Studies on physicochemical changes and volatile organic compounds released during storage of raw cow milk

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Abstract
This study aims to determine the changes in physicochemical properties along with the concentration of volatile organic compounds during storage of raw cow milk were discussed until spoilage. The physicochemical properties taken to study the raw milk quality are temperature, humidity, milk pH, milk acidity and concentration of volatile organic compounds. The raw milk sample was tested simultaneously for both the concentration of volatile organic compounds and other physicochemical parameters such as temperature, relative humidity, milk pH and acidity. The experiment was conducted for 10 hours regularly for 5 days. The result reveals that there is an increase in the concentration of total volatile organic acids from 139.50 ± 1.1180 μg/L to 482.25 ± 1.4790 μg/L. The increase in the concentration of volatile organic acids might be due to the metabolic activity of microorganisms such as Lactic acid bacteria, which is responsible for the decrease and increase of pH and acidity of the raw milk respectively. Hence, the concentration of total volatile organic compounds were correlated with milk pH and acidity. In which, the milk pH shows better correlation with the total volatile organic compounds with.

Keywords: Raw milk, spoilage, volatile gas sensor, volatile organic compounds, milk pH, milk acidity

Introduction
Milk is highly a nutritious and perishable food. In general, the cattle milk contains approximately 87% of water, 4.9% carbohydrates, 3.9% protein, 3.5% fat and 0.7% ash (Jenson, 1995) [13]. It contains almost all the nutrients includes hormones, nucleotides, immunoglobulins, peptides, polyamines, enzymes, cytokines, other bioactive peptides and growth factors (Haug, Høstmark, & Harstad, 2007) [10]. Hence, it is also called as 'complete food'.

Milk can be spoiled by many factors, but the most common spoilage in milk is due to microorganisms. The availability of higher nutrition, nearly neutral pH and high moisture content makes the milk as an excellent medium for the growth of microorganisms (Osman Erkmen, 2016) [22].

The spoilage caused by microorganisms were predominantly depends upon the source of contamination, storage temperature, humidity, pH and acidity of the product. The sources of contamination in raw milk are unclean milking environment, unsanitary equipment, dirt, flies, unhygienic milk handler, improper milking process etc. (Lu & Wang, 2017) [18] from which the microorganisms will transfer to the raw milk. Every microorganisms needs an optimum temperature for its growth.

Hence, storage temperature plays an important role in spoilage. The changes in temperature of raw milk will have an effect on microbial growth, pH, enzyme activity, etc (Jay, 1992) [12]. The amount of water vapour present in the atmosphere was known as humidity. The humidity present in the atmosphere will have direct effect on water activity of raw milk. Water activity determines the amount of water available for the growth of microorganisms (Liberty, Ugwuishiwu, Pukuma, & Odo, 2013) [15]. During spoilage, the microorganisms present in the raw milk consumes lactose sugar and it will covert in to lactic acid. This phenomenon will alters the pH and acidity of the raw milk. The changes in pH and acidity will indicates the milk spoilage (Tahmina Bilkis, Md. Manirul Islam, M.C. Sumy, Md. Nasim Ali Mandal and Gazi Md. Noor Uddin, 2013) [28].
Fresh milk has mild sweet taste, clean flavour and no aftertaste. The clean flavour of fresh raw milk is due to the volatile organic compounds obtained through the feed constituents and animal metabolism (Cadwallader, 2010) [3]. The development of off – flavour in milk is due to the production of volatile organic compounds during the growth of microorganisms and their enzymes reaction, which break down the components of the base materials such as carbohydrates, proteins and lipids. For example, during storage of milk for longer time, the fat molecules present in the milk will rise to the top and it forms the creamy layer. The exposure of lipids to the atmospheric oxygen results in oxidative rancidity, which produces off – flavour and decreases the shelf life of milk (Luana M, Juliano G, Luciano S, & Luis A, 2005) [19]. The Shelf life of milk is determined by the rate of production of volatile organic compounds, since the production of off – flavour is directly related with the activity of microorganisms (Rashid et al., 2019) [26]. Most of the detected volatile organic compounds were belongs to seven chemical families such as aldehydes, alcohols, ketones, hydrocarbons, esters, sulphur compounds and terpenes (Toso, Procida, & Stefanon, 2002) [31].

