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SHELF-LIFE ANALYSIS OF POTATO MILK AND POTATO YOGURT STORED AT DIFFERENT TEMPERATURES AND VIABILITY OF *Lactobacillus brevis* IN POTATO YOGURT

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ABSTRACT

When manufactured and stored, plant-based milks are unstable and tend to phase separate and degrade over extended periods. Studying the shelf life of milk and yogurt is crucial since it depends on whether they exhibit any physical, chemical, or sensory traits that make them unfit for human ingestion. The probiotics present in yogurt play a significant role in maintaining gut health. However, ensuring the probiotic's viability and assessing any physical property changes are crucial for fermented products containing probiotics, particularly when a combination of lactic acid bacteria is utilized as the probiotic or starter. Hence, the shelf-life of potato milk and yogurt was assessed by testing for the presence of total coliforms, total bacterial count, total fungal count, *Enterococcus sp., Clostridium sp., Pseudomonas sp., E. coli., V. parahemolyticus., V. cholerae., Shigella spp., Salmonella spp., S. aureus,* pH, total acidity, syneresis, total soluble solids, and the viability of potato yogurt stored at various temperatures was assessed using standard procedures. Potato milk and potato yogurt stored at room temperature had a shelf life of 1 day. At 15 °C, potato milk had a shelf life of 1 day, and potato yogurt had a shelf life of 2 days. At 10 °C, potato milk and yogurt had a shelf life of 2 days. At 5ºC, potato milk had a shelf life of 4 days, and potato yogurt had a shelf life of 8 days. At -2ºC, potato milk had a shelf life of 13 days, and potato yogurt had a shelf life of 16 days. The viability of *L. brevis* in potato yogurt stored at room temperature, 15 °C, 10 °C, 5 °C, and -2 °C remained the same between 10^7 and 10^8 cfu/ml.

Keywords: Shelf life, viability, *L. brevis*, potato yogurt, potato milk

INTRODUCTION

According to (MacBean, 2010), a product's shelf life is defined by how long it is safe to consume, how long its functional claims on the label or in compliance with regulations, and how long consumers find its sensory qualities acceptable. Their shelf life is carefully considered to ensure that potato milk and yogurt are suitable and safe for human consumption. Many factors affect shelf life, including how the product is prepared and heated, how it is packaged, and how the milk substitutes are kept to extend their shelf life and ensure the highest quality is delivered to the end users (*Glena & Caluza, 2019).*

There is a discernible loss of sensory qualities in the subsequent weeks of fresh milk or yogurt's shelf life. The three main microbiological agents, namely bacteria, yeast, and mold, are responsible for milk and yogurt spoiling. Yeast and mold can be found in fresh products due to contamination during processing (MacBean, 2010).

Particle size reduction, viscosity reduction, and microbe count reduction are three innovative technologies used to achieve stabilization. One key element influencing plant-based milk's stability is the size of the dispersed phase particles. When plant-based milks are stored for an extended period, they exhibit undesirable changes in their physicochemical characteristics and microbiological deterioration (Dhakal et al., 2014).

Microbiological techniques can minimize financial losses by identifying insufficient processing, packaging, or refrigeration early. This can be accomplished by monitoring the microbiological quality of bulk, finished, and raw sources during and after manufacturing. Microbiological measures are typically employed to confirm these conditions, particularly by enumerating total viable bacteria, coliforms, yeasts, and molds. According to (Yabaya & Idris, 2012), coliforms cause undesirable taints in products, making them of lower quality or even unmarketable. Yogurt spoiling is mainly caused by mold and yeasts, and the low pH creates a favourable environment for their growth (Fleet & Mian, 1987).

For probiotics to benefit the host's health, they must be alive. The sufficient quantity of living probiotic cells in a food product at consumption is called "viability." A minimum number of live microorganisms must be consumed to notice a beneficial health effect; this number typically ranges from 10^8 to 10^{11} cfu/ml, depending on the strains employed and the desired health effect (Vanderhoof & Young, 1998).

These recommendations have been established to offset the potential decrease in probiotic organism concentration during product manufacturing, storage, and transit through the upper and lower gastrointestinal tracts. Numerous studies have shown that probiotic bacteria do not develop well in milk, leading to low final concentrations in yogurt and even the loss of viability if storage conditions are improper or protracted. These bacteria must survive their shelf life until they are consumed (Ferdousi et al., 2013).

Probiotic yogurt needs to be stored in a refrigerator, as is often known, as storage temperature significantly impacts the vitality of probiotic bacteria. However, during industrial distribution, retailing, and residential storage, the goods, as mentioned earlier, may experience hours-long disruptions to the cold chain. This raises the question of whether the probiotic bacteria in the product diminish to the point where there are insufficient bacteria for it to be beneficial (Ferdousi et al., 2013).

Therefore, this paper aims to ascertain how quality variations in milk and yogurt are impacted by different storage conditions and determine the viability of lactic acid bacteria at various storage temperatures.

