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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) [5]. 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, & Luís 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, thermoduric 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

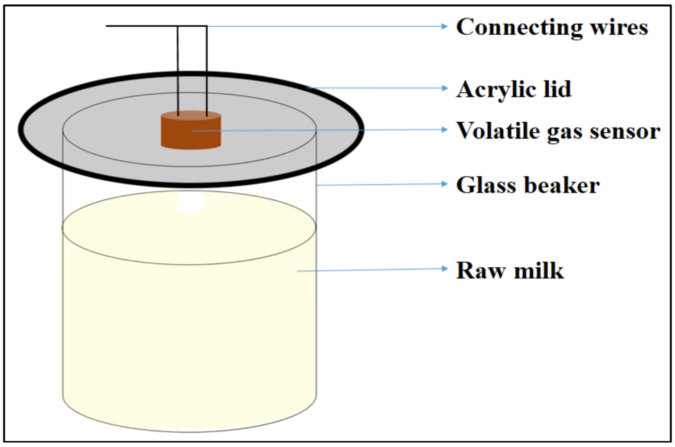

Fig 1: Schematic diagram of experimental setup

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 Draaiyer, Dugdill, Bennett, & Mounsey, 2009).

$$\text{Milk acidity (\%LA)} = \frac{9 \text{ x (ml of NaOH used)x (0.1N NaOH)}}{10 \text{ ml of milk sample}}$$

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) [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) [30]. (Robador, LaRowe, Finkel, Amend, & Nealson, 2018) [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 mesophillic microorganisms such as *Bacillus, Lactobacillus*, *Lactococcus etc*. These microorganisms can grow in 25 °C to 40°C (Luana M *et al*., 2005) [19]. The presence of these microorganisms are unavoidable, because it present in animal udder, milker's hand, utensils, etc (Jay, 1992) [12].

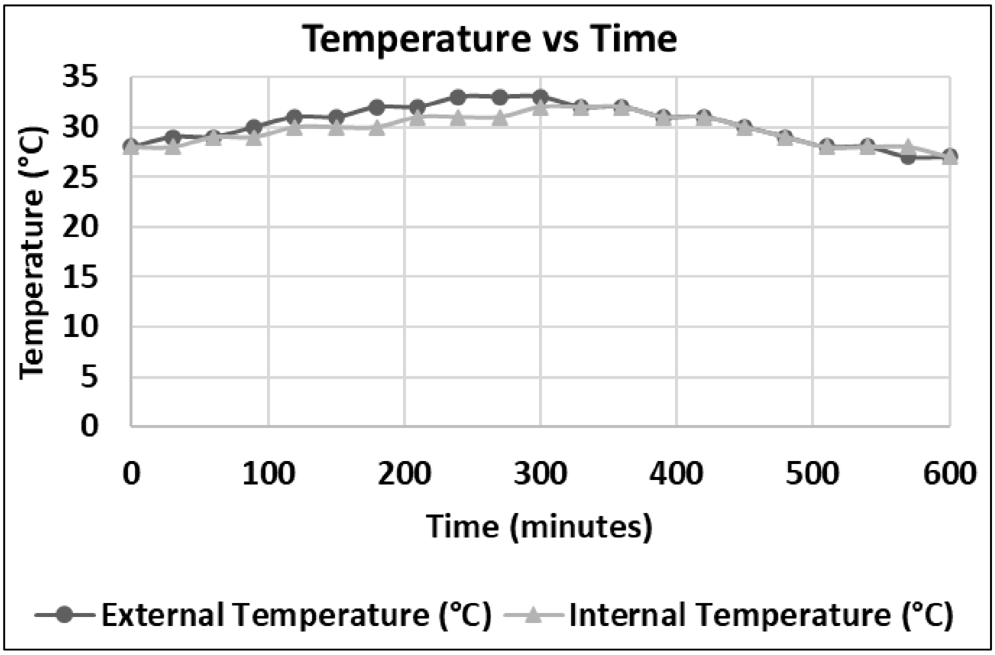

Fig 2: Changes in Temperature during storage

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) [15]. (Al-Muhtaseb, McMinn, & Magee, 2002) [1] also reported that high moisture foods will evaporate and forms condensation when the food closed with improper maintenance of temperature and airflow.