In most of the dairy industries, the quality of milk is determined using chemical, rapid and microbial tests. The chemical method includes determination milk acidity and milk pH. The rapid tests includes clot on boiling test (COB), methylene blue dye reduction test and organoleptic tests (sight, smell and taste). The microbial tests includes Standard plate count, coliform count, psychrotrophic count, thermudric count and yeast and mold count (Gandhi, Sharma, Brath, & Bimlesh, 2020; Lu et al., 2013) [8, 17]. Though so many effective methods were there to detect the milk quality, all the methods are destructive and time consuming. At the same time, the volatile organic compounds has its own degree of volatility. Hence, it can be used to assess the quality of milk.

(Alothman, Lusk, Silcock, & Bremer, 2018) [2] reported that, during milk spoilage there is an increase in the concentration of volatile organic compounds released. However, very less studies were conducted to determine the changes in the concentration of volatile organic compounds during milk spoilage. Therefore, the subject of this paper is study the changes in physicochemical properties of raw milk during storage along with changes in the concentration of volatile organic compounds.

2. Materials and Methods

2.1 Milk sample

Fresh raw cow milk sample was procured from the local farmers of Thanjavur, Tamil Nadu. It was ensured that the process of milking was carried in hygienic manner. The raw milk sample was examined with organoleptic tests (sight, smell and taste). The milk samples which failed in organoleptic tests were rejected from conducting the experiment. Within 2 hours of procurement the selected milk samples were used for experiment, until it was stored in a refrigerated condition at 4˚C.

2.2 Storage container

1 litre of glass beaker with 20% headspace volume was taken. It was closed with lid which is made of acrylic. The lid was fabricated with sensor slot, in order to hold the volatile gas sensor. The schematic diagram of the experimental setup was given in Fig 1.

2.3 Determination of physicochemical properties

The changes in physicochemical properties such as temperature, relative humidity, milk acidity, milk pH and total volatile organic compounds during storage was found until the milk was spoiled.

The procured raw milk sample was tested for total volatile organic compounds, milk pH and milk acidity. Temperature and humidity was monitored on both inside and outside of the milk storage container. The experiment was conducted for 600 minutes and it was repeated for 5 days.

2.3.1 Temperature and humidity

The changes in temperature and relative humidity on both inside (Internal Temperature) and outside (External Temperature) of the storage container was found using temperature-humidity sensor (DHT11).

The temperature – humidity sensor consists of negative temperature coefficient (NTC) thermistor and humidity sensing component. The humidity sensing component contains of two electrodes and moisture holding substrate. The conductivity of the substrate will change with respect to the changes in the humidity of the external environment. The temperature – humidity sensor was programmed to take readings for every 30 minutes.

2.3.2 Milk acidity

Milk acidity was determined for the rapid analysis of milk quality during spoilage. It was directly measured by titration method in terms of percentage lactic acid (%LA). About 10 ml of raw milk sample was mixed properly with 10 ml of distilled water in a conical flask.

In which, few drops of 0.5% phenolphthalein indicator solution was added. Then, the prepared mixture was titrated against 0.1 N NaOH (sodium hydroxide) until the sample colour turns in to pale pink colour as an end point (Jurjen Draaijer, Dugdill, Bennett, & Mounsey, 2009).

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\text{Milk acidity} \ (%\text{LA}) = \frac{9 \times (mL \text{ of NaOH used}) \times (0.1N \text{ NaOH})}{10 \ mL \text{ of milk sample}}
\]

Where

The molecular weight of lactic acid is 90.08 g/mol.

2.3.3 Milk pH

pH of the milk sample was measured using standard pH meter (SI – 144 microprocessor pH meter) at 30˚C. Before conducting the experiment the standard pH meter was calibrated using standard buffer solutions such as pH 4.0, pH 7.0 and pH 10. After calibration the raw milk sample was
directly tested for pH. About 10 ml of raw milk sample was taken in a small beaker, in which the pH electrode was placed to detect the pH value.

