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ORIGINAL ARTICLE

Electro-potential and Chemical Changes in Stored Paneer: A Potentiometric Approach

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ABSTRACT

The microbial and biochemical driven dynamics in terms of electro-potential and functional properties of stored paneer were investigated. Paneer samples were stored at 5, 10 and 15 °C for 15 days. The electrode potential of the samples was monitored throughout this period using a digital multimeter. Copper (anode), aluminium (cathode) rods and silver foil sheet (pseudo reference electrode) were employed as electrodes and connected to paneer samples. Physicochemical parameters such as pH, free fatty acids (FFA) content, and O – pthaldialdehyde (OPA) values were obtained on each temperature's 0, 5, 10, & 15th day of storage. All the samples showed slight voltage values with progressive storage duration. pH values decreased with increased storage days instead of FFA content and OPA value for all samples. The samples in storing temperatures between 5 and 10 exhibited acceptable pH, FFA, and OPA values up to five days of storage. Stored samples at 15 °C showed noticeable surface microbial growth within 42-74 h of storage marking spoilage. Based onthe relationship generated between physicochemical parameters and electro potential values of stored paneer, an operational amplifier based voltage divider network sensor circuit was developed which lights-up specific Light Emitting Diode (LED) positioned corresponding to electro-potential values indicating the quality of stored paneer. The electro-potential values with approximately less than 0.490 V are coherent with acceptable limits of microbial, biochemical and functional properties of stored paneer and could be considered as a range of voltage within which stored paneer remains acceptable for consumption. The higher the spoilage in the paneer sample, the higher the electro-potential experienced by potentiometric electrodes and respective LEDs positioned to indicate moderate and severe spoilage glows in the sensor circuit. The electro-potential range was observed as a quantitative measure to indicate the spoilage of paneer samples, and hence the quality assessment for the freshness of paneer sample is achieved by potentiometric electronic sensor circuit in real-time and with low cost.

Keywords: *Paneer, pH, Free Fatty Acid, OPA-Value, Electro-potential*

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INTRODUCTION

Paneer is a non-fermented, non-renneted and non-melting type of cheese that accounts for about 5% of Traditional Indian Dairy Products (TIDP)[1]. Recent advancements in techniques for producing and processing paneer supplemented with an improved cold supply chain are key factors driving the global demand for paneer. With the increasing expectancy of milk production in the country backed by advancements in methods of milk processing, the business of paneer has great potential for its elevated growth in the coming decades. Paneer of good quality has a white marble look, a solid, cohesive, and spongy body, a close-knit texture, and a sweetish-acidic-nutty flavor [2]. According to BIS (IS:1983), the composition of paneer should have moisture $\leq$60%, fat $\geq$ 50% in dry matter for paneer, total plate

count≤5×105, coliform count≤90 and yeast and mould≤ 250 CFU/g in paneer. The quality of the milk used to make paneer has a significant impact on its quality. The different types of milk such as buffalo, cow, goat milk etc. and varied preparation techniques result in wide variation in physicochemical, microbiological and sensory qualities of paneer [2, 3].Bhattacharya *et al.* reported the shelf life of paneer to be about 6 days at 10 °C though its freshness is lost after two or three days [4]. Aneja *et al.* reported that paneer becomes unfit for consumption after one day at room temperature [5]. The majority of the supply of paneer in the Indian market is contributed by the unorganized dairy sector and local sweet shops. The limited shelf life of the paneer sets a major bottleneck for its production at a commercial scale. However, traditional and inefficient production methodsin the unorganized sector accelerate the spoilage of paneer even at refrigeration temperature, which is a concern for suppliers and consumers. In general, the factors influencing the rate of spoilage of dairy products are moisture content, pH, processing parameters, the temperature of storage [6] and redox potential. The microenvironment of cheese is determined by redox potential, which is a physicochemical characteristic linked to temperature, pH, and ionic strength. It's a measurement of a biochemical/chemical system's tendency to lose or acquire electrons. According to Caldeo [7], the oxidation-reduction (redox) potential is a crucial physicochemical characteristic that influences the growth of microorganisms in dairy products and leads to a balanced flavour development in cheese. The oxidation-reduction potential (Eh) of milk measured over the years and, in aerobic conditions, at 25°C was found to be between +250 and +350 mV at pH 6.6-6.731 [8]. Redox potential can vary depending on the species' milk. One of the chemical transformations in milk caused by oxidation-reduction reactions is lipid oxidation [9]. Light, metallic ions and oxygen also catalyze the oxidation of fat molecules. Consequently, undesirable oxidized/metallic flavours can develop in dairy products.Potter was the first to notice a drop in the oxidation-reduction potential of a sterile media during bacterial growth, as measured potentiometrically [10].Hewitt [11] later reported on the dependence of microbial cell metabolism on different oxidation-reduction systems. In dairy products, the peptides linkages can be hydrolyzed by enzymes due to microbial growth causing undesirable flavour changes. The microbial activities, particularly that of psychrotrophic and bacteria that grow well at 15 °C, are known for producing enzymes that cause proteolysis and lipolysis. The degradation reactions usually cause the release of reducing agents [12].Nörnberg *et al.* also affirmed that the growth of coliform bacteria (psychro-tolerant) produces a lipolytic and proteolytic enzyme that leads to physical degradation and unacceptable product's sensory characteristics [13]. Many authors have carried out microbial enumerations of market paneer for the status of hygienic level [14,15]. Various methods for shelf life extension of the product have also been documented. However, there is no accurate, low cost and sensitive instrument developed yet to measure the electrical parameters of Paneer. Therefore, the present study aims to develop a simple and low-cost potentiometric sensor to ascertain the paneer quality and describe the relationship of electrical properties with functional properties. The study also includes determining a safe range of potential within which stored paneer can be indicated as acceptable for consumption.