MATERIALS AND METHODS

Production of potato milk using ultrasonication

The potatoes were peeled, cut into pieces, and soaked in sodium metabisulphite for 10 minutes, which serves as an antibrowning agent. Followed by subsequent washing with distilled water. They were then extracted using an electric mixer. The slurry obtained was treated with alpha-amylase for 30 minutes and then filtered to obtain potato extract. The obtained potato extract is then homogenized with the emulsifier in an ultrasonicator (LABMAN-PRO 650) for 10 minutes at 20 kHz and 100 W to obtain potato milk. The milk is then pasteurized for 25 seconds at 80°C.

Production of potato yogurt using lactic acid fermentation

Potato milk was heated to 42°C and inoculated with *Lactobacillus brevis* (10^8 cfu/ml), which was then fermented for roughly 18 hours at 37°C until the pH reached 4.5. To prevent further fermentation, the resulting potato yogurt was chilled at 4°C for 2 hours. The finished product was then stored at 5°C.

Preparation of sample

For further analysis, potato milk and yogurt were aseptically prepared and stored at various temperatures, including -2ºC, 5 °C, 10 °C, 15 °C, and room temperature.

Total bacterial count

The total bacterial count was enumerated using the pour plate method (IS 5402).1ml of the serially diluted sample is inoculated onto the respective petri dish, to which molten plate count agar is poured and allowed to set. The plates are incubated at 37ºC for 48 hrs. After incubation, the colonies were counted and recorded.

Total bacterial count (cfu/ml) = $\frac{No.of\ colonies\ X\ Total\ dilution\ factor}{Volume\ of\ sample\ plated\ in\ ml}$

Total fungal count (yeast and molds)

Total yeast and mold count was enumerated using the pour plate method (IS 5403).1 ml of the serially diluted sample is inoculated onto the respective petri dish, to which molten potato dextrose agar with 10% tartaric acid is poured and allowed to set. The plates are incubated at 25ºC for seven days. After incubation, the colonies were counted and recorded.

Total fungal count (cfu/ml) = $\frac{No.of\ colonies\ X\ Total\ dilution\ factor}{Volume\ of\ sample\ plated\ in\ ml}$

Total coliform

Total coliform was enumerated using the pour plate method (IS 5401: Part 1). 1 ml of the serially diluted sample is inoculated onto the respective petri dish, where molten violet red bile agar is poured and allowed to set. The plates are incubated at 37ºC for 48 hrs. After incubation, the colonies were counted and recorded.

Total coliform (cfu/ml) = $\frac{No.of\ colonies\ X\ Total\ dilution\ factor}{Volume\ of\ sample\ plated\ in\ ml}$

Determination of *Enterococcus* (*faecal streptococci*)

Enterococcus (*faecal streptococcus*) was identified according to the method prescribed in (IS 5887: Part 2). The sample is filtered through a sterile membrane filter (0.45 µm pore size), after which the filter is placed on ethyl violet azide dextrose agar plates and incubated at 37 °C for 48 hrs. Dark red colonies or colonies with red or pink centres indicate positive results.

Determination of *Pseudomonas spp.*

Pseudomonas was identified by the method prescribed in (IS 13428: Annex D).1 ml of sample is inoculated into 4 ml of double-strength asparagine proline broth and incubated for 48 hrs. After incubation, the broth is observed for the presence of fluorescence. If fluorescence is present, the broth is inoculated in milk agar plates at 42 °C for 24 hrs and observed for pigment production or casein hydrolysis.

Determination of *Escherichia coli*

Escherichia coli was identified by the method prescribed in (IS 5887: Part 1). 1 ml of sample is enriched in 0.1% peptone salt solution at 37 °C for 24 hrs. The enriched sample is inoculated onto eosin methylene blue agar and incubated at 37 °C for 24 hrs. The presence of a green metallic sheen indicates a positive result.

Determination of *Vibrio cholerae* and *Vibrio parahaemolyticus*

V. cholerae and *V. parahaemolyticus* were determined by the prescribed method (IS 5887: Part 5). 1 ml of sample is enriched in alkaline peptone water at 37°C for 6 to 8 hrs. The enriched culture is inoculated in Thio sulphate citrate bile salt sucrose agar and incubated at 37 °C for 24 hrs. Yellow colonies indicate positive for *V. cholerae*, and green colonies show positive for *V. parahaemolyticus*.

Determination of *Clostridium spp.*

Clostridium was identified by the method prescribed in (ISO 7937). A 2 ml sample was enriched in 20 ml of cooked meat medium and incubated at 37°C for 24 hrs. The presence of turbidity and gas production indicates positive tubes. The positive tube is inoculated into tryptose sulfite cycloserine agar and incubated at 37°C for 24 hrs. The presence of black colonies surrounded by a precipitate zone indicates a positive result.

Determination of *Salmonella spp.* and *Shigella spp.*

Salmonella spp. was identified by the method prescribed in (ISO 6579-1), and *Shigella spp.* was determined by the method defined in (ISO 21567). Pre-enrichment is done by inoculating 1 ml sample in 9 ml of buffered peptone water and incubating at 37°C for 24 hrs. Enrichment is done by inoculating the pre-enriched culture in 10 ml of Rappaport-Vassiliadis broth for *Salmonella* and 10 ml of selenite F broth for *Shigella* and incubating at 37°C for 24 hrs. The enriched culture is inoculated onto *xylose lysine deoxycholate agar and incubated* at 37°C for 24 hrs. Pink colonies with or without black centres indicate positive for *Salmonella spp.,* and red colonies for *Shigella spp*.