### 2.3.4 Total volatile organic compounds

The changes in the concentration of total volatile organic compounds (TVOC) in the headspace of the milk storage container were measured using volatile gas sensor in terms of ‘μg/L’. This volatile gas sensor consists of semi conductive metal oxide gas sensing layer. The conductivity of the gas sensing layer will change based on the concentration of volatile organic compounds adsorbed on it (Lin, Lv, Hu, Xu, & Feng, 2019) \cite{16}. This volatile gas sensor was placed at the top of the container and the changes in concentration of volatile organic compounds were recorded for every 30 minutes.

### 2.4 Statistical analysis

All the experimental tests were conducted with minimum 5 replications and confirmed for reproducibility. In each set of experimental tests, mean, standard deviations and standard errors revealed with 95% confidence level. All the statistical calculations were carried out using Microsoft Excel 2017.

### 3. Results and Discussion

The physicochemical properties of raw milk such as milk pH and milk acidity were compared with total volatile organic compounds released from raw milk during spoilage.

#### 3.1 Temperature

The changes in internal and external temperature during storage was given in Fig 2. From Fig 2, it was observed that the external temperature was increased during day time and it starts to decrease after noon time. Similarly, the same increase and decrease in temperature was followed inside the storage container. It indicates there is an influence of external temperature inside the container.

From Table 1, it was observed that the external and internal temperature becomes equal after 330 minutes. The increase in temperature inside the container might be due to the production of heat during the metabolic activity of microorganisms during its growth (Tarrand & Menard, 2012) \cite{30}, (Robador, LaRowe, Finkel, Amend, & Nealson, 2018) \cite{28} also reported that there is a heat dissipation during the growth of microorganisms and its metabolic activity. They also included the production of heat was higher during the logarithmic or exponential phase during the growth of microorganisms.

From Table 1, the recorded temperature on both the inside and outside of the storage container lies between 27°C to 33°C. It indicates the spoilage in the raw milk might be caused by mesophilic microorganisms such as *Bacillus*, *Lactobacillus*, *Lactococcus* etc. These microorganisms can grow in 25°C to 40°C (Luana M et al., 2005) \cite{19}. The presence of these microorganisms are unavoidable, because it present in animal udder, milker’s hand, utensils, etc (Jay, 1992) \cite{12}.

![Temperature vs Time](image)

**Fig 2:** Changes in Temperature during storage

#### 3.2 Humidity

The changes in internal and external humidity during storage was given in Fig 3. From Fig 3, the external humidity starts to decrease during day time and it gradually starts to increase after noon time. The increase and decrease in humidity was due to the changes in external room temperature. The internal humidity starts to increase during day time and decrease after noon time. It indicates the external humidity has very lesser effect on inside the storage container. The increase in internal humidity was due to the formation of condensation inside the storage container. The formation of the condensation might be due to the evaporation of the water molecules from the raw milk due to the increase in temperature on both inside and outside of the storage container. Since, the container was closed, air flow inside the container was very less and it also contributes to the increase in humidity (Liberty et al., 2013) \cite{15}. (Al-Muhtaseb, McMinn, & Magee, 2002) \cite{1} also reported that high moisture foods will evaporate and forms condensation when the food closed with improper maintenance of temperature and airflow.
3.3 Milk pH
From Table 1, the pH of the raw fresh milk was 6.66 ± 0.0053. It is nearly neutral pH (Chandrapala, McKinnon, Augustin, & Udabage, 2010; Helmenstine, 2020) [6, 11] also discussed that milk was slightly acidic and nearly neutral in pH. It could be due to the presence very trace amount of lactic acid. The presence of proteins, calcium and phosphates in raw milk perform as a strong buffer (Nwosu, Moscone, Falleschi, & Mascini, 1992) [21]. The changes in milk pH during spoilage was given in Fig 4. From Fig 4, it was evident that the pH of the milk sample will decrease during spoilage. It might be due to the production lactic acid by lactic acid bacteria (Poghsossian, Geissler, & Schöning, 2019) [24]. (Bouteille, Gaudet, Lecanu, & Thiis, 2013; Tamime, 2009) [4, 29] reported that the lactic acid bacteria consumes α- and β-lactoses and it will produce 2 lactates and 2 free protons as a result of homofermentation using Embden Mayerhoff glycolysis pathway. The changes in pH of the milk during storage was in accordance with the previous study conducted by (Guggilla, Rajeshwar, & Mache, 2016) [9].