MATERIAL AND METHODS

Sample preparation

A batch of paneer sufficient for the experimental study was procured from the local sweet shop in the industrial area in the Sonepat district of Haryana, India. The raw material used throughout the experimental study was from the same source to minimize experimental variation. Paneer (L×B×H = 12 cm×6.5 cm×7.5 cm) from the same batch was immediately wrapped in a sterile plastic bag to store the paneer samples immediately after procurement, and three replicas of each paneer samples were stored at three different temperatures (5°C (T1), 10°C (T2) and 15°C (T3) for 15 days. Paneer stored under each temperature was used to obtain potentiometric readings and evaluate the microbial, biochemical and functional properties of the respective samples.

Chemicals and Reagents

All the chemicals used for the analysis were of analytical grade. Anhydrous sodium sulphate, β-mercaptoethanol, L-Serine, O-pthaldialdehyde, petroleum ether, and potassium hydroxide were procured from SRL Private Ltd. Chloroform, Diethyl ether, Ethanol absolute, and methanol were procured from Fischer Scientific, UK [16, 17, 18].

Measurement of potential changes in paneer sample

The potentiometric voltage readings were measured throughout the experimental study for sample blocks T1, T2, and T3 samples at three different temperatures of 5°C, 10°C and 15°C for a storage duration of 15 days. The voltage reading was taken using a digital multimeter (MetroQ, MTS 888, Delhi) with copper (Cu) and aluminium (Al) rods (length = 50 mm; diameter = 0.5mm) acting as anode and

cathode electrodes connected to metal ends of the probes of multimeter and inserted into the paneer block as shown in Fig. 1, 3(a) and 3(b) to measure electrode potential for voltage reading for T1, T2, and T3 samples with the separation distance between the two electrodes were kept constant at 30 mm. The input impedance of the digital multimeter (MetroQ, MTS 888, Delhi) 10 M ohm is replaced by high input impedance operational amplifier in an electronic sensor circuit for impedance matching and to avoid reverse voltage. The silver foil sheet is placed under every paneer sample during electro-potential measurement, which acts as a pseudo-reference electrode to avoid reverse voltage. According to the literature, silver wires/ sheets are commonly used as pseudo- or quasi-reference electrodes in non-aqueous systems due to their simplicity, low ohmic resistance/ impedance effect, lack of a liquid junction potential requirement, and lack of contamination from solvent molecules or ions that a conventional reference electrode might transfer [19].However, there are certain limitations of using pseudo-reference electrodes discussed later. However, with reference to the scope of experimental work done in the present study, an attempt is made to develop an electro-potential based low cost and real-time sensor circuit for the determination of freshness of stored paneer which can be used as a prototype for futuristic development of more sophisticated, precise and commercially viable technique. The readings were taken in triplicates, and average values were reported. The potentiometric sensor circuit was developed with high input impedance operational amplifier to mitigate the effects of reverse voltage.

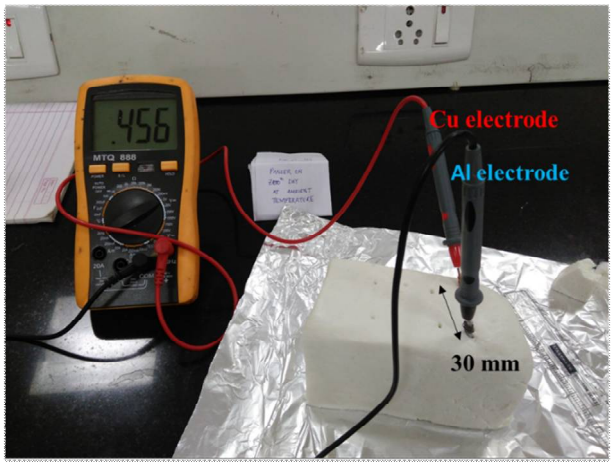


Figure 1; Experimental set-up for the electro-potential measurement of paneer sample

Sampling for chemical and microbial analysis

Before analysis, the paneer samples were tempered for 1 hour at 15.5°C. Paneer was sampled according to the technique outlined in ICAR Bulletin No. 70 (1951) for chhana.With a trier, 20 grams of paneer were extracted from various parts of the total bulk and pooled together.It was then transferred to screw-top sample vials for examination and kept refrigerated until the observation day's analysis.The experimental readings were taken in triplicates, and average values were reported.

RESULTS AND DISCUSSION

All the readings were taken triplicates and expressed as mean ±standard deviation. An errors bar in the plot represents the standard deviation. Correlation analysis between physicochemical parameters and electrode potential of stored paneer samples were performed using IBM SPSS Statistics.

