ORIGINAL ARTICLE Open Access

ISSN 2278-1404
International Journal of Fundamental & Applied Sciences (IJFAS)

IMPACT OF FERMENTATION TIME ON NUTRITIONAL AND FUNCTIONAL QUALITY OF PROBIOTIC CURD

Nuthana Jain V [1], Monisha T[1], Shruti Awasthi[2], Preethi Rajesh[3], Chethana V Chalapathy[2*]
[1]PG Student, Department of Life Sciences, Garden City University, Bengaluru
[2]Professor, Department of Life Sciences, Garden City University, Bengaluru
[3]Professor and Head, Department of Life Sciences, Garden City University, Bengaluru
***Corresponding author email address**: chethana.v@gcu.edu.in

Paper Received: 19.12.2025 | Revised: 14.03.2026 | Accepted: 30.04.2026

DOI: https://doi.org/10.59415/ijfas.334 | ARK: https://n2t.net/ark:/26340/IJFAS.v15i1.334

Abstract
Fermented dairy products are a good source for probiotics with health benefits that are aligned with sustainable development goals. This study understands the effect of fermentation time on the nutritional constitutes and functional quality of commercial probiotic curd. Inoculum curd was collected from retail outlets and was incubated at 37°C and analyzed at 4 different fermentation time intervals: 0, 6, 12 and 24 hours. Probiotic viability was evaluated by standard plate count (SPC) method on MRS agar and phytochemical parameters - pH, titratable acidity, syneresis were analysed using standard methods. Nutritional analysis - reducing sugar and proteins were determined by standard techniques like titration and colorimetric techniques. This analysis is mainly focused to identify the best fermentation time for a probiotic survival which contains desired nutritional and functional qualities. By observing the microbial growth pattern, acidity, nutrient components over time helped us to understand the optimal fermentation time required to maintain both the product quality and potential health benefits. These insights are intended to guide the producers in designing and optimizing the fermentation protocol to enhance consumer health while also focusing on keeping consumers informed about the voice of probiotic dairy consumption. This research highlights how science-driven optimization of traditional dairy processing can contribute to local food sustainability, consumer health, and broader public health goals.

Keywords: Probiotic curd, fermentation time, nutritional quality, functional properties, dairy sustainability, physicochemical properties, functional foods.

1. INTRODUCTION

Probiotics are live bacteria that improve the balance of the gut microbiome when eaten in the right amounts, which is good for the health of the host organisms. (Byakika, S., et al 2019) They have the ability to produce antimicrobial agents such as lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, antifungal compounds, and bacteriocins that prevent the growth of pathogenic bacteria in the gut. (Periyatt Veettil, S., 2025) They have applications in diet due to their higher resistance and better adhesion capabilities in the gut, thereby enhancing the gut health. (Kumar, R., et al 2020)

Fermented food products are identified as a principal component of ancestral diets throughout the world, especially in South Asia due to their nutritional value and probiotic efficiency. Among all these foods, curd stands one step ahead, as it is commonly included in everyday meals. Eventually, curd has advanced from a simple ancestral food to a peer-reviewed, nutrient-rich food due to its wide probiotic efficiency, where suitable amounts provide greater health benefits when consumed. They contribute to an increased potential to enhance gut health, intensify immunity, and also mitigate various diseases, preventing urinary, vaginal, gastrointestinal, and respiratory infections, and improving the health of the cardiovascular system. (Merenstein, D. J., et al 2024) Certainly, foods with enhanced probiotics are

gaining popularity across the global food industry. As such, probiotic curd is serving as an affordable, acceptable, and appetizing medium for providing these microbes to the human body.

The probiotic organisms are susceptible to various factors such as pH, temperature, acidity, oxygen and carbon dioxide content, and fermentation duration. (Terpou, A., et al 2019). Prolonged fermentation can negatively affect the microbial survival rate by altering the nutritional quality of the curd, and influencing the sensorial qualities such as texture, viscidity, and flavor which are crucial for consumption as well as therapeutics.

In spite of the wide availability of probiotic curd in the commercial market, the fermentation protocols for optimizing microbial viability, nutritional content, and desirable properties are not standardized. Most small-scale and some larger industries still depend on the fixed fermentation time without fully understanding the influence of time on probiotic viability.

This study aims to estimate the effect of fermentation time on the nutritional and functional properties of commercial probiotic curd by determining probiotic viability using the standard plate count (SPC), physiochemical characteristics such as pH, titratable acidity, viscidity, syneresis, and nutritional profile analysis, including moisture, reducing sugars, proteins, and fats, through standard titration and colorimetric estimation. The optimal fermentation time that maintains elevated levels of probiotic viability was identified by analyzing the microbial growth profile and nutritional changes during the fermentation process. This provides a clear understanding of how fermentation time affects product stability, health benefits, and overall quality.

