www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(10): 991-996

www.thepharmajournal.com
Received: 03-08-2023
Accepted: 07-09-2023

SR Badhe
Ph.D., Scholar, Department of Livestock Products Technology, Nagpur Veterinary College, Nagpur, Maharashtra, India

KS Rathod
Assistant Professor and Head, Department of Livestock Products Technology, Nagpur Veterinary College, Nagpur, Maharashtra, India

SP Chaudhari
Professor and Head, Department of Veterinary Public Health and Epidemiology Nagpur Veterinary College, Nagpur, Maharashtra, India

AP Dhok
Assistant Professor, Department of Animal Nutrition Nagpur Veterinary College, Nagpur, Maharashtra, India

SR Warke
Department of Veterinary Microbiology, Nagpur Veterinary College, Nagpur, Maharashtra, India

Anupama Kumar B
Department of Chemistry, Visvesvaraya National Institute of Technology, Nagpur, Maharashtra, India

BL Mundhe
Department of Livestock Products Technology, Nagpur Veterinary College, Nagpur, Maharashtra, India

Corresponding Author:
SR Badhe
Ph.D., Scholar, Department of Livestock Products Technology, Nagpur Veterinary College, Nagpur, Maharashtra, India

Paneer preservation puzzle: Unraveling the link between microbial spoilage and chemical clues in refrigerated storage

SR Badhe, KS Rathod, SP Chaudhari, AP Dhok, SR Warke, Anupama Kumar and BL Mundhe

Abstract
The aim of this study was to analyze the total microflora in aerobically packed Paneer stored under chiller conditions (4±1 °C) using chemical indicators of spoilage. Paneer samples were subjected to chiller storage and analyzed on alternate days, ranging from day 0 to day 8. Several chemical indicators, including pH, TBA (Thiobarbituric Acid), Tyrosin, TVBN (Total Volatile Basic Nitrogen), and Ammonia, were assessed. Total plate count, psychrophilic count, and pseudomonas count were also evaluated. In our findings, we observed a significantly positive correlation (p<0.01) between microbial and physicochemical parameters, except for pH. Notably, microbial parameters exhibited a strong positive correlation with TVBN and ammonia levels. The total plate count (TPC) demonstrated correlation coefficients of 0.815 and 0.833 with TVBN and ammonia, respectively. Psychrophilic count exhibited correlation coefficients of 0.804 and 0.789 with TVBN and ammonia, while pseudomonas count showed correlation coefficients of 0.803 and 0.789 with TVBN and ammonia, respectively. Based on these correlation coefficients for various physicochemical parameters, it becomes possible to predict the total plate count, psychrophilic count, and pseudomonas count in Paneer samples. This information is valuable for understanding the spoilage dynamics and quality assessment of refrigerated Paneer during storage.

Keywords: Refrigerated paneer, total plate count, pseudomonas count, psychrophillic count, TVBN, ammonia

Introduction
Paneer, a prominent coagulated dairy product in India, constitutes a substantial fraction of the nation's milk production, estimated at approximately 5%. The term "paneer" denotes a dairy item derived from cow or buffalo milk, or a combination thereof, precipitated using sour milk, lactic acid, or citric acid. It is characterized by a moisture content not exceeding 70.0% and a minimum milk fat content of 50.0% of the dry matter, in accordance with FSSAI standards (2011).

Paneer is distinguished by its distinctively mild acidic flavor complemented by a subtle sweetness. It possesses a firm, cohesive, and spongy texture with a closely knit, smooth consistency. However, akin to numerous indigenous dairy products, paneer is highly perishable, resulting in constrained shelf-life. When subjected to refrigeration and ambient temperatures, paneer typically retains its quality for up to 6 days and 3 days, respectively. In the contemporary milieu, the burgeoning emphasis on health-conscious living has heightened awareness of food safety concerns, significantly impacting consumer behavior. Bacterial spoilage presents a substantial economic challenge for the food industry, especially when dairy products fall prey to psychrotrophic microorganisms, which thrive in temperatures conducive to their growth.