Electro-potential dynamics

Electrode potential difference measured in volts, is generated due to the transfer of electrons between electrodes through a medium. In the present study, copper and aluminium metals rods were used as electrodes to measure the electromotive force (electro-potential), which is generated due to the potential difference between them when inserted in paneer sample blocks. It is widely available in the literature that fresh milk and dairy-based products contain oxygen which decreases with an increase in microbial activity, and thus, paneer also produces reducing agents with spoilage due to bacterial action. The potential of the paneer samples (T1, T2, and T3) was measured in volts for the entire storage duration of 15 days. The data obtained was analyzed to determine the voltage range at which physicochemical and microbiological data can indicate spoilage of paneer. The average readings of electro-potential for T1, T2 and T3 samples were measured for 15 days.

Potentiometric circuit

The LM3914, a monolithic integrated circuit with an adjustable reference resistance and 10-step voltage divider network, was used to create a potentiometric electronic sensor circuit.To prevent against reverse voltage signals and impedance matching, a high input impedance buffer operational amplifier is used at the input.The high input impedance buffer op-amp is connected in voltage follower configuration.

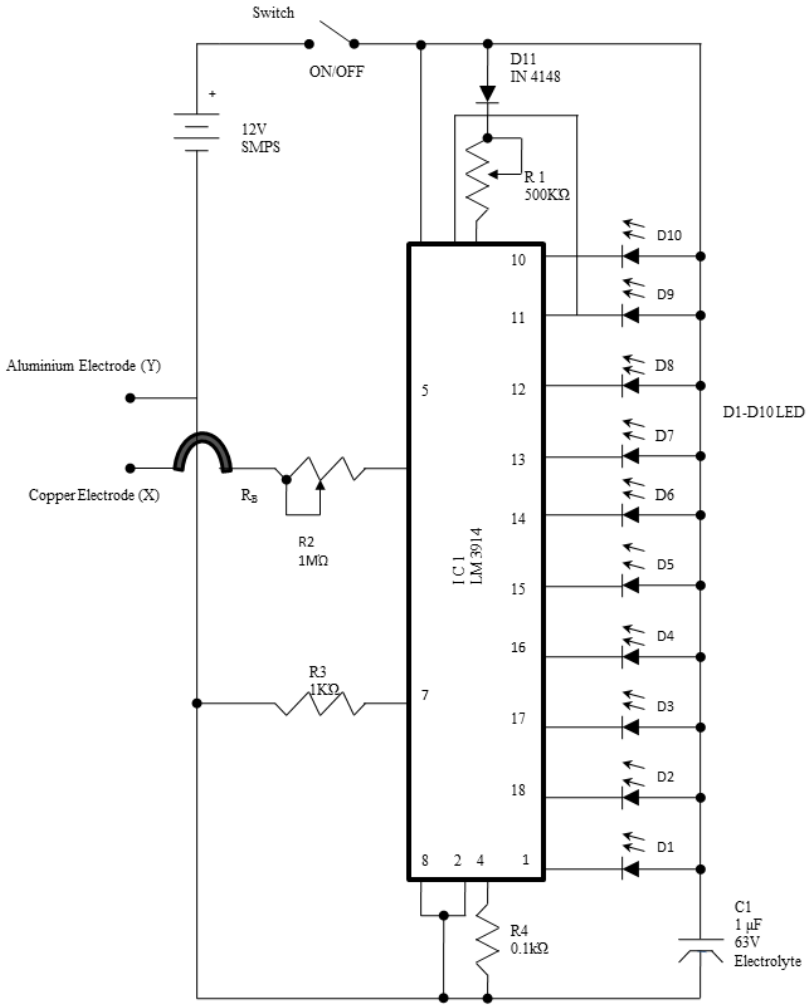
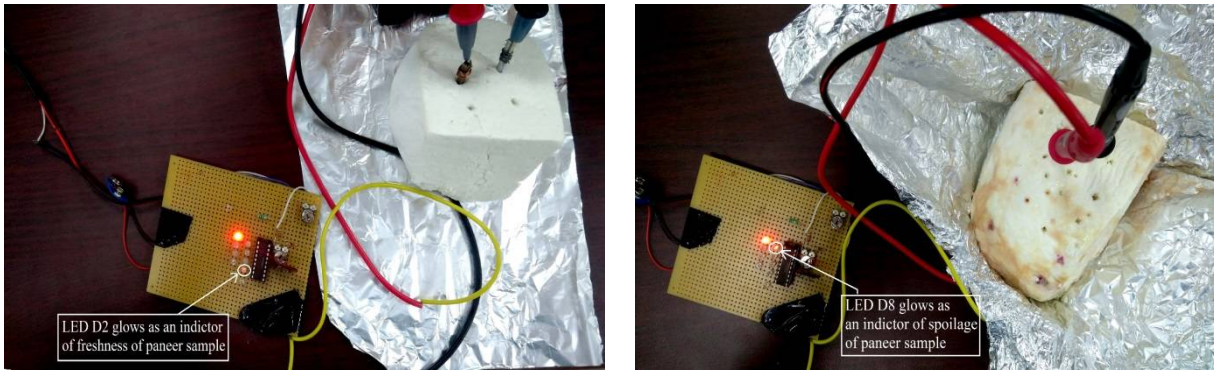