These discoveries enhance traditional fermentation techniques, promoting improvements in the efficacy, sustainability, and medicinal applications of fermented food items based on experimental evidence. This also provides better knowledge to the consumers about the nutritional properties and the health benefits of probiotic products.

2. MATERIALS AND METHODS

2.1 Experimental Design

This experiment was designed to examine the impact of fermentation time on the nutritional and functional quality of commercial probiotic curd. The study was undertaken by time-course approach in which the curd sample were incubated under controlled laboratory conditions and analyzed them at predetermined intervals of time 0, 6, 12 and 24 hours

Parameters such as - microbial viability, physiochemical properties and nutritional constitutes were measured using standard methods. This time-based experiment enables us to track the changes occurring during the time of fermentation, thereby identifying the best duration for the probiotic survival while retaining the desirable nutritional and functional characteristics.

2.2 Sample collection and preparation

2. 2.1 Source of sample

The sample used in this study were Commercial probiotic curd cups collected from retail outlets located in Bangalore. Care was taken to ensure the sample collected was fresh, good quality and not contaminated for experimental analysis. To minimize the variability during the analysis curd samples belonging to the same brand and same production batch were chosen. This helped to maintain uniformity in the microbial content and product composition.

After procurement, the probiotic curd samples were stored at 4°C to prevent uncontrolled fermentation and preserve microbial activity. The samples were processed within the 24 hours of purchase to ensure freshness and to maintain reproducibility in the experimental results.

2. 2.2 Preparation of Experimental Units

For setting up the experiment, the fresh collected probiotic curd samples were taken and gently mixed under aseptic

conditions to achieve uniform texture. From the **fresh curd (**Aseptically mixed) approximately 60g was weighed and transferred to a sterile beaker and was covered with a foil wrapper to avoid contamination and for good fermentation. 4 jars were sterilized and autoclaved. Each jar represents an independent experimental unit for individual fermentation intervals. For proper identification, the jars were labeled as 0h, 6h, 12h and 24h. The 0h served as the base line with respect to others under controlled incubation of 37°C. This preparation ensured that all units started from the same initial conditions, enabling reliable comparison of changes across fermentation intervals.

3. FERMENTATION/INCUBATION CONDITIONS

Cow milk was used as a substrate for fermentation. The milk was boiled for 10-15 min to remove contaminating microorganisms and later was cooled until the temperature was brought to 37- 40°C, which is an ideal temperature for the fermentation of curd bacterial activity. Once the milk was cooled to optimum temperature, 20 - 25% (w/v) of fresh curd (Aseptically mixed) which served as a starter culture was inoculated into the warm milk. The inoculum was mixed thoroughly aseptically to ensure uniform distribution of probiotic bacteria.

The inoculated milk was incubated in a controlled environment at 37 °C. This incubation will allow the starter cultures to actively ferment lactose into lactic acid. The progressive accumulation of lactic acidification of the medium leads to the coagulation of casein proteins, thereby forming curd. The fermented curd samples were collected at regular intervals of time of 0h, 6h, 12h and 24h for microbial , nutritional and functional quality analysis.

4. MICROBIOLOGICAL ANALYSIS

The microbial viability of probiotic curd was analysed by standard plate count (SPC) method. The curd samples fermented at different intervals (0h, 6h, 12h and 24h) were serial diluted in sterile water eightfolds and plated on MRS agar (5.5 to 5.7) , a selective media for lactic acid bacteria. Plates were incubated with 100µl of the inoculum using a spread plate method at 37 °C for 48h in the microbial incubator, distinct colonies were counted and calculated at colony - forming units per gram (CFU/g). This analysis enables the evaluation of probiotic survival and development processes linking the fermentation time with microbial variability and quality.

5. PHYSICOCHEMICAL ANALYSIS

5.1 pH Measurement

The pH of the probiotic curd sample was analysed using a digital pH meter instrument with standard buffer solution of pH 4.0 and 7.0 to ensure accuracy. For each measurement 20 g of sample was taken. Reading was taken and analysed.

5.2 Titratable Acidity (TA)

Titratable acidity of the curd sample was conducted to determine the degree of lactic acid produced in probiotic curd during different fermentation time intervals. This parameter is a direct indication of curd quality. The acidity values were expressed as the percentage of lactic acid at each fermentation interval. The titratable acidity measurement will provide insights on the acidity kinetics, texture impact, probiotic viability and flavor development.

5.4 Syneresis (whey separation)

Synersis was conducted to evaluate whey separation which is an important indicator for analysing the stability and water holding capacity of the fermented probiotic curd. This analysis was conducted by a centrifuge method. This examination provides information on the gel contraction and liquid loss during fermentation. Higher syneresis% indicates weaker gel structure. This parameter was analysed at all fermentation time to check the functional quality of curd.

