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Characterization of Pasteurized Milk Spoilage by Electronic Nose in Relation to its Selected Quality Parameters

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Abstract

Pasteurized fresh milk requires an accurate estimation of shelf life under various conditions to minimize the risk of spoilage and product losses. Milk samples were stored for 56 h in an oven at 25 °C and for 15 days in a refrigerator at 4 °C. Samples were analyzed using an electronic nose (e-nose), total bacterial count, titratable acidity and pH to determine the quality of milk. Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were used to analyze e-nose data of milk stored at 25 °C, and 4 °C. A clear shift in quality was identified by the e-nose, which also appeared in the total bacterial count after 24 h and 12 days for storage at 25 and 4 °C, respectively. On the other hand, titratable acidity exceeded the normal limits of 0.14 % - 0.21 % after 24 h for storage at 25 °C (0.247 ± 0.006 %) and after 15 days for storage at 4 °C (0.25 ± 0.01 %). If pH was a good indicator of quality for samples stored at 25 °C, it showed no clear trends for samples stored at 4 °C. Based on the microbial count data and e-nose output, the milk had a shelf life of 0.3 day (i.e. 8 h) when stored at 25 °C. Shelf life was extended to 9 days when stored at 4 °C.

Keywords: Pasteurized milk; Shelf-life; Spoilage; Electronic nose

1 Introduction

Beneficial bacteria are important in the production of fermented dairy products, while pathogens and spoilage bacteria have detrimental effects on milk quality and dairy products. The shelf life of pasteurized milk is around 14 days at chilled temperature and is affected by many factors, such as raw milk quality, production system, hygienic, and storage conditions (Bondoc, 2007). However, shelf life decreases with an increase in storage temperature. To determine the shelf life of pasteurized milk stored under different conditions, different quality indicators, such as microbial counts, titratable acidity (TA), pH and enzyme activity (i.e. lipase and protease) need to be performed (Ziyaina et al., 2018).
Food can be characterized by its aroma, which is related to the volatile organic compounds (VOCs). The mixture of VOCs represents the aroma characteristics of dairy products; therefore, consumers typically sniff milk to estimate its quality or freshness in the headspace of the milk container. The VOCs are thus a very important component of the sensory quality of processed and stored milk. Specific combinations of VOCs form a kind of fingerprint, which can be used as an indicator of food quality and safety.

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The VOCs in the headspace of milk were used to predict ageing and off-flavours development (Ali et al., 2003). The increase in the concentration of some aldehydes, ketones, alcohols, and esters during the storage of pasteurized milk indicates poor quality. Furthermore, Vallejocordoba and Nakai (1994a) found that the shelf life of pasteurized milk was better estimated by the analysis of the VOCs than by bacterial count.

Normally, the VOCs are qualitatively and quantitatively analyzed using gas chromatography (GC). Vallejocordoba and Nakai (1994a) successfully assessed the VOCs using dynamic headspace/GC to predict the shelf life of pasteurized milk. Recently, Rashid et al. (2019) found that acetone, butanone, pentanal, and ethanol were good indicators of spoilage of pasteurized milk during storage. This was done using headspace solid-phase micro-extraction (HS-SPME) coupled with a flame ionization detector (GC-FID).

Different types of chemical sensors and commercial systems (e.g. electronic-nose and electronic-tongue) can also be used to detect milk spoilage. Recently, electronic noses (e-nose) have been successfully used to monitor microbial growth and shelf life of milk (Kalit et al., 2014). Using an array of five sensors, rancidity of ultra-heat treated (UHT) and pasteurized milk showed a good correlation with ageing of milk (Capone et al., 2001). In addition, a gas-sensing system was successfully used to distinguish between unspoiled milk and contaminated milk with selected bacteria or yeasts (Magan et al., 2001). The e-nose signals of sterile milk inoculated with *Pseudomonas fluorescens or Bacillus coagulans* were correlated with both the microbial loads and sensory scores of the milk (Korel & Balaban, 2002). The e-nose has also been used to assess the bacterial growth and shelf life of milk when stored at 5 ^{o}C and at room temperature (i.e. 25 ^{o}C) (Labreche et al., 2005).

There are limited studies on the use of e-nose for the shelf life determination of milk. In addition, in some countries, the milk may be subjected to high temperature (i.e. 45-50 ^{o}C) during handling and storage. Therefore, predicting the exact shelf life of milk is necessary with varying temperatures of handling or storage (Ziyaina et al., 2018). It is also important to assure good quality of milk for the production of other products, such as yoghurt and cheese. In both cases, fast quality assessment methods are needed for milk. Therefore, the aim of this study was to evaluate the application of the e-nose in monitoring pasteurized milk spoilage during storage at 25 ^{o}C and 4 ^{o}C in comparison to traditional means of quality estimates.

2 Materials and Methods

2.1 Experimental Design

Pasteurized milk (i.e. 500 mL, Tetra Pak carton) spoilage was monitored using two storage temperatures, 25 ^{o}C and 4 ^{o}C. The first set of pasteurized milk samples was stored at 25 ^{o}C in a Labcon oven (LABOCON Corporation, USA) for 56 h (i.e. 0, 8, 12, 24, 32, 41, 48, 56 h) or 2.3 days (0, 0.3, 0.5, 1.0, 1.3, 1.7, 2.0, 2.3 days). The second set of pasteurized milk samples were stored at 4 ^{o}C in a refrigerator (Samsung, China) for 15 days and analyzed every three days (i.e. 0, 3, 6, 9, 15 days). The milk samples were analyzed using the e-nose, microbial count, pH, and TA. At the end of predetermined storage times, one milk carton was unsealed, and three sterile plastic containers were filled with 50 mL of milk to determine pH, TA and microbial count (i.e. triplicate analysis). For e-nose analysis, five vials (15 mL) were filled with 5 mL milk. Controls were used before the start of the storage period.

