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Studies on Microbial Fermentation and Production of Yogurt

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A B S T R A C T

This study was carried out to investigate microbial fermentation and yogurt production with special emphasis on lactic acid bacteria (LAB) and vegetable juice fortification. Yogurt samples were collected from different sources and analyzed for total microbial load using standard plate count methods. LAB were isolated and purified on MRS agar, and characterized using cultural, morphological, and biochemical techniques. Further, carrot and beetroot juices at varying concentrations (0.1%, 1%, 2%, and 5%) were incorporated into yogurt to evaluate their effect on physicochemical and sensory properties. Results showed that both Vijay curd (C1)(2.6×10^6 cfu/mL) and Hostel curd (C2)(5.3×10^6 cfu/mL) contained abundant LAB populations. Carrot juice fortification caused a gradual decrease in pH from 6.0 to 3.0, with increasing firmness, pale orange coloration, and stronger acidic odour. Beetroot juice addition led to a more pronounced drop in pH (1–3), accompanied by creamy to reddish-pink coloration and stronger flavour development. In both treatments, shelf life remained constant at 3 days, and replication trials showed reproducibility of results. Overall, 20% juice fortification was found to provide the best balance between flavour, acidity, colour, and texture. Between the two replications, C1 performed better than C2 in both carrot and beetroot yogurt samples, showing stronger flavour, lower pH, and improved overall fermentation quality. The findings highlight the potential of using LAB fermentation along with natural vegetable fortification to develop functional yogurts with enhanced nutritional and sensory attributes.

Introduction

Yogurt is a widely consumed fermented dairy product produced through the metabolic activity of lactic acid bacteria, primarily *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Tewari *et al.,* 2019; Rashed Rashid Anjum *et al.,* 2007). These microorganisms convert lactose into lactic acid, leading to a decrease in pH and coagulation of milk proteins, which imparts the characteristic texture and sour taste of yogurt - (Olatidoye *et al.,* 2017). The quality and characteristics of yogurt are influenced by several processing parameters, including the selection of starter cultures, heat treatment, inoculation procedures, incubation conditions, and preservation techniques (Rashed Rashid Anjum *et al.,* 2007). Additionally, the type and composition of milk used—commonly bovine milk—play a critical role in determining the physicochemical and sensory properties of the final product (Hank Thi Hong Nguyen *et al.,* 2014; Tamime and Robinson, 2007). Yogurt offers various health benefits, particularly for individuals with gastrointestinal disorders, as it supports digestion and promotes a balanced intestinal microflora. The lactic acid bacteria inhibit the growth of pathogenic organisms and help prevent gas formation in the gut (Nahar *et al.,* 2007; Olatidoye *et al.,* 2017). These functional properties make yogurt a valuable dietary component. Recent studies have focused on enhancing yogurt's nutritional profile by incorporating functional ingredients. One such innovation involves the addition of potato juice, which is rich in resistant starch, vitamin C, lutein, phenolic compounds, and chlorogenic acid—components known for their health-promoting properties (Aygun & Durmaz, 2017). The objective of this study is to develop a novel yogurt product by fermenting milk with potato juice and to evaluate its physicochemical and microbiological characteristics.

Yogurt

Yogurt is a fermented product resulting from the growth of lactic acid bacteria (LAB), lactobacillus delbrueckii ssp. Bulgaricus and streptococcus thermophiles, in milk (Adams and Moss, 2000).