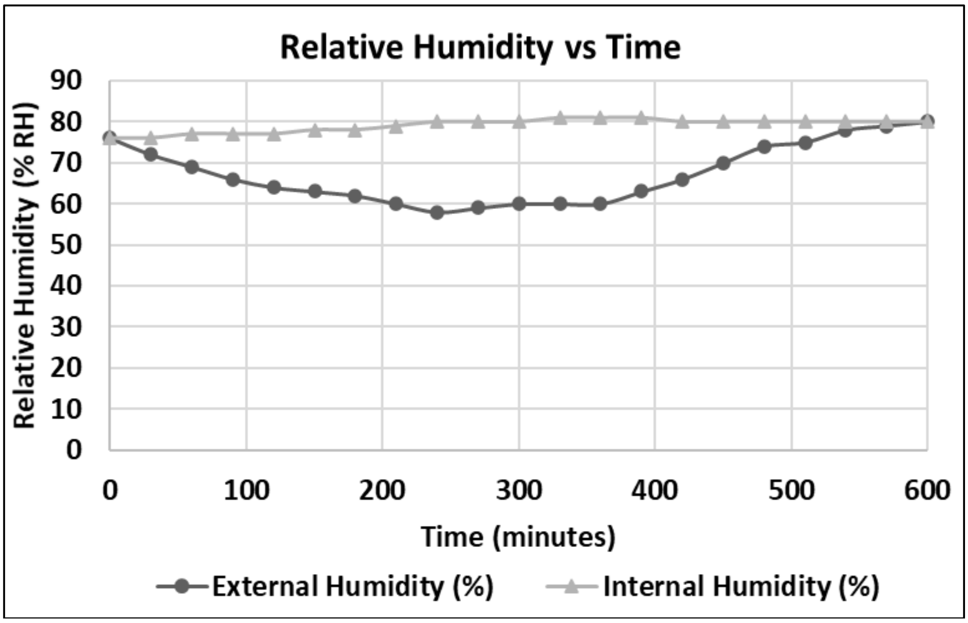

Fig 3: Changes in Relative humidity during storage

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 performs as a strong buffer (Nwosu, Moscone, Palleschi, & 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 (Poghossian, Geissler, & Schöning, 2019) [24]. (Bouteille, Gaudet, Lecanu, & This, 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, & Matche, 2016) [9]

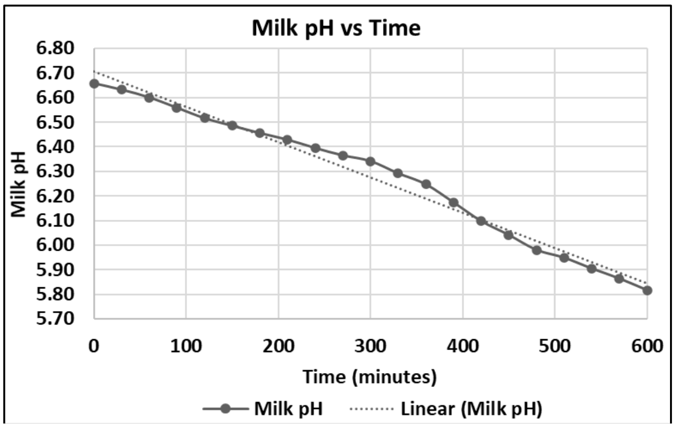

Fig 4: Changes in milk pH values during storage

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].

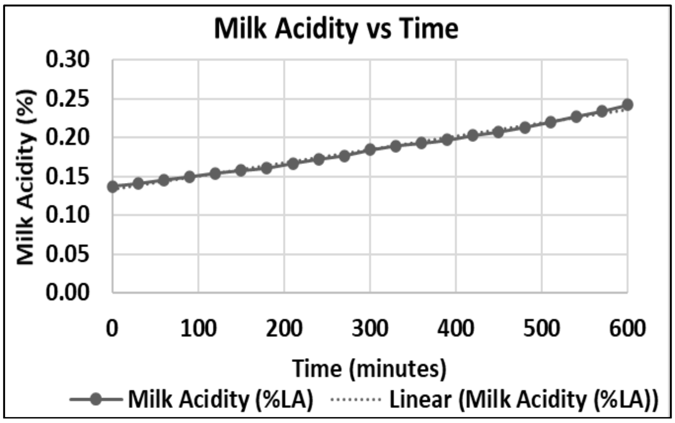

Fig 5: Changes in milk acidity during storage

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 ± 1.1180 µg/L. It might be due to the transformation of volatile organic compounds to raw milk through animal feed, rumen gases and animal metabolisms (Cadwallader, 2010; Toso *et al*., 2002) [5, 31]. After 10 hours, the concentration of TVOC was increased to 482.25 ± 1.4790 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 (Ziyaina, Rasco, Coffey, Ünlü, & Sablani, 2019) [33].

