Analysis of Organoleptic, physicochemical, and microbial properties of fermented cattle milk with year-round occurrence of lactic acid bacteria

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Abstract- This study aimed to isolate lactic acid bacteria (LAB) from a naturally fermented 1:1 blend of raw cow (Gir breed) and goat (Surti breed) milk collected from Surat city across three seasons—summer, monsoon, and winter. The fermented samples were evaluated through organoleptic, chemical, and microbial analysis. All samples exhibited acceptable sensory qualities, including sour aroma and semi-solid to watery consistency, without signs of spoilage. Chemical parameters such as pH, titratable acidity, protein, fat, total solids, and ash were assessed. LAB were present in all samples, with lower counts observed in winter. The findings confirm that naturally fermented milk is a reliable source of probiotic LAB and that seasonal variation influences microbial activity.

Keywords- *Lactic acid bacteria (LAB), Cow milk, Goat milk, Natural fermentation, Probiotic, Seasonal variation, Physicochemical analysis, Organoleptic analysis and Microbial analysis.*

I. Introduction

The milk of cow and goat has been a fundamental part of Indian nutrition since ancient times, valued not only for its rich supply of essential nutrients such as minerals and vitamins but also for its therapeutic and healing properties [1]. Both cow and goat milk provide important minerals, but they differ in their health benefits. Goat milk, in particular, is recognized for causing fewer allergic reactions and has been linked to improvements in gastrointestinal, cardiovascular, and stress-related conditions [2] Additionally, goat milk contains higher levels of magnesium, phosphorus, and calcium compared to cow and even human milk, making it an excellent source of nutrition that supports immune function and disease prevention [3].

In India, especially in Gujarat, specific breeds like the Gir cow and Mehsana goat are prominent dairy sources. The Gir breed, native to the Gir forest region of Saurashtra, is a well-established dairy cattle breed known for its adaptability and milk production [4]. Similarly, the Mehsana goat, adapted to the semi-arid climates of northern Gujarat, is valued for both milk and meat (Gujarat Livestock Census). These local breeds offer unique nutritional qualities that are significant for dairy-based research [5].

Lactic acid bacteria (LAB) are naturally present in raw milk and are known to be more abundant in raw milk than pasteurized milk, likely due to the richer macronutrient content that supports their survival [37]. LAB play a crucial role in milk fermentation by converting lactose into lactic acid, which curdles the milk and contributes to its preservation and enhanced flavour [6]. Beyond fermentation, LAB have important probiotic properties like: they inhibit harmful pathogens by producing organic acids, bacteriocins, and hydrogen peroxide, support intestinal microflora balance, improve nutrient absorption, and reduce lactose intolerance [36] [7].

Several studies have isolated various LAB species such as *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, and *Lactococcus lactis* from raw goat milk. Some of these strains have shown antimicrobial activity against pathogens like *Listeria monocytogenes* and even demonstrated anti-cancer potential in cell line studies.

Considering these factors, this study focuses on isolating LAB from a naturally fermented blend of raw cow (Gir breed) and goat (Surti breed) milk combined in equal proportions. The research aims to investigates the physicochemical, organoleptic, and microbial characteristics of the fermented milk to assess its suitability as a source of probiotic LAB, while also exploring how seasonal and environmental factors influence its microbial and chemical profile [36].

II. Materials and methods

Sampling of goat and cow milk:

A total of 30 goat and cow milk samples were collected from local dairy farms in and around Surat city over two years (2019–2020), during three seasons: Summer (March–June), Monsoon (July–October), and Winter (November–February). Goat milk, from the Surti breed, and cow milk, mainly from the Gir breed, were obtained based on availability.

Samples were collected in sterilized autoclavable plastic bottles, transported to the lab within 3 hours, and stored at -2 to -4°C. Equal volumes of goat and cow milk (1:1) were mixed and heated at 40–45°C for 10 minutes in a water bath to stimulate bacterial growth, following the method of [8].

