUCTION

The global food industry is based on a complex supply system of production, distribution, and consumption that is constantly subjected to economic pressures and quality standards. Meanwhile, the issue of food fraud has become a serious concern for both economic and public health reasons. Fraudulent practices are rooted in the pursuit of an economic profit paired with a low cost of production. The problem of food fraud is even more alarming in countries where laws are lax and food quality controls are scarce. For example, in a well-known health crisis reported in 2008 in China, manufacturers were found to have produced melamine-adulterated milk powder and infant formulas, resulting in serious health risks and several fatalities among infants and young children (Gossner et al., 2009).

The adulteration of milk involves a range of substances used to manipulate milk quality assessments (Azad and Ahmed, 2016). Water and whey are commonly added to increase the volume, while sugar and starch are used to increase the density of diluted milk (Kamthania et al., 2014). In addition, urea and melamine, which are nitrogen-rich compounds, are used to falsely elevate protein content (Liu et al., 2010, 2012). Animal and vegetable oils can increase milk fat content, and additives such as detergents and surfactants are used to emulsify the added oil, enhancing the milk's whiteness (Singuluri and Sukumaran, 2014). Furthermore, sodium carbonate and sodium hydrogen carbonate can neutralize the acidity in spoiled milk, and various chemicals, such as salt, hydrogen peroxide, formalin, boric acid, and salicylic acid, extend the milk's shelf life (Singh and Gandhi, 2015; Fehér Pindešová et al., 2022). Among adulterants, water and urea are particularly prevalent in adulterated milk samples. In some cases, high-value milk from 1 species may be adulterated with lower-value milk from another species. For example, owing to its seasonal production fluctuations and higher market price, goat milk is susceptible to adulteration with cow milk (Fan et al., 2023).

Although certain economically motivated adulterations, such as the addition of vegetable protein, milk from different species, whey, or water, may not carry severe health risks, other adulterants, such as urea, formalin, detergents, ammonium sulfate, boric acid, caustic soda, benzoic acid, salicylic acid, hydrogen peroxide, sugars, and melamine, can cause serious adverse health effects in consumers (Fischer et al., 2016; Kumar and Dash, 2021). The detection of adulterants in milk is challenging because each adulteration requires a separate technique.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

Furthermore, although qualitative assessments can be achieved through chemical reactions, the quantitative determination of adulterant concentrations is especially challenging because it is more complex (Azad and Ahmed, 2016). Conventional techniques, such as liquid chromatography (Chen et al., 2004; Sharma et al., 2009), ELISA (Sánchez et al., 2002; Hurley et al., 2004), and PCR (Bania et al., 2001; López-Calleja et al., 2004), are commonly employed in the detection of milk adulterants. However, these techniques have limitations in terms of specificity, speed, and adaptability, in addition to being time-consuming and expensive and requiring complex pretreatment of samples, specialized equipment, and qualified personnel (Azad and Ahmed, 2016).

Because of the limitations of conventional methods, researchers have been exploring innovative technologies for the detection of milk adulteration. For example, near-infrared spectroscopy has been used to quantify water and whey in cow milk (Kasemsumran et al., 2007), and other studies have compared near-infrared spectroscopy and medium-infrared spectroscopy for the detection of adulterants such as tap water, whey, hydrogen peroxide, synthetic urea, and urine (Santos et al., 2013; Mohammed and Shuming, 2021). Electrochemical impedance spectroscopy has also been used to identify adulteration of raw cow milk with urea (Minetto et al., 2022).

Water, one of the most commonly used adulterants to increase milk volume, poses unique challenges for detection. Traditional methods, such as freezing point determination, SNF content analysis, and lactometer readings, are useful but have limitations in terms of sensitivity and practicality (Kumar and Dash, 2021; Fehér Pindešová et al., 2022). The freezing point of milk decreases with the addition of water, making it a sensitive method for detecting water content when it exceeds 3%. Similarly, the SNF content decreases when water is added, providing another indicator of adulteration. The specific gravity of milk, measured by a lactometer, is also used to identify water adulteration (Kumar and Dash, 2021). Despite advances in detection technologies, their implementation can be challenging, particularly in rural areas where resources are limited. Furthermore, milk analyzers, a commonly used tool for detection, are expensive and not easily integrated into local industries that process a small number of samples.

This economic and technological gap highlights the need for easy, reliable, cost-effective, and user-friendly detection methods. For this purpose, electrochemical sensing mechanisms based on voltammetric, amperometric, potentiometric, or field-effect transistor detection have been reported. For example, an ionic polymer metal composite has been used as a sensor for detecting milk adulteration with water (Pal et al., 2023). In another study, a simple analytical method based on a copper sensor was used to monitor and quantify melamine in milk samples without the interference of organic substances that are normally present in this matrix (de Araujo and Paixão, 2014). In addition, a wide variety of sensing and biosensing approaches for the detection of urea adulteration in milk has been reported in recent years (Shalileh et al., 2023).