Determination of *Staphylococcus aureus*

Staphylococcus aureus was identified by the method prescribed in (Chapman,1945). 1 ml of sample was enriched in 0.1% peptone salt solution at 37ºC for 24 hrs. The enriched sample is inoculated onto mannitol salt agar and incubated at 37 °C for 24 hrs. The presence of yellow colonies indicates a positive result.

Determination of pH

The product response pH was estimated using the method 943.02 (Frederick et al., 1981). About 10 ml of sample is taken, in which the pH probe is placed and checked for its acidic or basic condition.

Determination of total solids

Total solids are measured by weighing the amount of solids in a known sample volume. About 10 g of the sample was taken, placed in the petri dish, spread evenly over the bottom, weighed, and recorded. The petri dish was then placed in the hot air oven to establish the optimum period required for complete drying. After drying, the sample was immediately removed from the oven and placed in a desiccator for cooling. After cooling, the petri dishes containing dry matter were weighed, and all weights were recorded. The samples were run in triplicate, and the percentage of total solids was determined (Frary & Jordan, 1940).

Total solids (%) = Weight of residue/Weight of the sample×100

Determination of titratable acidity

The method described in 942.15 (AOAC, 2000) was used to gauge the titratable acidity present. About 10 ml of the sample was taken in a conical flask, and an equal quantity of distilled water was added.3-4 drops of phenolphthalein indicator were added and titrated against 0.1 N NaOH solution until the endpoint pink color was obtained.

% acid= $\frac{\text{Normality of NaOH x Volume of NaOH used x Molecular weight of predominant acid}}{Sample\ size(ml)x\ 10}$

Determination of syneresis

According to the method proposed by (Habwalker & Kalab, 1986), the syneresis of the produced yogurt was estimated. About 10 g of yogurt was taken and filtered using a filtrate to remove the whey oozing out of the yogurt. The amount of syneresis was calculated as

Syneresis (%) = $\frac{Volume\ of\ whey\ drained}{Volume\ of\ yogurt\ sample\ taken}$x 100

Viability of *L. Brevis* on potato yogurt

Lactobacillus brevis was enumerated using the pour plate method (Kailasapathy et al., 2008). Potato yogurt stored at various temperatures is serially diluted in buffered peptone water.1 ml of the diluted sample is inoculated onto the respective petri dish, to which molten MRS agar is poured and allowed to set. The plates are incubated at 37ºC for 24 hrs. After incubation, the colonies were counted and recorded.

Viability of *L. brevis* (cfu/ml) = $\frac{No.of\ colonies\ X\ Total\ dilution\ factor}{Volume\ of\ sample\ plated\ in\ ml}$

Statistical analysis

Data were analyzed by Origin Pro 10.0 (Trial version). The results were expressed as means ± standard deviation of triplicate determinations. Statistical analysis was performed using analysis of variance (ANOVA), and the Duncan test was performed to test the mean deviation of readings from all analyses.

RESULTS AND DISCUSSION

Shelf-life analysis of potato milk

Table 1 Shelf-life analysis of potato milk stored at various temperatures

Parameters	Storage Temperature of Potato Milk									
	Room Temperature		15^{0}C		10^{0}C		5^{0}C		-2^{0}C	
	Day 0	Day 1	Day 0	Day 1	Day 0	Day 2	Day 0	Day 4	Day 0	Day 13
TBC	-	$2.9x10^4$	-	$6x10^4$	-	-	-	$1x10^5$	-	$2.3x10^4$
TFC	-	$4x10^4$	-	$1x10^4$	-	-	-	$1.6x10^3$	-	$5x10^3$
Total Coliform	-	-	-	$8.6x10^5$	-	-	-	-	-	-
Enterococcus spp.	-	-	-	-	-	-	-	-	-	-
Clostridium spp.	-	-	-	-	-	-	-	-	-	-
Pseudomonas spp.	-	-	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	Present	-	-	-	-
V.parahemolyticus	-	+	-	-	-	-	-	-	-	-
V.cholera	-	-	-	-	-	-	-	-	-	-
Shigella spp.	-	-	-	-	-	-	-	-	-	-
Salmonella spp.	-	-	-	-	-	-	-	-	-	-
S. aureus	-	Present	-	Present	-	-	-	Present	-	-
pH	7.1±0	6.7±0.8	7.1±0	7±0.87	7.1±0	6.9±1.3	7.1±0	7±0.1	7.1±0	7±0.5
TSS (%)	7.3±0	7.9±2.5	7.3±0	7.8±0.7	7.3±0	8.2±1.1	7.3±0	8±3.5	7.3±0	7.6±0.1

Values represent means ± standard deviation of replicate experiments (n=3)