(a) **(b)** **(c)**

Figure 1 (a) Milk collection of Gir cow **(b)** Cow and goat milk samples collected **(c)** Milk collection of Surti Goat

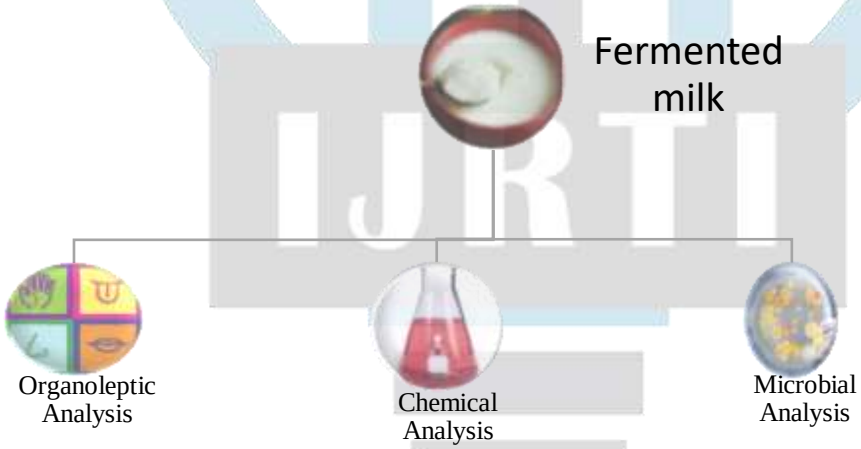

Figure 2 Three types of analysis of Fermented Milk

Analysis of Fermented Milk

Organoleptic analysis:

All test samples prepared were evaluated for consistency, colour, smell, and flavour through sensory analysis.

Chemical analysis:

Determination of pH, fat, protein, total solids, titratable acidity, and ash content was performed according to standard protocols outlined by the Food Safety and Standards Authority of India (FSSAI, 2022) and [38]. Prior to analysis, sample preparation was essential. Curdled milk samples were thoroughly blended to achieve a homogeneous mixture suitable for accurate chemical evaluation.

1. Estimation of Total Solids by Gravimetric method:

To determine the total solids content, the milk samples were dried in a hot air oven at 102 ± 2°C, and the residue weight was used for calculation. Initially, 10 mL of the milk sample was transferred to a beaker and gently warmed in a water bath at 35–40°C. The cream particles adhering to the sides were mixed slowly to ensure uniformity. The sample was then cooled to room temperature [9].

A clean dish and its lid were placed side by side in the oven and dried for at least one hour. Afterward, the dish was covered, placed in a desiccator to cool for 30 minutes, and then weighed (M_0). Subsequently, 5 mL of the curd sample was poured into the dish, covered with the lid, and weighed again (M_1). The dish, with the lid kept alongside, was placed in the oven for two hours. After drying, it was transferred immediately to the desiccator to cool and then weighed (M_2). This drying and weighing process was repeated adding one hour of oven time each cycle until the difference between two successive weights did not exceed 1 mg. The final weight was used to calculate the total solids content using the standard formula [10] [11].

Calculation

$$\text{Total Solid Content} = \frac{M2 - M0}{M1 - M0} \times 100$$

Where;

M0 = weight of dish in g + lid

M1 = weight of dish in g + lid and sample

M2 = weight of dish in g + lid and dried sample

2. Estimation of Fat in Milk by Rose-Gottlieb Method:

To determine the fat content in the curd sample, ammonia was used to dissolve the protein component, while ethyl alcohol facilitated protein precipitation. Fat extraction was carried out using diethyl ether and petroleum ether. After evaporation of all solvents, the remaining residue representing fat was weighed [12].

The procedure began with drying a clean beaker in a hot air oven at 102 ± 2°C for 2 hours. It was then cooled in a desiccator and weighed. This drying and weighing process was repeated until the difference between two successive measurements did not exceed 1 mg. Next, 10 g of the curd sample was placed into a Mojonnier extraction tube and mixed thoroughly with 1.25 mL of ammonia. This was followed by the addition of 10 mL of ethyl alcohol, which was also mixed thoroughly. Then, 25 mL of diethyl ether was added, and the tube was tightly sealed with a cork and shaken vigorously for approximately one minute. Afterward, 25 mL of petroleum ether was added, and the mixture was again shaken for one minute. The tube was left to stand until the upper ethereal layer completely separated and appeared clear [13].