The spoilage of paneer predominantly ensues from the proliferation of microorganisms, culminating in various physicochemical changes that manifest as off-flavors within the product. Microorganisms within milk introduce biochemical modifications in carbohydrates, proteins, and fats through their metabolic activity (Heeschen, 1972) [6].

Currently, the evaluation of Paneer's quality entails the assessment of its microbiological and sensory attributes over time. Given the financial and temporal constraints associated with microbial analyses, there exists a burgeoning interest in alternative methodologies predicated on the detection of chemical changes ensuing from microbial growth.

The demand for innovative technologies facilitating the rapid detection of spoilage and bacterial contamination in food products is steadily increasing (Scindia *et al*., 2007) [11].

Materials and Methods

Materials

All chemical reagents, including Plate Count Agar and Cetrimide (purchased from SRL, Virion Enterprises, Mumbai, Maharashtra, India), 0.5 N Sodium Hydroxide, Trichloroacetic Acid, Folin and Ciocalteu's Reagent (FC reagent, mixed with distilled water in a 1:2 ratio), L-Tyrosine, Magnesium Oxide, Ethanol, Sulphuric Acid, Ammonia, Boric Acid, Potassium Carbonate, and Phosphate Buffer Saline, were of analytical grade

Methods

Total Volatile Basic Nitrogen (TVBN)

TVBN was performed by micro diffusion technique described by Pearson (1968) [9]. Boric acid reagent 100 ml alcohol (95%) was stirred with 5 g boric acid and then 350 ml distilled water was added to it. After the acid has dissolved, 5 ml indicator (0.66% methyl red and 0.33% bromocresol green in alcohol) was added. Alkali (40% NaOH) was added until faint reddish colour was produced, and the mixture was made up to the volume with alcohol to 500 ml. 50 g of minced paneer sample was thoroughly triturated in a mortar with 2.5 g of powdered trichloroacetic acid. The mixture was allowed to stand as such at room temperature covered with aluminium foil for 30 minutes and filtered with muslin cloth. The filtrate thus obtained was re-filtered through a Whatmann filter paper No. 40 using glass funnel. Thoroughly cleaned, dried Conway micro diffusion unit was taken and 2 ml of boric acid reagent was added in its central compartment. One ml of paneer filtrate was accurately pipetted into its outer compartment. Then the cover lid was put in such a way that only small portion of outer compartment sufficient to insert the pipette remained open and 1ml of saturated potassium carbonate solution was then added through the gap. The lid was immediately closed without leaving any space and for better sealing petroleum jelly was applied over the rim. The lid was rotated manually to ensure proper mixing of paneer extract with saturated potassium carbonate solution and then incubated at 37 ºC for 3-4 hrs. During incubation the apparatus was again rotated 2 to 3 times.

After incubation, the boric acid solution in the central compartment (faint reddish colour of boric acid regent changed to green colour) was titrated with 0.02N sulphuric acid. The diffusion was carried out in triplicate along with blank. TVBN content was calculated using the following formula:

TVBN (mg/100 g) = Vol. of 0.02 N sulphuric acid consumed × Normality of acid used for titration × 14×100

Ammonia level

The ammonia level in paneer during storage period was determined as per the method prescribed by Sastry *et al*., (1999) [10]. 5 g of paneer sample was mixed with 50 ml of distilled water and homogenized for 2 min. at 10000 rpm. The homogenate obtained was filtered with Whatmann filter paper No. 1 and filtrate was obtained. The 40 ml of filtrate along with freshly prepared 10 ml of 10% Sodium hydroxide and magnesium oxide @ of 25 mg/ml of filtrate was added in distillation cylinder. The distillation was performed in a KEL plus distillation unit (classic DX, Pelican EquipmentPvt. Ltd., Chennai) and 25 ml of distillate was collected in a beaker having 25 ml of Tashiro indicator, the colour of which changed to green on collection of distillate having ammonia. The distillate thus obtained was titrated with 0.1N Sulphuric acid till the end, manifested by reappearance of pink colour. A blank was run in the same manner but without filtrate and titrated. The amount of ammonia was calculated by the formula as follows:

$$\text{Ammonia (mg/100 g) in Paneer} = \frac{100 \times Y \times (A-B) \times 0.0014}{X \times W}$$