6. NUTRITIONAL ANALYSIS

6.1 Reducing Sugars (DNS Method)

Reducing sugar in curd was estimated using a standard dinitrosalicylic acid (DNS) method. In this method the free aldehyde or the keto groups of the reducing sugar react with 3,5- dinitrosalic acid under heat of 90-100°C for 5-10 minutes to form a reddish - brown coloured complex. The intensity was measured at 540 nm in a colorimeter. The measurement is directly proportional to the concentration of reducing sugar in the sample.

6.2 Protein Content (Biuret Method)

Protein content in the fermented curd was analysed using the biuret method. Approximately 2 g of thoroughly mixed curd was diluted with 10 ml of distilled water and mixed evenly. 10%TCA (Trichloroacetic acid) was added to precipitate out protein from the sample. The sample was now centrifuged at 5000 rpm for 10 minutes to obtain supernatant. Biurate was prepared by dissolving copper sulfate and sodium potassium tartare in 0.2N sodium hydroxide with potassium iodide as stabilizer. 1 ml of sample was mixed with 4 ml of biurate reagent in the test tube and was incubated for 10 min at room temperature, the colour change to violet was measured at 540nm. OD was taken to analyse the protein content present in the given sample.

7. RESULT

7.1 Microbiological Analysis

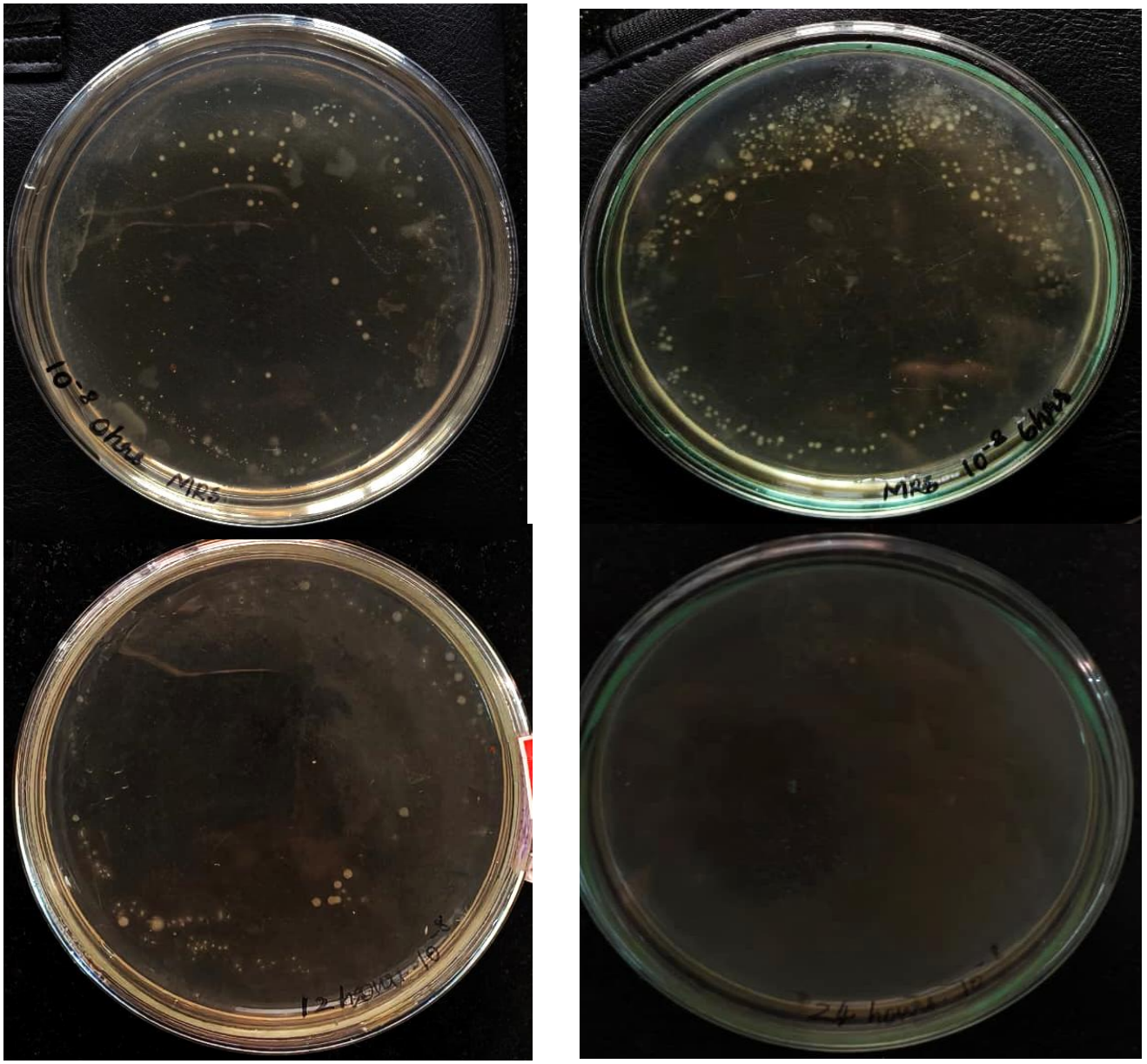
The standard plate count (SPC) showed a variation in the viability of the cell population of different time intervals. At 0h the probiotic count was 9.3×10^{10} CFU/ml showing the initial presence of probiotic bacteria in the fresh sample. A significant increase of 25.2×10^{10} CFU/ml was observed indicating rapid proliferation of probiotic organisms. By 12 h, variable count declined to 5.7×10^{10} indicating nutrient depletion and increase in acidity. At 24h the lowest count of 0.5×10^{10} was recorded which is a decline phase due to increased acidity.

