

## Effect of sugar concentration on acidification profile (pH) of *Streptococcus thermophilus* cultures in skim milk

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### Abstract

This study investigated the acidification profiles of ten different *Streptococcus thermophilus* cultures (C1-C10) in skim milk supplemented with varying sugar concentrations (0%, 5%, 10%, 15%, 20%, and 25%) over an 8-hour incubation period. The objective was to determine the influence of sugar content and incubation time on the pH reduction capability of these cultures. The pH of each culture was monitored at 0, 4, 6, 7, and 8 hours. Results indicated that for all cultures, pH decreased significantly ( $p < 0.05$ ) with increasing incubation time across all sugar concentrations. The initial pH, averaging between 6.00 and 6.31, dropped to final values ranging from 4.77 to 5.49 after 8 hours. The effect of sugar concentration on acidification was variable and dependent on the specific culture. Generally, the addition of sugar up to 15% enhanced the rate of acidification compared to the control (0% sugar). However, concentrations of 20% and 25% showed an inhibitory effect on some cultures (e.g., C1, C4, C8), resulting in a higher final pH compared to intermediate sugar levels. Conversely, other cultures (e.g., C5, C6) demonstrated high tolerance and effective acidification at these higher concentrations. Notably, significant variations ( $p < 0.05$ ) were observed among the ten cultures, with C3 and C6 emerging as the most robust acidifiers, achieving a pH of approximately 4.78. In contrast, cultures like C5 and C9 were slower acidifiers, maintaining a pH above 5.0 throughout the incubation. These findings underscore the critical importance of selecting specific *S. thermophilus* strains and optimizing sugar levels to control fermentation rates and achieve desired final pH in dairy products.

**Keywords:** Acidification profile, incubation period, sugar levels, dairy fermentation

### Introduction

*Streptococcus thermophilus* is a key thermophilic lactic acid bacterium extensively used as a starter culture in the production of fermented dairy products, particularly yogurt and certain cheeses. Its primary function in dairy fermentation is the rapid conversion of lactose into lactic acid, which leads to a decrease in pH and coagulation of milk proteins - an essential step in the development of texture, flavor, and microbial safety in dairy products (Tamime & Robinson, 2007) [3]. This acidification process is crucial not only for texture and flavor development but also for ensuring microbial safety and extending shelf life of the final product.

The acidification profile of *S. thermophilus*, typically measured through pH decline over time, is an important indicator of its metabolic activity and fermentation efficiency. Several intrinsic and extrinsic factors influence this process, including milk composition, sugar availability, temperature, and the physiological state of the culture (Zotta *et al.*, 2021) [4]. Skim milk, widely used in controlled fermentation studies, naturally contains lactose as the principal fermentable carbohydrate. However, supplementing milk with additional sugars such as sucrose or glucose may enhance fermentation by providing extra energy substrates, potentially shortening the lag phase and accelerating acid production (Donkor *et al.*, 2007) [2]. This can be particularly relevant in formulations aiming to achieve faster fermentation or modify sensory attributes. Moreover, sugar supplementation can influence not only acidification kinetics but also bacterial growth, exopolysaccharide (EPS) production, and flavor compound synthesis (Beal *et al.*, 1999) [1]. These attributes are

particularly important in the manufacture of stirred and set yogurts where texture and consistency are closely tied to acidification behavior and microbial activity.

Given the importance of rapid and controlled acidification in dairy fermentation, this study aims to evaluate the acidification profile of *Streptococcus thermophilus* in reconstituted skim milk supplemented with sugar. Monitoring pH changes over time in both supplemented and non-supplemented media provides insights into how sugar impacts the metabolic dynamics of *S. thermophilus*, with potential applications in optimizing industrial fermentation protocols. Understanding the acidification kinetics of *S. thermophilus* in sugar-enriched milk environments is essential for optimizing fermentation processes and tailoring the functional properties of fermented dairy products. This study aims to evaluate the pH profile of *S. thermophilus* in reconstituted skim milk supplemented with sugar, providing insights into how sugar addition influences acid production and fermentation performance.

### Objective

To assess the acidification behaviour of *Streptococcus thermophilus* during fermentation in reconstituted skim milk supplemented with sugar, monitoring pH decline over time.

### 1. Materials and Methods

#### 1. Milk Preparation

a. Reconstitute skim milk and sterilize by autoclaving (e.g., 121 °C for 15 min).