SHELF LIFE OF POTATO MILK STORED AT ROOM TEMPERATURE

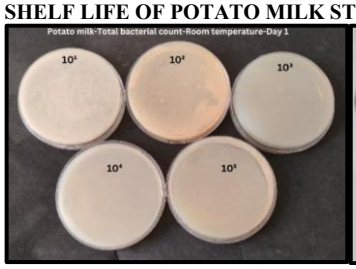


Figure 1 TBC of potato milk at day 1

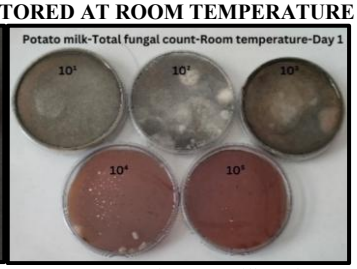


Figure 2TFC of potato milk at day 1

After being evaluated for total bacterial count on day 1 of storage, it was discovered that the potato milk kept at room temperature had $2.9x10^4$ cfu/ml (Figure 1). As per (FSSAI 1-110(3)/SP,2010), the highest allowable TBC is 1 x 10^9 cfu/ml. On day 1, potato milk, kept at room temperature, had a TFC of $4x10^4$ cfu/ml (Figure 2). The maximum allowable TFC is $1x10^3$ cfu/ml, per (FSSAI 1-110(3)/SP, 2010). The physicochemical properties that including pH and total soluble solids, showed no changes (Table 1).

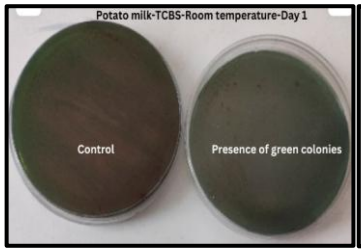


Figure 3 Presence of *V. parahemolytica* on day 1

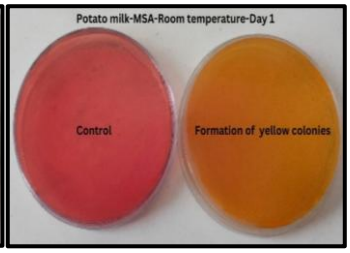


Figure 4 Presence of *S. aureus* on day 1

For food to be deemed edible, *Vibrio spp.* must be absent, per (FSSAI 1-110(3)/SP, 2010). *V. parahemolytica* was detected in potato milk held at room temperature on day 1 by developing green-colored colonies on TCBS agar (Figure 3). The maximum amount of *S. aureus* that can be present in food is zero (FSSAI 1-110(3)/SP, 2010); however, on day 1, potato milk kept at room temperature developed yellow colonies on MSA, indicating the presence of *S. aureus* (Figure 4). As a result, the shelf life of potato milk kept at room temperature was 1 day. There was no change in the physicochemical property (Table 1).

Shelf life of potato milk stored at 15 °c

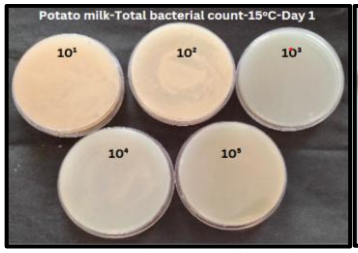


Figure 5 TBC of potato milk at day 1

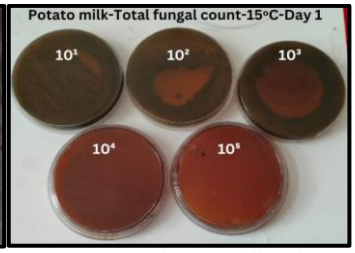


Figure 6 TFC of potato milk at day 1

After being evaluated for total bacterial count on day 1 of storage, it was discovered that the potato milk kept at 15 °C had $6x10^4$ cfu/ml (Figure 5). As per FSSAI 1-110(3)/SP,2010, the highest allowable TBC is 1 x 10^4 cfu/ml. On day 1, potato milk kept at room temperature had a TFC of $1x10^4$ cfu/ml (Figure 6). The maximum allowable TFC is $1x10^3$ cfu/ml, per (FSSAI 1-110(3)/SP, 2010). The physicochemical properties showed no changes (Table 1)

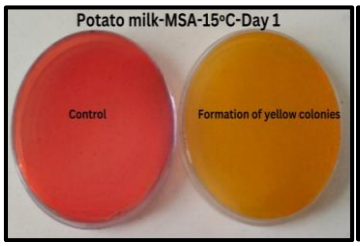


Figure 7 Presence of *S. aureus* at day 1

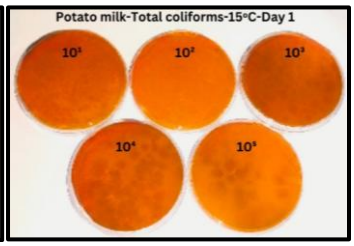


Figure 8 Presence of Total coliform at day 1

The maximum amount of *S. aureus* that can be present in food is zero (FSSAI 1-110(3)/SP, 2010); however, on day 1, potato milk kept at 15 °C developed yellow colonies on MSA, indicating the presence of *S. aureus* (Figure 7). The permissible amount of total coliform in food is zero (FSSAI 1-110(3)/SP, 2010); however, potato milk stored at 15°C on day 1 showed the presence of coliform on Violet red bile agar with $8.6x10^5$ cfu/ml (Figure 8). Hence, the shelf life of potato milk stored at 15 °C is 1 day. The physicochemical property remained the same till shelf life (Table 1).