Y= Volume (ml) of filtrate made up to
X= Volume (ml) of aliquot taken for distillation
A= Volume (ml) of 0.1N sulphuric acid used for titration of test sample
B= Volume (ml) of 0.1N sulphuric acid used for titration of blank
W= Weight (g) of sample taken for estimation

Tyrosine value

The procedure of Strange *et al*., (1977) [14] was followed for the estimation of tyrosine value. 20 g of paneer was blended with 100 ml of pre cooled 20% TCA solution for 2 min in homogenizer. The homogenate was filtered through the whatman filter paper No. 42 and TCA extract was collected in a test tube. To estimate tyrosine value, 2.5 ml of TCA extract was mixed with equal amount of distilled water and 10 ml of freshly prepared 0.5 N sodium hydroxide was added to it and kept for 10 min. Then 3 ml of Folin Ciocalteus reagent (FC reagent and distilled water in the ratio of 1:2) was added to it and shaken well. The mixture was then allowed to stand undisturbed for 30 minutes in a dark place for colour development. The optical density of mixture was measured at 730 nm. Tyrosine value was calculated as mg of tyrosine per 100 g paneer sample by referring to a standard graph, prepared as described below.

Standard graph: A stock solution of tyrosine (100 µg/ml) was prepared by dissolving 10 mg of L- tyrosine in 100 ml of distilled water. From the stock solution of tyrosine 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml of tyrosine was taken in different test tubes and final volume of all the test tubes was made up to 5 ml by the addition of requisite amount of distilled water. A blank was prepared by adding 5 ml of distilled water without tyrosine stock solution. To all the test tubes 2.5 ml of freshly prepared 0.5N sodium hydroxide was added and allowed to stand for 10 min. 3ml of Folin Ciocalteaus reagent (FC reagent and distilled water in the ratio of 1:2) was added and test tubes were kept in dark for development of greenish blue colour. After 30 minutes the O.D. was measured at 730 nm in a UV-1800 Shimadzu spectrophotometer against blank. A standard graph was prepared by potting the O.D. value on Y-axis and respective concentration of standard tyrosine on X-axis.

Total plate count

23.5 g plate count agar was suspended in 1000 ml distilled water and boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure at 121 ºC for 15 min. Final pH of the medium was 7.0±0.2. Duplicate sets of petri plates were inoculated aseptically with 1 ml of aliquots from appropriate dilutions. About 20 ml of plate count agar, melted and maintained at 44-46 ºC was poured gently. The

plates were incubated at 37±1 ºC for 48 hours. Plates showing 30-300 colonies were counted. The number of colonies were multiplied with reciprocal of the dilution and expressed as log_{10}cfu/g (BAM, 2001) [2].

Psychrophilic count

The plates were prepared similar to that of total plate count but incubated at 4±1 ºC for 14 days. The number of colonies were multiplied with reciprocal of the dilution and expressed as log10cfu/g.

Pseudomonas count

48.40 g of Pseudomonas agar base powder was dissolved in distilled water. To this 10 ml of glycerol was added and volume was made up to 1000 ml by addition of distilled water and agar was allowed to dissolve completely. It was then sterilized by autoclaving at 15 lbs pressure at 121 ºC for 15 minutes. The agar was then cooled to 50 ºC and sterile cetrimide was added to it. Duplicate sets of petri plates were inoculated aseptically with 1 ml of aliquots from appropriate dilutions. About 20 ml of Peudomonas agar, melted and maintained at 44-46 ºC was poured gently. The plates were incubated at 25±1 ºC for 48 hours. Plates showing 30-300 colonies were counted. The number of colonies were multiplied with reciprocal of the dilution and expressed as log_{10}cfu/g (IS 14843: 2000).