This upper ethereal layer, containing the extracted fat, was carefully drained into the pre-dried and weighed beaker. The delivery end of the extraction tube was rinsed with ether, and the washings were added to the same beaker. An addition15 mL of solvent was used to dissolve any residual extract in the Mojonnier tube. Petroleum ether was used to further rinse the beaker, ensuring complete transfer of fat while leaving behind any insoluble materials. The beaker was then dried in the oven and reweighed. The difference between the final and initial weights of the beaker represented the fat content extracted from the sample. The result was calculated using the standard formula [14] [15].

Calculation

$$Fat\ \%(w/w) = \frac{Weight\ of\ extracted\ fat}{Weight\ of\ sample} \times 100$$

3. Estimation of Total Ash content

To determine the total ash content in the curd sample, the organic matter was incinerated, and the remaining inorganic residue was quantified. This process involved two heating steps: initial gentle heating to remove moisture, followed by high-temperature charring at 550°C in a muffle furnace to completely oxidize the organic components [16].

The procedure began by labelling a clean crucible, drying it in a hot air oven for 30 minutes, cooling it in a desiccator, and recording its weight (M). Approximately 3 g of the curd sample was then accurately weighed into the pre-dried crucible, and the combined weight was recorded (M_1). The crucible with the sample was first gently heated on a hot plate to evaporate moisture and prevent spattering, and then transferred to a muffle furnace set at 550°C. The sample was converted to ash until a consistent grey residue was obtained, indicating complete combustion of organic matter [17].

The crucible was then placed in a desiccator to cool and then weighed. The heating, cooling and weighing cycle was repeated until the difference between two successive weights was less than 1 mg. The final weight (M_2) represented the inorganic residue or ash. The total ash content was then calculated using the appropriate formula [18].

Calculation

$$\text{Total ash \% by mass} = \frac{M2 - M}{100 - M0 \times M1 - M} \times 100$$

Where;

M = mass of the empty crucible in g;

M1 = mass of the crucible with curd sample and

M2 = mass of the crucible with ash in g

M0 = moisture, % by mass.

4. Estimation of total nitrogen content in milk samples by kjeldahl method

The Kjeldahl method, established by Johann Kjeldahl in 1883, remains the most widely accepted technique for determining organic nitrogen, which is used to calculate protein concentration in a sample. This method consists of two main stages: digestion and distillation, followed by titration. During digestion, proteins in the sample are broken down using concentrated sulfuric acid in the presence of a catalyst, oxidizing all organic matter except nitrogen, which is converted to ammonium sulfate. In the distillation step, ammonia is released from the digest, collected in boric acid, and quantified by titration with a standard acid using a mixed indicator. A reagent blank is used to account for nitrogen originating from reagents [19].

Reaction:

$$\text{Protein} \xrightarrow[\text{Heat}]{K_2SO_4, CuSO_4, H_2SO_4} (NH_4)_2SO_4$$

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ . H_2BO_4^-$$

$$\underset{\text{(Green)}}{NH_4^+ . H_2BO_4^-} + HCl \rightarrow \underset{\text{(Blue at pH} < 4.8\text{)}}{NH_4Cl + H_3BO_3}$$

All reagents employed in this procedure were of AR (Analytical Reagent) grade quality. For sample pretreatment, 5 mL of the curd sample was placed into a clean, dry Kjeldahl flask along with porcelain beads. To this, 15 g of potassium sulfate (K_2SO_4) and 1.0 mL of copper sulfate solution were added. The contents were mixed gently after the addition of 25 mL of concentrated sulfuric acid [20].