#### 2. Incubation

a. Incubate at 42 °C.

- b. Take pH measurements at 0, 4, 6, and 8 hours (or until pH drops to a significant amount).

**3. Controls**

- a. Include a control with no added sugar.
- b. Optional: use uninoculated milk to monitor spontaneous acidification or contamination.

**4. Acidification Profile in Skim Milk with Added Sugar**

Skim milk standardized to 12% total solids (TS) was supplemented with varying concentrations of sugar at 0%, 5%, 10%, 15%, 20%, and 25% (w/v). Each formulation was sterilized at 121°C for 15 minutes. Following sterilization

and cooling, the milk samples were inoculated with 2% (v/v) active *Streptococcus thermophilus* culture and incubated at 42°C. Acidification during fermentation was monitored by measuring the change in pH over time.

**5. pH Meter**

pH of the sample was measured with the help of pre-calibrated pH meter (ION2700, Labtek, Eutech, India) (Plate 1).

**Plate 1: Acidification profile (pH) of *Streptococcus thermophilus* in skim milk with added sugar**

Skim milk with added sugar (a) sample bottle for measuring acidification profile (pH) (b), Dahi prepared at departmental laboratory in incubator (c), pH measurement of sample(d).



**6. Statistical Significance**

Statistical analysis revealed that both incubation time and sugar concentration significantly affected acidification ( $p < 0.05$ ). The interaction between these two factors also influenced the pH outcomes, particularly during the later stages of fermentation. Superscript annotations in the table indicate that both time-dependent and concentration-

dependent variations were statistically significant.

**7. Results and Discussion**

The acidification profiles of ten distinct *Streptococcus thermophilus* cultures (C1–C10) in skim milk supplemented with varying sugar concentrations (0% to 25%) are detailed in the provided tables (Table 1 and 2).

**Table 1:** Acidification profile (pH) of *Streptococcus thermophilus* in skim milk with added sugar

Culture	Incubation(h)	Skim milk with added sugar %					
		0	5	10	15	20	25
	0	aA 6.31±0.003	aB 6.25±0.003	aAB 6.29±0.018	aB 6.26±0.003	aAB 6.28±0.020	C 6.18±0.013

