

# IMPACT OF NON NUTRITIVE SWEETENERS ON THE METABOLISM OF PROBIOTICS

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*Over the years, they have been increase in the consumption of non nutritive sweeteners. This type of sweeteners are present in various foods and drinks such as diet soda. By preserving the sugary taste without increasing calorie intake, they are used to lose weight and manage diabetes. Non nutritive sweeteners cause metabolic dysfunction, which could indirectly affect body-weight, glucose tolerance, hunger, and sense of taste. We investigated the influence of non nutritive sweeteners (aspartame, mannitol, saccharin, sucralose, and stevia) on the metabolism and growth rates of *Bifidobacteria sp.* and *Streptococcus stearothermophilus*. The two microbes (*Bifidobacteria sp.* and *Streptococcus stearothermophilus*) were grown in five different sugar substitutes in a litmus milk media and checked repeatedly for growth. After 48 hours of incubation, aspartame, stevia, saccharine, and sucralose grown with *Streptococcus stearothermophilus* showed an increase in metabolism and growth. However, in mannitol, *Streptococcus stearothermophilus* metabolic activity was similar to the control group when compared to the metabolism and growth of *Streptococcus stearothermophilus* which was grown with glucose (control). *Bifidobacteria sp.* grown with aspartame, saccharin, and stevia showed a decrease in metabolism when compared to *Bifidobacteria sp.* cultured with glucose after 48 hours of incubation. The metabolism of *Bifidobacteria sp.* with mannitol was increased after 48 hours of incubation, while sucralose did not affect the *Bifidobacteria sp.* metabolism. In conclusion, our findings reveal that non-nutritive sweeteners can alter the metabolism of *Bifidobacteria sp.* and *S. stearothermophilus*.*

## Introduction

Sugar intake has increased worldwide over the past couple of years. The increase in sugar consumption has led to the growing concern of its effect on the cardiovascular health and metabolism (1). As these non nutritive sweeteners (NNSs) have become increasingly popular, they are being promoted as a diet tool that helps people lose weight by replacing sugar (2). But emerging studies show that NNSs can impact human metabolism, and they are also associated with glucose intolerance and weight gain due to an increase in appetite (3). NNSs are used in a variety of foods and drinks. Consumable items with this type of sugar are often labeled “diet” and “sugar-free.” Commercially available NNSs can be divided

into two forms. The first form is synthetic, which includes sucralose, acesulfame potassium, saccharin, and aspartame (1). The second form is natural sugar (NS). A common example of this type of sugar is stevia, which comes from the plant “*Stevia rebaudiana*” (2).

In recent studies, the gut microbiome community has been shown to respond differently to natural and synthetic NNSs (4). This is because the dynamics of the human gut microenvironment are more complicated, with a high level of “inter-species synergy” and “cross-feeding”(5). While the effect of NNSs has been rigorously studied on how they can affect host health, they have ignored the influence they have on gut microbiota (5). Intestinal microbes influence human health and disease since they are involved in the metabolism, growth, fermentation of carbohydrates, and immunity (6). In a batch culture experiment, the researchers fermented fecal samples with NNSs (aspartame-based sweeteners, sucralose, and stevia). The results from the experiment showed that stevia and sucralose shifted the microbiome community structure, while aspartame-based sweeteners promoted the growth of *Bifidobacterium* (5).

Although the gut microbiome is comprised of a diverse community, three genera of bacteria, *Bifidobacteria*, *Lactobacillus*, and *Streptococcus*, have been commonly used as probiotics and are thought to be involved in the digestion of NNSs (7). Since the gut microbiome is responsible for approximately 10 percent of our daily caloric intake, sugar substitutes could significantly slow the metabolism and increase the caloric intake. The aim of this study is to analyze the influence of non nutritive sweeteners on two strains of human gut microbes (*Bifidobacteria* and *Streptococcus stearothermophilus*) associated with bacteria growth and metabolism.

## Method

### Preparation of Test Tube Culture

Non nutritive sweeteners (aspartame, saccharin, stevia, sucralose, and mannitol) and glucose with a concentration of 10 g/100 ml were added to Litmus Milk media (BD). Yogurt diluted to a 10<sup>1</sup> dilution was inoculated into the Litmus Milk media and then incubated at 37°C. Signs of growth were checked after 24 and 48 hours. Growth was determined by a drop in pH as indicated by the colorimetric change of azolithium in the media from purple to pink and

finally white. All incubation was performed in triplicate. 1.09g of culture from glucose control was diluted with 11ml of saline. The diluted culture and non nutritive sweeteners were added to a new set of Litmus Milk media (BD). After adding the sweeteners, the media was incubated at 37°C and signs of growth were checked after 24 and 48 hours.