Statistical analysis

The experiments were repeated three times and the data generated for different quality characteristics were compiled and analyzed using SPSS (version 17.0 for windows; SPSS, Chicago, III., U.S.A.) with three replicates (n=3). The data were subjected to analysis of variance, (one way ANOVA for paneer quality parameters at refrigerated storage), Pearson coefficient of correlation (to ascertain correlation between selected parameters) and regression analysis (for regression coefficient and equation) and the level of significance was reported at (P<0.05).

Results and Discussion

Total plate count, Psychrophillic count, pseudomonas count, pH, Tyrosin, TVBN ammonia and Thiobarbituric acid value of paneer stored under refrigerated condition (4±1ºC) were evaluated on day 0, day 2, day 4, day 6 and day 8. Total plate count of paneer increased throughout the storage period of paneer from 3.9 to 7.27 log_{10} cfu/g, as shown in Table 1 and Figure 1. The association between total plate count and chemical parameters was established by analyzing the Pearson coefficient correlation. TPC showed a strong negative correlation of -0.927 with pH, a positive correlation of 0.857 with tyrosine, 0.815 with total volatile basic nitrogen, 0.833 with ammonia, and 0.916 with TBA. All other parameters showed positive correlations with the total plate count, except for pH, which exhibited a negative correlation, as shown in Table 5. The pH of paneer decreased significantly (p<0.05) throughout the refrigerated storage period, as presented in Table 3. Similar findings were reported by Trupti *et al*. (2019) [16] and Karunamay *et al*. (2020) [8]. TPC of paneer samples on the sixth day of refrigerated storage exceeded the recommended microbiological criterion of 5.34 log_{10} cfu/g, as set by the Food Safety and Standards Authority of India (FSSAI). On the eighth day, the TPC value reached 7.27±0.06 log10 cfu/g, exceeding the recommended microbial limit as per FSSAI. Microbial spoilage is a significant concern for paneer, as it has a limited shelf life under refrigerated conditions. The first indication of spoilage in paneer is the production of off-odors, which become apparent when microbial numbers reach approximately 6.83 log_{10} cfu/g.

The association between psychrophilic count and chemical parameters was established through the analysis of Pearson correlation coefficients. The psychrophilic count exhibited a correlation coefficient of -0.910 with pH, 0.903 with tyrosine, 0.804 with total volatile basic nitrogen, 0.789 with ammonia, and 0.893 with TBA. All other parameters showed positive correlations with the psychrophilic count, except for pH, which exhibited a negative correlation, as shown in Table 5. A similar positive correlation was recorded by Didolkar (2018) [3] in their study on chicken meat, where they observed a significant positive correlation between psychrophilic count, tyrosine, TVBN, and ammonia.

The association between *pseudomonas* count and chemical parameters was established through analyzing pearson coefficient correlation. *Pseudomonas* count showed a correlation coefficient of -0.909 with pH, 0.904 with tyrosin, 0.803 with total volatile basic nitrogen, 0.799 with ammonia and 0.902 with TBA. All other parameters are positively correlated with *pseudomonas* count except pH which is negatively correlated with *pseudomonas* count as shown table 5. Similar positive correlation recorded by Didolkar (2018) [3] studied on studied on chicken meat observed significant positive correlation between pseudomonas count with tyrosin, TVBN and ammonia.