2.2 Materials

Cartons of pasteurized milk (500 mL each) were collected from the College of Agricultural and Marine Sciences pilot dairy plant, Sultan Qaboos University, Oman. The high temperature short time pasteurization (HTST) was applied in the dairy plant (72 ^{o}C for 15 s). Milk samples were collected from the dairy plant after production and transferred to the Food Processing Laboratory on the day of production.

2.3 E-nose Analysis

A Cryanose 320 (Sensigent Company, California, USA), equipped with 32 sensors, was used in this

study. Five mL of pasteurized milk sample was placed in a 15 mL glass vial (Supleco, Bellefonte, PA, USA) sealed with a rubber septum cap. Vials were kept at 4 ^{o}C until analysis. First, the e-nose sensors were calibrated with air and the e-nose parameters were optimized using similar procedures as Rahman et al. (2018): baseline purge (10 s), sample draw (5 s), air intake purge (20 s), and sample purge (40 s). To measure the volatile profile of the stored milk headspace, the vial septum was punctured with a needle connected to the e-nose. This initiated the collection and transfer of the volatiles from the headspace to the sensors of the e-nose. Five replicates were done for each storage time.

2.4 Optimization of Volatile Compounds Collection

The optimization procedure included four predetermined times (5, 15, 30 and 50 min) and three predetermined temperatures (30, 35, and 40 ^{o}C) of extraction. A water bath (Fisher Scientific (Cambridge) Ltd, England) was used to control the temperature of the vials during the extraction. The vials with milk samples took 5 and 3 min to reach the desired temperature from their storage temperature, i.e. 4 and 25 ^{o}C, respectively. The milk sample stored at 4 ^{o}C was considered as a standard. The experiment was replicated ten times, with each vial considered as a single replicate.

2.5 pH measurement and Titratable Acidity

Measurements of the milk pH were taken using pH/Mv/^{o}C meter from EUTECH Instruments, Singapore. The TA analysis was performed in triplicate. Approximately 50 g of stored milk samples in glass bottles were used for TA analysis. The milk sample (9 g) was placed in a conical flask and three drops of phenolphthalein solution (1 %) were then added. It was then titrated using 0.1 N NaOH (Sigma-Aldrich, Switzerland) until a pink colour appeared. The titration volume (i.e. V) of NaOH was recorded and TA was then calculated (Wehr et al., 2004) according to the following formula.

$$\% \ acidity = \frac{N(NaOH) \times V(NaOH) \times (0.09)}{Sample \ weight} \times 100 \quad (1)$$

2.6 Microbial Count

Milk samples were placed in sterilized plastic containers and transported immediately to the laboratory for measuring the total plate count. The procedures were performed according to Wehr et al. (2004). Peptone maximum recovery diluent was used. Under sterile conditions, several dilutions of milk samples were prepared, and triplicate dishes were prepared for every dilution. The Petri dishes were placed in the incubator (BINDER, Germany) at 32 ^{o}C for 48 h. After 48 h, total bacterial colonies were counted using a colony counter (Gallenkamp Co. Ltd., England).

2.7 Statistical Analysis

E-nose data were analyzed using Principal Component Analysis (PCA) (Past Software version 2.17 c) (Hammer et al., 2001) and Linear Discriminant Analyses (LDA) with R package (RStudio, 2019). The microbial count and TA were assessed using ANOVA followed by Tukey's posthoc test ($\alpha = 0.05$) for mean separation in R. In order to determine the optimal volatile release conditions, a non-metric Multidimensional Scaling (MDS) was used.

3 Results and Discussion

3.1 Total Bacterial Count and Acidity

Total Bacterial Count (TBC) (expressed as $\log_{10}$ cfu mL^{-1}) showed no significant change until 8 h (0.3 d) of storage at 25 ^{o}C when total microbial count was 2.42 $\pm$ 0.20 $\log_{10}$ cfu mL^{-1} (Fig. 1A) (p <0.05, Tukey's test). This initial low microbial count was below the acceptable limit of pasteurized milk of 4.3 $\log_{10}$ cfu mL^{-1} (Food and Drug Administration, 2017). A significant increase in the TBC (7.7 $\pm$ 0.07 $\log_{10}$ cfu mL^{-1})

was observed after 24 h, which then exceeded the spoilage limit of 5 log10 cfu mL^{-1}(p <0.05) (Ziyaina et al., 2019). Similar values of TBC for pasteurized milk stored at ambient temperature after 24 h of storage was observed as 8.18 $\log_{10}$ cfu mL^{-1} (Labreche et al., 2005). The shelf life of the pasteurized milk used in this study was shown to be approximately 8 h (i.e. 0.3 d) at 25 ^{o}C, which was similar to the results of Lucknakhul et al. (2014) (i.e. 0.35 d at the 25 ^{o}C). In contrast, pasteurized milk stored at 19, 15 and 13 ^{o}C had an increased shelf life of 24 h (1 d), 36 (1.5 d) and 72 h (3 days) respectively when the microbial count exceeded the spoilage limit of 5 $\log_{10}$ cfu mL^{-1} (Ziyaina et al., 2019). Considering the Gompertz model for microbial growth curves, the lag periods (t_L) of the current study were 6.0 and 0.3 day (s) for storage temperatures 4 and 25 ^{o}C, respectably, while maximum growths (μ_{max}) were 0.6 and 12.4 ($\log_{10}$ cfu mL^{-1}1) day, respectively.