C1	4	bA 5.73±0.020	bB 5.47±0.003	bC 5.41±0.009	bD 5.35±0.010	bC 5.43±0.006	C 5.42±0.003
	6	cA 5.56±0.007	cB 5.18±0.009	cD 5.12±0.003	cE 5.08±0.009	cC 5.15±0.006	D 5.13±0.003
	7	dA 5.50±0.003	dB 5.11±0.006	dC 5.02±0.006	dD 4.99±0.010	dC 5.04±0.006	C 5.03±0.006
	8	eA 5.47±0.006	eB 5.06±0.019	eD 4.93±0.003	eE 4.90±0.010	eC 4.97±0.003	C 4.99±0.007
C2	0	aA 6.31±0.003	aB 6.24±0.015	aA 6.30±0.003	aB 6.24±0.007	aB 6.22±0.006	aB 6.23±0.018
	4	bB 5.38±0.009	bC 5.32±0.006	bC 5.31±0.007	bD 5.24±0.009	bD 5.24±0.013	bA 5.74±0.009
	6	cA 5.17±0.006	cB 5.06±0.010	cB 5.06±0.003	cC 4.97±0.027	cC 4.99±0.007	cC 4.97±0.006
	7	dA 5.12±0.010	dB 4.99±0.003	dB 5.00±0.006	cD 4.88±0.009	dD 4.87±0.009	dC 4.92±0.006
	8	eA 5.02±0.009	eB 4.92±0.003	eB 4.92±0.003	eB 4.80±0.062	eB 4.83±0.006	eB 4.84±0.006
C3	0	aA 6.24±0.003	aB 6.15±0.000	aA 6.20±0.003	aB 6.13±0.000	aC 6.07±0.033	aBC 6.11±0.007
	4	bA 5.26±0.007	bB 5.20±0.003	BC 5.16±0.006b	bC 5.14±0.037	bC 5.15±0.006	bB 5.20±0.003
	6	cA 5.05±0.026	cB 4.99±0.006	cB 4.95±0.009	cB 4.93±0.006	cB 4.96±0.019	cB 4.97±0.030
	7	dA 5.01±0.006	dB 4.89±0.007	dC 4.87±0.007	dC 4.86±0.006	dC 4.87±0.003	dB 4.91±0.006
	8	eA 4.92±0.006	eB 4.86±0.006	eD 4.78±0.006	eD 4.79±0.007	eD 4.79±0.012	eC 4.83±0.007
C4	0	aA 6.28±0.003	aBC 6.20±0.003	aB 6.21±0.010	aC 6.18±0.010	aC 6.18±0.003	aD 6.14±0.006
	4	bC 5.21±0.006	bD 5.16±0.006	bD 5.16±0.035	bB 5.27±0.003	bA 5.33±0.013	bA 5.37±0.006
	6	cB 5.04±0.023	cC 4.95±0.026	cC 4.95±0.029	cB 5.02±0.015	cAB 5.07±0.018	cA 5.12±0.006
	7	dB 5.00±0.003	dD 4.88±0.006	dE 4.85±0.003	dC 4.93±0.015	dA 5.03±0.006	dAB 5.02±0.006
	8	dA 4.98±0.006	dD 4.84±0.006	dE 4.80±0.000	eC 4.89±0.010	eB 4.92±0.006	eA 4.97±0.003
C5	0	aB 6.23±0.006	aA 6.27±0.009	aB 6.21±0.006	aC 6.14±0.007	aB 6.20±0.006	aC 6.12±0.019
	4	bC 5.76±0.030	bB 5.81±0.007	bC 5.76±0.003	bC 5.75±0.003	bA 5.86±0.003	bB 5.81±0.003
	6	cB 5.47±0.003	cA 5.51±0.009	cBC 5.45±0.003	cC 5.44±0.009	cA 5.50±0.006	cC 5.43±0.007
	7	cA 5.43±0.007	dA 5.44±0.015	dB 5.37±0.006	dC 5.32±0.009	dB 5.39±0.006	dD 5.29±0.006
	8	dC 5.25±0.006	eA 5.37±0.006	eC 5.27±0.003	eD 5.22±0.006	eB 5.32±0.009	eD 5.21±0.006

Values are means of three independent trials with SE; a, b, c: Different superscript in each column denotes significant

differences (p<0.05) of pH with incubation hour; A, B, C: Different superscript in each row denotes significant difference(p<0.05) between sugar level.

**Table 2:** Acidification profile (pH) of *Streptococcus thermophilus* in skim milk with added sugar

Culture	Incubation (h)	Skim milk with added sugar %					
		0	5	10	15	20	25
C6	0	aA 6.20±0.018	aC 6.10±0.015	aBC 6.13±0.015	aB 6.15±0.000	aE 6.00±0.007	aD 6.05±0.003
	4	bA 5.54±0.006	bB 5.46±0.006	bBC 5.41±0.010	bC 5.39±0.047	bC 5.36±0.006	bBC 5.41±0.007
	6	cA 5.17±0.007	cB 5.06±0.006	cC 4.99±0.006	cD 4.96±0.010	cE 4.93±0.003	cD 4.96±0.006
	7	dA 5.14±0.003	dB 4.98±0.006	dC 4.90±0.009	dC 4.89±0.006	dD 4.83±0.007	dD 4.85±0.019
	8	eA 5.10±0.006	eB 4.94±0.006	eC 4.83±0.006	eC 4.81±0.003	eE 4.77±0.007	eD 4.79±0.007
	0	aA 6.30±0.003	aB 6.23±0.006	aB 6.22±0.003	aC 6.19±0.003	aD 6.17±0.006	aE 6.11±0.006