Lactic Acid Bacteria

Lactic acid bacteria are gram positive, non-spore forming, catalysenon producing, and non-motile microoraganisms (Aktas *et al.,* 2024). The main characteristic of these bacteria is lactic acid production, which is a key to fermentation products. In addition, they have a high tolerance to acidic pH, are aerotolerant, and can appear as cocci or rods (Aguirre-Garcia *et al.,* 2024). Even though LAB can be present naturally in some dairy products, they are often deliberately incorporated as a starter culture, or sometimes as ingredients or additives to amplify the product's functionality, particularly their probiotic potential (Agagunduz *et al.,* 2021). Lactic acid bacteria (LAB) are considered one of the greatest possibilities for assembling for manufacturing "natural food", benign and healthy. These microorganisms are a different group of Gram-positive bacteria, non-sporulation bacteria that produce lactic acid as the most important end product of carbohydrate fermentation. They are a heterogeneous group of bacteria, normally exhibit in naturally rich nutrient environments such as decomposing plants and milk products common among bacteria populating skin and mucosal tissues of humans and animals (Mangiapane *et al.,* 2015). According to the FDA, to be considered yogurt, dairy ingredients must be cultured with LAB. Yogurt cannot contain less than 3.25% milk fat, less than 8.25% milk solids that are not fat, are have a titratable acidity that is less than 0.9%. It must be pasteurized or ultra-pasteurized before the addition of bacterial culture. Flavors, colours, stabilizers and vitamins are optional (FDA, 2020).

General Position of Specific Lab

Lactobacillus

The l*actobacilli* are rods, usually long and slender, that form chains in most species. They are microaerophilic, but some strict aerobes are known, catalase – negative and gram – positive, and they ferment sugars to yield lactic acid as the main product. They ferment sugar chiefly to lactic acid if they are homofermeatative, with small amounts of acetic acid, carbon dioxide, and trace products, if they are heterofermentative, they produce appreciable amounts of volatile products, including alcohol, in addition to lactic acid. Most species of this non spore forming bacterium ferment glucose into lactate hence the name *Lactobacillus*. The most common application of *Lactobacillus* is industrial production of dairy products.

Colony and cultural characteristics

Isolated LAB from raw milk, observing colonies that were smooth, creamy-white, circular, and showed no proteolytic halos on skim milk agar, supporting earlier observations on limited extracellular protease activity El Ahmadi *et al.,* (2025). Liu *et al.,* (2024) noted that *Lactobacillus fermentum* strains showed weak but detectable proteolytic activity when tested in fermented goat milk formulations, attributing this to intracellular or surface-bound enzymes rather than extracellular diffusion. Çetin *et al.,* (2025) confirmed that most LAB isolates did not form clear zones on casein-containing agar, reinforcing the understanding that proteolysis is usually a localized, contact-based process in LAB.

Growth studies- effect of temperature

El Ahmadi *et al.,* (2025) demonstrated that fermentation at higher temperatures (43–45°C) accelerates acid production by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, but leads to increased whey separation and reduced gel strength. This results in a yogurt with weaker texture and lower viscosity. Conversely, fermenting at lower temperatures (32–39°C) slows acidification but improves sensory properties such as creaminess and mouth feel.

Çetin *et al.,* (2025) reported that temperature influences the volatile metabolite composition of yogurt, affecting flavor development. Fermentation at 42°C favored accumulation of ketones and other desirable flavor compounds, whereas 37°C fermentation increased short-chain fatty acids, altering taste profiles. Zhang *et al.,* (2024) observed that higher fermentation temperatures (46°C) speed up microbial growth and pH decline, shortening fermentation time. However, this rapid acidification can compromise yogurt texture and stability. Conversely, lower temperatures extend fermentation but yield firmer, more stable gels.

Effect of pH

The typical pH range for yogurt is between 4.0 and 4.6. This acidic environment is primarily generated by the metabolic activity of starter cultures, mainly *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Kumar *et al.,* 2023). Ramesh *et al.,* (2024) reported that yogurts with pH close to 4.2 exhibited superior gel strength and reduced syneresis compared to yogurts with higher pH (~4.6) Excessive acidification (pH < 4.0), however, can cause over-tightening of the gel network, resulting in a brittle texture and poor mouthfeel (Singh & Patel, 2023).

The acid profile and flavor compounds in yogurt are strongly influenced by pH. A moderately acidic pH (around 4.2–4.4) optimizes the production of desirable flavor metabolites such as acetaldehyde and diacetyl, which contribute to the characteristic yogurt aroma (Fernandes *et al.,* 2022). Kim and Cho (2024) observed that yogurts with slower pH decline during refrigerated storage had improved texture stability and longer shelf life. Strategies such as selecting specific starter cultures or controlling fermentation conditions to modulate final pH have been suggested to mitigate excessive post-acidification.