$$\text{CFU/ml} = \frac{colonies\ counted \times dilution\ factor}{volume\ plated\ (ml)}$$

Dilution factor = reciprocal of the dilution plated

Fermentation time	Colonies number	Volume plated	CFU/ml
0h	93	0.1	9.3×10^{10}
6h	252	0.1	25.2×10^{10}
12h	57	0.1	5.7×10^{10}
24h	5	0.1	0.5×10^{10}

Table 1: Colony count (CFU/ml)



(C) (D)

Figure 1: Colony morphology and growth pattern of probiotic isolates on MRS agar plates at different fermentation times and dilutions.
(A) Plate showing microbial colonies from 10^{-8} dilution at 0 h (baseline, before fermentation).
(B) Plate showing microbial colonies from 10^{-8} dilution at 6 h of fermentation, with a higher number of visible colonies indicating active microbial growth
(C) Plate showing microbial colonies from 10^{-8} dilution at 12 h of fermentation, with moderate colony growth.
(D) Plate showing microbial colonies after 24 h of fermentation, with very few visible colonies, indicating decline in viable count.

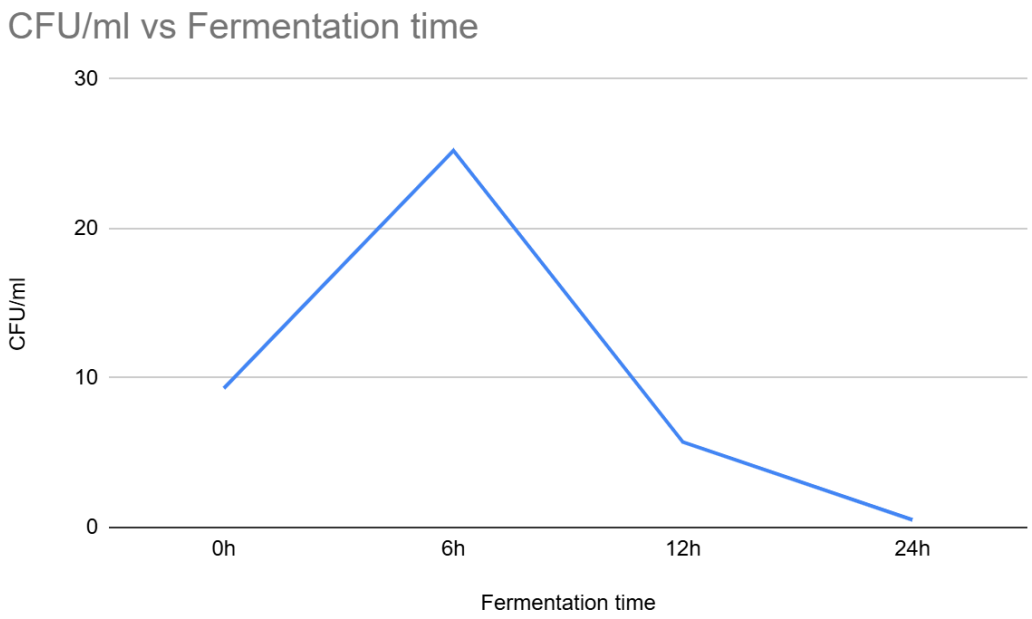


Figure 2 : Comparative study of probiotic viability (CFU/ml) in curd at different fermentation intervals (0, 6, 12, and 24 hours). The colony forming units increased at 6 hours of fermentation, followed by a standard decrease at 12 hours and 24 hours.