The degree of autolysis and bacterial proteolysis in paneer can be quantified by measuring the tyrosine value, which reflects the presence of amino acids, specifically tyrosine and tryptophan, in a paneer extract. The increase in tyrosine value during refrigerated storage may be primarily attributed to intrinsic (autolysis) changes in paneer and partly to bacterial activity. No significant difference (p<0.05) was observed between the 0 and 2nd day, 4th and 6th day, and 6th and 8th day of storage. However, a significant (p<0.05) difference in tyrosine value was noted between the 2nd and 4th day and the 4th and 8th day results are presented in Table 3 and Figure 2. Similar findings were observed in a study conducted by Shukla (2014) [4], where the tyrosine value of refrigerated (4±1 ºC) beef, packaged with a bromophenol blue-based indicator sensor, significantly increased (p<0.05) during the storage period.

TVBN values resulting from the spoilage of paneer were 0, 0, 0, 12.13±0.9, and 14±0.0 on days 0, 2, 4, 6, and 8, respectively. A significant difference was observed between day 6 and day 8, as indicated in Table 3 and Figure 2. Silva and Gloria (2002) reported that the increase in TVBN levels is attributed to the formation of ammonia and other volatile amines. Fraqueza *et al*. (2008) [5] also linked TVBN as one of the primary chemical parameters influencing the growth of microorganisms, including Pseudomonas. The significant (p<0.05) increase in TVBN values observed during the storage of paneer can be attributed to the heightened deamination of amino acids by the bacterial flora.

The ammonia content of packaged paneer under refrigerated storage increased significantly (p<0.05) during the storage period from day 0 to day 8. No significant differences were observed between day 0, day 2, and day 4, but significant differences were observed between day 6 and day 8, as illustrated in Table 3 and Figure 2. This increase in ammonia production can be attributed to the deamination of amino acids during spoilage. The accumulation of easily hydrolysable nitrogen over the storage period results from protein hydrolysis. Proteins from paneer can be partially or completely degraded into simple compounds such as CO_2, H_2O, NH_3, H_2S, etc. Significant differences (p<0.05) were observed throughout the storage period. These research

findings align with Surmei *et al*., (2013) [15] study, which reported an increase in easily hydrolysable nitrogen from 15.10 mg NH_3/100g to 35.58 mg NH_3/100g in thigh muscle packaged in stretch trays with a modified atmosphere and stored for a period of 10 days under refrigerated conditions."
The TBA value of packaged paneer increased significantly (p<0.05) during the storage period. This increase in TBA value is likely attributed to the growth of lipolytic organisms and the oxidation of fatty acids.

Sensory evaluation: The sensory qualities of paneer during refrigerated storage were assessed based on appearance, color, odor, and sliminess, and the results were recorded and are presented in Table 6. These assessments were conducted alongside the measurement of physicochemical and microbial parameters, as shown in Table 1 and Table 3.

Comparative study
A clear correlation was observed between the microbial quality of stored paneer and the chemical indicators derived from the stored paneer concerning sensory evaluation, as presented in Table 8. A positive and significant (p<0.01) correlation was identified between chemical indicators such as TVBN, tyrosine, ammonia levels, and microbial growth, in relation to the sensory evaluation of paneer. Adams and Moss (2000) [1] also reported a similar positive correlation between total plate count and pH, tyrosine, ammonia, TVBN, psychrophilic count, and Pseudomonas count, with a notably strong positive correlation observed with Pseudomonas count. Additionally, when microbial numbers reached levels of approximately 10^8cfu/cm², another indication of spoilage became evident, characterized by visible slime. This finding aligns with the observations made by Shukla (2015) [12].

Table 1: Microbial analysis of Paneer packaged with synthetic Indicator sensor stored under refrigerated condition (4±1°c)

Parameter	0 Day	2 Day	4 Day	6 Day	8 Day
Total Plate count	3.9 ± 0.08^{d}	5.16 ± 0.07^{c}	5.87 ± 0.3^{b}	6.83 ± 0.2^{a}	7.27 ± 0.06^{a}
Psychrophilic count	4.1 ± 0.06^{d}	5.1 ± 0.08^{c}	6.1 ± 0.04^{b}	6.6 ± 0.3^{b}	7.2 ± 0.05^{a}
Pseudomonas	3.6 ± 0.15^{d}	4.8 ± 0.31^{c}	6.2 ± 0.07^{b}	6.9 ± 0.26^{a}	7.5 ± 0.12^{a}

