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Assessment of paneer quality during refrigerated storage

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Abstract

This study aimed to assess the physico-chemical and microbial quality of paneer during refrigerated storage at 4 ± 1 °C over an 8-day period. Various chemical parameters including pH, TBA (Thiobarbituric Acid), Tyrosin, TVBN (Total Volatile Basic Nitrogen), and Ammonia were monitored at alternate intervals from day 0 to day 8. Additionally, microbial analyses including Total Plate Count (TPC), Psychrophilic count, and Pseudomonas count were conducted. Results revealed a consistent increase in TPC, psychrophilic count, and pseudomonas count throughout the storage period. Significant increases ($p < 0.05$) were observed in Tyrosin value, TBA value, Total Volatile Basic Nitrogen, and Ammonia levels, while pH remained relatively stable. The TPC value exceeded recommended limits, reaching $6.04\pm 0.02 \log_{10}$ CFU/g by the end of the storage period. Microbial spoilage became evident as off-odors were noticeable when microbial numbers reached approximately $6.4 \log_{10}$ CFU/g. Sensory analysis scores of paneer showed a significant decrease ($p < 0.05$) with the passage of the storage period. These findings underscore the importance of proper refrigerated storage to maintain the quality and safety of paneer.

Keywords: Refrigerated paneer, total plate count, pseudomonas count, psychrophilic count, TVBN, ammonia, TBA

Introduction

Paneer, renowned for its mild acidic flavor and delicate sweetness, boasts a distinctive texture characterized by firmness, cohesiveness, and a spongy consistency with a smooth finish. Despite its culinary allure, paneer faces a notable challenge due to its inherent perishability, resulting in a limited shelf-life. Under refrigerated conditions, paneer typically maintains its quality for around 6 days, while exposure to ambient temperatures shortens this period to approximately 3 days. This susceptibility to spoilage has prompted heightened attention to food safety concerns, particularly in light of the growing trend towards health-conscious living, which influences consumer behavior.

In addressing these challenges, the physico-chemical and microbial analysis of paneer emerges as a critical area of study. The composition and properties of paneer undergo dynamic changes during storage, influenced by factors such as temperature, pH, and microbial activity. Physico-chemical parameters, including pH levels, moisture content, and lipid oxidation, play pivotal roles in determining the quality and safety of paneer. Additionally, microbial analysis is indispensable in assessing the proliferation of spoilage microorganisms, notably psychrotrophic bacteria

that thrive in refrigerated environments.

The spoilage of paneer primarily arises from the rapid growth of microorganisms, which leads to a cascade of physicochemical alterations resulting in the development of off-flavors. Microbial activity within paneer initiates biochemical transformations in its carbohydrates, proteins, and fats, contributing to the degradation of the product's quality (Heeschen, 1972) ^[9].

Presently, the quality assessment of paneer involves monitoring its microbiological, Physico-chemical and sensory characteristics throughout its storage period.

Materials and Methods

Materials

All chemical reagents, such as Plate Count Agar and Cetrimide (procured from SRL, Virion Enterprises, Mumbai, Maharashtra, India), 0.5 N Sodium Hydroxide, Trichloroacetic Acid, Folin and Ciocalteu's Reagent (FC reagent, diluted 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

TVBN was conducted using the micro-diffusion technique outlined by Pearson (1968) [14]. To prepare the sample, 50 g of minced paneer was thoroughly triturated in a mortar with 2.5 g of powdered trichloroacetic acid. The mixture was left to stand at room temperature, covered with aluminum foil, for 30 minutes and then filtered using muslin cloth. The filtrate was further filtered through Whatman filter paper No. 40 using a glass funnel.