Electronic tongues, or e-tongues, are being developed for use in milk sample analyses because they are reliable and simpler, faster, and more economical than other methods. Research in this area has investigated cross-sensitive solid contact electrodes for the detection of goat milk adulteration with cow milk (Dias et al., 2009); a disposable voltammetric e-tongue to detect milk adulteration with hydrogen peroxide (Paixão and Bertotti, 2009); a portable voltammetric e-tongue device based on an electrochemical sensor array of polypyrrole to evaluate the discrimination of samples of fresh milk adulterated with starch (Arrieta-Almario et al., 2018); and a voltammetric e-tongue composed of metallic electrodes to detect the residues of 6 different antibiotics in cow milk (Wei and Wang, 2011) and to monitor the quality and storage time of pasteurized milk samples (Wei et al., 2013). Moreover, many studies have focused on nanosensor platforms for the detection of adulterants in milk (Himshweta and Singh, 2023). When using e-tongues, extracting relevant information from the electrochemical data is a crucial step, and for this purpose, applying different chemometric tools is necessary to reliably discriminate between samples (Grassi et al., 2022).

In this study, a simple and portable potentiometric e-tongue based on polyvinyl chloride (**PVC**) membranes modified with different plasticizers and additives was used to identify water adulteration in cow milk. Experimental samples represented various processing stages, such as raw milk, pasteurized milk, and milk from a cow medicated for mastitis. Moreover, the potentiometric device was also used to identify the adulteration of goat milk with cow milk. Multivariate statistical analyses were employed to analyze the potentiometric data obtained from milk. In particular, principal component analysis (**PCA**) was used to study the discrimination capacity of the potentiometric sensors, and partial least squares (**PLS**) analysis was carried out to establish prediction models that correlated the data obtained with the potentiometric e-tongue and chemical parameters obtained by traditional analysis of milk, such as fat, proteins, and lactose.

MATERIALS AND METHODS

Reagents

All chemicals were of analytical grade and were used without further purification. They were purchased from

Table 1. Plasticizers and additives used to prepare the polymeric membranes

Item	Nomenclature	Compound	Chemical formula
Plasticizers	A	Bis(1-butylpentyl) adipate	$C_{24}H_{46}O_4$
	B	Tris(2-ethylhexyl) phosphate	$C_{24}H_{51}O_4P$
	C	Dibutyl sebacate	$C_{18}H_{34}O_4$
	D	2-Nitrophenyl-octyl ether	$C_{14}H_{21}NO_3$
	E	Dioctyl phenylphosphonate	$C_{22}H_{39}O_3P$
Additives	1	Octadecylamine	$C_{18}H_{39}N$
	2	Oleyl alcohol	$C_{18}H_{36}O$
	3	Tridodecylmethylammonium chloride	$C_{37}H_{78}ClN$
	4	Oleic acid	$C_{18}H_{34}O_2$

Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared in MilliQ deionized water (Merck, KGaA, Darmstadt, Germany).

Potentiometric E-Tongue

The potentiometric sensors used in this study were based on polymeric membranes composed of high-density PVC. The PVC was combined with different plasticizers and additives, and the mixture was homogenized using tetrahydrofuran as the solvent to form a smooth matrix. The weight percentages of the components were about 32% (wt/wt) for PVC, 3% (wt/wt) for additives, and 65% (wt/wt) for plasticizers. Table 1 lists the plasticizers and additives used to manufacture the polymeric membranes. Twenty different membrane combinations were created for this study, and the e-tongue was therefore developed based on a total of twenty potentiometric sensors (Table 2). The final device was assembled according to an earlier work (Pérez-González et al., 2021). The polymeric membranes were placed on the top of a silver epoxy resin layer (EPO-TEK, Billerica, USA) and inserted in a methacrylate tube with 0.3-cm-diameter holes (Figure 1).

The measurements were carried out using an Ag/AgCl reference electrode and a multiplexer Agilent 39704A switching unit (Agilent Data Acquisition Switch Unit 34970A). Each membrane was connected to the multiplexer via electrical copper wires. Glass beakers containing 250 mL of a standard solution or milk were used for the measurements. The sensors were immersed in the liquid, and the potentiometric apparatus was used to record the potential (Figure 1). Data acquisition started after a 5-min wait to stabilize the signal. To guarantee the repeatability of the analysis, each milk sample was measured 10 times using various aliquots.