Materials and Methods

Collection of samples

Though curd from various commercial producers were available in local markets, frequently consumed curd varieties were collected from different sources such as Vijay curd(C1), Hostel (C2)

Enumeration of bacteria

Total bacterial load in the samples were determined by serial dilution-pour plate method. Samples were diluted and aliquots were plated on to plate count agar media. After incubation at 37°C for 24 hours, total bacterial load was determined in the form of cfu/mL.

Isolation and purification of lab

De Man, Rogosa and Sharpe (MRS) agar media was used for selective isolation of *Lactobacillus.* Bacterial colonies isolated from curd samples were streaked across the two selected media and incubated at 37°C for 48hrs. After incubation colonies developing with characteristic growth characters were isolated and purified.

Characterization and identification of lab

With a view to identify the isolates upto species level cultural, morphological, physiological and biochemical characteristics of the isolates were investigated, The observed characters were compared to Bergey's Manual of Determinative Bacteriology, 8th edition

Experiment and Result

Examination of microbial load in curd sample – Total lactic acid bacteria

The total lactic acid bacteria in the two curd samples were enumerated and presented in Table 1. The population of total lactic acid bacteria ranged from 2.6 to 5.3 x 10^5 cfu m1 $^{-1}$). The higher total lactic acid bacteria population 5.3 x 10^5 cfu ml^{-1} in C2 and lower count 2.6 x 10^5 cfu ml^{-1}) was recorded in C1.
The effect of various growth temperatures on the growth of four LAB strains was studied and the results are presented in Table 2. The growth the LAB strains was studied at different temperature viz., 35, 36, 38, 40 ºC in a BOD incubator.

As the concentration increases from 0.1% to 5%, the temperature also increases steadily. At 0.1% concentration, the temperature is 35°C, and it gradually rises to 39°C at 5% concentration. This shows that higher concentrations lead to higher temperatures. Among the values tested, the 5% concentration gives the highest temperature of 39°C, indicating it is the most effective in this context

The effect of various growth temperatures on the growth of four LAB strains was studied and the results are presented in Table 3. The growth the LAB strains was studied at different temperature viz., 35, 36, 38, 40 ºC in a BOD incubator. The data shows a consistent increase in temperature with increasing concentration. At 0.1% concentration, the temperature is 35°C, and it gradually rises to 40°C at 5% concentration. This indicates a positive correlation between concentration and temperature. Among all tested values, the 5% concentration produces the highest temperature of 40°C, making it the most effective**.**

The effect of various pH on the growth of four LAB strains was studied and results are presented in Table 4. The data compares the effectiveness of different concentrations (0.1%, 1%, 2%, and 5%) across two conditions (C1 and C2), with a control value of 7. As the concentration increases, the observed values decrease. At 5% concentration (referred to as 3pH), both C1 and C2 show the lowest values (3), indicating the highest effectiveness. Therefore, the 3 pH (5% concentration) is the best, as it produces the most significant reduction compared to other concentrations and the control.

The effect of various pH on the growth of four LAB strains was studied and results are presented in Table 5. The results show the effect of different concentrations (0.1%, 1%, 2%, and 5%) on two conditions (C1 and C2), compared to a control value of 6. Although some lower concentrations show variations, the 5% concentration provides consistent values (C1: 3, C2: 3)**,** which are significantly lower than the control. This indicates stable and effective performance. Therefore, 5% concentration is considered the best**,** showing a reliable reduction in both C1 and C2 compared to other groups.

The effect of various levels of inoculums load of LAB for dahi fermentation was studied and the results are presented in Table 6. The effect of various inoculums concentration (0.1%, 1%, 2%,5%) and the juice concentration(5%,10%,15%and 20%). Based on the analysis of carrot juice concentrations ranging from 0.1 ml (5%) to 5 ml (20%), it is clear that the flavour intensity, consistency, and acidic odour progressively increase with higher juice concentrations. However, at the highest concentration of 5 ml (20%), the carrot flavour becomes high, consistency turns highly firm, and the acidic odour becomes strong, indicating robust fermentation. Though the texture is firmer and the odour more intense, these changes enhance the overall sensory impact. Therefore, 5 ml (20%) is considered the best concentration, delivering the strongest flavour, ideal firmness, and maximum sensory appeal for carrot yogurt fermentation.