The TA increased exponentially as a function of storage time (R^2: 0.971) (Fig. 1B) and reached 0.25 ± 0.01 % after 24 h. Similarly, Ziyaina et al. (2018) noticed an increase (< 0.20 %) in TA (above 5 $\log_{10}$ cfu mL^{-1} of TBC) after 24 h of storage at 19 ^{o}C. On the other hand, the pH decreased linearly during storage (Fig. 1B) and after 24 h, decreased to 6.43, below the normal pH range of milk (6.6-6.8). Similar findings were reported by Lucknakhul et al. (2014) at 25 ^{o}C. On the other hand, after 24 h the TA increased (i.e. 0.34 ± 0.0 % - 0.53 ± 0.02 %) above the normal range 0.14 to 0.21 % (Walstra et al., 2005). A similar result was also observed by Lucknakhul et al. (2014). The sourness of pasteurized milk stored at ambient temperature was attributed to either the activity of mesophilic microorganisms, which normally survive pasteurization. Alternatively, the putrefaction of pasteurized milk could cause sourness due to the presence of psychrotrophic bacteria (such as *Pseudomonas* species), which may be the result of a re-contamination after pasteurization (Al-Qadiri et al., 2008).

There were no significant changes in TBC for the milk stored at 4 ^{o}C until the sixth day (i.e. 2.48 ± 0.04 $\log_{10}$ cfu mL^{-1}) of cold storage (Fig. 2A). Then TBC increased significantly in the ninth day (4.89 ± 0.21 $\log_{10}$ cfu mL^{-1}) exceeding the permitted limit of grade A pasteurized milk (i.e. 4.3 $\log_{10}$ cfu mL^{-1}) (Food and Drug Administration, 2017). However, spoilage onset is typically considered when the bacterial count exceeds 5.0 $\log_{10}$ cfu mL^{-1} (Ziyaina et al., 2018). Others reported that the milk reached the end of its shelf life when the total bacterial counts reached values of 6.0-7.0 $\log_{10}$ cfu mL^{-1} (Harding, 1995; McAuley et al., 2016). Our milk had a bacteriological shelf life of approximately 9 days at 4 ^{o}C. In contrast, the shelf life of pasteurized milk stored at 6 ^{o}C was 4 days as observed by Lucknakhul et al. (2014).

Recently, Rashid et al. (2019) studied the shelf life of pasteurized milk at different temperatures (e.g. 4 and 7 ^{o}C) for 19 days. The bacterial count after 9 days of storage at 4 and 7 ^{o}C were 3.27 and 3.64 $\log_{10}$ cfu mL^{-1}, respectively. Furthermore, the bacterial count increased to 3.72, 4.41, and 5.46 $\log_{10}$ cfu mL^{-1} at 4 ^{o}C after storage for 12, 14, and 16 days, respectively. Another study by Labreche et al. (2005) showed that pasteurized milk stored at 5 ^{o}C for 9.2 days reached TPC of 7.1 $\log_{10}$ cfu mL^{-1}. Their finding is higher than our measured microbial count after 9 days of storage (i.e. 4.89 ± 0.21 $\log_{10}$ cfu mL^{-1} at 4 ^{o}C). The variations of the reported results may be due to the varied sources of milk, processing conditions, and post-handling of pasteurized milk and the different temperature conditions. In addition, microbial count, heat stable enzymes (i.e. lipase and protease produced by psychotropic bacteria) of raw milk, and the activity of native plasmin and lipoprotein could play a role in determining the shelf life of milk.

The pH and TA values of milk stored at 4 ^{o}C are presented in the Figure 2B. The pH values showed no significant changes until it reached 6.70 at the end of day 15. It was within the normal pH range of fresh milk 6.6-6.8 (Walstra et al., 2005). Similarly, at 5 ^{o}C storage, the change of the pH value was within the acceptable range at 16 (Ziyaina et al., 2018) and 14 days (Sadhu, 2018). At higher storage temperatures, such as 13, 15, and 19 ^{o}C, the pH reached at 6.5 within 3, 2, and 1 day(s) storage, respectively (Ziyaina et al., 2018).

The TA is a better indicator of shelf life of pasteurized milk compared to pH (Ziyaina et al., 2018). The current study showed insignificant changes in the TA (Figure 2B) until day 9 (i.e.

from 0.160 ± 0.01 to 0.177 ± 0.11 %). This finding is within the acceptable range of TA (i.e. 0.14 to 0.21 %) of fresh milk (Walstra et al., 2005). Similarly, Slewa and Azhar (2018) reported 0.16 % and 0.17 % TA after 1 and 3 days of storage at 6 ^{o}C. However, a significant increase in TA to 0.18 and 0.25 %, was observed in the current study after 12 and 15 days, respectively and this trend was similar to the bacterial counts. The sharp increase in acidity after 12 days was likely due to the fermentation process of lactose to lactic acid.

3.2 Optimization of VOCs Headspace Collection

The optimum conditions of volatiles released in the headspace are illustrated in Figure 3. All increased duration and increased temperatures of extraction enhanced the response of all sensors compared to the standard. The standard response was considered as the pasteurized milk stored at 4 ^{o}C. Each plot shows the effect of storage time with extraction at 30 ^{o}C (Fig. 3A), 35 ^{o}C (Fig. 3B) and 40 ^{o}C (Fig. 3C), respectively. The overall responses of the sensors increased with time, and 30 min at 40 ^{o}C showed the highest responses (i.e. optimum) (Fig. 3C and Fig. 4), but longer volatile release time (i.e. 40 and 50 min) at 40 ^{o}C did not show any further increase in the response (Fig. 3C). Therefore, 30 min and 40 ^{o}C were considered as the optimal conditions for volatile release. Published research shows that temperatures of 20-50 ^{o}C and times of 3-90 min were usually used for volatile release. For example, Oliveros et al. (2005) used 40 ^{o}C for 30 min in order to create a homogeneous headspace, and they mentioned that 38 – 40 ^{o}C allowed for easier comparisons. In this study, temperatures higher than 40 ^{o}C were not used due to the possibility of generating new volatiles as a result of overheating. Vazquez-Landaverde et al. (2005) also pointed out that higher temperatures (45 ^{o}C to 75 ^{o}C) could lead to generating new volatiles in milk as an effect of heating.