Figure 2; Potentiometric Circuit to determine the quality of Paneer

A 12V SMPS is used to convert 230V AC input supply from the switchboard, and current consumption is restricted to about 10mA, even when LED is in an 'on' state. A preset resistance of 1MΩ is applied from non-inverting input to ground, which provides paths for input bias current, which in turn sets the op-amp IC's input impedance for impedance matching and avoids reverse or false voltage. Another preset resistance of 500KΩ is applied across non-inverting input and the ground, which determines the proportional offset voltage for the combination of linear resistors and potentiometric electrodes. The sensor circuit's sensitivity and range of deviation can be adjusted using these two preset resistance. The copper (X) and aluminium (Y) electrodes act as anode and cathode, respectively, to detect voltage developed in the paneer sample. The output voltage of the buffer operational amplifier is directly connected to the inverting inputs of comparators. In the absence of any input voltage across X and Y, the offset voltage is maximum, and hence by adjusting the preset resistance, the LED D10 can be made to glow, which is an indicator of the circuit working properly. Any voltage reading other than the open resistance across X and Y brings the offset voltage down, which drives LEDs positioned corresponding to electro-potential measurements to glow. The position of glowing LED changes proportionally with the changes in the electro-potential voltage across X and Y and thereby indicative of freshness of paneer sample.



a b

Figure 3; LED indication (a) D2 indicates fresh paneer sample (b) D8 indicates spoiled paneer sample

The higher the spoilage in the paneer sample, the higher the electro-potential experienced by potentiometric electrodes, which is approximately greater than 0.590V for almost all spoiled samples and accordingly LED D7 to D9 glows as illustrated in Fig. 3b. In contrast, for a less spoiled sample of paneer, mid-range electro-potential voltage is experienced for moderately spoiled paneer samples which experience electro-potential voltage ranging from approximately 0.490 V-0.590 V and thus proportionally mid-range LEDs glows, i.e. LED D4 to D6. For a fresh sample of paneer, the electro-potential voltage range experienced by potentiometric electrodes is approximately less than 0.490 V for almost all relatively fresh or good quality samples, and accordingly, LEDs D1 to D3 glows as shown in Fig. 3a. Hence, the quality assessment of the freshness of paneer sample is achieved by potentiometric electronic sensor circuit in real-time and with low cost. The components used in the circuit are mentioned in Table 1.

Change in electro-potential

The fresh market paneer sample was found to have an electro-potential value of 0.46±0.01 V. From Fig. 4, and it was observed that all the samples exhibited an increase in electro-potential values with remarkable variations progressively for about ten days of storage period and from then downturned till 15 days of storage. The increase in electro-potential values of T1 and T2 samples was found comparatively slower than that of the T3 sample. The electro-potential values of T1 and T2 samples up to five days of storage were in the range of 0.469 to 0.484 V. After that period, T1 and T2 samples exhibited slightly higher potential differences. T1 and T2 samples reached a maximum of 0.565±0.027 V and 0.575±0.023 V during the tenth day of the storage, respectively. After that, electro-potential values were found to decrease in both T1 and T2 samples whose values by the 15th day were 0.511±0.006 V and 0.518±0.006 V, respectively. In the case of the T3 sample, the initial electro-potential value was the same as the T1 and T2 samples. A drastic increase (0.681±0.012 V in 10 days) followed by a steep decrease in electro-potential values (0.506±0.019 V in 15 days) were observed. T3 sample further exhibited a comparatively higher electro-potential value (0.681±0.018 V) in 10 days, followed by a decline in potential difference. The fluctuation in electro-potential values over the storage duration was more pronounced in the T3 sample than in T1 and T2 samples. The fluctuation could indicate alteration in the number of mobile ions available for transport phenomenon for generation of electro-potential difference in the sample resulting from the production of reducing agents/oxidants caused by microbial growth and other associated chemical reactions such as redox reactions with the increase in storage period. The microbial actions, particularly that of psychrotrophic and bacteria that grow well at 15°C, are known for the production of enzymes that causes proteolysis and lipolysis. The degradation reactions usually result in the release of reducing agents [12].

Weak acids or organic acids from the bacterial action do not ionize completely for the redox reaction [20], which can otherwise aid in developing electro-potential differences in products. However, the potential difference depends on the availability of number and mobility of ions which transport charges across the two electrodes, resulting in the generation of electro-potential difference between two electrodes. This could explain the drastic fluctuations observed in all samples' electro-potential values and do not necessarily indicate the absence of decomposed products. Certainly, the number of available charged ions and their associated mobility in a sample depends on the nature of decomposition by-products of bacterial activity and other associated chemical reactions. The progression of bacterial activity is, in turn, the function of storage temperature and storage period. Thus, the development of higher potential difference across the electrodes can be considered dependent on progressive microbial growth thriving under favourable conditions resulting in a change in pH levels and the number of available charged ions

and their associated mobility in the respective paneer sample. T3 samples stored under 15 °C provided more favourable thriving conditions for microbial growth, which was reflected in the steeper fluctuations of electro-potential values compared to T1 and T2 samples. Lower electro-potential values up to five days of storage period for T1 and T2 samples could manifest retarded microbial growth assisted by chiller storage temperature. The electro-potential values obtained for different samples over the storage period can be further analyzed with appropriate physicochemical parameters to establish a more precise range within which freshness can be assured.