The effect of various levels of inoculums load of LAB for dahi fermentation was studied and the results are presented in Table 7. The various inoculums concentration (0.1%, 1%, 2%, 5%) and the juice concentration (5%, 10%, 15% and 20%). Based on the analysis of inoculum and beetroot juice concentrations ranging from 0.1 ml (5%) to 5 ml (20%), it is observed that the flavour intensity, consistency, and acidic odour gradually increase with higher juice concentrations. The lower concentrations like 0.1 ml and 1 ml, the beetroot flavour remains low to medium, with a slightly fermented aroma, creamy pink colour, and slightly firm texture. The highest concentration of 5 ml (20%), the beetroot flavour remains intense, consistency becomes highly firm, and the acidic odour becomes strong, suggesting robust fermentation. The colour shifts to a deeper reddish pink, enhancing the visual appeal. Therefore, 5 ml (20%) beetroot juice is considered the most effective concentration, providing the best flavour, ideal firmness, and overall sensory appeal for fermented beetroot yogurt.

Results and Discussion

In the present study, yogurt was prepared separately using carrot and beetroot to assess their individual contributions to taste, appearance, and overall quality. Carrot is a good source of antioxidants and beta-carotene, but due to its low natural sugar content, the yogurt made with carrot had a mild flavor and was less appealing in taste. Aly *et al.,* (2004) reported that carrot juice up to 15% improved the sensory and microbial quality of yogurt, though the flavor remained subtle. In contrast, beetroot is naturally sweeter and rich in pigments like betalains, which not only enhanced the yogurt's color but also improved its taste. According to Adjei *et al.,* (2024), beetroot puree at around 2.03% significantly increased consumer acceptance without negatively impacting pH, acidity, or texture. In our study as well, the yogurt prepared with beetroot had a more desirable taste and was better accepted compared to the carrot-based yogurt. Yogurt samples were prepared with varying concentrations of carrot juice (0.1%, 1%, 2%, and 5%) and beetroot juice (0.1%, 1%, 2%, and 5%) and tested in two replications (C1 and C2) to ensure consistency and reliability. For carrot-based yogurt, both C1 and C2 showed highly similar results across all concentrations, with a progressive increase in carrot flavour and texture firmness as the concentration increased. At lower concentrations (0.1% and 1%), the yogurt had low carrot flavour, pale orange colour, slightly fermented consistency, pH around 5–6, and low acidic odour. At 2%, a medium carrot flavour and firmer consistency were observed, with a pH of 5.

The highest concentration (5%) resulted in strong carrot flavour, highly firm texture, pale orange colour, a sharp drop in pH to 3, and a noticeably high acidic smell. In the beetroot-based yogurt, C1 and C2 again showed consistent results in terms of flavour, colour, consistency, and odour, with only minor pH differences at lower concentrations. At 5 ml (20%) beetroot juice concentration, the yogurt displayed the best overall characteristics including intense beetroot flavour, reddish-pink colour, highly firm consistency, and a strong acidic aroma making it the most suitable and preferred formulation in this study. Yang *et al.,* states that the effects of fermentation at temperatures of 30°C, 37°C, 40°C, 42°C, and 45°C on the quality of yogurt produced using *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Janiszewska-Turak *et al.,* (2023) stated that a consistent and rapid decrease in pH from fresh carrot and beetroot juices during lactic acid fermentation.

Specifically, pH dropped below 4.5 a typical threshold for microbial stability with the lowest pH observed in beetroot juice after fermentation. At the end, between these two replications C1 (vijay curd) performed better than C2 (hostel curd) in both carrot and beetroot yogurt samples, showing stronger flavour, lower pH, and improved overall fermentation quality.