A clean and dry Conway micro-diffusion unit was utilized, into which 2 ml of the boric acid reagent was added to the central compartment. One ml of the paneer filtrate was accurately pipetted into the outer compartment. The cover lid was positioned to leave only a small opening sufficient for the pipette, through which 1 ml of saturated potassium carbonate solution was added. The lid was then tightly closed, and petroleum jelly was applied around the rim for better sealing. The lid was manually rotated to ensure proper mixing of the paneer extract with the saturated potassium carbonate solution, and the unit was incubated at 37 °C for 3-4 hours. During incubation, the apparatus was rotated 2 to 3 times. After incubation, the boric acid solution in the central compartment (faint reddish colour of boric acid reagent 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:

$$\text{TVBN (mg/100 g)} = \text{Vol. of 0.02 N sulphuric acid consumed} \times \text{Normality of acid used for titration} \times 14 \times 100$$

The ammonia content in paneer during the storage period was determined following the method outlined by Sastry *et al.* (1999) [16].

To estimate the tyrosine value, 2.5 ml of the TCA extract was mixed with an equal amount of distilled water, and then 10 ml of freshly prepared 0.5 N sodium hydroxide solution was added. This mixture was allowed to stand for 10 minutes. Subsequently, 3 ml of Folin Ciocalteus reagent (prepared by mixing FC reagent and distilled water in a 1:2 ratio) was added, and the mixture was shaken well. It was then left undisturbed for 30 minutes in a dark place for color development. The optical density of the mixture was measured at 730 nm. The tyrosine value was calculated as milligrams of tyrosine per 100 grams of paneer sample by referring to a standard graph, which was prepared as described below.

A stock solution of tyrosine (100 µg/ml) was prepared by dissolving 10 mg of L-tyrosine in 100 ml of distilled water. From this stock solution, varying volumes of tyrosine (0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml) were pipetted into different test tubes, and the final volume in each test tube was adjusted to 5 ml by adding the appropriate amount of distilled water. A blank solution was prepared by adding 5 ml of distilled water without the tyrosine stock solution.

To each test tube, 2.5 ml of freshly prepared 0.5N sodium hydroxide solution was added and allowed to stand for 10 minutes. Subsequently, 3 ml of Folin Ciocalteus reagent (prepared by mixing FC reagent and distilled water in a 1:2 ratio) was added to each test tube, and the tubes were placed in the dark for the development of a greenish-blue color. After 30 minutes, the optical density (O.D.) of each solution was measured at 730 nm using a UV-1800 Shimadzu spectrophotometer against the blank.

Microbial analysis

Plate count agar was formulated by dispersing 23.5 grams of agar in 1000 ml of distilled water, then boiling until fully dissolved. The medium underwent sterilization through autoclaving at 15 lbs pressure at 121 °C for 15 minutes. The pH of the medium was subsequently adjusted and maintained at 7.0±0.2. Duplicate sets of petri plates were aseptically inoculated with 1 ml aliquots from appropriate dilutions. Approximately 20 ml of melted plate count agar, kept at 44-46 °C, was poured gently into each plate. Following incubation at 37±1 °C for 48 hours, colonies within the range of 30 to 300 were enumerated. Colonies ranging from 30 to 300 were counted on each. The colony count was multiplied by the reciprocal of the dilution factor expressed as log₁₀ cfu/g (BAM, 2001) [2].

The plates were prepared similarly to those for the total plate count, but they were incubated at 4±1 °C for 14 days. Colonies were counted, and the number was multiplied by the reciprocal of the dilution and expressed as log₁₀ cfu/g.

48.40 grams of Pseudomonas agar base powder was dissolved in distilled water. Then, 10 ml of glycerol was added, and the volume was adjusted to 1000 ml with distilled water to ensure complete dissolution of the agar. The mixture was sterilized by autoclaving at 15 lbs pressure at 121 °C for 15 minutes. After sterilization, the agar was cooled to 50 °C, and sterile cetrimide was added.

Duplicate sets of petri plates were aseptically inoculated with 1 ml aliquots from appropriate dilutions. Approximately 20 ml of melted Pseudomonas agar, kept at 44-46 °C, was gently poured into each plate. The plates were then incubated at 25±1 °C for 48 hours. Colonies ranging from 30 to 300 were counted on each plate. The number of colonies was multiplied by the reciprocal of the dilution and expressed as log₁₀ cfu/g (IS 14843: 2000) [10].