Shelf life of potato milk stored at 10 °c

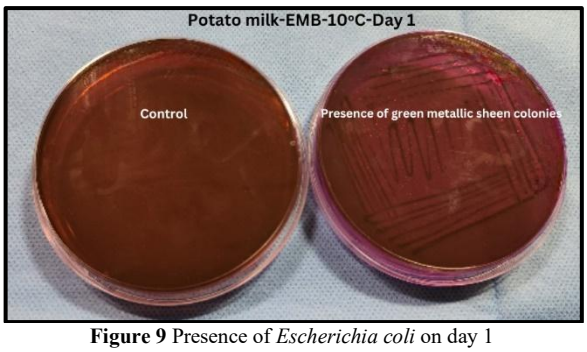


Figure 9 Presence of *Escherichia coli* on day 1

According to (FSSAI 1-110(2)/SP, 2010), the permissible limit of *E. coli* is zero. Potato milk stored at 10 °C on day 2 showed the presence of green metallic sheen on EMB agar (Figure 9); hence, the shelf life of potato milk stored at 10 °C is 2 days. There was a mild difference in the physicochemical properties (Table 1).

Shelf life of potato milk stored at 5°c

Figure 10 TBC at day 4

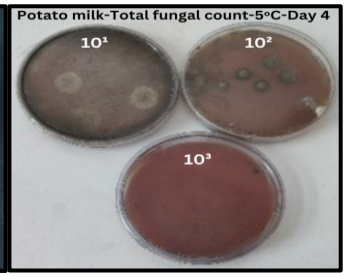


Figure 11 TFC at day 4

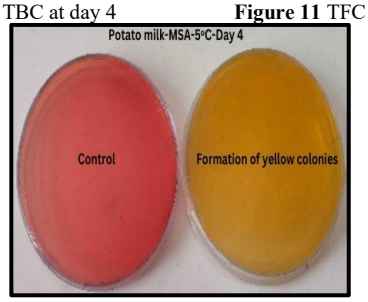
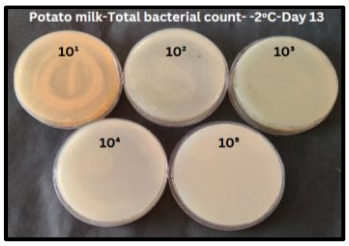


Figure 12 *S. aureus* on day 4

After being evaluated for the total bacterial count on day 4 of storage, it was discovered that the potato milk kept at 5ºC had $1x10^5$ cfu/ml (Figure 10). As per FSSAI 1-110(3)/SP,2010, the highest allowable TBC is 1 x 10^4 cfu/ml. On day 4, potato milk kept at 5ºC, had a TFC of $1.6x10^3$ cfu/ml (Figure 11). The maximum allowable TFC is $1x10^3$ cfu/ml, per (FSSAI 1-110(3)/SP, 2010). The maximum amount of *S. aureus* that can be present in food is zero (FSSAI 1-110(3)/SP, 2010); however, on day 4, potato milk kept at 5 °C developed yellow colonies on MSA, indicating the presence of *S. aureus* (Figure 12). Hence, the shelf life of potato milk stored at 5ºC is 4 days. A mild difference was observed in the physicochemical properties (Table 1).

Shelf life of potato milk stored at -2ºc



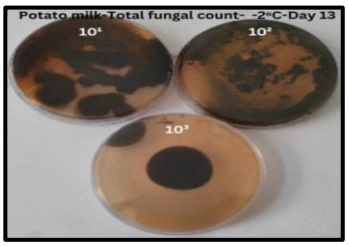


Figure 13 TBC of potato milk at day 13 **Figure 14** TFC of potato milk at day 13

After being evaluated for total bacterial count on day 13 of storage, it was discovered that the potato milk kept at -2 °C had 2.3×10^4 CFU/ml (Figure 13). As per (FSSAI 1-110(3)/SP,2010), the highest allowable TBC is 1 x 10^4 cfu/ml. On day 13, potato milk kept at -2ºC had a TFC of $5x10^3$ cfu/ml (Figure 14). The maximum allowable TFC is $1x10^3$ cfu/ml, per (FSSAI 1-110(3)/SP, 2010). Hence, the shelf life of potato milk stored at -2ºC is 13 days. There was a minor difference in the physicochemical properties (Table 1).

Shelf-life analysis of potato yogurt

Table 2 Shelf-life analysis of potato yogurt stored at various temperatures

Parameters	Storage Temperature of Potato Yogurt									
	Room temperature		15⁰C		10⁰C		5⁰C		-2⁰C	
	Day 0	Day 1	Day 0	Day 2	Day 0	Day 2	Day 0	Day 8	Day 0	Day 16
TFC	-	$5x10^1$	-	$1x10^3$	-	$2x10^2$	-	-	-	-
Total Coliform	-	-	-	-	-	-	-	-	-	-
Enterococcus spp.	-	-	-	-	-	-	-	-	-	-
Clostridium spp.	-	-	-	-	-	-	-	-	-	-
Pseudomonas spp.	-	-	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	Present	-	-	-	-
V.parahemolyticus	-	-	-	-	-	-	-	-	-	-
V.cholera	-	Present	-	Present	-	-	-	-	-	-
Shigella spp.	-	-	-	-	-	-	-	-	-	-
Salmonella spp.	-	-	-	-	-	-	-	-	-	-
S. aureus	-	Present	-	Present	-	-	-	Present	-	Present
pH	4.9±0	7.4±1.1	4.9±0	5±2.9	4.9±0	5.2±0.1	4.9±0	5.2±1.1	4.9±0	4.9±0.5
T. Acidity (%)	0.6±0	0.6±2.5	0.6±0	0.7±0.7	0.6±0	0.6±1.1	0.6±0	0.7±3.5	0.6±0	0.8±0.1
Syneresis (%)	22±0	22±1.9	22±0	23±2.1	22±0	22±1.3	22±0	22±2.0	22±0	23±3.2