Means with different superscripts indicate significant (P<0.01) difference

Table 2: Analysis of variance for microbial analysis of paneer packaged with indicator synthetic indicator sensor at refrigerated storage (4±1ºC)

Parameters	Between groups		
	D. F.	MSS	F-Value
TPC	4	5.337	44.618
Psychrophilic	4	4.303	58.261
Pseudomonas	4	7.451	60.032

Table 3: Physicochemical analysis of paneer packed with synthetic indicator sensor stored under refrigerated condition (4±1°c)

Parameters	0 Day	2 Day	4 Day	6 Day	8 day
pH	6.6 ± 0.00^{a}	6.4 ± 0.06^{b}	6.3 ± 0.1^{c}	6.1±0.03c	5.8 ± 0.0^{d}
Tyrosin	0^{c}	0^{c}	8.7 ± 0.3^{b}	9 ± 0.3^{ab}	10 ± 0.5^{a}
TBA	0.03 ± 0.009^{d}	0.06 ± 0.003^{c}	0.08 ± 0.0^{b}	0.17 ± 0.007^{a}	0.19 ± 0.003^{a}
TVBN	0^{c}	0^{c}	0^{c}	12.13 ± 0.9^{b}	14 ± 0.0^{a}
Ammonia	0^{c}	0^{c}	0^{c}	12.3 ± 0.3^{b}	15.3 ± 0.7^{a}

Means with different superscripts indicate significant (p<0.05) difference

Table 4: Analysis of variance for physico-chemical parameters of Paneer packaged with indicator synthetic indicator sensor at refrigerated storage (4±1 ºC)

Parameters	Between groups		
	D. F.	MSS	F-Value
pH	4	0.358	9.017
Tyrosine	4	77.267	210.727
TVBN	4	154.971	296.500
Ammonia	4	175.600	439.000
TBA	4	0.014	184.000

Table 5: Correlation between physico-chemical and microbiological parameters of paneer packaged with Synthetic indicator sensor under refrigeration temperature

	TPC	PSY	PSEUDO	pH	TYROSIN	TVBN	AMMONIA	TBA
TPC	1	.917(**)	.932(**)	-.927(**)	.857(**)	.815(**)	.833(**)	.916(**)
PSY	.917(**)	1	.972(**)	-.910(**)	.903(**)	.804(**)	.789(**)	.893(**)
PSEUDO	.932(**)	.972(**)	1	-.909(**)	.904(**)	.803(**)	.799(**)	.902(**)
pH	-.927(**)	-.910(**)	-.909(**)	1	-.805(**)	-.839(**)	-.859(**)	-.891(**)
TYROSIN	.857(**)	.903(**)	.904(**)	-.805(**)	1	.709(**)	.707(**)	.819(**)
TVBN	.815(**)	.804(**)	.803(**)	-.839(**)	.709(**)	1	.991(**)	.961(**)
AMMONIA	.833(**)	.789(**)	.799(**)	-.859(**)	.707(**)	.991(**)	1	.958(**)
TBA	.916(**)	.893(**)	.902(**)	-.891(**)	.819(**)	.961(**)	.958(**)	1

Table 6: Sensory evaluation of paneer packed with synthetic indicator sensor stored under refrigerated condition (4±1 °C)

Attributes	0 Day	2 Day	4 Day	6 Day	8 Day
Appearance	5 ± 0^{a}	4 ± 0^{b}	3.3 ± 0.3^{c}	2 ± 0^{d}	1 ± 0^{e}
Colour	5 ± 0^{a}	4 ± 0^{b}	3 ± 0^{c}	1.6 ± 0.6^{d}	1 ± 0^{d}
Odour	5 ± 0^{a}	4 ± 0^{b}	3.3 ± 0^{c}	2.3 ± 0.6^{d}	1 ± 0^{e}
Sliminess	5 ± 0^{a}	4.6 ± 0.3^{a}	3.6 ± 0.3^{b}	1.3 ± 0.3^{c}	1 ± 0^{c}