Statistical analysis

The experiments were replicated three times, and the data obtained for various quality characteristics were compiled and analyzed using SPSS (version 17.0 for Windows; SPSS, Chicago, Illinois, USA) with three replicates (n=3). The data underwent analysis of variance (one-way ANOVA for paneer quality parameters during refrigerated storage), Pearson coefficient of correlation (to determine correlation between selected parameters), and regression analysis (for regression coefficient and equation). The significance level was set at $p < 0.05$.

Results and Discussion

The pH of the paneer, packaged with the natural indicator sensor, showed a significant decrease ($p < 0.05$) during refrigerated storage (see Table 1). These results are consistent with the findings of Karunamay *et al.* (2020) [11]. Walstra and Jenness (1984) [21] explained that the addition of acid to hot milk leads to a decrease in pH and the solubilization of calcium ions. This process destabilizes casein micelles, ultimately resulting in the formation of a coagulum.

Bhattacharya *et al.* (1971) [3] noted that paneer typically possesses high moisture content (53–55 g/100 g) and falls within a pH range of 5.4–5.9, characteristics that make it prone to rapid spoilage and limit its shelf-life to 1–2 days at room temperature and 5–6 days under refrigeration. The

decrease in pH observed during paneer storage can be attributed to the fermentation of lactose into lactic acid.

The tyrosine value of paneer packaged under refrigerated conditions significantly increased throughout the storage period, as indicated in Table 1. The degree of autolysis and bacterial proteolysis in paneer can be quantified by measuring the tyrosine value, which reflects the concentration of amino acids, specifically tyrosine and tryptophan, in a paneer extract. The observed rise in the tyrosine value during refrigerated storage primarily stems from intrinsic changes (autolysis) within the paneer matrix and, to some extent, bacterial activity (Elango *et al.*, 2010; Rai *et al.*, 2008; Sindhu *et al.*, 2000) [5, 15, 18].

Remarkably, no significant differences ($p < 0.05$) were observed between the 0 and 2nd day, the 4th and 6th day, or the 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 between the 4th and 8th day of storage, as illustrated in Table 1.

The increase in TV (Total Volatile Basic Nitrogen) in the control sample during storage can be attributed to the elevated microbial load and the enhanced production of proteolytic enzymes during the late logarithmic phase of microbial growth, leading to autolysis and bacterial proteolysis (Dainty *et al.*, 1996) [4]. These findings align with those of Karunamay *et al.* (2020) [11], who demonstrated that the tyrosine content value of stored paneer progressively increased throughout the storage period. Similarly, Rai *et al.* (2008) [15] investigated the effects of Modified Atmosphere Packaging (MAP) and Vacuum packaging on the storage ability of paneer. They observed a progressive increase in the tyrosine content value, with values rising from 12.61 (mg/100 g) to 34.80, 29.18, and 33.21 for samples packaged under vacuum, MAP (100% CO₂), and MAP (100% N₂) conditions, respectively. Similarly, Sindhu *et al.* (2000) [18] reported an increase in tyrosine content (mg/g) from 0.14 to 0.42 for buffalo milk paneer and from 0.12 to 0.47 for cow milk paneer when stored for 30 days at temperatures below 10 °C. This rise in tyrosine content indicates proteolysis resulting from microbial spoilage.

The TVBN content of paneer packaged under refrigerated conditions significantly increased during storage, as depicted in Table 1. Silva and Gloria (2002) [22] suggested that the elevation in TVBN levels is linked to the formation of ammonia and other volatile amines. Additionally, Fraqueza *et al.* (2008) [23] associated TVBN with microbial growth, particularly by microorganisms such as *Pseudomonas*. The significant ($p < 0.05$) increase in TVBN values during paneer storage can be attributed to the heightened deamination of amino acids by the bacterial flora.