### Isolation of *Bifidobacteria* and *Streptococcus Stearothermophilus* from yogurt

To isolate *Bifidobacteria sp.* and *Streptococcus stearothermophilus*, the glucose culture was plated on milk agar plates containing 100 g/L of dehydrated milk. Plates were incubated at 37°C for 5 days. The isolated colonies were first confirmed to be pure via gram staining, and then each of the colonies was transferred to a sterile microcentrifuge tube containing 1 mL of saline solution. Colony and cell morphology were used to confirm the identification of the strains.

### Determining the effects of artificial sweeteners on *Bifidobacteria sp* and *Streptococcus Stearothermophilus*

To determine the effect of the NNSs on *Bifidobacteria sp* and *Streptococcus Stearothermophilus*, 0.02 ml of the inoculum with glucose, aspartame, saccharin, stevia, sucralose, or mannitol were added to a fresh Liquid Milk media and then incubated at 37°C. Signs of growth were observed by the colorimetric change of azolithium in the media due to pH change.

## Result

### Sugar substitute affects the growth and metabolism of probiotics in yogurt culture

First, we analyzed the effects of sucralose, saccharin, stevia, aspartame, and mannitol on the metabolism of probiotics found in yogurt. The growth and metabolism of the probiotics were assessed by measuring the amount of acid production that occurred during fermentation in the Litmus Milk media. Saccharin and stevia increased the metabolism of the microorganisms, while aspartame and mannitol decreased the metabolism compared to the control (glucose) (Figure 1). Saccharin and stevia had the most impact on the metabolism of the probiotics in the Litmus Milk media, with a 1.6% increase (P = 2.5, P = 1.70) in metabolism when compared to the control (Table 1). In the case of aspartame and mannitol, there was a decrease in the growth and metabolism of the microorganisms in Litmus Milk media. When compared to glucose, aspartame exhibited a 1.6% decrease (P = 0.225) in metabolism and growth, while mannitol had a 4.4%

decrease in metabolism and growth (Table 1).

Sweeteners	Percent pH change	Percent litmus reduction	P-value Litmus reduction
Glucose (control)	30.0 ± 1.4	70.0 ± 1.4	
Aspartame	31.6 ± 1.6	68.4 ± 1.6	0.225
Mannitol	34.4 ± 1.9	65.6 ± 1.9	0.192
Saccharin	28.4 ± 2.5	71.6 ± 2.5	0.529
Stevia	28.4 ± 1.7	71.6 ± 1.7	0.438
Sucralose	28.6 ± 1.6	71.4 ± 1.6	0.497

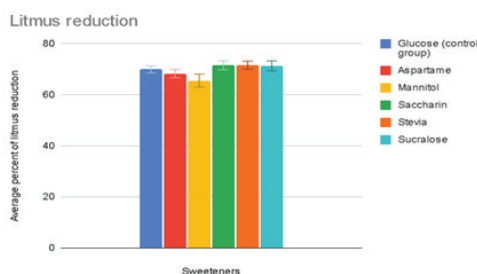
**Table 1.**

*Average fermentation of both Bifidobacteria sp. and Streptococcus stearothermophilus after 24 hours of incubation.*

The percent pH change indicates lactose was fermented and acid was produced, while the percent litmus reduction indicates the amount of litmus that was reduced in the absence of oxygen. Percent pH change and percent litmus reduction were used to compare the metabolism of the microbe.

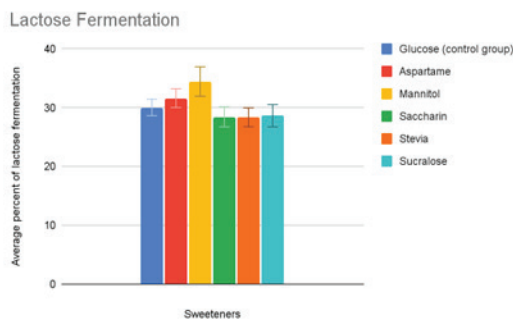
**Figure 1.**

*Average percent growth rates of both Bifidobacteria sp. and Streptococcus stearothermophilus with different sweeteners.*



(A) Litmus reduction

*Each bar represents the average percent of litmus in the litmus milk media that has been reduced after 24 hours. The error bars represent the standard deviation.*



(B) Lactose fermentation

*Each bar represents the average percent of fermented lactose in the media after 24 hours. The error bars represent the standard deviation.*