Means with different superscripts indicate significant (p<0.01) difference

Table 7: Analysis of variance for sensory evaluation of Paneer packaged with synthetic indicator sensor at refrigerated storage (4±1 ºC)

Parameters	Between groups		
	D. F.	MSS	F-Value
Appearance	4	7.567	113.500
Colour	4	8.067	30.250
Odour	4	7.100	53.250
Sliminess	4	10.433	52.167

Table 8: Correlation between chemical indicators and microbial parameters with respect sensory evaluation of stored paneer under refrigerated condition.

Days	TPC (log 10 CFU/g)	Psychrophilic (log 10 CFU/g)	Pseudomonas (log 10 CFU/g)	Tyrosine (mg/100 g)	Ammonia (mg/100 g)	TVBN (mg/100 g)	Sensory evaluation
0	3.9±0.08	4.1±0.06	3.6±0.15	0	0	0	Accepted
2	5.16±0.07	5.1±0.08	4.8±0.31	0	0	0	Accepted
4	5.87±0.3	6.1±0.04	6.2±0.07	8.7±0.3	0	0	Accepted
6	6.83±0.2	6.6±0.3	6.9±0.26	9±0.3	12.3±0.3	12.13±0.9	Rejected
8	7.27±0.06	7.2±0.05	7.5±0.12	10±0.5	15.3±0.7	14±0.0	Rejected

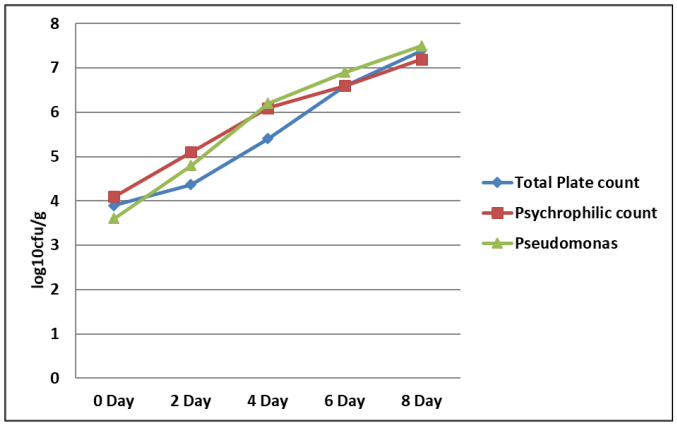

Fig 1: Microbial analysis of paneer packed with synthetic indicator sensor stored under refrigerated condition (4±1 °C)

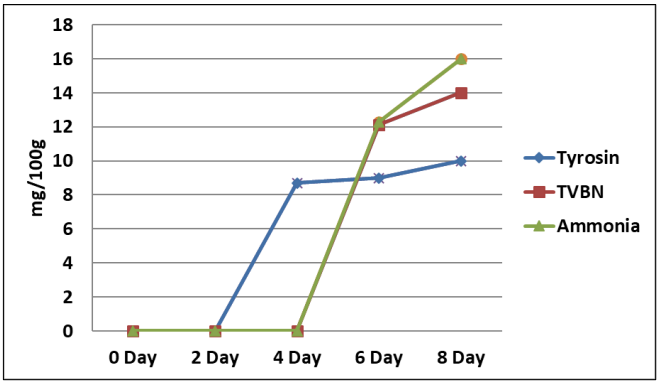

Fig 2: Physicochemical analysis of paneer packaged with synthetic indicator sensor under refrigerated storage

Conclusion

A significant correlation was observed between the microbiological quality and the concentration of chemical indicators in paneer concerning sensory evaluation during refrigerated storage. Microbial analyses are known to be both costly and time-consuming. Utilizing alternative methods based on chemical indicators can significantly reduce the time and cost required for the microbial analysis of paneer stored under refrigerated conditions.