3.4 Milk acidity
The changes in milk acidity during storage was given in Fig 5. From Fig 5, the acidity of the milk increases with increase in spoilage of the milk during storage. Since, pH and acidity was interrelated with each other, the increase in milk acidity might be due to the production of lactic acid by lactic acid bacteria during the fermentation of lactose (Lakade, Sundar, & Shetty, 2016; Lu & Wang, 2017) [14, 18].

3.5 Total volatile organic compounds
The changes in the concentration of total volatile organic compounds in headspace of the container during storage was given in Fig 6. From Fig 5, it was evident that during spoilage the concentration of total volatile organic compounds were increased as mentioned in (Toso et al., 2002; Villeneuve et al., 2013) [32]. From Table 1. The fresh milk has TVOC concentration of about 139.50 ± 1180 μg/L. It might be due to the transformation of volatile organic compounds to raw milk through animal feed, rumen gases and animal metabolisms (Cadamwallader, 2010; Toso et al., 2002) [5, 31]. After 10 hours, the concentration of TVOC was increased to 482.25 ± 1479 μg/L. This gradual increase in concentration of TVOC might be due to the production of volatile organic compounds such as aldehydes, ketones, hydrocarbons, alcohols, etc. as a result of microbial metabolism (Rankin, Lopez-Hernandez, & Rankin, 2011) [25]. Most of the aldehydes, ketones and alcohols are the metabolic by-products during microbial spoilage of milk. These Volatile organic compounds can also be formed by the oxidation of lipids, it may be a chemical oxidation, either through a free radical triggered or enzyme catalysed reaction on lipids (Ziyania, Rasco, Coffey, Unlü, & Sablani, 2019) [33].
3.6 Correlation of physicochemical properties of raw milk with concentration of TVOC

Correlation between TVOC concentrations with Milk acidity and Milk pH was given in Fig 7 and Fig 8 respectively. The physicochemical changes during raw milk spoilage and correlation of TVOC with other physicochemical parameters was given in Table 1 and Table 2 respectively.

From Table 2, the concentration of TVOC shows better correlation with milk pH of having an $R^2$ value of 0.9661. Whereas, with milk acidity it shows an $R^2$ value of 0.9646. The strong correlation between pH, acidity and volatile organic compounds might be due to the activity of lactic acid bacteria (Mozzi, Raya, Vignolo, & Love, 2016) [20].

During lactose fermentation, these microorganisms will produce lactic acid and also some free radicals. The lactic acid and free radicals will react with amino acids, free fatty acids it produces volatile organic compounds (Gadaga, Viljoen, & Narvhus, 2007) [7].

For example, the unsaturated fatty acids present in the raw milk will get oxidised in the presence of free radicals, it forms hydroperoxides, which will rapidly decompose in to hexanal or unsaturated aldehydes. These unsaturated fatty acids will also form methyl ketones by decarboxylation. Similarly, alcohols were the terminal end product in the breakdown of glucose and catabolism of amino acids (Bernalier, Dore, & Durand, 1999; Pan, Wu, Peng, Zeng, & Li, 2014; Ziyaina et al., 2019) [3, 23, 33].