## Effect of sugar substitutes on the metabolism of *Bifidobacteria*

Each sweetener has different effects on the metabolism of *Bifidobacteria*. When compared to the control, *Bifidobacteria* grown with stevia showed a 5% decrease after 48 hours (Figure 2B). *Bifidobacteria* grown with aspartame showed a decrease ( $P < 0.05$ ) in metabolism after 48 hours (Table 2A). This was also the case in *Bifidobacteria* grown with saccharin. After 48 hours of incubation, there was a significant decrease ( $p = 0.045$ ) in the bacteria metabolism  $k$  (Figure 3). On the other hand, when compared to the control group, *Bifidobacteria* grown with mannitol first experienced an 8% decrease in metabolism, and after 48 hours, the metabolic activity increased 3% (Figure 3A). Sucralose was the only sugar substitute that did not affect the metabolic and growth rates of *Bifidobacteria* when compared to the control (Figure 2,3).

Sweeteners	Percent pH change	Percent litmus reduction	P-value Litmus reduction
Glucose (control)	88 ± 5.1	12 ± 5.1	
Aspartame	97 ± 5.2	3.0 ± 5.2	0.00010
Mannitol	96 ± 1.6	4.0 ± 1.6	0.052
Saccharin	96 ± 3.6	4.1 ± 3.6	0.085
Stevia	79 ± 2.3	21 ± 2.3	0.12
Sucralose	88 ± 5.2	12 ± 5.2	0.42

**Table 2A.**

*Average fermentation rate of Bifidobacteria after 24 hours of incubation*

The percent pH change indicates lactose was fermented and acid was produced, while the percent litmus reduction indicates the amount of litmus that was reduced in the absence of oxygen. Percent pH change and percent litmus reduction were used to compare the metabolism of the microbe.

	Percent pH change	Percent litmus reduction	P-value Litmus reduction
Glucose(control)	23 ± 1.4	77 ± 1.4	
Aspartame	29 ± 6.6	71 ± 6.6	0.17
Mannitol	20 ± 1.6	80 ± 1.6	0.12
Saccharin	30 ± 2.1	70 ± 1.3	0.045
Stevia	28 ± 4.1	72 ± 4.1	0.15
Sucralose	23 ± 1.4	77 ± 1.4	0.42

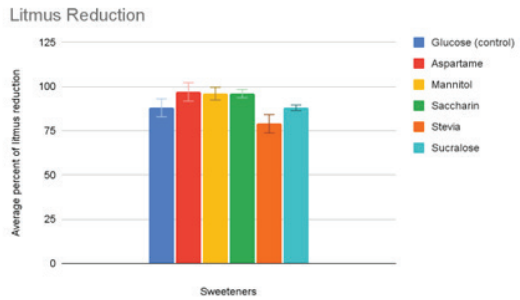
**Table 2B.**

*Average fermentation rate of Bifidobacteria after 48 hours of incubation*

The percent pH change indicates lactose was fermented and acid was produced, while the percent litmus reduction indicates the amount of litmus that was reduced in the absence of oxygen. Percent pH change and percent litmus reduction were used to compare the metabolism of the microbe.

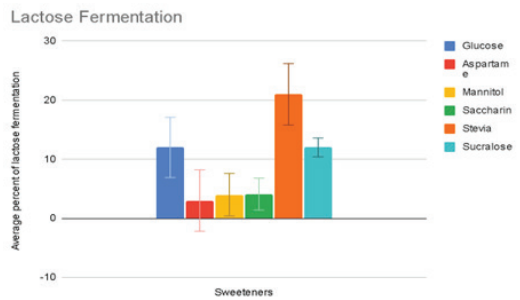
**Figure 2.**

*Average percent growth rate of Bifidobacteria with different sweeteners.*



**(A) Litmus reduction**

*Each bar represents the average percent of litmus in the litmus milk media that has been reduced after 24 hours. The error bars represent standard deviation.*

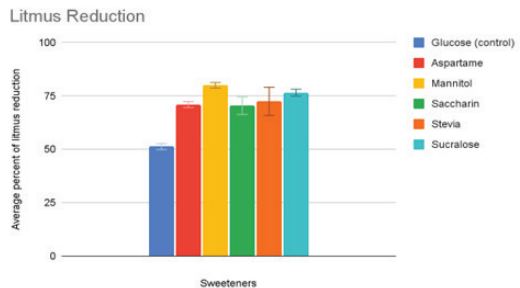


**(B) Lactose fermentation**

*Each bar represents the average percent of fermented lactose in the media after 24 hours. The error bars represent standard deviation.*