Table.1 Enumeration of microorganism from collection of curd sample (MRS agar medium)

Curd samples	Average total bacterial population in (cfu) per ml of curd
Vijay curd (C1)	26,00,000
Hostel (C2)	53,00,000

Table.2 Effect of temperature on the growth of lactic acid bacteria (Carrot Yogurt)

Concentration (%)	Temperature (°C)
0.1	35
1	36.5
2	37.5
5	39

Table.3 Effect of temperature on the growth of lactic acid bacteria (Beetroot Yogurt)

Concentration (%)	Temperature (°C)
0.1	35
1	36
2	38
5	40

Table.4 Effect of pH on the growth of lactic acid bacteria (Carrot Yogurt)

Concentration (ml)	Replication	pH
0.1	C1	6
	C2	5
1	C1	6
	C2	6
2	C1	5
	C2	5
5	C1	3
	C2	3
Control	-	7

Table.5 Effect of pH on the growth of lactic acid bacteria (Beetroot Yogurt)

Concentration	Replication	pH
0.1	C1	1
	C2	3
1	C1	1
	C2	1
2	C1	3
	C2	5
5	C1	3
	C2	3
Control	-	6

Figure.1

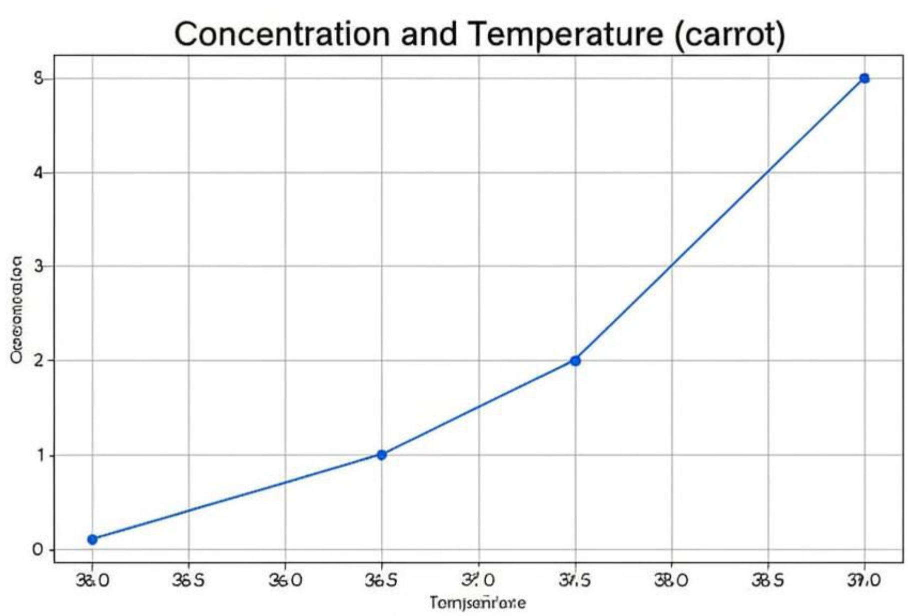

Table.6 Estimation of different inoculums load of LAB for yogurt fermentation (Carrot)

Concentration (ml)	Replication	Juice concentration (%)	shelf life	Taste	Ph	colour	consistency	smell
0.1	C1	5	3	Low carrot flavour	6	Pale orange	Slightly fermented	Low acidic odour
	C2		3	Low carrot flavour	5	Pale orange	Slightly fermented	Low acidic odour
1	C1	10	3	Low carrot flavour	6	Pale orange	Slightly firm	Low acidic odour
	C2		3	Low carrot flavour	6	Pale orange	Slightly firm	Low acidic odour
2	C1	15	3	Medium carrot flavour	5	Pale orange	firm	Moderate acidic odour
	C2		3	Medium carrot flavour	5	Pale orange	firm	Moderate acidic odour
5	C1	20	3	High carrot flavour	3	Pale orange	Highly firm	High acidic odour
	C2		3	High carrot flavour	3	Pale orange	Highly firm	High acidic odour
Control	-	-	3	No carrot flavour	7	Creamy white	Slightly firm	Low acidic odour