Values represent means ± standard deviation of replicate experiments (n=3)

Shelf life of potato yogurt stored at room temperature

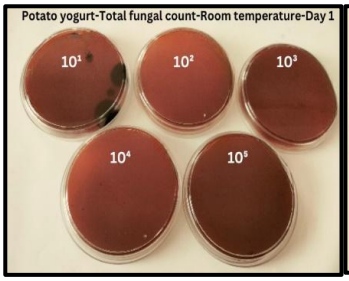


Figure 15 TFC at day 1

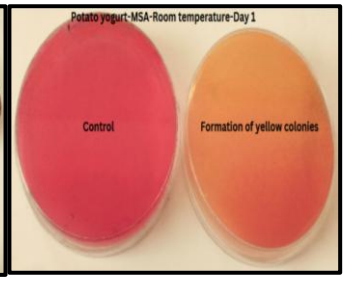


Figure 16 *S. aureus* at day 1

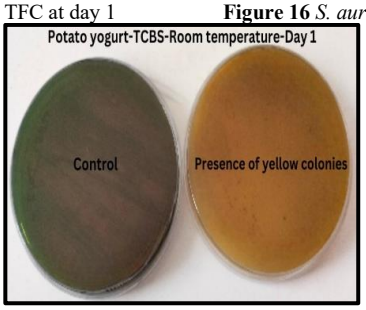


Figure 17 *V. cholerae* at day 1

The maximum allowable TFC is $1x10^2$ cfu/ml, per (FSSAI 1-110(3)/SP, 2010). On day 1, the TFC of potato yogurt was within the permissible limit of $5x10^1$ cfu/ml (Figure 15). The maximum amount of *S. aureus* that can be present in food is zero (FSSAI 1-110(3)/SP, 2010); however, on day 1, potato yogurt kept at room temperature developed yellow colonies on MSA, indicating the presence of *S. aureus* (Figure 16). For food to be deemed edible, *Vibrio spp.* must be absent, per (FSSAI 1-110(3)/SP, 2010). *V. cholerae* was detected in potato yogurt held at room temperature on day 1 by developing yellow-colored colonies on TCBS agar (Figure 17). As a result, the shelf life of potato yogurt kept at room temperature was 1 day. The pH drastically increased to an alkaline condition, while titratable acidity and syneresis remained the same (Table 2).

Shelf life of potato yogurt stored at 15 °c

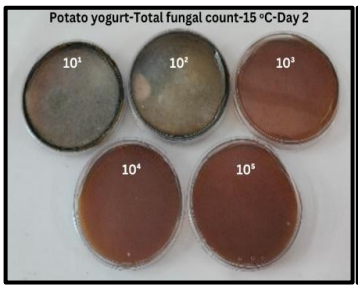


Figure 18 TFC at day 2

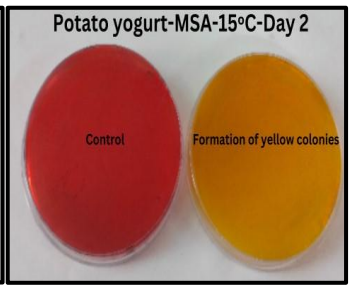


Figure 19 *S. aureus* at day 2

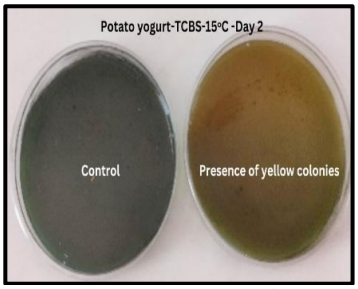


Figure 20 *V. cholerae* at day 2

The maximum allowable TFC is $1x10^2$ cfu/ml, per (FSSAI 1-110(3)/SP, 2010). At day 2, the TFC of potato yogurt was $1x10^3$ cfu/ml (Figure 18). The maximum amount of *S. aureus* that can be present in food is zero (FSSAI 1-110(3)/SP, 2010); however, on day 2, potato yogurt kept at 15 °C developed yellow colonies on MSA, indicating the presence of *S. aureus* (Figure 19). For food to be deemed edible, *Vibrio spp.* must be absent, per (FSSAI 1-110(3)/SP, 2010). *V. cholerae* was detected in potato yogurt held at 15°C on day 2 by developing yellow-colored colonies on TCBS agar (Figure 20). As a result, the shelf life of potato yogurt kept at 15 °C was 2 days. There was no change in the physicochemical property (Table 2).