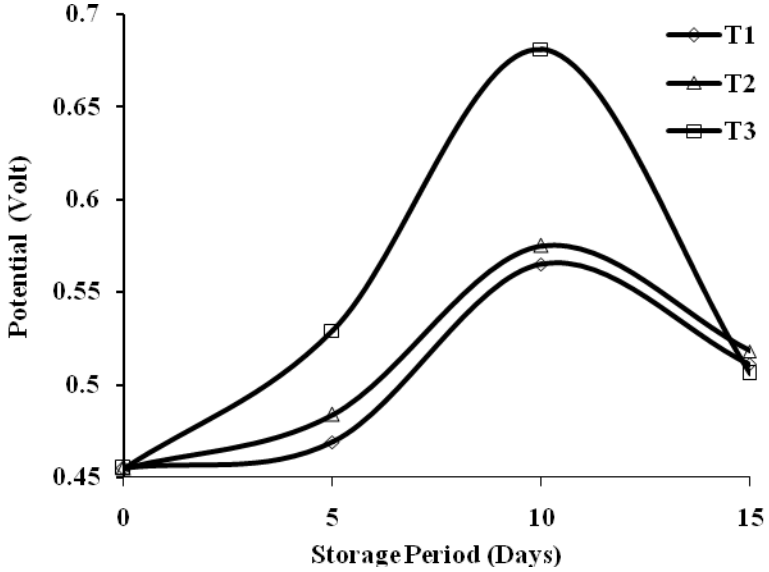


Figure 4; Effect of storage period on electro-potential of paneer samples.

Change in pH

The acidity of the food sample is usually expressed inpH. The pH of the fresh market paneer was found to be 5.716±0.044. It can be seen from Fig. 5 that T1 and T2 samples showed a gradual decrease in their pH values with an increase in the number of storage days till the 15th day. The pH of the T1 sample was decreased to 5.51±0.025 in 5 days, to 5.287±0.047 in 10 days, and 5.183±0.025 in 15 days, while the pH of the T2 sample was decreased to 5.38±0.01 in 5 days, to 5.20±0.053 in 10 days and to 5.177±0.015 in 15 days. T3 sample showed a steep decrease in pH from initial 5.716±0.044 to 5.34±0.015 in 5 days, to 5.093±0.015 in 10 days and slightly increase to 5.22±0.025 on 15th day (Fig.5).Similar observation reported by Singh *et al.* [21] showed a steady decrease in pH of paneer sample up to 12 days and increased rapidly after that when paneer was wrapped in sorbic acid-coated paper and stored at 5 °C. The slight increase in pH could be due to the utilization of lactic acid for the formation of its metabolites and subsequent liberation of non-acidic amino compounds by proteolytic bacteria [3]. The pH of the T3 (15 °C) paneer sample was found comparatively lower than T1 (5 °C) and T2 (10 °C) samples till the 10th day. This could be due to the more rapid conversion of lactose to lactic acid by fermenting bacteria at comparatively higher storage temperatures (15 °C). Bacterial action might have slowed down at 5 and 10 °C temperatures at which T1 and T2 samples were stored. Similar results of a decrease in pH with subsequent storage were reported by Bhattacharya *et al.* [4],Yadav [22], Jadhavar[23], and Pal [24]. Bhattacharya *et al.* found a decrease in pH of paneer (prepared from standardized buffalo milk with 5% fat) from 6.60 to 5.80 on the sixth day of storage at 10 °C [4]. Pal [24] also indicated a decrease in pH value of fresh paneer (6.21) during storage at 8 ± 2 °C to 6.04, 5.78, and 5.73 on the 5th, 10th, and 15th day, respectively. The higher initial microbial load in the market paneer could be responsible for more acidic pH values during storage due to acidic decomposition products (lactic acid from lactose) produced upon fermenting bacterial growth. The higher initial microbial load could indicate more contamination in the procured market sample. Arora and Gupta [25] and Bhamba [26] similarly explained the subsequent decrease in pH during storage due to the development of lactic acid. Kumar and Bector [27] also reported a progressive increase in acidity of all paneer samples stored at 5 and 15 °C temperatures. Therefore, the resulting trend of decreasing pH with an increase in storage duration was in agreement with that of reports by several authors.

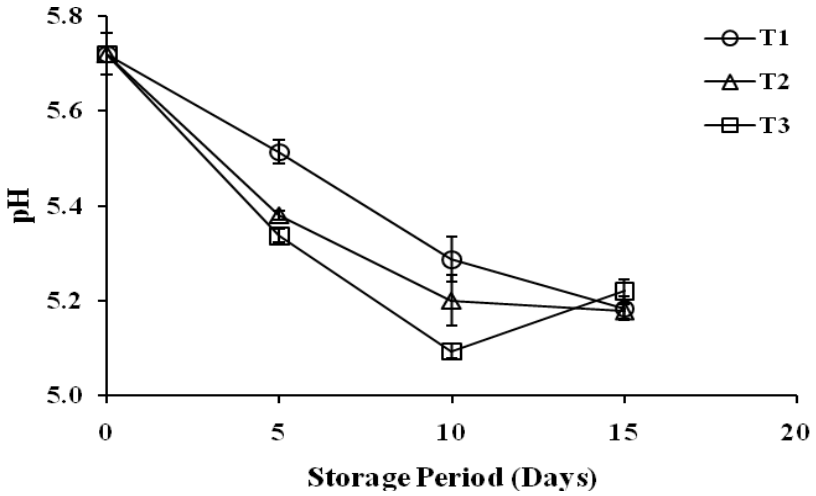


Figure 5; Effect of storage temperature and storage duration on pH and freshness of paneer.