Figure.2

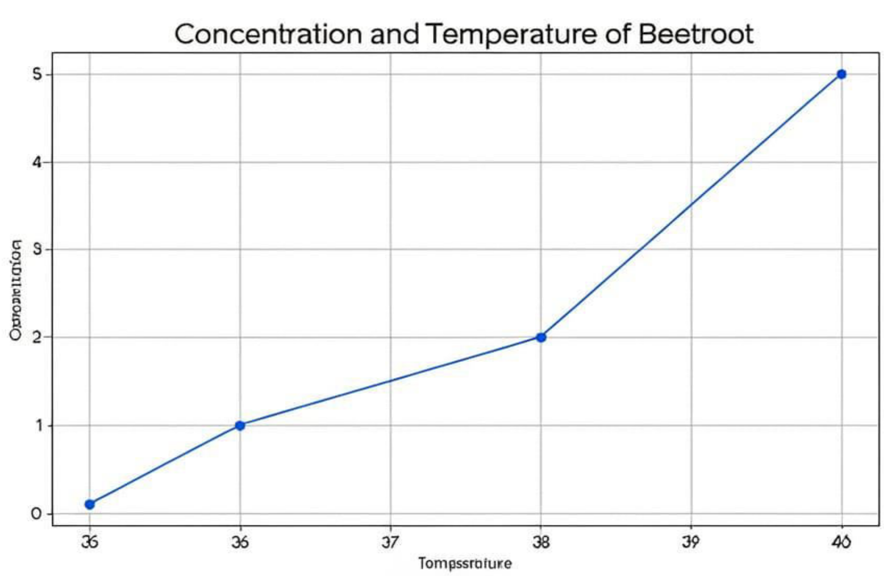

Table.7 Estimation of different inoculum load of LAB for yogurt fermentation (beetroot)

Concentration	Replication	Juice concentration (%)	shelf life	Taste	Ph	colour	Consistency	Smell
0.1	C1	5	3	Low beetroot flavour	1	Creamy pink	Slightly fermented	Low acidic odour
	C2		3	Low beetroot flavour	3	Creamy pink	Slightly fermented	Low acidic odour
1	C1	10	3	Medium beetroot flavour	1	Creamy pink	Slightly firm	Low acidic odour
	C2		3	Medium beetroot flavour	1	Creamy pink	Slightly firm	Low acidic odour
2	C1	15	3	High beetroot flavour	3	Creamy pink	Firm	Moderate acidic odour
	C2		3	High beetroot flavour	5	Creamy pink	Firm	Moderate acidic odour
5	C1	20	3	High beetroot flavour	3	Reddish pink	Highly firm	High acidic odour
	C2		3	High beetroot flavour	3	Reddish pink	Highly firm	High acidic odour
Control	-	-	3	No beetroot flavour	6	Creamy white	Slightly firm	Low acidic odour

In this study, the fermentation and quality characteristics of yogurt were investigated based on the inoculation concentration of LAB extending shelf-life and enhancing the safety of food products. Carrot and beetroot is safe for public health and used as vitaminized food supplement. Yoghurt with 15% carrot or beetroot juice is best for production of flavoured yoghurt Good quality, long shelf life (i.e) It could be kept at 4 ºC for 11 days without significant microbial growth or loss of the product colour and texture as well as 77% aflatoxin degradation during manufacture and storage.

Author Contributions

P. Elamparithi: Investigation, formal analysis, writing—original draft. A. Harini: Validation, methodology, writing—reviewing. S. Kartheeswari:—Formal analysis, writing—review and editing. V. Mukesh Kumar: Investigation, writing—reviewing. S. Poojegan: Resources, investigation writing—reviewing. T. Priyadharshini: Validation, formal analysis, writing—reviewing. M. Rexcy Blesslin: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. K. Sivasakthi: Investigation, formal analysis, writing—original draft. A. Sowbaraniga: Validation, methodology, writing—reviewing. M. Subalakshmi:—Formal analysis, writing—review and editing. S. Sujitha: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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