Shelf life of potato yogurt stored at 10 °c

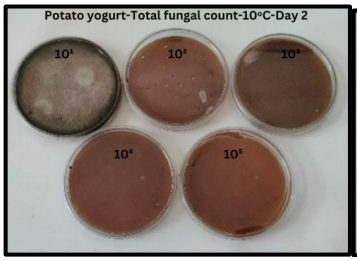


Figure 21TFC at day 2

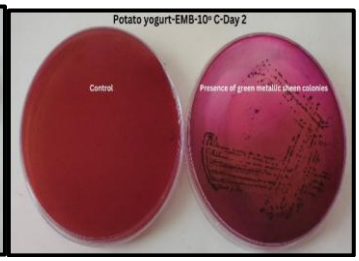


Figure 22 *E. coli* on day 2

The maximum allowable TFC is $1x10^2$ cfu/ml, per (FSSAI 1-110(3)/SP, 2010). On day 2, potato yogurt stored at 10 °C had a TFC of $2x10^2$ cfu/ml (Figure 21). According to (FSSAI 1-110(2)/SP, 2010), the permissible limit of *E. coli* is zero. Potato yogurt stored at 10 °C on day 2 showed the presence of green metallic sheen on EMB agar (Figure 22); hence, the shelf life of potato yogurt stored at 10 °C is 2 days. The physicochemical properties of potato yogurt showed no changes (Table 2).

Shelf life of potato yogurt stored at 5°c

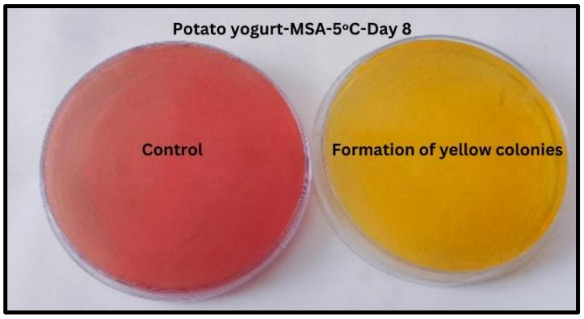


Figure 23 *S. aureus* at day 8

The maximum amount of *S. aureus* that can be present in food is zero (FSSAI 1-110(3)/SP, 2010); however, on day 8, potato yogurt kept at 5 °C developed yellow colonies on MSA, indicating the presence of *S. aureus* (Figure 23). Hence, the shelf life of potato yogurt stored at 5°C is 8 days. There was no drastic change observed during the shelf life (Table 2).

Shelf life of potato yogurt stored at -2°c

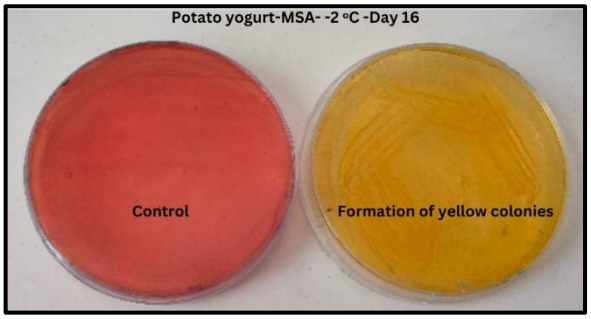


Figure 24 *S. aureus* at day 16

The maximum amount of *S. aureus* that can be present in food is zero (FSSAI 1-110(3)/SP, 2010); however, on day 16, potato yogurt kept at -2 °C developed yellow colonies on MSA, indicating the presence of *S. aureus* (Figure 24). Hence, the shelf life of potato yogurt stored at -2°C is 16 days. The physicochemical properties remained the same throughout the entire shelf life (Table 2).