Samples Analyzed

Standard Solutions. The response of the potentiometric e-tongue was evaluated in different standard solutions to calibrate the system and to analyze its ability to detect compounds usually found in milk. For this purpose, 5 salts (KCl, $CaCl_2$, $MgCl_2$, NH_4Cl, and NaCl), 3 sugars (galactose, glucose, and lactose), 3 organic acids (citric acid, lactic acid, and monosodic L-glutamic acid), and 1 nitrogen-based compound (urea) were analyzed. All standard solutions except salts were prepared at the following concentrations: 0.0001, 0.001, 0.01, and 0.1 *M*. The salt solutions were prepared at concentrations of 0.01, 0.1, 0.2, 0.3, 0.4, and 0.5 *M*.

Milk Samples. The study was carried out using 17 types of milk with different origins or adulterations (Table 3). The samples included raw and pasteurized cow milk from 2 different local cattle farms, milk from a cow medicated for mastitis, and raw goat milk from a local farm. The adulterated samples used these milks with deionized water added to achieve different dilution ratios: dilution 1:1, with 50% milk and 50% water by volume, and dilution 1:3, with 25% milk and 75% water

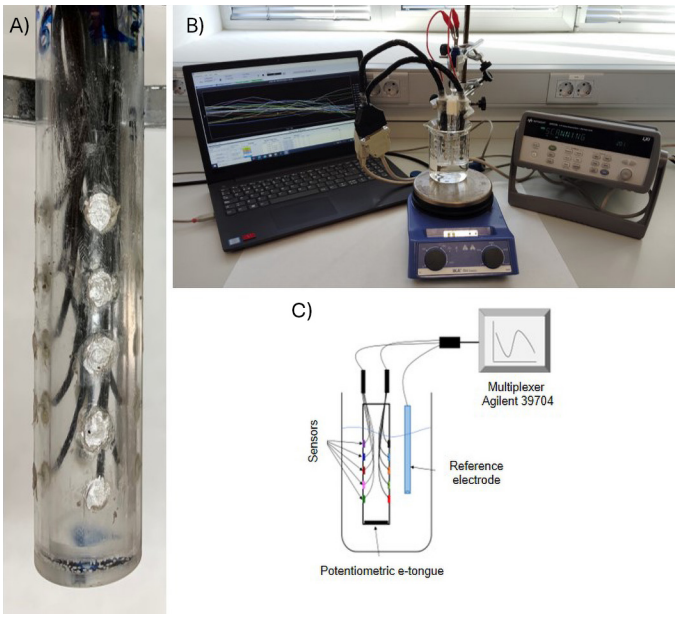

Figure 1. (A) Membranes on the device used as a potentiometric e-tongue. (B) Image of the potentiometric e-tongue device connected to the multiplexer Agilent 39704. (C) Scheme of the assembly showing the sensor system, the reference electrode, and data acquisition.

Table 2. Plasticizer and additives used for each membrane in the potentiometric e-tongue

	Membrane																			
Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Combination (plasticizer + additive)	1A	1B	1C	1D	1E	2A	2B	2C	2D	2E	3A	3B	3C	3D	3E	4A	4B	4C	4D	4E

by volume. The final sample analyzed was a blend of raw cow milk and raw goat milk to study adulteration of goat milk with cow milk (each 50% by volume). Each milk sample was measured 10 times using various aliquots.

Traditional standard chemical procedures were used to evaluate the milk: HPLC was used to determine the lactose content (International Organization for Standardization, 2007); the Tritatrion method (International Organization for Standardization, 2012), for acidity; the Gravimetry Röse-Gottlieb method (International Organization for Standardization, 2010a), for fat content; and the Kjeldahl method (International Organization for Standardization, 2014), for protein content. The samples were also examined for nonfat dry matter (**NFDM**; International Organization for Standardization, 2010b). Table 4 presents a summary of the physicochemical data.

Chemometric Analysis

Principal component analysis was used to discriminate between the samples based on the results obtained from the potentiometric e-tongue (used as the input data matrix). Partial least squares regression was employed to establish linear correlations between the physicochemical parameters and the responses of the potentiometric sensors. The multivariate data analysis was performed by using The Unscrambler X (version 10.4, CAMO Software, Oslo, Norway).

RESULTS AND DISCUSSION

Potentiometric E-Tongue Response to Standard Solutions and Milk Samples

The differences found between the standard solutions confirmed the cross-selectivity of the sensors and their efficiency as part of an e-tongue. Moreover, Figure 2 illustrates the responses obtained to the milk samples under study, once more showing a degree of cross-selectivity of the membranes forming the array that can be used to discriminate between them.

PCA Results

Discrimination of Standard Solutions. The PCA of the responses obtained for standard solutions is shown in Figure 3. Principal components (**PC**)1 and PC2 explained 74% of the total covariance of the data. The compounds were grouped based on their chemical nature, confirming the ability of the multisensor system to discriminate according to different "tastes."