8. PHYSICOCHEMICAL ANALYSIS

8.1 pH Measurement

The pH of the curd sample showed decrease in pH with increase in fermentation time, reflecting the lactic acid production by probiotic microorganisms. At the initial stage of fermentation 0h the ph was nearly (pH 6.15) indicating fresh milk. At 6h the pH decreased to (pH 5.54), while 12h the ph was (pH 5.05), corresponding to the formation of the curd firm matrix. A ph of (pH 4.4) was recorded which is the lowest ph representing the maximum acidification and stabilization of curd in the experiment. Overall result confirms that extended fermentation time will directly link to increase in lactic acid in curd and decline in pH.

Time (h)	pH
0	6.15
6	5.54
12	5.05
24	4.4

Table 2: pH measurement

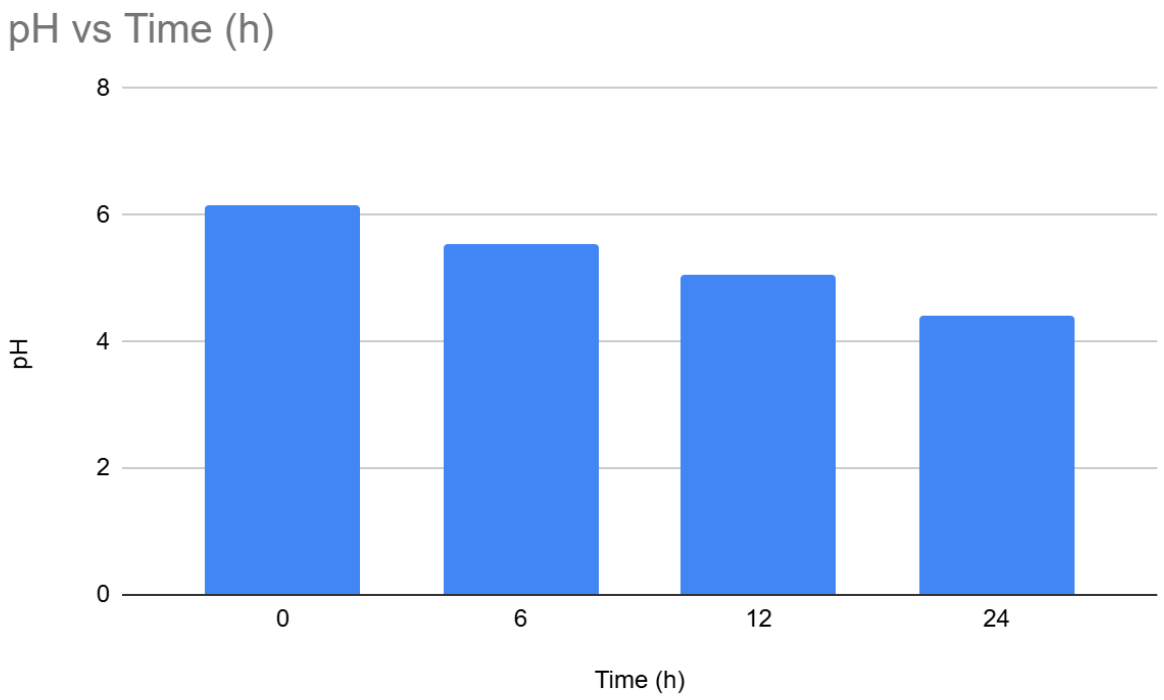


Figure 3: Graph on the effect of fermentation time on the pH. The pH was observed to decrease steadily with extended fermentation, showing an increase in the accumulation of lactic acid produced by the probiotics.

8.2 Titratable Acidity (TA)

The titratable acidity of the fermented curd showed increased acidity with prolonged fermentation time. At initial the acidity was relatively low corresponding to fresh milk with minimal microbial activity. As the fermentation started acidity rose consistently showing lactose conversion to lactic acid. In the mid and late stage of fermentation the acidity persistently increased. The observation of increase in the acidity is responsible for curd texture and flavor. Early acidification promoted coagulation and gel formation in curd , later acidification led to firmer texture , increased syneresis and tangy taste.

Titratable Acidity % = $\frac{V \times N \times Eq.wt \times 100}{sample\ volume\ (\ ml\ ) \times 1000}$

V = Volume of NaOH used

N= normality of NaOH

Fermentation time	Volume of NaOH (ml)	Normality of NaOH	Sample volume (ml)	Titratable acidity %
0h	1.3	0.1	1	1.17%
6h	2.3	0.1	1	2.07%
12h	2.5	0.1	1	2.25%
25h	2.7	0.1	1	2.43%

Table 3: Titratable Acidity

8.3 Syneresis (whey separation)

Syneresis of the fermented probiotic curd sample gradually increased with fermentation time , reflecting the change in gel matrix formation and water holding capacity. 20g of curd was used for analysis At the initial stage of 0h , whey separation was negligible (1.5 ± 0.2%) . Indicating a fresh and stable matrix. At 6h and 12h of fermentation %syneresis increased enhancing lactic acid production and maintaining protein network in the sample (4.8 ± 0.2% and 8.6 ± 0.6%) . The highest %syneresis was recorded at 24h fermentation indicating whey expulsion and weakened gel stability (13.2 ± 0.8%). This results brings a view on the impact of fermentation duration on the curd structure, effect on prolonged fermentation time of probiotic curd which leads to increased whey loss and reduced consumers acceptance.