Digestion began with slow heating of the flask on a digestion apparatus for 20 minutes until white fumes appeared. The heat was then increased to 50% for 15 minutes, followed by maximum heat for an additional 1 to 1.5 hours until the digest turned a clear light blue-green, indicating complete breakdown of organic matter. The digest was allowed to cool to room temperature for about 25 minutes. After cooling, 300 mL of distilled water was added to the flask to dissolve the contents completely.

Distillation was initiated by activating the condenser water flow. Then, 75 mL of 50% sodium hydroxide solution (by mass) was carefully added down the tilted neck of the Kjeldahl flask to avoid premature mixing. This created two distinct layers. The flask was immediately connected to the distillation apparatus, with the outlet of the condenser submerged in 50 mL of boric acid solution containing a mixed indicator (methyl red and bromocresol green) in a 500 mL conical flask. The flask's contents were then vigorously mixed to eliminate visible layer separation, and the mixture was heated to boiling.

Distillation continued until irregular boiling (bumping) began, at which point approximately 150 mL of distillate had been collected, and the total volume in the receiving flask reached around 200 mL. All released ammonia was captured by the boric acid, turning its color from purple to green. The efficiency of the condenser ensured that the temperature in the receiving flask did not exceed 35°C. If less than 150 mL of distillate was collected, it typically indicated that less than 300 mL of water had been added during dilution [20].

Titration was performed using 0.1 N hydrochloric acid. The boric acid solution containing the ammonia (green in color) was titrated until a faint purple endpoint was observed. The burette reading was recorded to determine the nitrogen content.

A **blank test** was also conducted in parallel using the same procedure, but replacing the milk sample with 5 mL of distilled water and approximately 0.85 g of sucrose to simulate organic material. This control helped account for any nitrogen contributed by the reagents themselves.

Calculations

Calculate the nitrogen content, expressed as a percentage by mass, and results were noted by following formula

$$\mathrm{Wn} = \frac{1.4007 \times (\mathrm{VS} - \mathrm{VB}) \times \mathrm{N}}{W}$$

Wn = nitrogen content of sample, expressed as a percentage by mass;

VS = volume in mL of the standard hydrochloric acid used for sample;

VB = volume in mL of the standard hydrochloric acid used for blank test;

N = Normality of the standard hydrochloric acid expressed to four decimal places;

W = mass of test portion in g, expressed to nearest 0.1 mg.

The crude protein content was expressed as a percentage by mass, was obtained by multiplying the nitrogen content by 6.38.

5. Estimation of Titrable acidity:

Titratable acidity is a measure of the total acidity present in milk and curd, influenced primarily by bacterial fermentation and enzymatic lipolysis. The presence of proteins and salts significantly contributes to titratable acidity, as they can effectively bind hydroxyl ions [21]. To determine the titratable acidity, 10 g of the curd sample was placed into a clean flask and mixed with 1 mL of phenolphthalein indicator solution. This mixture was then titrated against a standard sodium hydroxide (NaOH) solution until a

faint pink endpoint was reached, indicating neutralization. The volume of NaOH used was recorded, and the acidity was calculated accordingly [22].

6. Estimation of pH

The pH of the curd sample was measured using a digital pH meter (Equiptronics), which operates on the potentiometric principle. This method measures the voltage difference generated by hydrogen ion activity between the pH electrode and a reference electrode immersed in the sample. Prior to measurement, the pH meter was calibrated using standard buffer solutions. The electrode was then gently immersed in the sample, ensuring it did not contact the sides or bottom of the container. The sample was lightly stirred to ensure uniformity, and the reading was allowed to stabilize before the pH value was recorded from the digital display.

Microbial Analysis:

Isolation and identification of LAB

Enrichment of samples: Cow and goat milk were collected from local areas of Surat city and mixed in equal proportion and allowed to ferment naturally at room temperature for 24 h. Then obtained fermented milk was used for isolation of LAB. One gram of each sample was mixed with sterile saline solution (0.9% NaCl) and vortexed [23]. Then, 100 µL of the suspensions were plated on the De Man Rogosa Sharpe (MRS) agar (Hi media) and incubated at 37 °C for 2 days. Colonies with LAB-like morphology were selected and reinoculated on fresh MRS plates. Catalase tests with 3% hydrogen peroxide and Gram staining confirmed the isolates as LAB. Various other biochemical tests were performed to confirm LAB [24].