Table 3. Milk samples analyzed based on their origin and adulteration

Adulteration	Animal origin	Type of milk	Code
Unadulterated	Cow	Raw cow milk-1	RC1
		Raw cow milk-2	RC2
		Pasteurized cow milk-1	PaC1
		Pasteurized cow milk-2	PaC2
		Medicated cow milk	MC
	Goat	Raw goat milk	RG
Water-adulterated	Cow	Raw cow milk-1 (dilution 1:1)	RC1.1
		Raw cow milk-2 (dilution 1:1)	RC2.1
		Pasteurized cow milk-1 (dilution 1:1)	PaC1.1
		Pasteurized cow milk-2 (dilution 1:1)	PaC2.1
		Raw cow milk-1 (dilution 1:3)	RC1.2
		Raw cow milk-2 (dilution 1:3)	RC2.2
		Pasteurized cow milk-1 (dilution 1:3)	PaC1.2
		Pasteurized cow milk-2 (dilution 1:3)	PaC2.2
	Goat	Raw goat milk (dilution 1:1)	RG.1
		Raw goat milk (dilution 1:3)	RG.2
Goat milk adulterated with cow milk	Goat and cow	Mix of raw cow milk-1/raw goat milk (50% of each in volume)	MX

Table 4. Physicochemical parameters of the milk samples analyzed

Samples	Fat, % (wt/wt)	Protein, % (wt/wt)	Lactose, % (wt/wt)	NFDM, %	SCC, cells/mL	Cryoscopic P, °C	Acidity, dronic
RC1	3.54	3.17	4.84	8.76	208,000	−0.522	15.4
PaC1	3.64	3.19	4.97	8.91	948,000	−0.537	14.6
RC2	3.82	3.12	4.8	8.67	1,355,000	−0.517	14.6
PaC2	3.88	3.17	4.93	8.85	989,000	−0.533	14.8
MC	4.18	3.34	4.61	8.7	10,539,000	−0.523	16.8

Discrimination of Cow Milks. Figure 4 shows the 2-dimensional score plot of the results obtained from raw milk and pasteurized milk, with PC1 and PC2 explaining 67% and 18% of the covariance, respectively. The diagram shows that pasteurized samples could be clearly discriminated from raw milk samples. In addition, pasteurized samples are located on the left of the diagram, while raw samples appear on the right, confirming the capability of the potentiometric e-tongue.

Figure 5 shows the 2-dimensional score plot of the results obtained from raw milk, pasteurized milk, and milk from a cow medicated for mastitis. In this case, PC1 and PC2 explained 81% of the covariance. The milks are easily discriminated and are located in different areas of the graph. Pasteurized samples appear close to each other with negative PC1 and PC2 values; raw milks are located close to each other with negative PC2 values; and the medicated milk sample has positive PC1 and PC2 values.

Comparison Between Unadulterated and Water-Adulterated Cow Milks. A PCA was carried out to discriminate unadulterated cow milk samples from corresponding water-adulterated samples. As shown in Figure 6, water-adulterated samples are clearly distinguished from the original raw milk samples, with PC1 and PC2 explaining 59% and 25% of the total covariance, respectively. Moreover, the samples appear to group separately according to the degree of adulteration. In addition, PCA also confirmed the discrimination of the pasteurized samples (Figure 7) with a total covariance of 94% (PC1 and PC2), confirming, once again, the abilities of the potentiometric e-tongue. Finally, Figure 8 shows the discrimination of all the cow milk samples and their water-adulterated counterparts. In this case, the first 2 PC explained 71% of the covariance of the data (58% by PC1 and 13% by PC2). Unadulterated milks (raw and pasteurized) appear on the left of the graph, water-adulterated (1:1 dilution) milks are in the middle, and water-adulterated (1:3 dilution) milks are on the right.

Adulteration of Goat Milk with Cow Milk. Goat milk presents a higher nutritional and economic value than cow milk. For this reason, it is sometimes adulterated by the addition of cow milk. To evaluate the capability of the potentiometric e-tongue to distinguish between milk samples from different animals and between mixtures of such samples, cow and goat milks and mixtures of goat and cow milks were analyzed. As shown in Figure 9, milks could be discriminated according to the animal species, with cow milk samples appearing on the left of the diagram and goat milk on the lower right side. The goat milk adulterated with cow milk appears on the top right side. Overall, PC1 and PC2 explained 76% and 14% of the total covariance, respectively, for a total of 90%.