%Syneresis= $\frac{W1}{W2}$×100

W1= weight of whey separated (g)

W2= initial curd weight (g)

Fermentation time	Initial weight (g)	Whey weight (g)	%syneresis
0h	20	0.30g	1.5%
6h	20	0.96g	4.8%
12h	20	1.72g	8.6%
24h	20	2.64g	13.2%

Table 4: Values of syneresis

9. NUTRITIONAL ANALYSIS

9.1 Reducing Sugars (DNS Method)

The analysis of reducing sugar using the DNS method showed consistent decline throughout the fermentation period which indicated the lactose utilization by probiotic microorganisms to convert curd. At initial 0h , the sample contained high reducing sugar present, indicating natural lactose and partial glucose / galactose present in the commercial curd. At 6h there was a noticeable reduction in the lactose content from the sample with an increase in the microbial growth. The preceding fermentation time of 12h decreased the reducing sugar present in the sample, suggesting minimal metabolic activity of microbial growth of converting sugar to lactic acid. At 24h the OD reading was low indicating the reduction of sugar and complete utilization of sugar by the probiotic microorganisms. The progressive decline in sugar concentration directly corresponded with the observed decrease in pH and increase in titratable acidity, confirming efficient carbohydrate metabolism by lactic acid bacteria. However, excessive reduction of sugars beyond 12 h may compromise sweetness and overall palatability. Thus, fermentation duration directly determines both functional and organoleptic quality of probiotic curd.

Fermentation time	Reducing sugar OD at 540nm
0h	1
6h	0.85
12h	0.67

24h	0.46

Table 5: OD of Reducing Sugars (DNS Method)

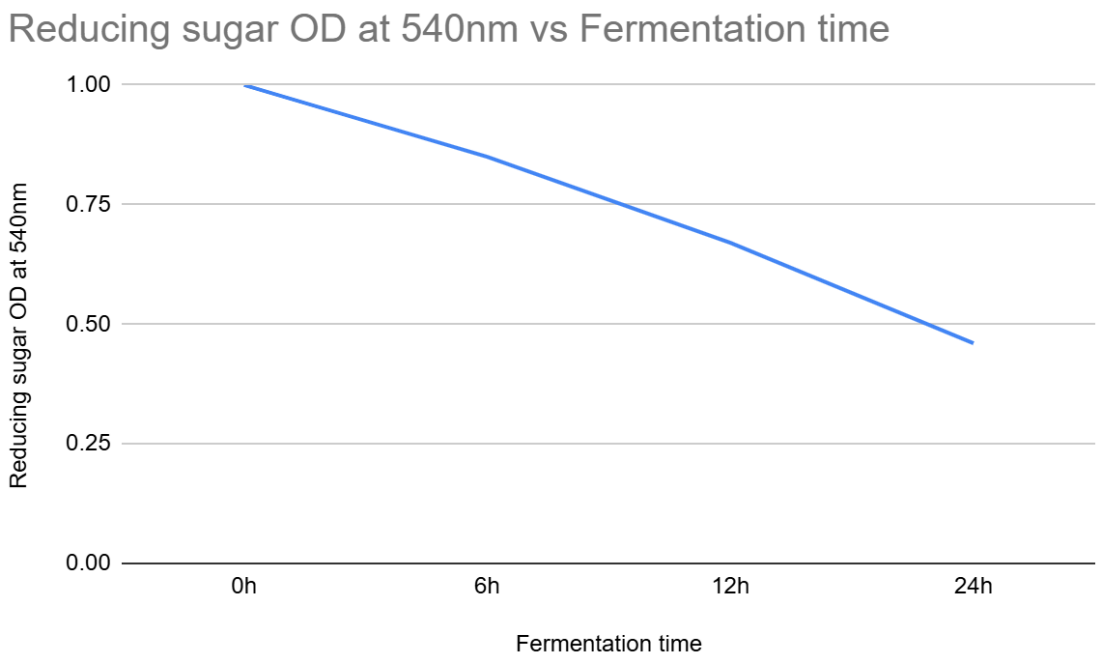


Figure 4 : Effect of fermentation time on Reducing sugar concentration (OD at 540 nm) in probiotic curd. The steady decrease in reducing sugars from 0 h to 24 h, showed that lactose was utilized by probiotic bacteria for lactic acid production.