The ammonia content of paneer packaged under refrigerated conditions increased significantly throughout the storage period. This observed increase in ammonia production can be attributed to the deamination of amino acids during spoilage. The accumulation of easily hydrolysable nitrogen with prolonged storage results from protein hydrolysis, where proteins from milk and milk products are partially or completely degraded into simpler compounds such as NH₃, H₂S, and amines (Osman and Faruk, 2016; Dainty, 1996) [13, 4]. Notably, a significant ($p < 0.05$) difference in ammonia

content was observed throughout the storage period, as depicted in Table 1 and Fig 1.

The TBA (Thiobarbituric Acid) value of paneer packaged under refrigerated conditions increased significantly throughout the storage period. Similar findings were reported by Wagh *et al.* (2014) [24], who observed that as storage progressed, cheddar cheese samples exhibited significantly higher TBA values and a faster oxidation rate ($p < 0.05$). These results are consistent with the findings of Karunamay *et al.* (2020) [11], who also reported an increase in TBA value throughout the storage period.

The Total Plate Count (TPC) values of paneer ranged from 3.6±0.07 on day 0 to 6.04±0.02 on day 8, as presented in Table 3 and Fig 2. The TPC values for refrigerated paneer significantly increased ($p < 0.05$) as the storage duration advanced. By the sixth day, the TPC value reached 5.02. It's worth noting that even on the sixth day, the TPC of paneer samples stored under refrigeration remained below the recommended microbiological criterion of 5.34 log₁₀ CFU/g set by the Food Safety and Standards Authority of India (FSSAI). However, by the eighth day, the TPC value exceeded this recommended limit, reaching 6.04±0.02 log₁₀ CFU/g. Microbial spoilage is a significant concern for paneer, as it has a limited shelf life under refrigerated conditions, with off-odors becoming noticeable when microbial numbers reach approximately 6.4 log₁₀ CFU/g.

The psychrophilic count of refrigerated paneer showed a significant increase ($p < 0.05$) as the storage days passed. However, the increase in psychrophilic count was non-significant up to day 4; thereafter, the psychrophilic count significantly increased on day 6. Interestingly, no significant ($p < 0.05$) difference was observed between day 0 and day 2, or between day 2, day 4, and day 6. However, significant differences were observed between day 0 and day 6, as well as day 8, as indicated in Table 3 and Fig. 2.

It's worth noting that the water used for dipping paneer can serve as an important source of psychrotrophic microorganisms. Bambha (1988) [25] reported a steady increase in psychrotrophic, proteolytic, and lipolytic counts in market paneer samples during storage at 7 °C for up to 7 days. Subsequently, the counts increased tremendously. These findings were well-supported by the findings of Karunamay *et al.* (2020) [11] who reported a significant increase in psychrophilic count in paneer during refrigerated storage conditions.

The *Pseudomonas* count of refrigerated paneer showed a non-significant difference ($p < 0.05$) up to the 6th day of storage. However, it was found to be significant on the 8th day (see Table 3 and Fig. 2). These results are in line with the findings of Lu and Wang (2017) [26], who observed milk spoilage within five days under temperatures ranging from 3-7 °C, even if the milk initially contained only one *Pseudomonas* per milliliter. These bacteria are known to produce extracellular heat-stable lipases, proteinases, and phospholipases, which can impact the quality of various milk products such as UHT milk, butter, cheese, and fluid milk products.

The appearance score of paneer decreased significantly ($p < 0.05$) as the storage period progressed, with changes in the color of the indicator sensor showing significant ($p < 0.05$) differences throughout the storage period (see Table 5 and Fig. 3). Osman and Faruk (2016) [13] stated that

residual or reactivated heat-stable photolytic enzymes can cause instability of casein micelles and the appearance of bitter flavors in milk products. Gas formation may result in small spherical shiny appearances on the cheese.

Singh *et al.* (2014) [19] observed that the storage period significantly impacted the flavor, color, appearance, body, and texture scores of paneer. Similarly, Kristensen *et al.* (2001) [12] investigated the effect of temperature on the flavor, color, and oxidative stability of processed cheese,

while Hamid (2014) [27] studied significant variations in the color, flavor, taste, and texture of control and cumin oil-treated cheese during the storage period.