Finally, PCA of adulterated goat milk and cow milk revealed that the goat milk samples (unadulterated and adulterated) could be separated from the cow milk samples, as shown at the bottom of the graph in Figure 10. Additionally, the cow milk samples in the graph are grouped according to their degree of adulteration, with the 1:1 dilution samples being grouped separately from the 1:3 dilution samples. Moreover, the 1:1 dilution goat and cow milk samples are closer to the raw milk samples than the 1:3 dilution samples. Finally, the cow-goat mixture sample appears closer to the original goat sample in the graph, which could be because goat milk has a more intense flavor than other types of milk because of its unique composition and structure; therefore, goat milk would have a strong influence in the mixture. In this case, PC1 accounted for 53% of the covariance and PC2 accounted for 20%, explaining 73% of the total covariance of the data.

PLS Regression Results. Once the e-tongue's performance was verified by discriminating between different types of milk and their origins and detecting adulteration, a mathematical model was designed to determine the typical chemical values of milk using the e-tongue.

The initial strategy to accomplish this task used PLS to generate correlations with the physical parameters and included a full cross-validation function as internal validation for the mathematical model. In this analysis, the calibration fit the model to the available data, while the validation checked the model using new data. The potential values obtained through the e-tongue for the cow milk samples (pasteurized, raw, and medicated) were used as input data for the model, creating a matrix of the predictors (X), while the physicochemical values formed a matrix of the expected responses (Y).

The values of R^2, along with their associated errors, showed the great effectiveness of the model in calibrat-

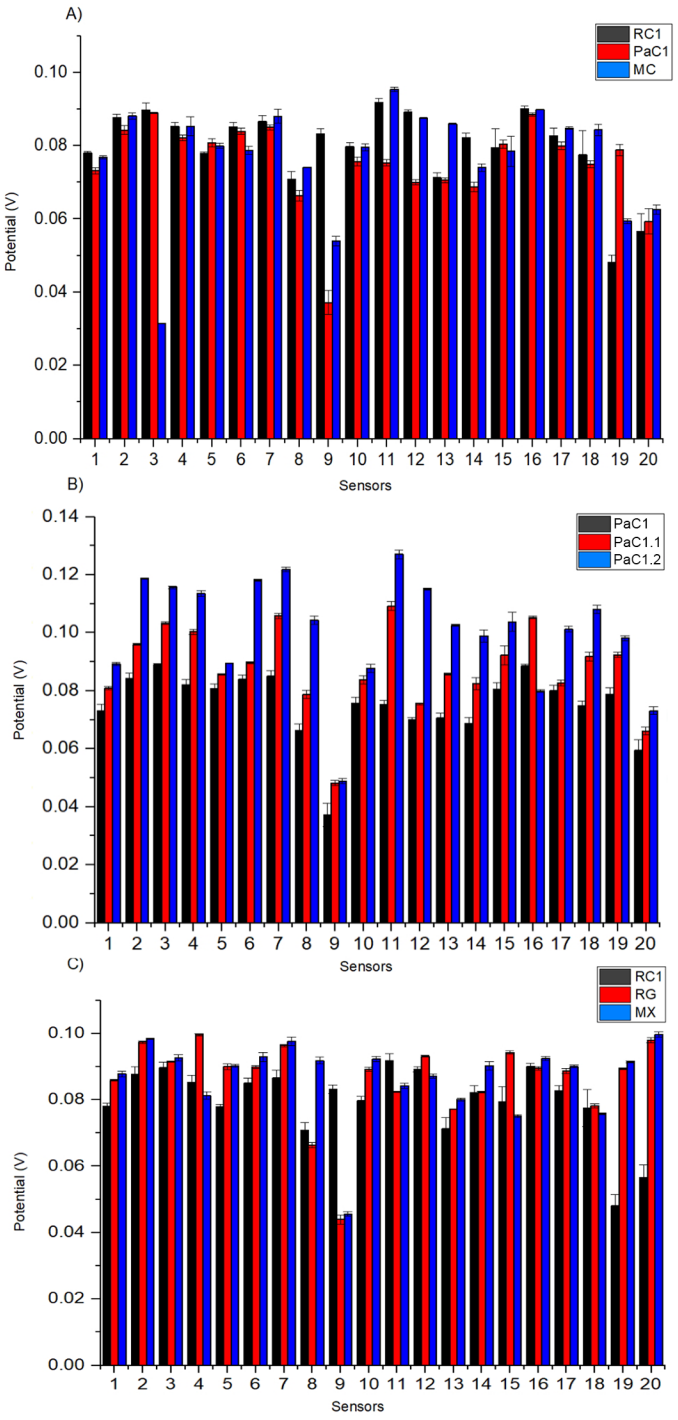

Figure 2. (A) Response of the e-tongue to raw and pasteurized cow milks and milk from a cow medicated for mastitis. (B) Response of the e-tongue to pasteurized cow milk and water-adulterated pasteurized cow milk. (C) Response of the e-tongue to raw cow milk, goat milk, and the mixture of the 2 milks.