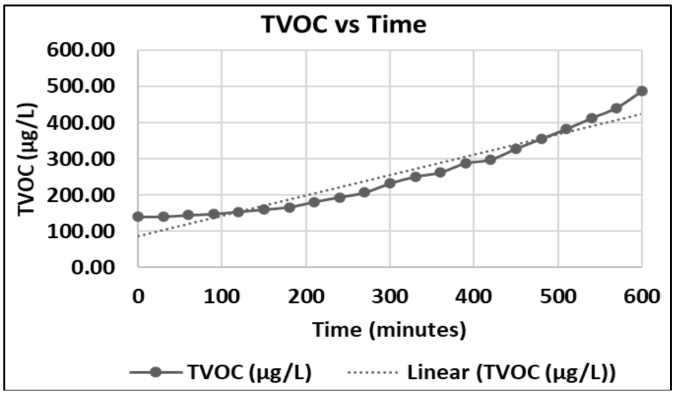

Fig 6: Changes in TVOC during spoilage

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 reacts 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 gets 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].

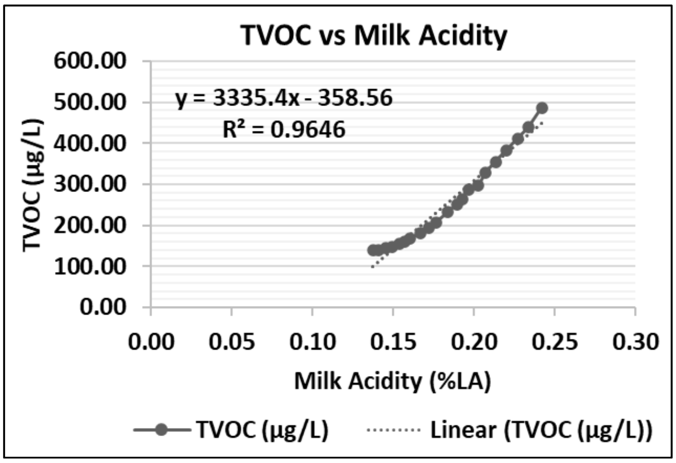

Fig 7: Correlation between TVOC concentrations with Milk acidity

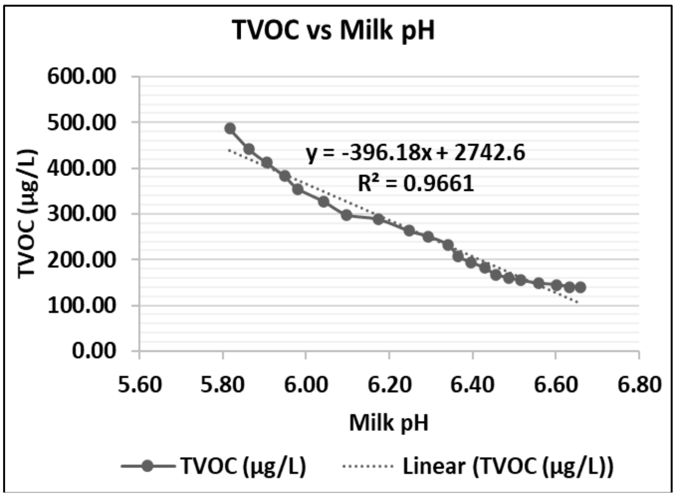

Fig 8: Correlation between TVOC concentrations with Milk pH

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 ± 0.0053	139.50 ± 1.1180
2	30	29	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 R^2 = 0.9646 Where, y = Total volatile organic compounds (µg/L) x = Milk acidity (%LA)	y = -396.18x + 2742.6 R^2 = 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 R^2 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, this quality assessment method was non-destructive, low cost and less time and energy consuming comparing with all the chemical, rapid and microbiological tests. Future research work can be done after connecting the sensor setup with Internet of Things (IoT).

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