C7	4	bA 5.53±0.024	bB 5.42±0.009	bC 5.34±0.020	bD 5.19±0.003	bD 5.21±0.007	bD 5.22±0.006
	6	cA 5.37±0.006	cB 5.33±0.003	cC 5.22±0.009	cD 4.95±0.003	cD 4.95±0.006	cD 4.97±0.009
	7	dA 5.31±0.003	cA 5.31±0.009	dB 5.16±0.006	cC 4.92±0.015	dC 4.86±0.003	cdC 4.89±0.087
	8	eA 5.27±0.006	dA 5.27±0.006	dB 5.12±0.013	cC 4.87±0.063	eC 4.81±0.006	dC 4.83±0.006
C8	0	aA 6.27±0.034	aB 6.20±0.003	aB 6.22±0.006	aB 6.19±0.007	aB 6.18±0.003	aC 6.11±0.009
	4	bC 5.79±0.007	bE 5.60±0.003	bD 5.71±0.010	bD 5.69±0.007	bB 5.88±0.006	bA 5.98±0.010
	6	cB 5.37±0.013	cD 5.22±0.006	cC 5.32±0.015	cC 5.29±0.010	cB 5.37±0.009	cA 5.55±0.007
	7	dB 5.22±0.006	dE 5.11±0.006	dC 5.19±0.003	dC 5.15±0.007	dD 5.18±0.006	dA 5.30±0.006
	8	eB 5.16±0.006	eD 5.03±0.006	eC 5.06±0.006	eC 5.06±0.006	eE 5.01±0.007	eA 5.22±0.000
C9	0	aAB 6.06±0.006	aAB 6.04±0.030	aB 5.99±0.059	aA 6.13±0.006	aAB 6.10±0.055	aA 6.13±0.007
	4	bA 5.73±0.003	bA 5.73±0.006	bB 5.63±0.006	bC 5.58±0.009	bC 5.57±0.003	bC 5.57±0.006
	6	cA 5.59±0.013	cB 5.42±0.007	cC 5.32±0.003	cD 5.24±0.003	cD 5.26±0.009	cD 5.26±0.006
	7	dA 5.52±0.007	cB 5.39±0.007	dC 5.20±0.003	dD 5.16±0.003	dD 5.15±0.028	dD 5.15±0.003
	8	eA 5.49±0.006	dB 5.29±0.006	eC 5.09±0.003	eC 5.04±0.006	dD 5.08±0.009	eD 5.05±0.007
C10	0	a 6.24±0.020	a 6.19±0.007	a 6.20±0.006	a 6.12±0.010	a 6.11±0.003	a 6.13±0.006
	4	b 5.57±0.003	b 5.55±0.007	b 5.34±0.007	b 5.34±0.007	b 5.56±0.006	b 5.88±0.006
	6	c 5.32±0.003	c 5.19±0.010	c 5.22±0.009	c 5.16±0.007	c 5.31±0.006	c 5.51±0.007
	7	d 5.24±0.009	d 5.10±0.006	d 5.18±0.007	d 5.06±0.003	d 5.17±0.003	d 5.29±0.006
	8	e 5.18±0.006	e 5.01±0.006	e 5.05±0.019	e 4.99±0.007	e 5.09±0.003	d 5.27±0.012

Values are means of three independent trials with SE; a, b, c: Different superscript in each column denotes significant differences ( $p < 0.05$ ) of pH with incubation hour; A, B, C: Different superscript in each row denotes significant difference ( $p < 0.05$ ) between sugar level.

- Effect of Incubation Time on Acidification** For all ten cultures, a progressive and statistically significant ( $p < 0.05$ ) decrease in pH was observed over the 8-hour incubation period, irrespective of the sugar concentration. The initial pH of the milk samples, recorded at hour 0, ranged from 5.99 to 6.31. By the end of the 8-hour incubation, significant acidification had occurred in all treatments, with final pH values dropping to as low as 4.77 (Culture C6, 20% sugar).
- Effect of Sugar Concentration on Acidification** The addition of sugar had a significant and varied impact on the acidification profiles of the different *S. thermophilus* cultures.
  - Strong Acidifiers (e.g., C2, C3, C6, C7):** These cultures demonstrated robust acidification, generally reaching a pH below 5.0 after 6-8 hours. Cultures C3 and C6 were particularly effective, achieving some of the lowest pH values. For instance, after 8 hours, C3 reached a pH of 4.78 at 10% sugar, and C6 reached 4.77 at 20% sugar. These strains showed enhanced or

stable acidification performance at moderate to high sugar concentrations.