### Table 1: Physicochemical changes during raw milk spoilage

| S. No | Time (minutes) | Temperature (°C) | Humidity (%) | Milk acidity (% LA) | Milk pH | TVOC (μg/L) |
|-------|----------------|-----------------|--------------|-------------------|--------|-------------|
|       |                | External | Internal | External | Internal |               |             |               |
| 1     | 0              | 28    | 28     | 76       | 76     | 0.14 ± 0.0012 | 6.66 ± 0.0051 | 139.50 ± 1.1180 |
| 2     | 30             | 28    | 28     | 72       | 76     | 0.14 ± 0.0023 | 6.63 ± 0.0053 | 141.75 ± 0.8292 |
| 3     | 60             | 29    | 29     | 69       | 77     | 0.15 ± 0.0031 | 6.60 ± 0.0058 | 144.25 ± 0.8292 |
| 4     | 90             | 30    | 29     | 66       | 77     | 0.15 ± 0.0023 | 6.56 ± 0.0046 | 149.50 ± 0.5000 |
| 5     | 120            | 31    | 30     | 64       | 77     | 0.15 ± 0.0020 | 6.52 ± 0.0000 | 153.75 ± 0.4330 |
| 6     | 150            | 31    | 30     | 63       | 78     | 0.16 ± 0.0012 | 6.49 ± 0.0012 | 158.50 ± 1.1180 |
| 7     | 180            | 32    | 30     | 62       | 78     | 0.16 ± 0.0023 | 6.46 ± 0.0012 | 167.75 ± 1.4790 |
| 8     | 210            | 32    | 31     | 60       | 79     | 0.17 ± 0.0023 | 6.43 ± 0.0050 | 179.00 ± 1.8708 |
| 9     | 240            | 33    | 31     | 58       | 80     | 0.17 ± 0.0000 | 6.39 ± 0.0012 | 191.75 ± 1.0897 |
| 10    | 270            | 33    | 31     | 59       | 80     | 0.18 ± 0.0023 | 6.36 ± 0.0090 | 211.00 ± 1.5811 |
| 11    | 300            | 33    | 32     | 60       | 80     | 0.18 ± 0.0035 | 6.34 ± 0.0162 | 230.75 ± 1.4790 |
| 12    | 330            | 32    | 32     | 60       | 81     | 0.19 ± 0.0058 | 6.29 ± 0.0053 | 249.00 ± 1.4142 |
| 13    | 360            | 32    | 32     | 60       | 81     | 0.19 ± 0.0050 | 6.25 ± 0.0058 | 265.75 ± 1.9203 |
| 14    | 390            | 31    | 31     | 63       | 81     | 0.20 ± 0.0050 | 6.17 ± 0.0100 | 284.25 ± 1.2990 |
| 15    | 420            | 31    | 31     | 66       | 80     | 0.20 ± 0.0050 | 6.10 ± 0.0053 | 300.25 ± 1.4790 |
| 16    | 450            | 30    | 30     | 70       | 80     | 0.21 ± 0.0050 | 6.04 ± 0.0050 | 325.75 ± 1.9203 |
| 17    | 480            | 29    | 29     | 74       | 80     | 0.21 ± 0.0101 | 5.98 ± 0.0058 | 353.75 ± 1.4790 |
| 18    | 510            | 28    | 28     | 75       | 80     | 0.22 ± 0.0092 | 5.95 ± 0.0092 | 376.75 ± 2.5860 |
| 19    | 540            | 28    | 28     | 78       | 80     | 0.23 ± 0.0050 | 5.90 ± 0.0110 | 409.25 ± 1.9203 |
| 20    | 570            | 27    | 28     | 79       | 80     | 0.23 ± 0.0035 | 5.86 ± 0.0100 | 441.00 ± 1.8708 |
| 21    | 600            | 27    | 27     | 80       | 80     | 0.24 ± 0.0020 | 5.82 ± 0.0012 | 482.25 ± 1.4790 |

All the data are represented as the mean ± SD for n=5
Table 2: Correlation of total volatile organic compounds with other physicochemical parameters

| Correlation | Milk Acidity | Milk pH |
|-------------|--------------|---------|
| Total volatile organic compounds (μg/L) | y = 3335.4x - 358.56  
R² = 0.9646  
Where,  
y = Total volatile organic compounds (μg/L)  
x = Milk acidity (% L) | y = -396.18x + 2742.6  
R² = 0.9661  
Where,  
y = Total volatile organic compounds (μg/L)  
x = Milk pH |

4. Conclusion
From the obtained results it was clear that the concentration of total volatile organic compounds will increase during spoilage. It might be due to the action of the metabolic activity of the lactic acid bacteria during lactose fermentation. Hence, the pH of the milk shows better correlation with the concentration of total volatile organic compounds with an R² value of 0.9661. Therefore, the quality of the raw milk during spoilage can be assessed using volatile organic compounds present in the headspace of the container. At the same time, spoilage can be assessed using volatile organic compounds with an R² value of 0.9661. Hence, the pH of the milk shows better correlation with the concentration of total volatile organic compounds with an R² value of 0.9661.