9.2 Protein Content (Biuret Method)

The protein concentration of the fermented curd sample was determined by standard biuret method, and showed minor changes throughout the fermentation period. At 0h the level of protein was high, reflecting the natural milk protein present in the curd matrix. As the fermentation begins slight reduction of protein was observed in early hours, indicating the proteolytic activity of lactic acid bacteria. In the mid fermentation stage, the protein concentration showed more noticeable decline. Towards the end of 24h of fermentation protein degradation had already occurred and no significant changes were taking place. The result shows that the protein content is stable in early fermentation and reduces in the later stage which highlights the microbial proteases in nutritional and biochemical profile shaping.

Fermentation time	Protein concentration (biurate) at 540nm
0h	1.09
6h	1.04
12h	1.02
24	0.11

Table 6: OD of protein (biurate)

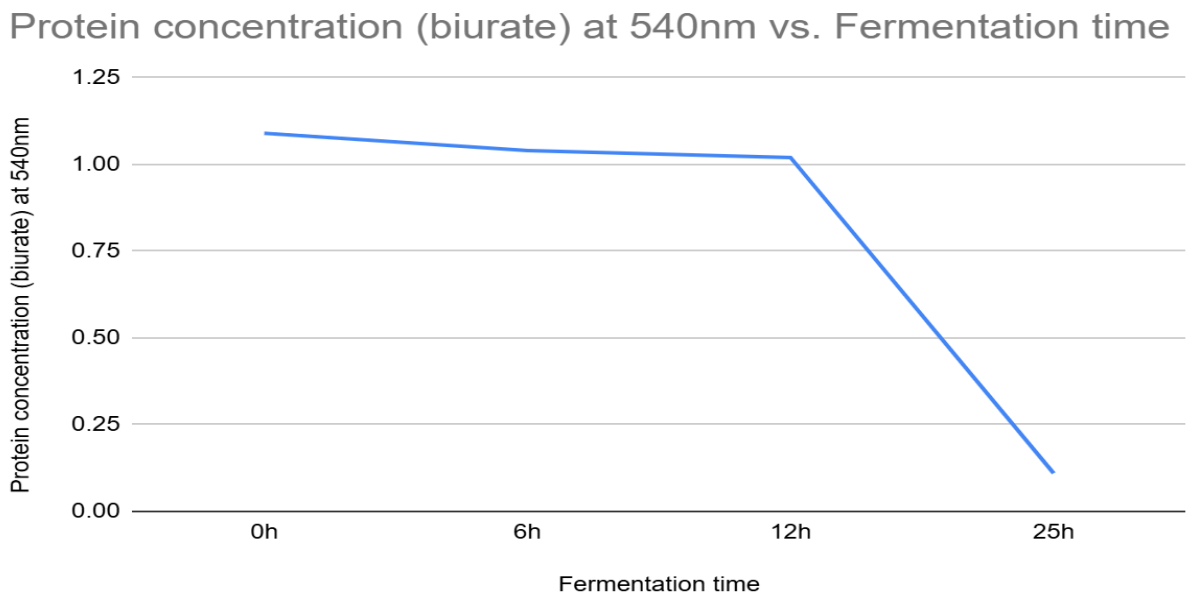


Figure 5: Effect of fermentation time on protein concentration (OD at 540 nm) in probiotic curd. Protein content was stable in the early hours and decreased gradually at 24 hours due to the proteolytic activity of the organisms.

10. CONCLUSION

This study shows an insight on how fermentation time affects the nutritional and functional qualities of commercial probiotic curd. The viability of probiotics increased rapidly at 6 hours which indicates this as the optimal fermentation time for the highest viability, after which there was a standard decline at 12 and 24 hours. This is likely due to the depletion of nutrients and increase in the acidity. The physiochemical parameters such as titratable acidity and syneresis showed an increase in percentage and pH decreased rapidly. The nutrient analysis, such as reducing sugar, affects the lactose fermentation which leads to the tanginess of the curd after 12 hours of fermentation. Protein content decreased with the increase in fermentation time indicating the proteolytic activity of lactic acid bacteria that has an effect on the biochemical properties of the curd. These findings show that the 6 hours of fermentation supports the maximum survival of probiotics by maintaining the physiochemical properties and the nutrient levels. Extended fermentation time led to the increased metabolic stress due to the accumulation of acid, loss of nutrients and increased syneresis. Thus, optimizing fermentation time is necessary for the maximum viability of probiotics, thereby enhancing local food sustainability and consumer health.