Viability of *L. Brevis* in potato yogurt stored at various temperatures

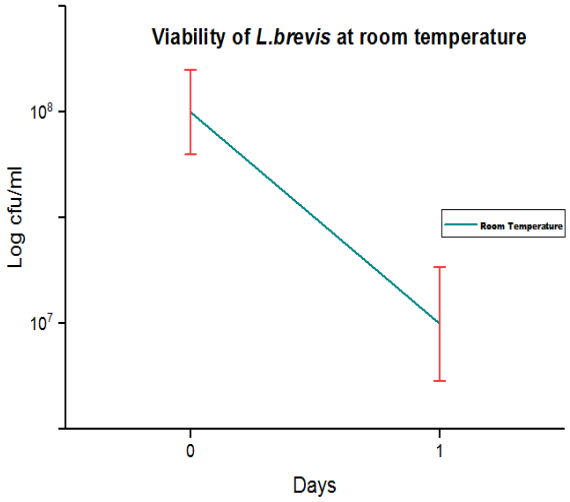


Figure 25 Viability of *L. brevis* at RT

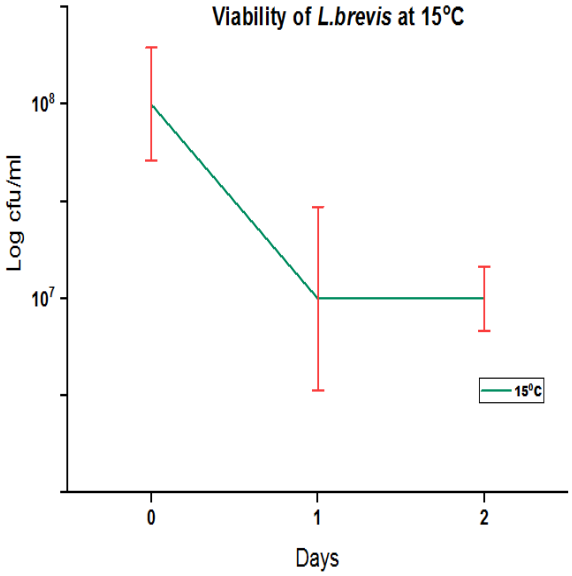


Figure 26 Viability of *L. brevis* at 15 °C

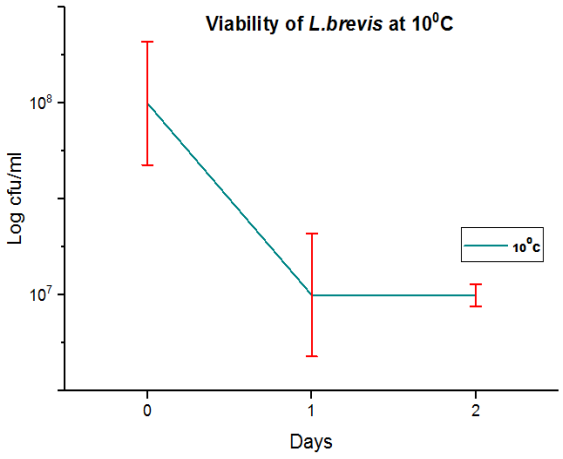


Figure 27 Viability of *L. brevis* at 10 °C

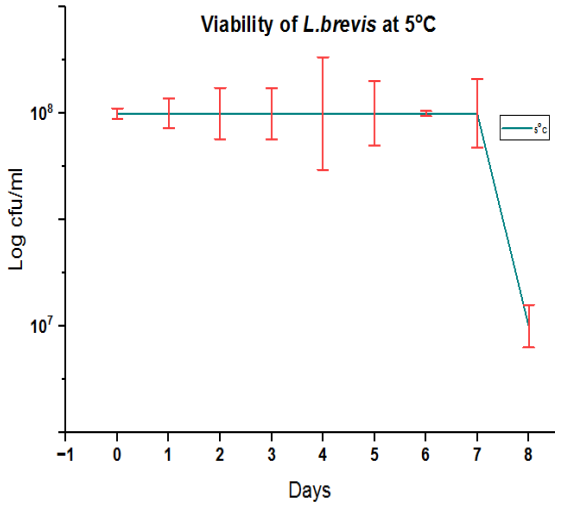


Figure 28 Viability of *L. brevis* at 5°C

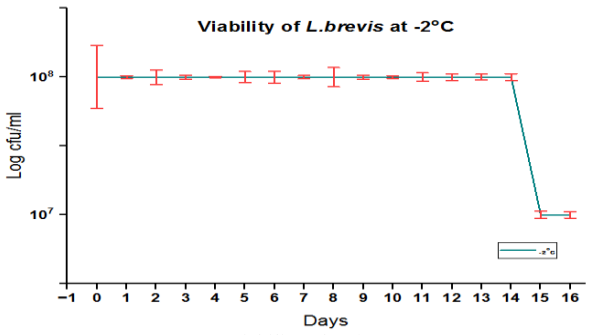


Figure 29 Viability of *L. brevis* at -2 °C

L. brevis present in potato yogurt stored at various temperatures was assessed for its viability until its respective shelf life. At room temperature, 15 °C, 10 °C, and 5 °C, the viability of *L. brevis* was between 10^7 and 10^8 until its shelf life (Figure 25) (Figure 26) (Figure 27) (Figure 28) (Figure 29). This was in accordance with (Parra et al.,2013), where it was stated that a good probiotic culture should contain between 10^6 and 10^8 viable cells per ml.

CONCLUSIONS

The shelf-life analysis of potato milk and yogurt, and the viability of potato yogurt stored at various temperatures, were assessed using standard procedures. Potato milk and potato yogurt stored at room temperature had a shelf life of 1 day. Notably, at room temperature, potato yogurt exhibited a drastic increase in pH to an alkaline level, indicating a significant physicochemical change that compromises product safety and sensory quality. This rise in pH may be attributed to microbial spoilage, particularly the growth of proteolytic or alkaline-producing contaminants such as *Staphylococcus aureus* and *Vibrio cholerae*, which were detected during storage. At -2ºC, potato milk had a shelf life of 13 days, and potato yogurt had a shelf life of 16 days. Notably, storage at 5 ºC greatly enhanced the lifespan to 3 days for potato milk and 7 days for potato yogurt. This conclusion was drawn as the presence of a pathogen was not detected, and the total bacterial count and fungal count remained within the acceptable limits. The physicochemical property also remained stable, and the viability of *L. brevis* remained the same throughout the shelf-life period. The viability of *L. brevis* in potato yogurt stored at room temperature, 15 °C, 10 °C, 5 °C, and -2 °C remained the same between 10^7 and 10^8 cfu/ml.

In conclusion, while extremely low temperatures, -2ºC have proven to slow down the microbial growth, 5 °C has provided a proper balance between microbial safety and functional probiotic safety, offering the longest storage period among the temperatures tested. These findings recommend 5 °C as the optimal storage condition for potato-based milk and yogurt alternatives to ensure product safety, quality, and efficacy over time.

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