The response of the sensors was also analyzed by non-metric Multidimensional Scaling (MDS) (using a Euclidean distance) to quantify the optimum conditions. If we consider as optimal the largest possible distance in the nose-response space, the optimum is reached when the distance is the largest on the MDS plot, which was 40 ^{o}C and 30 min (Fig. 5). The high distances, from the standard (marked as S) in the MDS biplot, indicates high response. Similarly, Groenen and Borg (2015) used MDS to visualize the optimum conditions (i.e. time and temperature) of volatile release.

3.3 E-nose Output of Fresh Pasteurized Milk Stored at 25 ^{o}C

The 6 days of measurements (i.e. 0, 0.3, 1, 1.3, 2, and 2.3) formed 6 natural clusters, marked A-F on the PCA coordinate plot (Fig. 6). The visual clusters B, C, D, E, and F shifted to the left compared to the initial storage (i.e. cluster A). The cluster B for 0.3 day showed only a marginal shift from cluster A, while other clusters (i.e. C, D, E, and F) showed considerable shifts. This change in position in the PCA plot could be correlated with the significant increase in the microbial count observed in the samples corresponding to the additional storage duration, which may have led to the formation of additional volatiles. The e-nose response of cluster D shifted down compared to cluster C followed by further lift up (i.e. clusters E and F). There was a minimal change in microbial count between clusters C, D, and E compared to cluster F. Similarly, using PCA, Capone et al. (2001) identified three classes of milk as rancidity increased during storage. They also observed that the classes of milk shifted to the left as rancidity occurred. Similarly, in the case of fish fillet storage, Di Natale et al. (2001) was unable to classify samples within 1 and 7 days, while at day 11 a clear separation of the samples was observed on the PCA plots.

3.4 E-Nose Output of Pasteurized Milk Stored at 4 ^{o}C

On the PCA plot (Fig. 7), two distinct groups of points emerged (A and B). There were no particular trends in the location of all the data points

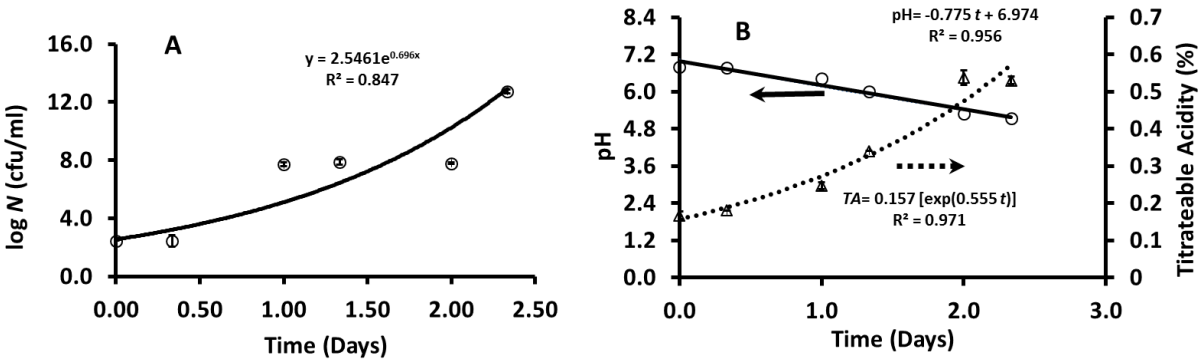

Figure 1: Total Bacterial Count (A) Titratable acidity and pH (B) of fresh pasteurized milk stored at 25 ^{o}C for 56 hours (2.3 days). (Data are plotted as means ± 2SD).

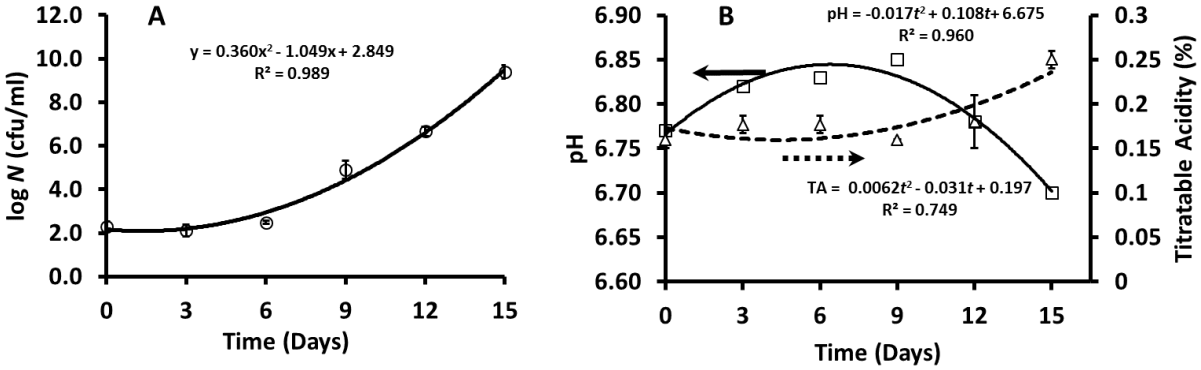

Figure 2: Total Bacterial Count (A) Titratable acidity and pH (B) of fresh pasteurized milk stored at 4 ^{o}C for 15 days. (Data are plotted as means ± 2SD).