Free Fatty Acid

Lipids in milk products undergo hydrolysis (lipolysis) through microbial enzymes [28]. Lipolysis leads to an increase in free fatty acids (FFA) content and thereby changes the flavour characteristics of the product. Estimating FFA content thus determines the extent of lipolytic changes in fat-rich dairy products. Oleic acid is considered the most abundant of the unsaturated fatty acid residues (about 70%). Thus, FFA content may be represented in terms of oleic acid content. The free fatty acid (FFA) content of fresh market paneer was found to be 0.583±0.008 (% oleic acid).

The FFA of all samples increased with a longer storage duration. T1 and T2 samples showed a slower rate of increase in FFA content than T3 samples until the end of the storage period. The FFA of T3 samples was sharply increased to 3.043±0.047 (% Oleic acid) in 5 days from the initial value (0.583±0.008) to 3.297±0.018 (% Oleic acid) in 10 days and to 4.363±0.113 (% Oleic acid) in 15 days (Fig.6). The slow rise in FFA content in T1 and T2 samples can be attributed to lower storage temperatures, i.e., 5 and 10 °C, respectively. The lower temperature might have slowed down the growth of fat splitting bacteria or yeast and mould during storage and consequently lowered the rate of liberation of FFA. Therefore, initial fat content, active enzymes, microbial enzymes, storage temperature and storage period could be considered factors influencing the rate of increase in FFA content during storage. Based on the analytical data of various parameters such as pH and microbial quality in association with FFA content, it can be inferred that FFA content of less than 1% oleic acid can be considered acceptable in stored paneer.

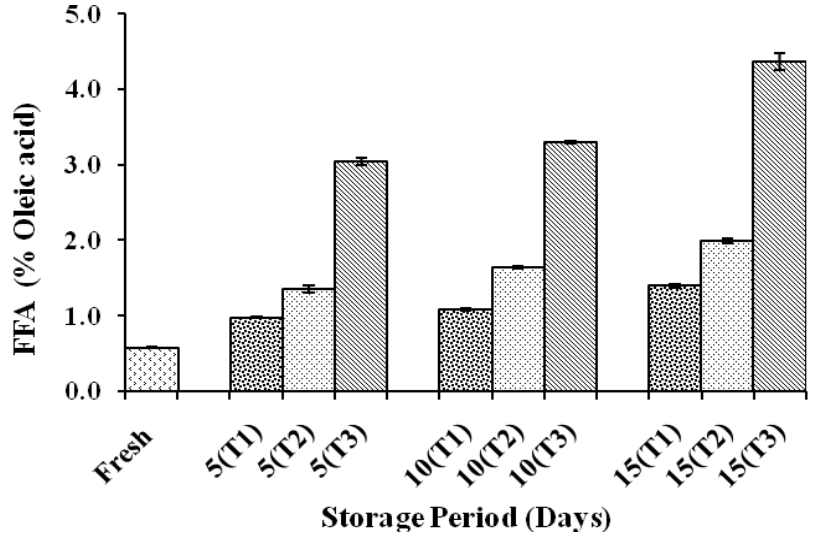


Figure 6; Effect of storage temperature and storage duration on free fatty acid (FFA) and freshness of paneer.

In a similar study, Boghra*et al.* [29] observed the marginal release of FFA in paneer up to 2 days followed by a comparatively higher increase in the content after that period. The enzymatic action that acts normally or enhanced otherwise was reported to be responsible for the change of pattern in FFA in

paneer. Kumar and Bector [27] found that the FFA of all treated paneer samples increased during storage with the increase in storage duration, Khatkar*et al.* found a highly significant (P0.01) rise in free fatty acid in all samples for both control and cinnamon treated paneer samples [30, 31].When packed with MP, NP, and LDPE, the free fatty acid in the control sample increased considerably (P0.01) from 0.175 to 0.517 in 10 days, 0.491 in 8 days, and 0.541 in 5 days.The lipolysis action was reported to be responsible for an increase in FFA. Solanki *et al.* [32] also reported similar results stating an increase in FFA in control burfi and different wattage-time combinations of microwave treated burfi samples during storage. Thus, the results obtained in this study are in line with the results of other authors who worked under similar conditions.

Proteolysis

Proteolysis is the breakdown of proteins into smaller polypeptides or amino acids. In dairy products, the peptides linkages can be hydrolyzed by enzymes due to microbial growth causing undesirable flavour changes [12]. The extent of proteolytic changes is determined by estimating the OPA (O – pthaldialdehyde) value. The OPA value of fresh market paneer samples was found to be 38.871±1.284 µg (L-serine)/g. A progressive increase in OPA values was observed in all the samples stored at different temperatures until the end of storage duration. The rate of increase of the OPA value of the T3 sample was found comparatively faster than T1 and T2 samples throughout the storage duration (Fig. 7). This can be attributed to the active breakdown of proteins with more microbial growth under a comparatively favourable temperature of 15 °C. T1 sample showed a relatively slower rate of increase in OPA value. OPA value of the T1 sample in 5 days was 61.89±1.818 µg/g which gradually increased to 88.17±0.699 µg/g in 10 days and 98.31±1.107 µg/g in 15 days. The increase was more substantial in T2 and T3 samples. T2 sample reached 73.23±0.632 µg/g OPA value in 5days, which increased to 143.75±1.61 µg/g and 156.15±0.661 µg/g in 10 and 15 days, respectively. In the case of the T3 sample, OPA value rapidly reached 113.169±0.484 µg/g in 5 days from the initial OPA value (38.871±1.284 µg/g) to 162.374±0.882 µg/g and 260.724±0.793 µg/g within 10 and 15 days, respectively. This could be due to the presence of active microbial growth in the T3 sample resulting in more rapid conversion of proteins and subsequent release of free amino acids, even by the 5th day under the 15 °C temperature. Similarly, Kumar and Bector16 reported that the storage temperature had a significant effect on the proteolytic changes. The comparative chiller temperatures (5 and 10 °C) of T1 and T2 samples could have possibly slowed down the activity of microbial growth and retarded the production of protease enzymes required for proteolytic changes up to the 5th day.