The scores for sliminess exhibited a significant decrease ($p < 0.05$) as the storage period progressed. Slimy appearance of paneer might be due to the growth of psychrophilic bacteria, according to Goyal and Goyal (2016) [8], who recorded a slimy appearance at the top of the paneer samples after 6 days of storage.

Table 1: Physicochemical analysis of paneer packed under refrigerated condition (4±1 °C)

Parameters	0 Day	2 Day	4 Day	6 Day	8 Day
pH	6.6±0.03 ^a	6.4±0.03 ^b	6.2±0.0 ^c	6.1±0.03 ^c	5.7±0.0 ^d
Tyrosin Mg/100 g	0 ^c	0 ^c	8.6±0.3 ^b	9.3±0.3 ^{ab}	10±0.0 ^a
TVBN Mg/100 g	0 ^c	0 ^c	0 ^c	7.4±0.93 ^b	11.2±0.0 ^a
Ammonia Mg/100 g	0 ^b	0 ^b	0 ^b	12.3±0.3 ^a	16±0.7 ^a
TBA Mg/1000 g	0.05±0.008 ^b	0.06±0.01 ^b	0.1±0.02 ^b	0.012±0.03 ^b	0.20±0.01 ^a

Means ± SE with different superscripts in a row indicate significant ($p < 0.05$) difference

Table 2: Analysis of variance Physicochemical analysis of paneer packed under refrigerated condition (4±1 °C)

Parameters	Between groups		
	D. F.	MSS	F-Value
pH	4	0.328	122.87
Tyrosine	4	44.26	4
TVBN	4	78.92	151
Ammonia	4	233.06	43.70
TBA	4	0.011	7.08

Table 3: Microbial analysis of paneer packaged under refrigerated condition (4±1 °C)

Parameter	0 Day	2 Day	4 Day	6 Day	8 Day
Total Plate count	4.1±0.07 ^c	4.2±0.03 ^{b c}	4.46±0.2 ^{bc}	4.6±0.2 ^b	5.3±0.02 ^a
Psychrophilic count	4.19±0.06 ^c	4.31±0.03 ^{bc}	4.98±0.3 ^{abc}	5.06±0.3 ^{ab}	5.75±0.3 ^a
Pseudomonas	3.9±0.02 ^b	4.1±0.03 ^b	4.6±0.3 ^b	4.6±0.2 ^b	6±0.6 ^a

Means ± SE with different superscripts in a row indicate significant ($p < 0.05$) difference

Table 4: Analysis of variance for microbial analysis of paneer packaged under refrigerated condition (4±1 °C)

Parameters	Between groups		
	D. F.	MSS	F-Value
TPC	4	0.761	9.283
Psychrophilic	4	1.204	5.874
Pseudomonas	4	1.824	5.569

Table 5: Sensory evaluation of paneer packed with natural indicator stored under refrigerated condition (4±1 °C)

Attributes	0 Day	2 Day	4 Day	6 Day	8 Day
Appearance	5±0 ^a	4.3±0.3 ^b	3.6±0.3 ^c	2±0 ^d	1±0 ^e
Colour	5±0 ^a	4.3±0.3 ^b	3±0 ^c	3±0 ^c	1±0 ^d
Odour	5±0 ^a	4.3±0.3 ^b	3.3±0.3 ^c	2±0 ^d	1±0 ^e
Sliminess	5±0 ^a	4.6±0.3 ^{ab}	4±0 ^b	3±0.6 ^c	1±0 ^d

Means ± SE with different superscripts in a row indicate significant ($p < 0.05$) difference

Table 6: Analysis of variance for sensory evaluation of Paneer packaged with natural indicator sensor at refrigerated storage (4±1 °C)

Parameters	Between groups		
	D. F.	MSS	F-Value
Appearance	4	8.267	62.000
Colour	4	7.067	106.000
Odour	4	8.100	60.750
Sliminess	4	7.767	29.125