Seasonal occurrence of LAB in naturally fermented milk sample.

The prevalence of LAB in naturally fermented milk samples was investigated across three different seasons. Milk samples were collected and allowed to ferment naturally. The analysis was conducted using the serial dilution technique, followed by identification procedures as described earlier. This method allowed for the assessment of LAB populations in the fermented milk samples throughout the seasonal variations [25].

III. Result and discussion:

Table 1. Details of cow and goat milk collection.

Milk sample collection												
Sr. no	Sample no.	Date	Time	Location	Season	Temp. (°C)	Humidity	Cattle	Breed	Weight (kgs)	Age (years)	Food
1	C1	27-06-2020	04:37 PM	Vanz Village	Monsoon	33	69%	Cow	Gir	320	10	Green grass and Godrej cattle feed
2	C2	27-06-2020	04:45 PM	Vanz Village	Monsoon	33	69%	Cow	Gir	305	5	Green grass and Godrej cattle feed
3	C3	27-06-2020	05:00 PM	Vanz Village	Monsoon	33	69%	Cow	Gir	200	3	Green grass and Godrej cattle feed
4	C4	28-06-2020	05:15 PM	Vanz Village	Monsoon	33	69%	Cow	Gir	200	3	Green grass and Godrej cattle feed
5	C5	27-06-2020	05:24 PM	Vanz Village	Monsoon	33	69%	Cow	Gir	318	8.5	Green grass and Godrej cattle feed
6	G1	29-07-2020	01:46 AM	Hazira	Monsoon	32	79%	Goat	Surti	20	5	vegetable waste, leaves and cattle feed
7	G2	29-07-2020	01:50 AM	Hazira	Monsoon	32	79%	Goat	Surti	16	4	vegetable waste, leaves and cattle feed
8	G3	29-07-2020	01:55 AM	Hazira	Monsoon	32	79%	Goat	Surti	34	4	maize powder, green banyan leaves, wheat bran
9	G4	29-07-2020	01:58 AM	Hazira	Monsoon	32	79%	Goat	Surti	12	3.5	vegetable waste, leaves and cattle feed
10	G5	29-07-2020	02:15 AM	Hazira	Monsoon	32	79%	Goat	Surti	14	4	vegetable waste, leaves and cattle feed
11	C6	12-12-2020	05:27 PM	Vanz Village	Winter	26	45%	Cow	Gir	322	8	Green grass and Godrej cattle feed
12	C7	13-12-2020	05:05 AM	Pal gam	Winter	27	45%	Cow	Gir	220	11	Green grass and Godrej cattle feed
13	C8	13-12-2020	05:15 AM	Pal gam	Winter	27	45%	Cow	Gir	310	6	Green grass and Godrej cattle feed
14	C9	08-01-2021	04:05 AM	Adajan gam	Winter	25	51%	Cow	Gir	348	9	Green grass and Godrej cattle feed
15	C10	08-01-2021	05:05 AM	Adajan gam	Winter	25	51%	Cow	Gir	210	11	Green grass and Godrej cattle feed
16	G6	29-01-2020	02:00 AM	Hazira	Winter	22	51%	Goat	Surti	14	6	vegetable waste, leaves and cattle feed
17	G7	02-02-2020	04:25 AM	Hazira	Winter	24	46%	Goat	Surti	22	6	vegetable waste, leaves and cattle feed
18	G8	02-02-2020	04:28 AM	Hazira	Winter	24	46%	Goat	Surti	21	6	maize powder, green banyan leaves, wheat bran
19	G9	03-02-2020	04:42 AM	Hazira	Winter	24	46%	Goat	Surti	12	2.5	vegetable waste, leaves and cattle feed
20	G10	03-02-2020	05:02 AM	Hazira	Winter	24	46%	Goat	Surti	10	3.5	vegetable waste, leaves and cattle feed
21	C11	09-04-2021	04:30 AM	Pal gam	Summer	35	56%	Cow	Gir	253	6.5	Green grass and Godrej cattle feed
22	C12	09-04-2021	05:15 AM	Pal gam	Summer	35	56%	Cow	Gir	237	7	Green grass and Godrej cattle feed
23	C13	14-05-2021	04:15 AM	Pal gam	Summer	37	60%	Cow	Gir	258	5	Green grass and Godrej cattle feed
24	C14	14-05-2021	04:25 AM	Pal gam	Summer	37	60%	Cow	Gir	270	4.5	Green grass and Godrej cattle feed
25	C15	15-05-2021	04:00 AM	Pal gam	Summer	37	60%	Cow	Gir	220	7	Green grass and Godrej cattle feed
26	G11	19-05-2021	04:05 AM	Pal gam	Summer	39	60%	Goat	Surti	11	3	maize powder, green banyan leaves, wheat bran
27	G12	19-05-2021	04:18 AM	Pal gam	Summer	39	63%	Goat	Surti	26	11	maize powder, green banyan leaves, wheat bran
28	G13	19-05-2021	04:25 AM	Pal gam	Summer	39	63%	Goat	Surti	24	5	maize powder, green banyan leaves, wheat bran
29	G14	19-05-2021	04:35 AM	Pal gam	Summer	39	63%	Goat	Surti	21	10	maize powder, green banyan leaves, wheat bran
30	G15	20-05-2021	04:35 AM	Pal gam	Summer	40	63%	Goat	Surti	21	10	maize powder, green banyan leaves, wheat bran