5. References
1. Al-Muhtaseb AH, McMinn WAM, Magee TRA. Moisture sorption isotherm characteristics of food products: A review. Food and Bioproducts Processing: Transactions of the Institution of Chemical Engineers, Part C 2002:80(2):118-128. https://doi.org/10.1205/09603080252938753
2. Alothman M, Lusk KA, Silcock PJ, Bremer PJ. Relationship between total microbial numbers, volatile organic compound composition, and the sensory characteristics of whole fresh chilled pasteurized milk. Food Packaging and Shelf Life, 15(November) 2018, 69-75. https://doi.org/10.1016/j.fpsl.2017.11.005
3. Bernalier A, Dore J, Durand M. Biochemistry of Fermentation. In Colonic Microbiota, Nutrition and Health (Third edit) 1999. https://doi.org/10.1007/978-94-017-1079-4_3
4. Bouteille R, Gaudet M, Lecanu B, This H. Monitoring lactic acid production during milk fermentation by in situ quantitative proton nuclear magnetic resonance spectroscopy. Journal of Dairy Science 2013;96(4):2071-2080. https://doi.org/10.3168/jds.2012-6092
5. Cadwallader K. Instrumental measurement of milk flavour and colour. In Improving the Safety and Quality of Milk: Improving Quality in Milk Products 2010. https://doi.org/10.1533/9781845699437.2.181
6. Chandrapala J, McKinnon I, Augustin MA, Udapage P. The influence of milk composition on pH and calcium activity measured in situ during heat treatment of reconstituted skim milk. Journal of Dairy Research 2010;77(3):257-264. https://doi.org/10.1017/S0022029910000026
7. Gadaga TH, Viljoen BC, Narvhus JA. Volatile organic compounds in naturally fermented milk and milk fermented using yeasts, lactic acid bacteria and their combinations as starter cultures. Food Technology and Biotechnology 2007;45(2):195-200.
8. Gandhi K, Sharma R, Brath P, Bimlesh G. Chemical Quality Assurance of Milk and Milk Products 2020. https://doi.org/10.1007/978-981-15-4167
9. Guggilla M, Rajeshwar B, Mateche S. Lactic Acid Based Spoilage Indicator for Milk. Indian Journal of Advances in Chemical Science 2016;1(1):68-72. Retrieved from www.ijacsikos.com
10. Haug A, Hostmark AT, Harstad OM. Bovine milk in human nutrition – a review 2007;16:1-16. https://doi.org/10.1186/1476-511X-6-25
11. Helmenstine AM. What Is the Acidity or pH of Milk? Retrieved from Thought Co 2020. website: https://www.thoughtco.com/what-is-the-ph-of-milk-603652
12. Jay JM. Modern Food Microbiology. Modern Microbiology Handbook 2012. https://doi.org/10.1007/978-94-011-6480-1
13. Jenson RG. Handbook of milk composition 1995. https://doi.org/10.1016/B978-0123844340-9-50003-2
14. Lakade AJ, Sundar K, Shetty PH. Nanomaterial-based sensor for the detection of milk spoilage. LWT - Food Science and Technology 2016. https://doi.org/10.1016/j.lwt.2016.10.031
15. Liberty JT, Ugwuishiwu BO, Pukuma SA, Odo CE. Principles and Application of Evaporative Cooling Systems for Fruits and Vegetables Preservation. International Journal of Current Engineering and Technology 2013;3(3):1000-1006.
16. Lin T, Lv X, Hu Z, Xu A, Feng C. Semiconductor Metal Oxides as Chemosensitive 2019. https://doi.org/10.3390/s19020233
17. Lu M, Shiau Y, Wong J, Lin R, Kravis H, Blackmon T, Wang NS, et al. Milk Spoilage: Methods and Practices of Detecting Milk Quality. Food and Nutrition Sciences 2013;04(07):113–123. https://doi.org/10.4236/fns.2013.47a014
18. Lu M, Wang NS. Spoilage of Milk and Dairy Products. In The Microbiological Quality of Food: Foodborne Spoilers 2017. https://doi.org/10.1016/B978-0-08-100502-6.00010-8
19. Luana MP, Juliano GP, Luciano SB, Luís AN. The Microbiology of Raw Milk. In Dairy Microbiology Handbook 2005, pp. 39-90. https://doi.org/10.1002/0471723959.ch2
20. Mozzi F, Raya RR, Vignolo GM, Love JC. Biotechnology of Lactic Acid Bacteria—Novel Applications 2016. Retrieved from https://febs.onlinelibrary.wiley.com/doi/pdf/10.1002/9783527007796
21. Nwosu TN, Moscone D, Palleschi G, Mascini M. L-Lactate Electrochemical Biosensor: Performance Evaluation and the Application in Monitoring of Milk Microbial Attack. In Biosensors ’92 Proceedings 1992. https://doi.org/10.1016/B978-1-85617-161-8.50077-x
22. Osman Erkmen TF. Spoilage of Milk and Milk Products (first edit; T. F. B. Osman Erkmen, Ed.). John Wiley & Sons, Ltd 2016.
23. Pan DD, Wu Z, Peng T, Zeng XQ, Li H. Volatile organic compounds profile during milk fermentation by Lactobacillus pentosus and correlations between volatiles
flavor and carbohydrate metabolism. Journal of Dairy Science 2014;97(2):624–631. https://doi.org/10.3168/jds.2013-7131