11. REFERENCES

1. Merenstein DJ, Tancredi DJ, Karl JP, Krist AH, Lenoir-Wijnkoop I, Reid G, et al. Is there evidence to support probiotic use for healthy people? Adv Nutr. 2024;15(8):100265. https://doi.org/10.1016/j.advnut.2024.100265
2. Terpou A, Papadaki A, Lappa IK, Kachrimanidou V, Bosnea LA, Kopsahelis N. Probiotics in food systems: significance and emerging strategies towards improved viability and delivery. Nutrients. 2019;11(7):1591. https://doi.org/10.3390/nu11071591
3. Byakika S, Mukisa IM, Byaruhanga YB, Muyanja C. A review of criteria and methods for evaluating probiotic potential of microorganisms. Food Rev Int. 2019;35(5):427–466. https://doi.org/10.1080/87559129.2019.1584815
4. Periyatt Veettil S, Presanna Kumar S, Venkatesh T. Characterization and molecular identification of probiotic bacteria and proliferation analysis. Food Biosci. 2025;69:106809. https://doi.org/10.1016/j.fbio.2025.106809
5. Kumar R, Sood U, Gupta V, et al. Recent advancements in development of modern probiotics. Indian J

Microbiol. 2020;60:12–25. https://doi.org/10.1007/s12088-019-00808-y

6. Kumari M, Patel HK, Kokkiligadda A, Bhushan B, Tomar SK. Development of fermented soymilk with improved properties. LWT. 2022;154:112827. https://doi.org/10.1016/j.lwt.2021.112827
7. Wilkinson MG. Flow cytometry for measuring probiotic viability: a review. Trends Food Sci Technol. 2018;78:1–10. https://doi.org/10.1016/j.tifs.2018.05.006
8. Meng C, Bai C, Brown TD, Hood LE, Tian Q. Human gut microbiota and gastrointestinal cancer. Genomics Proteomics Bioinformatics. 2018;16(1):33–49. https://doi.org/10.1016/j.gpb.2017.06.002
9. Neffe-Skocińska K, Rzepkowska A, Szydłowska A, Kołożyn-Krajewska D. Trends in probiotic use in food production. In: Holban AM, Grumezescu AM, editors. Handbook of Food Bioengineering. Academic Press; 2018. p. 65–94.
10. Knorr D. Technology aspects related to microorganisms in functional foods. Trends Food Sci Technol. 1998;9(8–9):295–306. https://doi.org/10.1016/S0924-2244(98)00051-X
11. Hansen LT, Allan-Wojtas PM, Jin YL, Paulson AT. Survival of microencapsulated probiotics. Food Microbiol. 2002;19(1):35–45. https://doi.org/10.1006/fmic.2001.0452
12. Clark PA, Cotton LN, Martin JH. Selection of bifidobacteria for dietary use. Cult Dairy Prod J. 1993;28(4):11–14.
13. Akan E. Effect of fermentation time on probiotic goat yogurts. An Acad Bras Cienc. 2022;94(3):e20210875.
14. Gomes AMP, Malcata FX. Probiotic bacteria properties. Trends Food Sci Technol. 1999;10(4–5):139–157. https://doi.org/10.1016/S0924-2244(99)00033-3
15. Dave RI, Shah NP. Viability of probiotic bacteria in yoghurt. Int Dairy J. 1997;7(1):31–41. https://doi.org/10.1016/S0958-6946(97)00017-6
16. Saarela M, Mogensen G, Fondén R, Mättö J, Mattila-Sandholm T. Probiotic microbes: from markets to mechanisms. Curr Opin Biotechnol. 2000;11(5):483–487.
17. Charteris WP, Kelly PM, Morelli L, Collins JK. Development of methodology for probiotic tolerance. J Appl Microbiol. 1998;84(5):759–768.
18. Peng Y, Horne DS, Lucey JA. Impact of fermentation time on yogurt properties. J Dairy Sci. 2009;92(7):2977–2990. https://doi.org/10.3168/jds.2008-1221
19. Ranadheera CS, Baines SK, Adams MC. Importance of food in probiotic efficacy. Food Res Int. 2012;48(2):946–954.
20. Shori AB. Influence of food matrix on probiotic viability. Food Biosci. 2016;13:1–8. https://doi.org/10.1016/j.fbio.2015.11.001
21. Talwalkar A, Kailasapathy K. Role of oxygen in probiotic viability. Curr Issues Intest Microbiol. 2004;5(1):1–8.
22. Shah NP. Probiotic bacteria in dairy foods. J Dairy Sci. 2000;83(4):894–907.
23. Kailasapathy K. Survival of probiotic bacteria and sensory effects. LWT Food Sci Technol. 2006;39(10):1221–1227.
24. Vinderola G, Bailo N, Reinheimer J. Survival of probiotic microflora in yoghurt. Food Res Int. 2000;33(2):97–102.