- Moderate Acidifiers (e.g., C1, C4, C9):** These cultures showed effective acidification, though generally less rapid than the strong acidifiers. Culture C1's acidification was significantly enhanced at 10%, 15%, and 20% sugar levels compared to the control (0% sugar). However, for cultures C4 and C9, higher sugar concentrations (20-25%) resulted in a significantly slower pH drop compared to intermediate concentrations, indicating potential inhibition. For example, at 8 hours, the pH for C4 at 25% sugar was 4.97, significantly higher than the 4.80 observed at 15% sugar.
- Slow Acidifiers (e.g., C5, C8):** Cultures C5 and C8 exhibited the slowest rates of acidification. C5 was the least effective overall, with its final pH remaining above 5.20 across all sugar levels after 8 hours. Culture C8 displayed a clear inhibitory effect at the highest sugar concentration; its final pH at 25% sugar was 5.22, significantly higher than at any other sugar level.
- Neutral Response (C10):** For culture C10, no statistically significant difference ( $p > 0.05$ ) in pH was noted between the different sugar concentrations at any

given time point. The culture showed a consistent, time-dependent drop in pH regardless of the added sugar.

### Comparison Among Cultures

There was notable variability in acidification capacity among the different *S. thermophilus* strains. Cultures C1, C6, C7, and C9 showed the fastest acidification rates, achieving lower pH values more rapidly across all sugar concentrations. In contrast, cultures such as C4 and C5 exhibited relatively slower acidification, which may be attributed to differences in sugar uptake efficiency or metabolic activity. The highest acidification potential was observed in Culture C6, which consistently achieved the lowest pH values across all sugar concentrations. Conversely, Culture C5 exhibited the least reduction in pH, indicating a comparatively milder acidification profile. This study reveals that the acidification performance of *Streptococcus thermophilus* is not only strain-dependent but is also significantly modulated by the concentration of added sugar. The universal decrease in pH across all cultures is attributed to the fermentation of lactose present in the skim milk into lactic acid, a hallmark characteristic of this species.

The varied responses to sugar supplementation highlight the diverse metabolic capabilities among the different *S. thermophilus* strains. The enhanced acidification observed in cultures like C3 and C6 at moderate to high sugar levels suggests that these strains can efficiently metabolize sucrose in addition to lactose, leading to greater lactic acid production and a faster pH drop. This trait is highly desirable for the manufacturing of sweetened fermented dairy products, such as yogurt, as it ensures a consistent and rapid fermentation process.

Conversely, the results for cultures C4, C8, and C9 demonstrate a clear case of osmotic stress. High concentrations of sugar increase the osmotic pressure of the medium, which can negatively impact bacterial growth and metabolic activity. This leads to the observed inhibition of acidification at 20% and 25% sugar levels. The bacterial cells likely divert energy from fermentation to osmoregulation to survive, slowing the rate of acid production. Culture C5, being a slow acidifier even without added sugar, appears to be naturally less vigorous or more sensitive to the experimental conditions.

The behavior of culture C10, which showed no significant response to added sugar, is intriguing. It suggests that this strain's rate of acidification is primarily limited by factors other than carbohydrate availability, or that its lactose metabolism is so efficient that the presence of sucrose does not significantly alter its fermentation rate under these conditions. The findings align with previous reports that carbohydrate availability enhances lactic acid production in *S. thermophilus* through increased glycolytic activity. The variability among cultures may reflect genetic differences influencing sugar transport systems, enzymatic activities (e.g., lactate dehydrogenase), and buffer capacity of the media.

Enhanced acidification at moderate sugar concentrations (10–15%) suggests an optimal range for industrial fermentation processes, where over-supplementation may not yield proportional acidification gains and may instead inhibit bacterial activity. These insights are valuable for optimizing starter cultures in yogurt and other fermented dairy products, where precise control over acidification is

critical to product quality, safety, and organoleptic properties.

### Conclusion

The findings underscore the critical importance of starter culture selection based on the specific formulation of a dairy product. For products with high sugar content, selecting a sugar-tolerant, fast-acidifying strain like C3 or C6 is crucial for achieving the desired product quality and texture in a timely manner. In contrast, strains like C4 or C8 would be unsuitable for such applications due to their susceptibility to inhibition by high sugar levels. This research provides a practical framework for the dairy industry to optimize culture selection and improve the consistency of sweetened fermented products.

This study demonstrated that the acidification behavior of *Streptococcus thermophilus* in skim milk is significantly influenced by both the level of added sugar and the specific bacterial strain used. All tested cultures exhibited a time-dependent decrease in pH, indicating active lactic acid production during fermentation. The addition of sugar enhanced acid production up to an optimal concentration, particularly between 10% and 15%, beyond which the benefit either plateaued or declined—likely due to osmotic stress or self-limiting acid accumulation.

### Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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