after 0, 3, 6, 9, and 12 days of storage but there was a large jump after 15 days (Fig. 7). A linear discriminant analysis (LDA) was applied to analyze the e-nose data (i.e. multiple regression of day as a function of 32 sensors' signals). Four discriminant functions (LD1, LD2, LD3, and LD4) were obtained, which described variances as 72.96, 14.79, 7.11, and 1.78 % respectively. Based on the values of the coefficients, sensors 5, 18, 1, 31, 25, 23, and 20 showed high contribution to separate the clusters (i.e. highest responses to VOCs responsible for spoilage). Figure 8 shows the bar plot of the first discriminate scores as a function of storage days. Samples on day 0, day 3, and day 6 had a negative contribution to the discriminate function (DF) (i.e. there was negligible response of spoilage sensors). In contrast, samples at day 12 and day 15 had a positive contribution to the DF (i.e. major spoilage occurred). On day 9, the responses contributed to the discriminate function both positively and negatively; and this could be considered perhaps a transition phase of the major volatiles (Fig. 8). On the biplot of LDA as a function of LD1 and LD2 (Fig. 9), six clusters (i.e. A, B, C, D, E, and F) were identified each representing different storage days (i.e. 0, 3, 6, 9, 12, and 15 days, respectively). Clusters A, B, C, and D were clearly separated from the clusters E and F (i.e. moved from left to right). The clusters E

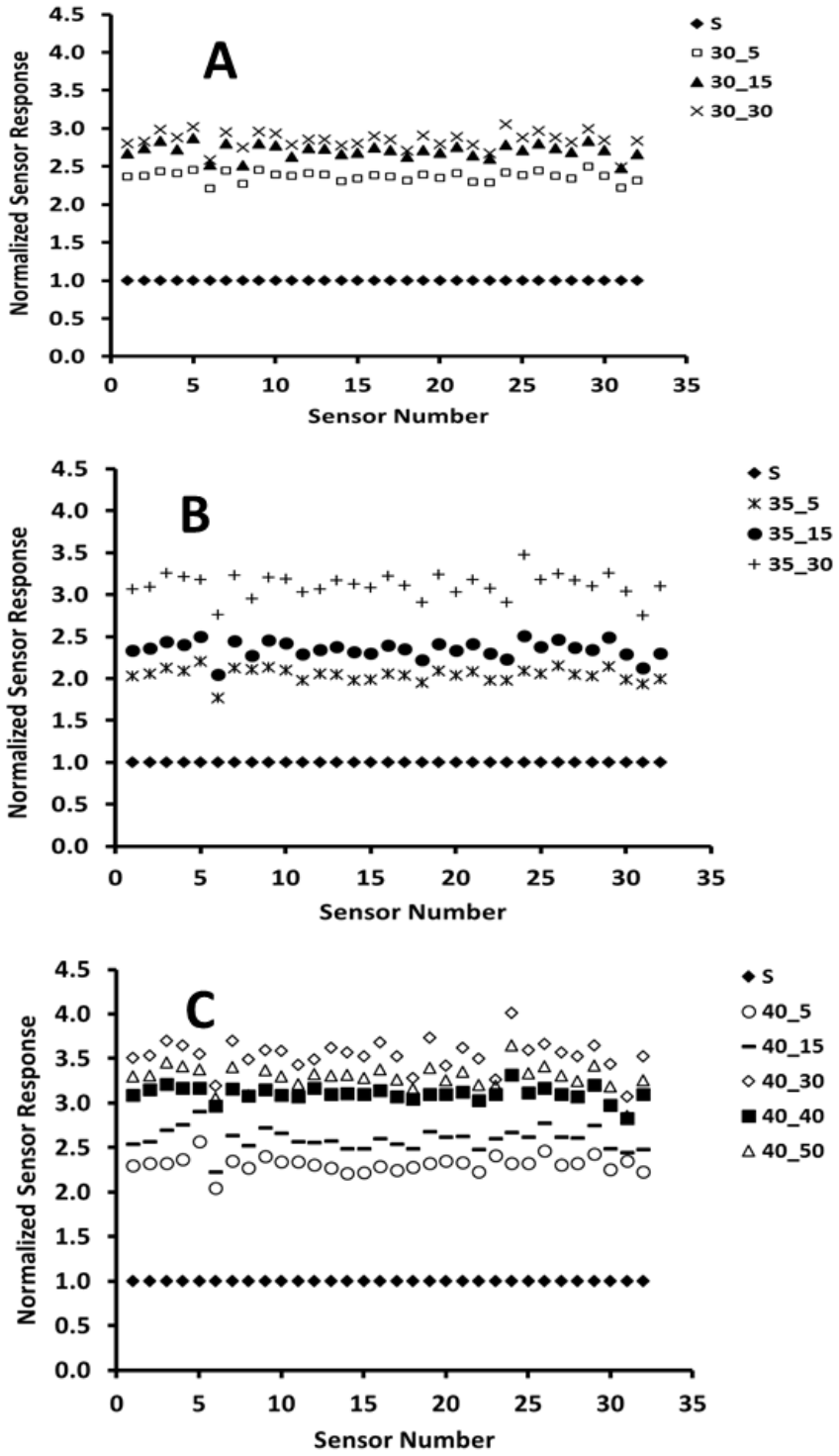

Figure 3: Normal analysis of e-nose signals of milk heated for specific times at specific temperatures: (A) 30 ^{o}C, (B) 35 ^{o}C and (C) 40 ^{o}C.