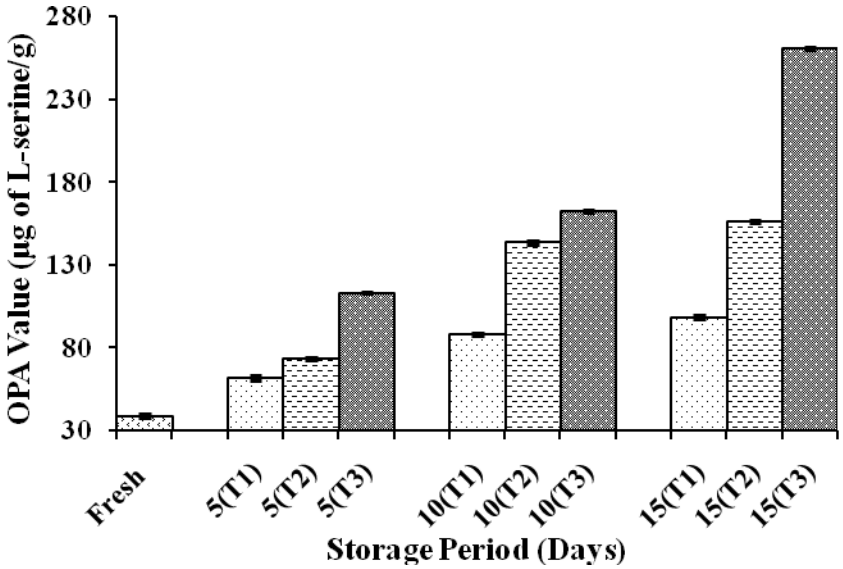


Figure 7; Effect of storage temperature and storage duration on proteolysis and freshness of paneer.

Bamba*et al.* studied proteolytic changes in paneer samples from NDRI, India and the nearby local market during storage at 7 °C for 28 days [26]. He discovered that paneer can be maintained at room temperature for up to a week without losing significant biological activity. Following that period, an increase in proteolysis was found due to psychrotrophic protease synthesis .In his study, the average initial tyrosine value of 10g/g in NDRI paneer samples was increased to 134.8g/g after 28 days of storage, indicating that the level of proteolysis was initially modest, rose gradually throughout storage, and increased at a higher pace after 28 days [4, 29]. An increase in OPA value during storage is considered a

sure indication of degradation of proteins which results in the release of free amino acids. Therefore, the extent of the increase of OPA value is directly proportional to the extent of degradation of proteins. Khatkar*et al.* also observed that the tyrosine content of the control sample was increased steeply with the increase in storage period. Proteolysis was reported to affect the steep increase in tyrosine content during the storage of paneer [30].

RELATIONSHIP BETWEEN ELECTRO-POTENTIAL AND CHEMICAL PARAMETERS THROUGH STATISTICAL ANALYSIS

A negative correlation was observed between pH values with FFA content, OPA value, and electrode potential (Fig. 8). The results indicate that more proteolysis and lipolysis reflected in terms of increasing FFA content and OPA value for all samples understudy during the storage period lead to a decrease in pH value. The decrease in pH value of paneer with progressive storage period was found to be inversely proportional to the strength of electrode potential developed in stored paneer. However, a slight variation exists depending on the chemical species generated and involved in redox reaction during subsequent spoilage and the mobility of ions involved in the development of potential differences between two selected electrodes. In contrast, a positive correlation was observed between electrode potential and FFA content and OPA value, with the progressive growth of spoilage microorganisms, particularly psychrotrophic and activities of heat resistant enzymes such as protease in stored paneer, FFA content and OPA value increase which is an indication of lipolysis and proteolysis during storage. The generation of chemical species and ions will then increase over time resulting from chemical reactions between inherent enzymes and enzymes of microbes with protein and fats of paneer. Thus, the positive correlation between electrode potential of stored paneer with associated FFA and OPA values could be due to more availability of disintegrated proteins and fats into free amino acids and free fatty acids respectively in stored paneer, which could have generated more mobile ions available for transport phenomenon resulting in the generation of more electro-potential as compared to the fresh paneer with proteins and fats molecules intact.