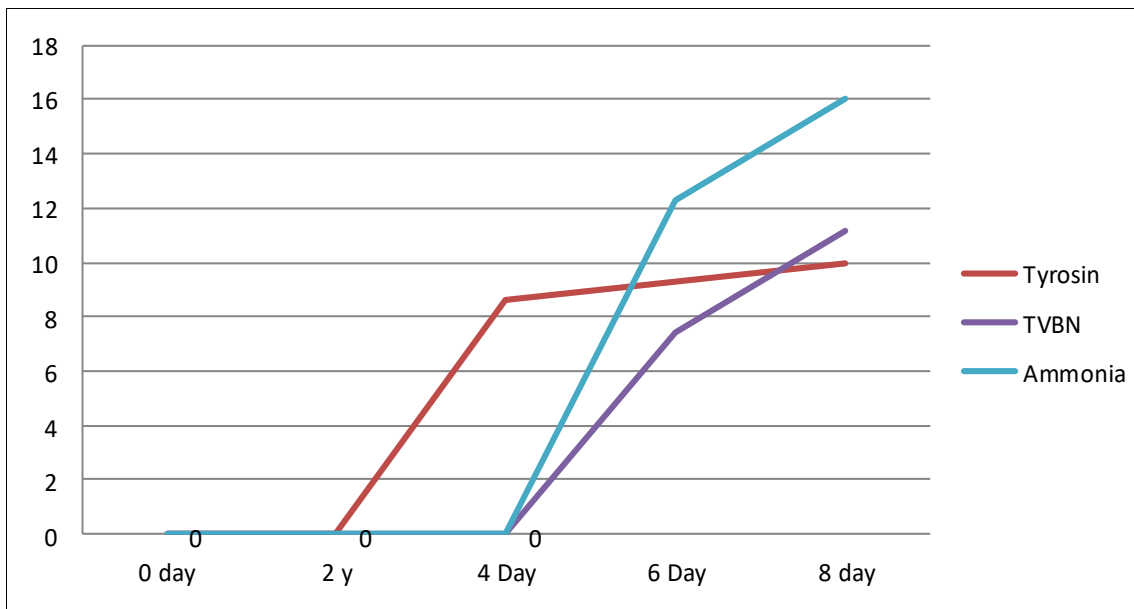


Fig 1: Physico-chemicals analysis of paneer packed under refrigerated temperature

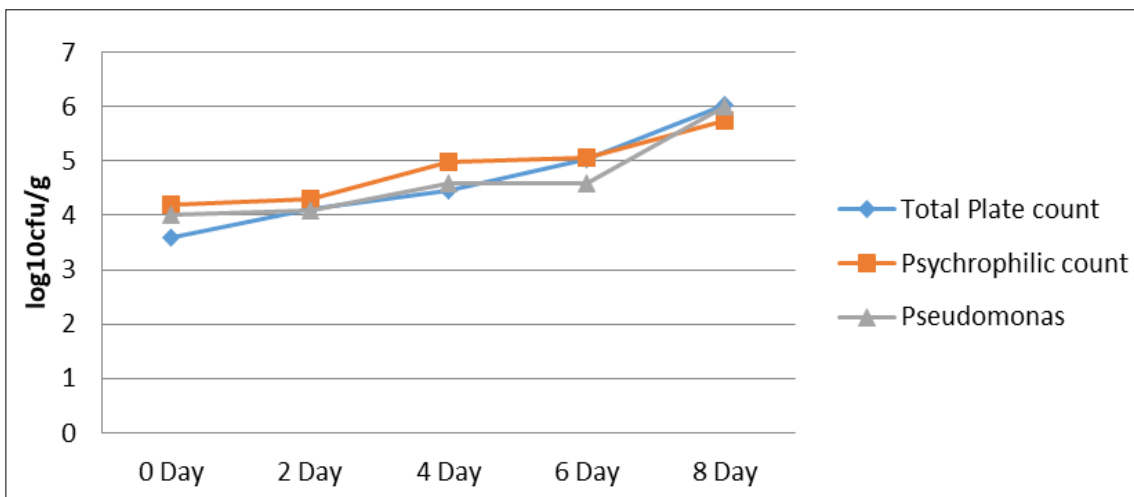


Fig 2: Microbial analysis of paneer packaged under refrigerated condition (4±1°C)