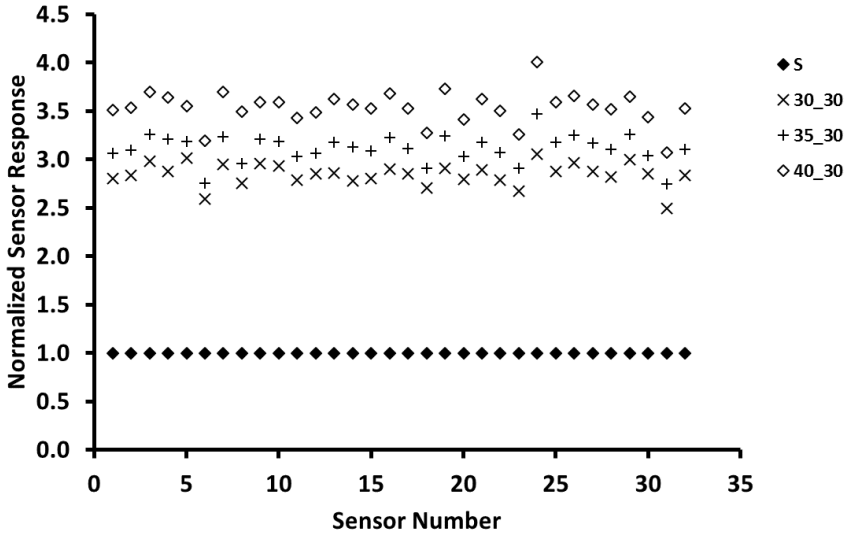

Figure 4: Normal analysis of e-nose signals of milk heated for 30 min at specific temperatures.

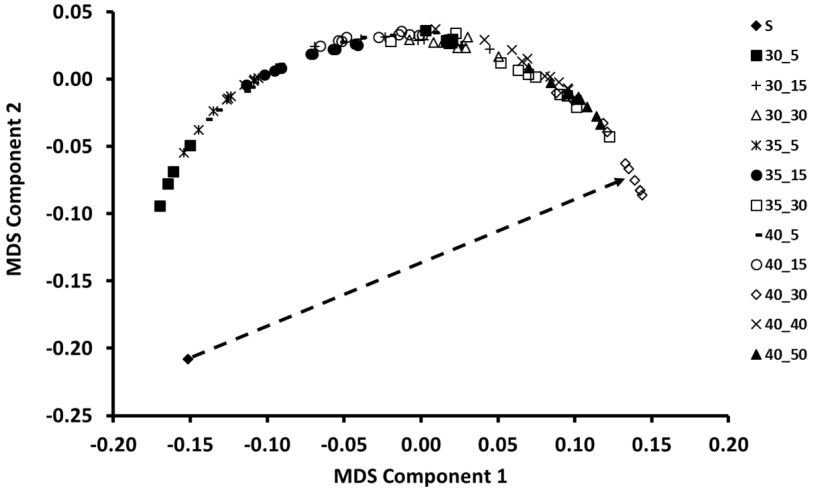

Figure 5: Biplot of MDS (Euclidean) analysis of e-nose signals of milk heated for specific times at specific temperatures (Arrow shows the distance between the reference and optimum measurement condition).

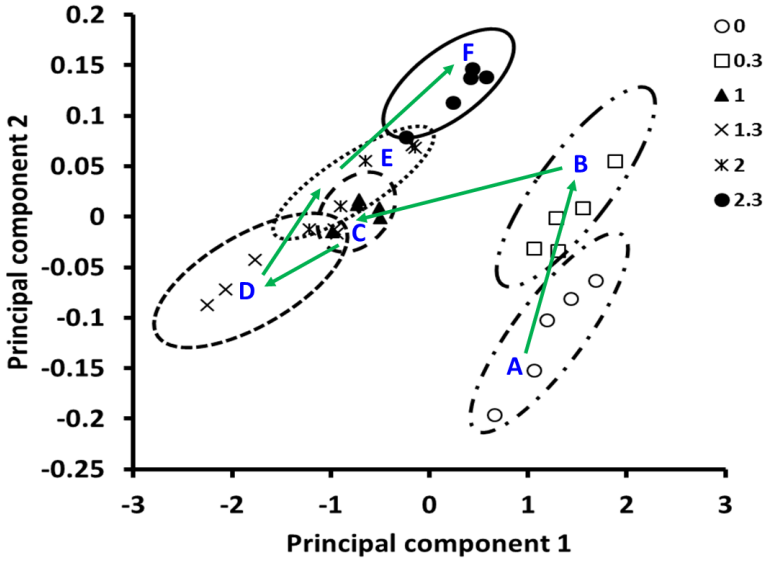

Figure 6: Plot of 2 first principal components of the e-nose signals of milk stored at 25 ^{o}C as a function of time (56 hours or 2.3 days of storage) (Circles show the groupings).

and F showed an onset shift from day 9, which could be related to the beginning of changes in the volatile profile of milk. The same transition was also observed when the microbial count indicated the onset of spoilage. Thereafter, a clear and considerable shift of clusters to the right side of the biplot was observed for samples collected on days 12 and 15. This shift was related to the significant increase in the bacterial count, which was likely due to the formation of new volatile compounds. Rashid et al. (2019) detected six compounds in fresh pasteurized milk stored at 4 ^{o}C (i.e. 2 ketones, 2 alcohols, and 2 aldehydes). The numbers of compounds increased to 12 (3 ketones, 7 alcohols, and 2 aldehydes) at the end of a 19 days storage period. In the present study, a significant change in volatiles was observed on day 12 instead of day 19. This variation between the current study and the literature data presented above could be due to the initial microbial contamination (types and microbial counts) in the fresh milk.