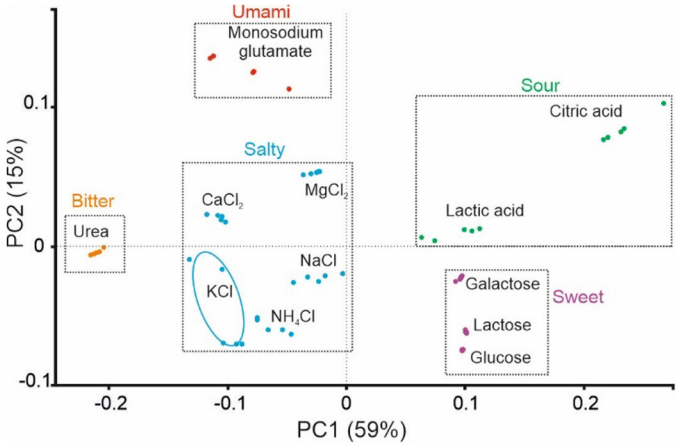

Figure 3. Principal component analysis score plot corresponding to the classification of standard solutions.

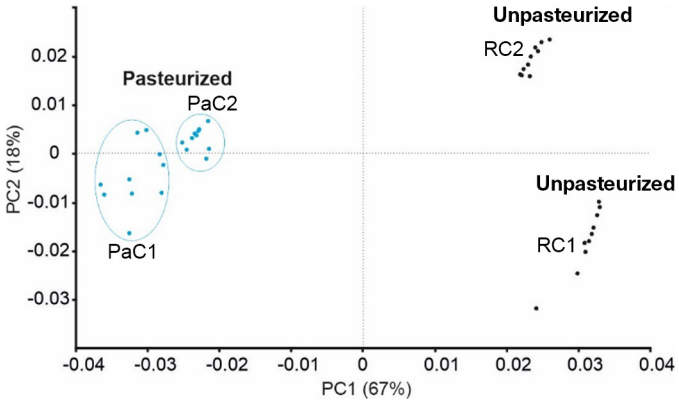

Figure 4. Principal component analysis score plot corresponding to the classification of raw and pasteurized milk.

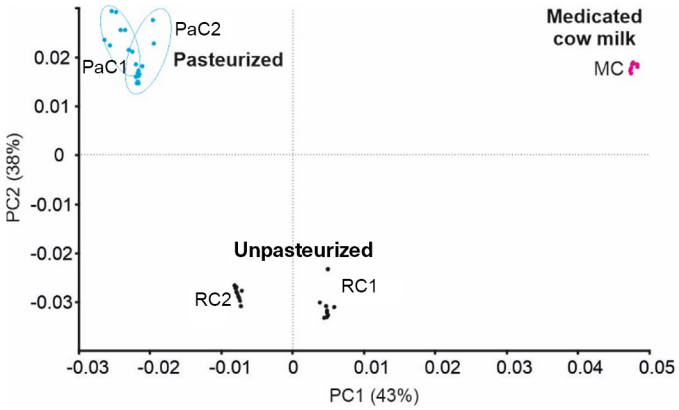

Figure 5. Principal component analysis score plot corresponding to the classification of raw milk, pasteurized milk, and milk from a cow medicated for mastitis.

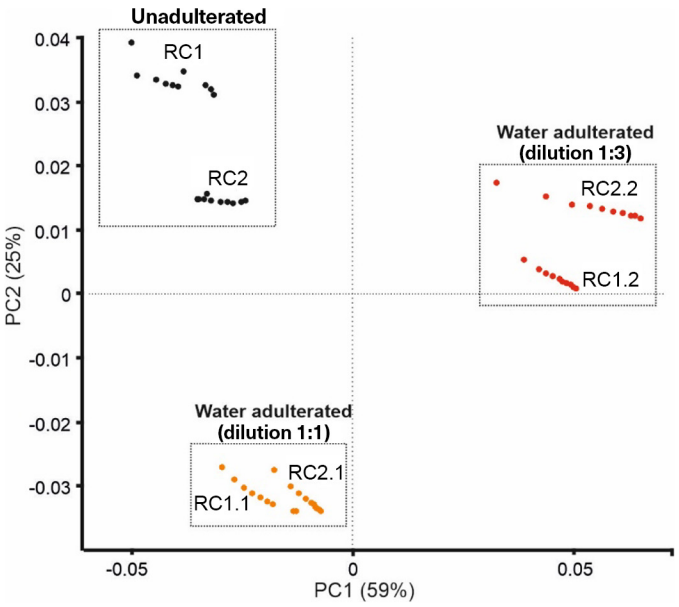

Figure 6. Principal component analysis score plot corresponding to the classification of raw cow milks and water-adulterated raw cow milks.