Sample collection:

A comprehensive collection of 30 raw milk samples was undertaken from diverse local areas in Surat city. Among these, 15 samples comprised of cow milk and the remaining 15 were of goat milk. The sampling was further stratified, with 5 milk samples from each of the cow and goat categories, resulting in a total of 10 milk samples collected during each season- summer, monsoon and winter. For specific details pertaining to the sample collection process, refer to Table 1.

Analysis of Fermented Milk

Organoleptic analysis:

Table 2. Organoleptic analysis.

Sample	Consistency	Color	Smell	Flavour
T1	loose	pale yellow	too sour	no abnormal flavour
T2	soft watery	cream	sour	no abnormal flavour
T3	soft watery	white	sour	no abnormal flavour
T4	loose	cream	sour	no abnormal flavour
T5	soft watery	cream	sour	no abnormal flavour
T6	soft watery	off white	sour	no abnormal flavour
T7	loose	cream	too sour	no abnormal flavour
T8	loose	cream	sour	no abnormal flavour
T9	soft watery	white	sour	no abnormal flavour
T10	loose	pale yellow	too sour	no abnormal flavour
T11	soft watery	cream	too sour	no abnormal flavour
T12	soft watery	white	sour	no abnormal flavour
T13	loose	cream	sour	no abnormal flavour
T14	loose	white	sour	no abnormal flavour
T15	soft watery	white	sour	no abnormal flavour

The organoleptic properties of fermented milk samples, prepared from the combination of cow and goat milk, are displayed in Table 2. The consistency of the prepared samples was characterized as loose and watery due to the absence of a starter culture inoculation in milk. Instead, fermentation occurred naturally through the LAB present in the raw milk, as described by [26]. The colouration of the test samples appeared to be pale yellow, cream, white and off white. According to [27] cow milk is supposed to be pale yellow in colour due to presence of carotene. All fermented samples emitted a sour smell, indicative of lactose fermentation and acid production. No abnormal flavours were detected in any of the samples.

Chemical analysis:

Table 3. The physicochemical analysis of fermented milk samples.