It was clearly observed that LDA was a better classifier compared to the PCA. This was due to the linear assumption of PCA which is not designed to separate groups. It is only designed to show the directions of maximum variability along with a series of the independent axis (i.e. it removes the correlations between variables). However, LDA is a linear classifier and calculates the best linear combination of variables in separating the known groups. Therefore, LDA has the ability to handle complex linear responses of e-nose signals. In addition, it improves the discrimination by maximizing the separation between the groups while minimizing the variance within the groups and within the inter-groups (Tohidi et al., 2018). Furthermore, the LDA was successfully used to predict the shelf life of pasteurized milk (Vallejocordoba & Nakai, 1994b).

3.5 Quality Grade Assessment of Pasteurized Milk

The e-nose could also be used to classify different quality grades of milk in addition to the discriminating milk during storage periods. In this study, three grades of pasteurized milk (i.e. stored at 4 ^{o}C) were proposed based on the volatiles measured by the e-nose and the bacterial count (Table 1 and Fig. 10): excellent, good,

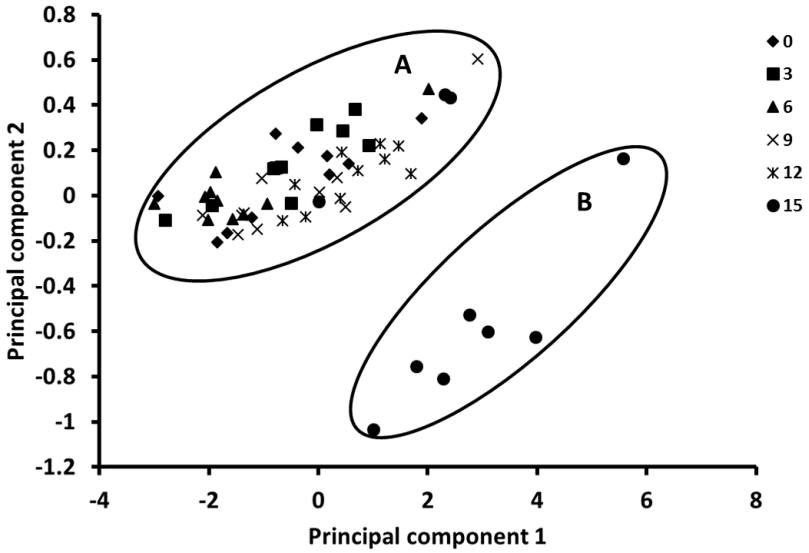

Figure 7: Biplot of principal components analysis of e-nose signals of milk stored at 4 ^{o}C as a function of time (15 days of storage). (Circles show the groupings).

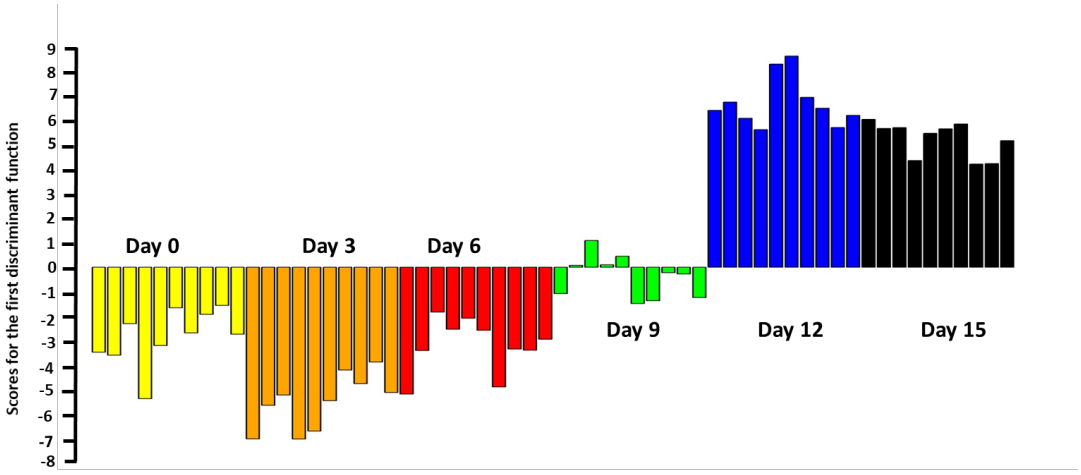

Figure 8: First discriminant scores (LD1) as a function of time (days).

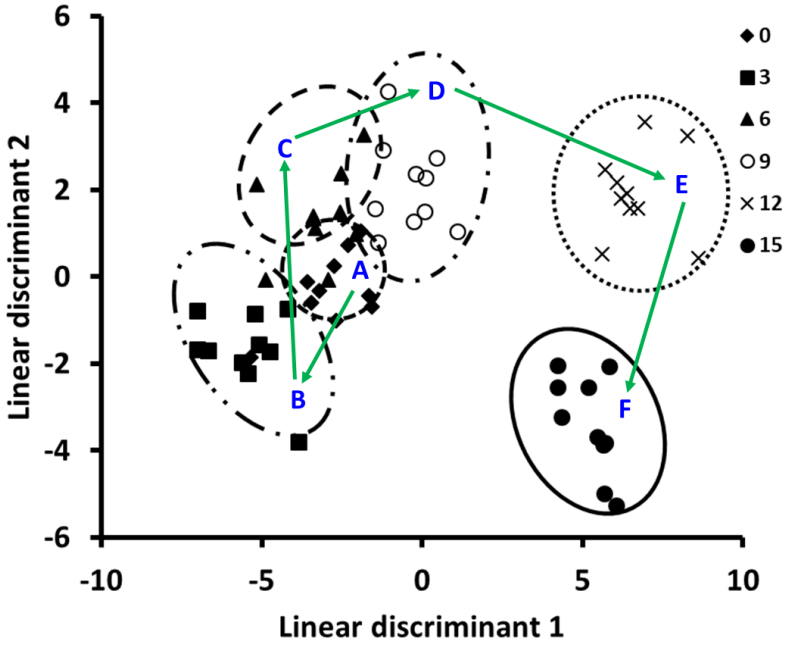

Figure 9: Biplot of Linear Discriminant Analysis of e-nose signals of fresh pasteurized milk stored at 4 ^{o}C as a function of time (15 days). (Circles show the groupings).