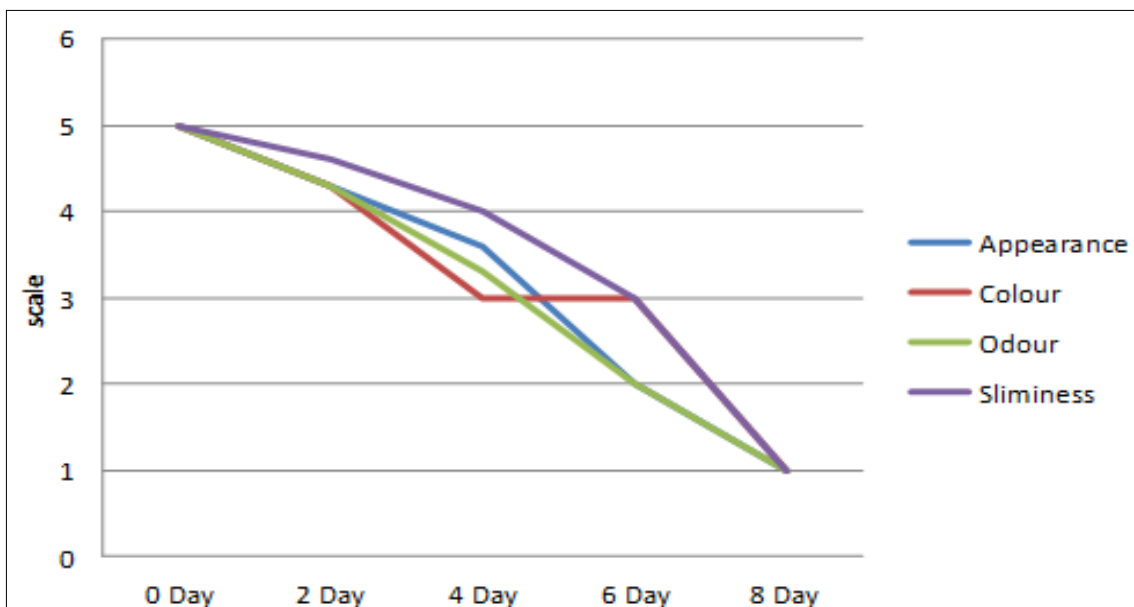


Fig 3: Sensory evaluation of paneer packed under refrigeration condition

Conclusion

In conclusion, the evaluation of paneer's quality during refrigerated storage revealed significant changes in various physicochemical and microbial parameters. The pH of paneer decreased significantly over time, correlating with previous studies. This decrease is attributed to the fermentation of lactose into lactic acid, resulting in the destabilization of casein micelles and coagulum formation. The increase in tyrosine value and TVBN content indicated autolysis and bacterial proteolysis, while the rise in ammonia content suggested amino acid deamination during spoilage. Additionally, the TBA value increased significantly, indicating lipid oxidation, while microbial counts, particularly psychrophilic and *Pseudomonas* counts, exhibited significant increases with storage duration. These findings underscore the susceptibility of paneer to microbial spoilage and the impact of storage conditions on its quality attributes.

Furthermore, sensory evaluation revealed a significant deterioration in appearance and sliminess scores over time, indicative of microbial growth and enzymatic activities. The observed changes in sensory attributes highlight the importance of monitoring paneer's quality throughout its shelf-life to ensure consumer satisfaction and safety. Overall, these findings emphasize the need for effective storage practices and quality control measures to prolong the shelf-life and maintain the quality of refrigerated paneer. The authors gratefully acknowledge the generous financial support provided by 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

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