Sr No.	Sample No.	pH	Titrable acidity %	Fat %	TS %	Ash %	Protein %
1	T1(C1+G1)	4.37±0.06	0.99±0.01	4.12±0.01	11.7±0.4	0.82±0.01	4.04±0.09
2	T2(C2+G2)	4.14±0.06	1.14±0.06	3.8±0.03	12.94±0.09	0.83±0.02	3.52±0.08
3	T3(C3+G3)	4.61±0.01	0.86±0.01	3.89±0.16	13.53±0.64	0.85±0.02	4.51±0.03
4	T4(C4+G4)	4.41±0.04	0.97±0.02	4.24±0.08	10.98±0.52	0.8±0.01	4.78±0.14
5	T5 (C5+G5)	4.37±0.06	1.02±0.01	4.43±0.03	11.77±0.06	0.81±0.02	4.22±0.01
6	T6 (C6+G6)	4.47±0.06	0.96±0.02	4.11±0.01	11.66±0.01	0.81±0.03	3.89±0.01
7	T7 (C7+G7)	4.31±0.1	0.96±0	4.13±0.05	11.15±0.04	0.88±0.04	3.92±0.06
8	T8 (C8+G8)	4.16±0.05	0.96±0.02	3.37±0.04	11.97±0.03	0.82±0.02	3.73±0.04
9	T9 (C9+G9)	4.11±0.05	1.02±0.06	3.99±0.04	11.63±0.07	0.9±0.01	3.89±0.01
10	T10 (C10+G10)	4.04±0.03	1.06±0.04	4.11±0.06	11.21±0.09	0.77±0.01	2.57±1.72
11	T11 (C11+G11)	4.01±0.03	1.12±0.04	4.18±0.03	12.02±0.01	0.77±0.01	4.09±0.05
12	T12 (C12+G12)	4.24±0.06	0.93±0.04	4.22±0.02	10.92±0.07	0.89±0.01	3.08±0.05
13	T13 (C13+G13)	4.13±0.05	0.94±0.04	4.13±0.11	10.12±0.12	0.83±0.02	3.47±0.04
14	T14 (C14+G14)	4.27±0.06	0.99±0.01	4.68±0.06	11.93±0.08	0.83±0.01	3.72±0.02
15	T15 (C15+G15)	4.57±0.06	0.88±0.01	4.73±0.08	12.85±7.42	0.8±0.01	3.52±0.04

Values are expressed as mean ± SD of three replicates.

1. **Determination of Total Solids by Gravimetric method:**

Figure 3. Total solid contents of test samples

2. **Determination of Fat in Milk by Rose-Gottlieb Method:**

A B

Figure 4. Fat extraction from test samples by Rose Gottlieb method (A) Mojonnier fat extraction tube (B) Fat extracted from the test samples.

3. Determination of Total Ash:

Figure 5. Ash of samples from muffle furnace

4. Determination of total nitrogen content in test samples by Kjelda.hl method

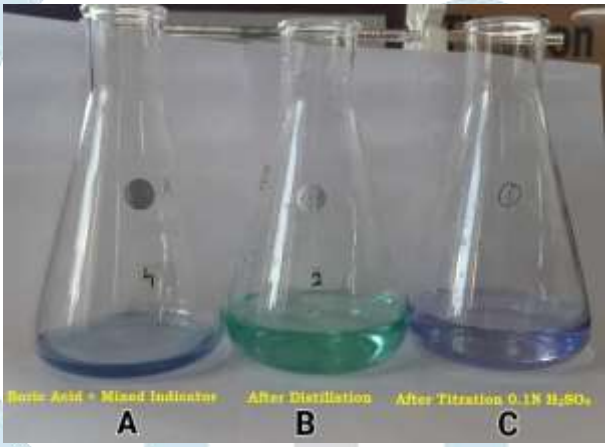

Figure 6. Determination of Nitrogen content in samples (A) Purple color observed is of boric acid and mixed indicators acidic pH (B) after distillation purple color changed to green color when ammonia gas mixed with boric acid and indicating alkaline pH (C) After titration green to purple color measured the protein in test samples

Titrable acidity

Figure 7. Titrable acidity of test sample

Results of Titrable acidity of all the samples was determined by observing the burette readings as noted in Table 3.