Table 1: Quality classes of pasteurized milk during common storage (4 ^{o}C).

Quality Class	Storage (days)	Total Bacterial Count ($\log_{10}$ cfu mL^{-1})
Excellent	0-6	$\leq 2.48 \pm 0.04$
Good	9	4.89 ± 0.21
Poor	12-15	$\geq$ *5.00

* Spoilage onset

Table 2: Quality classes of pasteurized milk during room temperature storage (25 ^{o}C)

Quality Class	Storage (days)	Total Bacterial Count ($\log_{10}$ cfu mL^{-1})
Non-spoiled	0-0.3	$\leq 2.42 \pm 0.20$
Spoiled	1-2.3	$\geq$ *5.00

* Spoilage onset

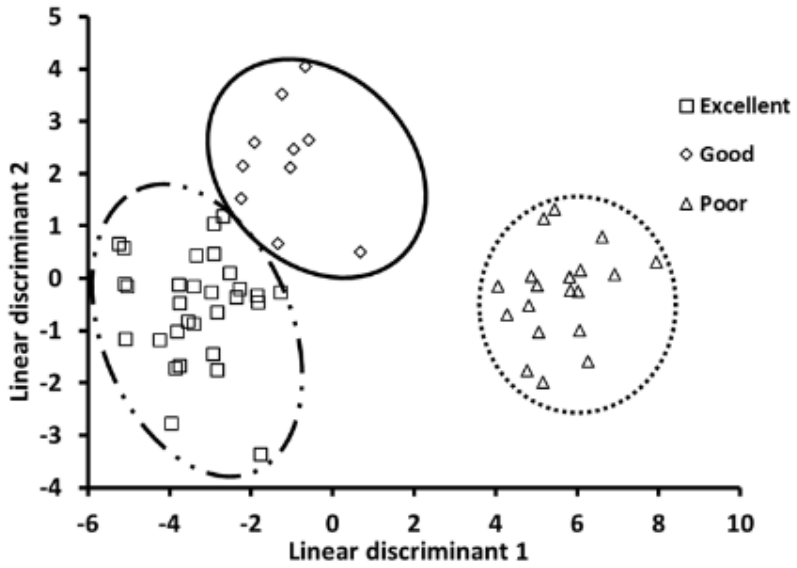

Figure 10: Quality classes of fresh pasteurized milk stored at 4 ^{o}C as a function of time (15 days) (Circle show the groupings).

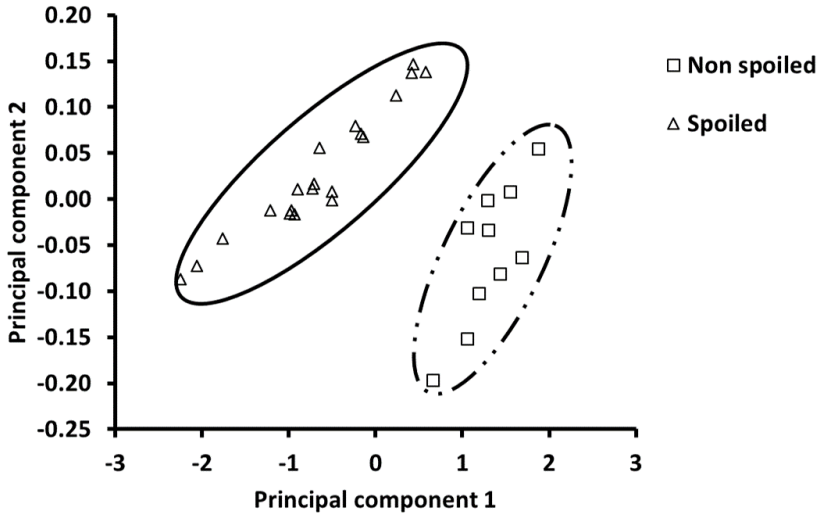

Figure 11: Quality classes of fresh pasteurized milk stored at 25^{o}C as a function of time (2.3 days).

and poor compared to two quality classes (i.e. non-spoiled and spoiled) of milk stored at 25 °C (Table 2 and Fig. 11). In general, the e-nose was a good classifier of the volatile profiles of pasteurized milk stored both at 4 °C and 25 °C. Similarly, Vallejocordoba and Nakai (1994b) classified the quality of pasteurized milk into three classes, good, marginal, and poor using LDA.

4 Conclusions

The current study investigated the possibility of using the e-nose for shelf life determination of milk during storage (i.e. at 25 °C and 4 °C). The electronic nose responses were correlated with the shift in the bacterial count of the tested milk samples. In addition, the e-nose was a good classifier of the aroma print of pasteurized milk at both room temperature and during refrigerated storage. Furthermore, milk quality was successfully classified using e-nose sensor responses and microbial data into three classes namely excellent, good, and poor. Early detection of milk spoilage is very important in reducing economic loss and the health risks of produced milk. Aroma-based detection of milk spoilage was very effective and could express both milk ageing and end of shelf life.

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