Acknowledgement

The authors would like to extend their heartfelt gratitude to Dr. R. J. Zende, Principal Investigator of the National Agricultural Higher Education Project at the Indian Council of Agricultural Research, Centre for Excellence for Advanced Research on Animal Food Safety, Mumbai Veterinary College, Parel, Mumbai, Maharashtra Animal and Fishery Sciences University, Nagpur, India, for their invaluable financial support. Furthermore, the authors wish to express their sincere appreciation to the Department of Livestock Products Technology, Department of Veterinary Public Health, Department of Animal Nutrition, and Department of Veterinary Microbiology at Nagpur Veterinary College, Nagpur, for their invaluable contributions to this research. We also acknowledge the support provided by Visvesvaraya National Institute of Technology (VNIT Nagpur), India, which has played a crucial role in the success of this work. Once again, the authors extend their heartfelt thanks and appreciation for this support.

References

1. Adams MR, Moss MO. Food microbiology. Royal society of chemistry; c2000.
2. BAM F. Bacteriological analytical manual. Aerobic plate count, 2001, 3.
3. Didolkar DA, Badhe SR, Shukla VH, Yadav SR. Development of bromocresol purple based indicator sensor for monitoring broiler meat quality under refrigerated storage. Indian Veterinary Journal. 2018;95(8):70-73.
4. Food safety and standards (food products standards and food additives) regulations; c2011.
5. Fraqueza MJ, Ferreira MC, Barreto AS. Spoilage of light

(PSE-like) and dark turkey meat under aerobic or modified atmosphere package: Microbial indicators and their relationship with total volatile basic nitrogen. British Poultry Science. 2008;49(1):12-20.
6. Heeschen W, Tolle A, Wernery H, Reichmuth J. Determination of metabolites of bacterial activity in milk. Advances in Automated Analysis, Proceedings of the 1972 Technician International Congress. 1973;7:5-14.
7. Indian Standard Meat And Meat Products - Enumeration Of Pseudomonas SPP. IS 14843: 2000 ISO 13720: 1995
8. Karunamay S, Badhe SR, Shulka V, Jaiswal S. Effect of essential oil of clove and oregano treated edible packaging film in extending the shelf life of paneer. J Pharm Innov. 2020;9(7):317-322.
9. Pearson D. Methods related to protein breakdown. J Sci. Food Agri. 1968;19:366-369.
10. Sastry VRB, Kamra DN, Pathak NN. Estimation of ammonia nitrogen. Laboratory manual of Animal Nutrition; c1999.
11. Scindia Y, Silbert L, Volinsky R, Kolusheva S, Jelinek R. Colorimetric detection and fingerprinting of bacteria by glass-supported lipid/polydiacetylene films. Langmuir. 2007;23(8):4682-4687.
12. Shukla V, Kandeepan G, Vishnuraj MR. Development of on-package indicator sensor for real-time monitoring of buffalo meat quality during refrigeration storage. Food Analytical Methods. 2015;8:1591-1597.
13. Silva CM, Glória MBA. Bioactive amines in chicken breast and thigh after slaughter and during storage at 4±1 C and in chicken-based meat products. Food chemistry. 2002;78(2):241-248.
14. Strange ED, Benedict RC, Smith JL, Swift CE. Evaluation of rapid tests for monitoring alterations in meat quality during storage: I. Intact meat. J. Food Prot. 1977;40(12):843-847.
15. Surmei, Elena, *et al*. The quality of poultry meat in various type of packaging stored under refrigeration conditions. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Animal Science & Biotechnologies, 2013, 70(2).
16. Trupti P, Wanjari Shekhar Badhe, Vivek Shukla, Satish R Yadav, Vilas M Vaidya. Evaluation of Garlic Extract in Extending Shelf Life of Paneer: I. J of Livestock Research. 2019;9(7):100-110.