5. Determination of pH

The pH of all samples was measured using a digital pH meter. Results, as presented in Table 3, ranged from 4.01 to 4.61. This acidic pH is attributed to the production of organic acids by LAB during the fermentation process which results in clumping of proteins in milk. The pH values for each sample were recorded directly from the digital display of the pH meter.

The physicochemical properties of all fermented milk samples are summarized in Table 3. From the standard deviations obtained, it appears that the results do not differ significantly and are therefore relatively dependable. The range of pH in fermented milk samples was 4.01- 4.6, The range of Titrable acidity in fermented milk sample was 0.86 -1.14%. The range of fat content in fermented milk sample was 3.37- 4.73%. The range of TDS content in fermented milk sample was 10.12-13.53%. The range of protein content in fermented milk sample was 3.83-4.43%. The range of ash content in fermented milk sample was 0.77- 0.90%. As per earlier studies by [28] Fat content of cow and goat milk was recorded as 3.90 and 5.46. Protein content of cow and goat milk was documented as 2.41 and 3.32 by [29]. [30] jotted down the ash content of cow and goat milk as 0.64 and 0.8.by. According to [31] and [29] Total solid content of cow and goat milk was 2.0 and 2.10. Fermented goat milk sample had high fat and protein content as compared to cow milk. But combination cow and goat milk showed low fat and high protein contents [32]. Amalgamation of cow and goat milk helps to obtain better sensory characteristcs and increased nutritional content. Goat milk has higher calories compared to cow milk.

Microbial Analysis:

Isolation and Identification of LAB

Lactic acid producing bacteria were isolated from total 15 milk samples prepared. A total of 71 bacterial cultures were initially isolated from naturally fermented raw milk samples of cow and goat. These isolates were further subjected to physiological tests. Out of total isolates 22 of them were presumed as LAB on the basis of their morphological and colony characteristics. Only Gram positive, rod or cocci, catalase negative, non-spore forming and non- motile isolates were further identified by biochemical and carbohydrate fermentation tests. Furthermore, isolation of LAB from cow and goat milk, as conducted in this study, exhibit analogous findings with [33], [34] and [35].

Seasonal occurrence of LAB in naturally fermented milk sample.

Table 4. The Rate of occurrence of LAB in different season.

Sr. no.	Seasons	(CFU/mL)
1	Sumner	$10.74 \times 10^2 \pm 0.07$
2	Monsoon	$10.73 \times 10^2 \pm 0.08$
3	Winter	$07.74 \times 10^2 \pm 0.36$

Values are expressed as mean ± SD n=5.

Rate of occurrence of LAB in curdled milk samples of cow and goat during summer winter and monsoon season has been shown in Table 4.

Mean of LAB isolated in summer season in fermented milk samples were 10.74×10^2 CFU/mL. Mean of LAB isolated in monsoon season in fermented milk samples were 10.73×10^2. Mean of LAB isolated in winter season in fermented milk samples were 07.74×10^2 CFU/mL respectively. The data undoubtedly displays that a lesser number of LAB were observed in winter compared to monsoon and summer. In Surat city temperature ranges from 16 °C to 37 °C which is not too extreme. The difference in temperature from season summer to monsoon or monsoon to winters is not as much as it is from winters to summer, therefore a significant difference in the occurrence of LAB is detected from winters to summers. The ecological condition in summer that is high temperature and humidity supports the bacterial growth in milk. Significant difference may be a result of this vast temperature variation.

IV. Conclusion

This study demonstrated that LAB are naturally present in raw cow and goat milk across all seasons, though their abundance decreases during winter. A 1:1 mixture of Gir cow and Surti goat milk was fermented without the addition of external cultures, relying solely on native LAB. The fermented samples underwent organoleptic, chemical, and microbial analysis, confirming proper fermentation with desirable sensory attributes and no signs of spoilage. Chemical analysis provided insight into key milk properties such as pH, titratable acidity, protein, fat, and ash content. Seasonal sampling from various regions of Surat city allowed for a balanced evaluation of how environmental conditions influence milk quality and microbial activity. Overall, naturally fermented milk from local breeds proves to be a consistent and promising source for isolating probiotic LAB.

V. References

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