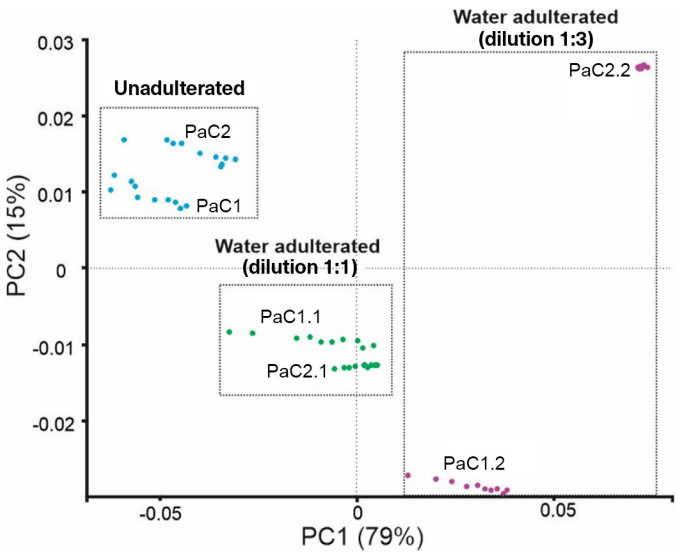

Figure 7. Principal component analysis score plot corresponding to the classification of pasteurized cow milks and water-adulterated pasteurized cow milks.

ing and validating the parameters of interest with values between 0.91 and 0.99. For example, the fat parameter had an R^2 of 0.9381 with an error of 0.0549 in calibration and an R^2 of 0.9266 with an error of 0.0610 in validation. Table 5 shows the effectiveness of the system developed for determining the main components of milk with high coefficients of correlations and low residual errors and with a lower number of latent variables (factors).

This effectiveness is also illustrated in the supplemental material (see Notes), which presents the explained variance versus the number of factors for the PLS-1 models, along with plots of predicted Y-values versus the true (measured) reference Y-values. The models performed well, as indicated by the residual variance curves (calibration and validation) being close together. As shown in Supplemental Figures S2.1 to S2.7 (see Notes), the mod-

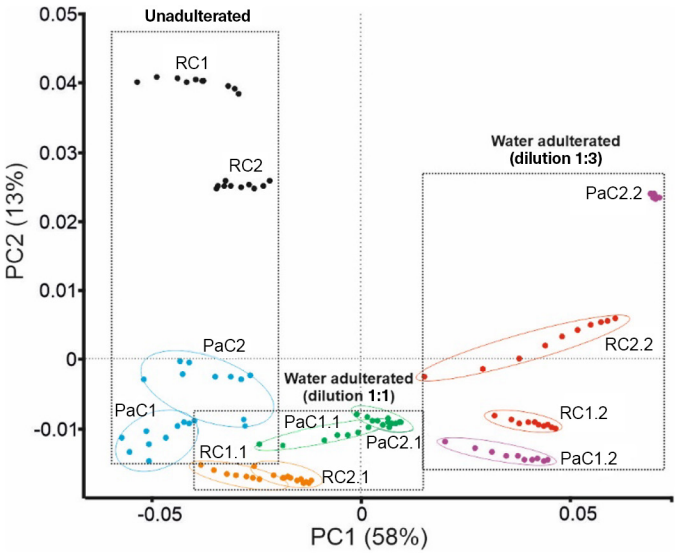

Figure 8. Principal component analysis score plot corresponding to the classification of cow milks and water-adulterated cow milks.

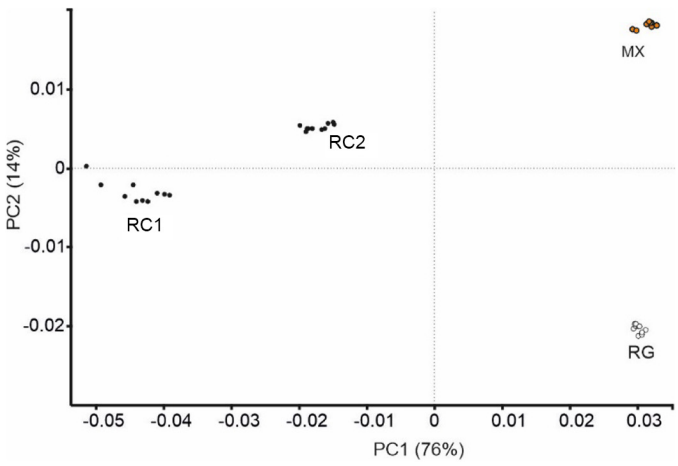

Figure 9. Principal component analysis score plot corresponding to the classification of raw cow milk, raw goat milk, and the mixture of cow and goat milks.