24. Poghossian A, Geissler H, Schöning MJ. Rapid methods and sensors for milk quality monitoring and spoilage detection. Biosensors and Bioelectronics. https://doi.org/10.1016/j.bios.2019.04.040

25. Rankin SA, Lopez-Hernandez A, Rankin AR. Liquid Milk Products: Liquid Milk Products: Super-Pasteurized Milk (Extended Shelf-Life Milk). Encyclopedia of Dairy Sciences: Second Edition 2011, 281-287. https://doi.org/10.1016/B978-0-12-374407-4.00281-8

26. Rashid A, Javed I, Rasco B, Sablani S, Ayaz M, Ali MA, et al. Measurement of off-flavoring volatile compounds and microbial load as a probable marker for keeping quality of pasteurized milk. Applied Sciences (Switzerland) 2019;9(5). https://doi.org/10.3390/app9050959

27. Robador A, LaRowe DE, Finkel SE, Amend JP, Nealson KH. Changes in microbial energy metabolism measured by nanocalorimetry during growth phase transitions. Frontiers in Microbiology 2018;9(FEB):1–7. https://doi.org/10.3389/fmicb.2018.00109

28. Tahmina Bilkis Md, Manirul Islam MC, Sumy Md, Nasim Ali Mandal, Gazi Md, Noor Uddin, et al. Rapid Estimation of Quality of Raw Milk for its Suitability for Further Processing in the Dairy Industries of Bangladesh. International Journal of Dairy Science 2013;(8):1-11. https://doi.org/10.3923/ijds.2013.1.11

29. Tamime AY. Milk Processing and Quality Management. In Milk Processing and Quality Management 2009. https://doi.org/10.1002/9781444301649

30. Tarrand J, Menard K. Metabolic Heat Associated with Viable Escherichia Coli. 4 2012. Retrieved from https://www.perkinelmer.com/lab-solutions/resources/docs/APP_009942_01_Metabolic_Heat_Associated_with_Viable_Escherichia_Coli.pdf

31. Toso B, Procida G, Stefanon B. Determination of volatile compounds in cows’ milk using headspace GC-MS. Journal of Dairy Research 2002;69(4):569-577. https://doi.org/10.1017/s0022029902005782

32. Villeneuve MP, Lebeuf Y, Gervais R, Tremblay GF, Vuillemard JC, Fortin J, et al. Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. Journal of Dairy Science0 2013;96(11):7181-7194. https://doi.org/10.3168/jds.2013-6785

33. Ziyaina M, Rasco B, Coffey T, Ünlü G, Sablani SS. Colorimetric detection of volatile organic compounds for shelf-life monitoring of milk. Food Control 2018;100:220–226. https://doi.org/10.1016/j.foodcont.2019.01.018