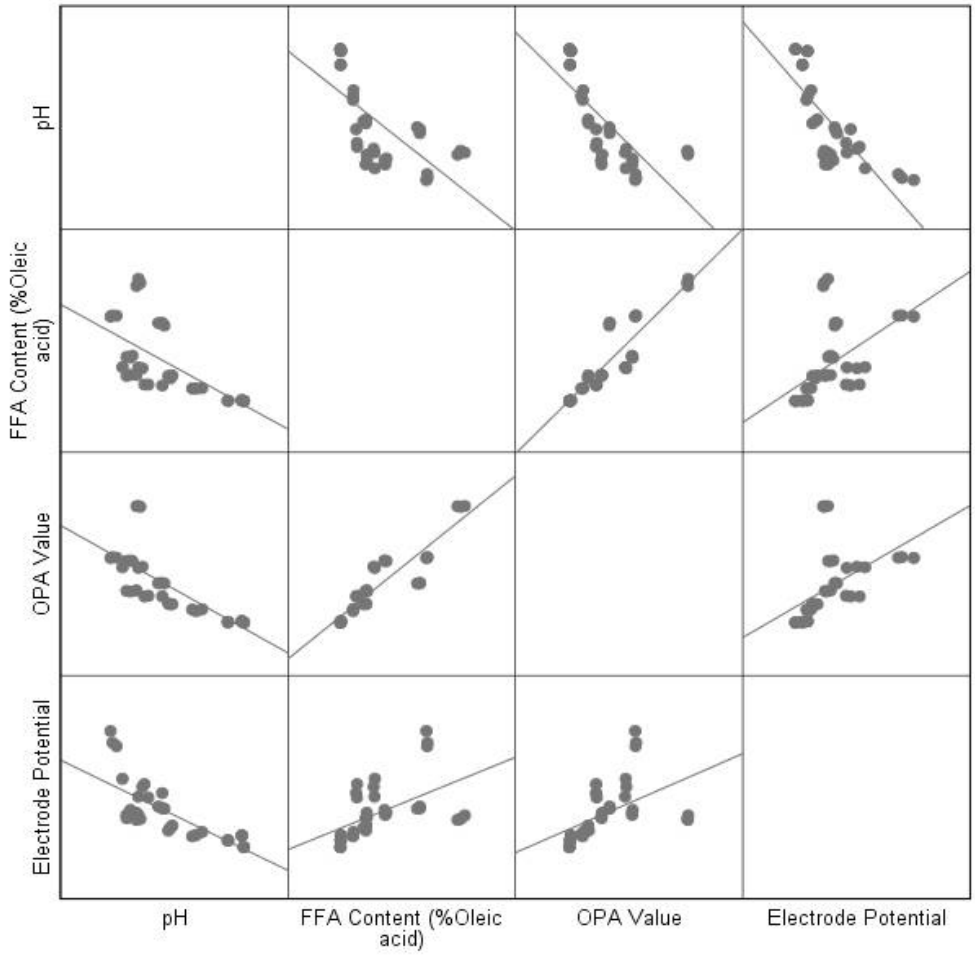


Figure 8; Correlation between electrode potential and pH, FFA content and OPA value.

CONCLUSIONS

Physico-chemical and microbial parameters (MC, pH, FFAs content, OPA Value, colour change) from three sets of paneer samples (5°C (T1), 10°C (T2) and 15°C (T3)) stored at different temperatures were analyzed for their relations with their associated electro-potential observed during the storage period of 15 days. With progressive storage duration, the FFAs content and OPA values were found to increase, whereas pH was found to decrease for all samples (T1, T2, and T3 samples), and the results of the present study were found to be in accord to similar work available in the literature. T3 samples less than 15 °C showed noticeable surface colour change caused by spoilage by the 10th day. The pH, FFA content and OPA value of samples were found to be acceptable limits when electro-potential values are approximately less than 0.490 V in the sensor circuit and thus, could be considered as a range of voltage within which stored paneer remains acceptable for consumption. The higher the spoilage in the paneer sample, the higher the electro-potential was experienced by potentiometric electrodes for relegated quality and spoiled samples.

Progressive microbial growth under a favourable environment during storage enhances redox reactions and other associated chemical reactions. The composition of paneer, initial microbial load, storage temperature and storage duration are the factors responsible for the extent of proteolysis and lipolysis degradation during storage and resulting in variation in electro-potential of panner sample, which is used as a quantitative indicator of the quality of stored paneer in the present study. However, the product's composition and initial microbial load will vary from one manufacturing unit to another. Thus, the range of electro-potential values obtained in the present study might not represent all paneer samples, and the present study could be further studied upon with other known compositions and initial microbial load of paneer. There are several disadvantages of using pseudo- or quasi-reference electrodes, such as the silver foil sheet used in the present study as it lacks thermodynamic equilibrium, and thus determination of reference electrode potential is not possible.

Moreover, most pseudo-reference electrodes function within a narrow range of circumstances, such as pH or temperature, and measurements outside of that range cause the electrodes to behave in unforeseen ways [19]. In light of the limitations and scope of this experimental study, the sensor circuit developed in the study could be used as an initial attempt and prototype version to develop an improved and sophisticated model for quality assessment of paneer for real-time, quantitative and low-cost spoilage detection techniques. A non-linear model and simulation can be adapted to develop sensor for high accuracy and precision. In the present study, the electro-potential range is observed as a quantitative measure to indicate the spoilage of paneer and hence the shelf life quality assessment of the freshness of paneer sample is achieved by the potentiometric electronic sensor in real-time and with low cost.

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