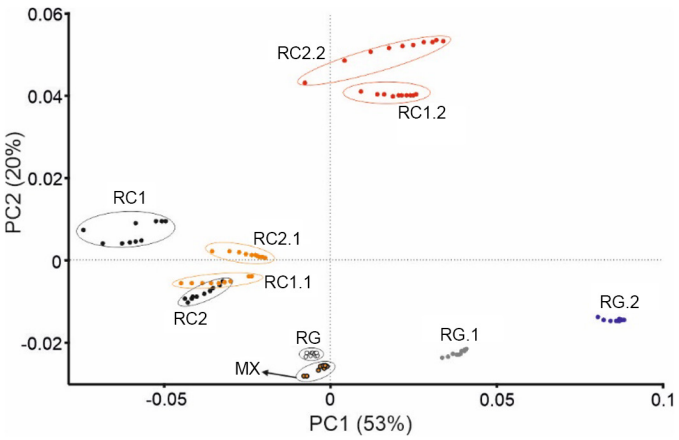

Figure 10. Principal component analysis score plot corresponding to the classification of the different samples of cow and goat milks under study.

els required only 2 factors (or latent variables) to explain more than 90% of the variance. The similarity between the calibration and validation curves corroborates the high quality of the model.

One of the key strengths of the e-tongue lies in its ability to discriminate between different types of milk and detect common adulterants with remarkable accuracy. Through a combination of polymeric membranes and chemometric analyses, the e-tongue successfully distinguished raw, pasteurized, and medicated cow milk, as well as goat milk. Moreover, it effectively identified adulterants, such as water, in cow and goat milks and cow milk in goat milk. This capability is crucial for ensuring the integrity and quality of milk products in the food industry, safeguarding consumer health and trust.

Furthermore, the e-tongue demonstrated promising results in establishing correlation models between sensor responses and traditional milk parameters. By correlating potentiometric sensor data with physicochemical parameters such as fat, protein, and lactose content, the system provided valuable insights into milk composition. The models exhibited strong predictive power, highlighting the potential of the e-tongue for rapid milk quality assessment.

Table 5. Results obtained from PLS regression analysis[1]

Physicochemical parameter	$RMSE_C$	R^2_C	$RMSE_V$	R^2_V
Fat	0.0549	0.9381	0.0610	0.9266
Protein	0.0088	0.9862	0.0095	0.9844
Lactose	0.0144	0.9869	0.0170	0.9824
NFDM	0.0241	0.9286	0.0262	0.9187
Cells	38.5750	0.9901	44.5900	0.9873
Cryoscopy point	0.0018	0.9419	0.0020	0.9314
Acidity	0.0781	0.9912	0.0867	0.9896

[1]R^2_C = calibration R^2 value; R^2_V = validation R^2 value; $RMSE_C$ = calibration root mean square error; $RMSE_V$ = validation root mean square error.

The results of this study confirm that the e-tongue is a valuable and reliable instrument for quickly and accurately assessing milk quality in dairy monitoring.

CONCLUSIONS

The development of the potentiometric e-tongue represents a notable advance in the detection and quantification of milk adulteration. This innovative system offers several advantages over traditional detection methods, including rapid analysis, cost-effectiveness, and high sensitivity to various adulterants. Overall, the potentiometric e-tongue offers a versatile and reliable solution for detecting milk adulteration, addressing a critical challenge faced in the food industry. Its ability to quickly identify adulterants and establish correlations with key milk parameters underscores its potential for ensuring food safety and quality. As research in this field continues to evolve, the e-tongue holds promise for broader applications in food authentication and quality control, contributing to a more transparent and trustworthy food supply chain.

NOTES

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Nonstandard abbreviations used: MC = medicated cow milk; MX = mix of raw cow milk-1/raw goat milk (50% of each in volume); NFDM = nonfat dry matter; PaC1 = pasteurized cow milk-1; PaC1.1 = pasteurized cow milk-1 (dilution 1:1); PaC1.2 = pasteurized cow milk-1 (dilution 1:3); PaC2 = pasteurized cow milk-2;

PaC2.1 = pasteurized cow milk-2 (dilution 1:1); PaC2.2 = pasteurized cow milk-2 (dilution 1:3); PC = principal component; PCA = principal component analysis; PLS = partial least squares; PVC = polyvinyl chloride; R^2_C = calibration R^2 value; R^2_V = validation R^2 value; RC1 = raw cow milk-1; RC1.1 = raw cow milk-1 (dilution 1:1); RC1.2 = raw cow milk-1 (dilution 1:3); RC2 = raw cow milk-2; RC2.1 = raw cow milk-2 (dilution 1:1); RC2.2 = raw cow milk-2 (dilution 1:3); RG = raw goat milk; RG.1 = raw goat milk (dilution 1:1); RG.2 = raw goat milk (dilution 1:3); $RMSE_C$ = calibration root mean square error; $RMSE_V$ = validation root mean square error.

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