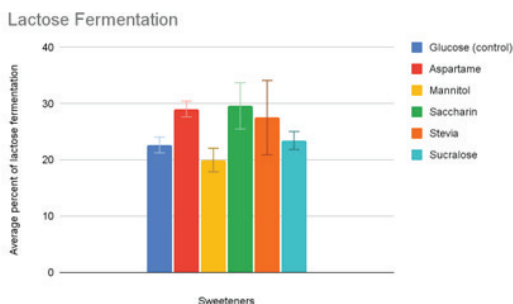
**Figure 3.**

*Average percent growth rate of Bifidobacteria sp. with different sweeteners.*



**(A) Litmus reduction**

*Each bar represents the average percent of litmus in the litmus milk media that has been reduced after 48 hours. The error bars represent standard deviation.*



(B) Lactose fermentation  
Each bar represents the average percent of fermented lactose in the media after 48 hours. The error bars represent standard deviation.

### Effect on the metabolism of *Streptococcus stearothermophilus*

After 48 hours of incubation, there was an increase in the average metabolism of *S. stearothermophilus* across all sugar substitutes except mannitol when compared to control (Figure 5). *S. stearothermophilus* grown with mannitol was the only one that experienced similar metabolism to the control group after 48 hours (Table 3B). For the other sugar substitutes (aspartame, sucralose, stevia, and saccharin), *S. stearothermophilus* had an increase in metabolism compared to the control group after 48 hours. *S. stearothermophilus* grown with aspartame had the highest metabolic and growth rates (Figure 5A).

Sweeteners	Percent pH change	Percent litmus reduction	P-value Litmus reduction
Glucose (control)	100 ± 0.0	0.0 ± 0.0	
Aspartame	93.8 ± 5.3	6.2 ± 5.3	0.18
Mannitol	100.0 ± 0.0	0.0 ± 0.0	0
Saccharin	100 ± 0.0	0.0 ± 0.0	0
Stevia	93.8 ± 3.0	6.2 ± 3.0	0.070
Sucralose	100 ± 0.0	0.0 ± 0.0	0

**Table 3A.**  
Average percent fermentation rate of *Streptococcus stearothermophilus* after 24 hours of incubation.

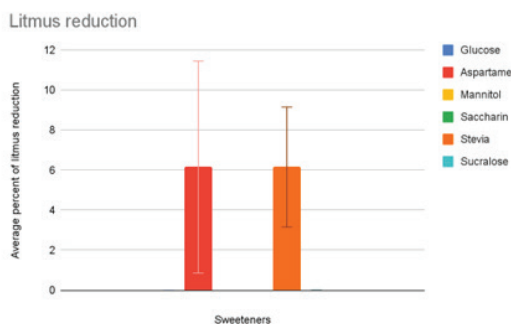
The percent pH change indicates lactose was fermented and acid was produced, while the percent litmus reduction indicates the amount of litmus that was reduced in the absence of oxygen. Percent pH change and percent litmus reduction were used to compare the metabolism of the microbe.

Sweeteners	Percent pH change	Percent litmus reduction	P value Litmus reduction
Glucose (control)	57.0 ± 2.0	43.0 ± 2.0	
Aspartame	42.1 ± 2.6	57.9 ± 2.6	0.023
Mannitol	56.6 ± 1.7	43.4 ± 1.7	0.85
Saccharin	47.4 ± 0.9	52.6 ± 0.9	0.024
Stevia	49.0 ± 5.8	51.0 ± 5.8	0.12
Sucralose	51.5 ± 0.1	48.5 ± 0.1	0.043

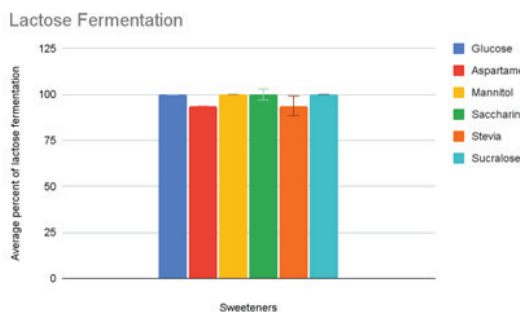
**Table 3B.**  
Average percent fermentation rate of *Streptococcus stearothermophilus* after 48 hours of incubation

The percent pH change indicates lactose was fermented and acid was produced, while the percent litmus reduction indicates the amount of litmus that was reduced in the absence of oxygen. Percent pH change and percent litmus reduction were used to compare the metabolism of the microbe.

**Figure 4.**  
Average percent growth rate of *Streptococcus stearothermophilus* with different sweeteners.



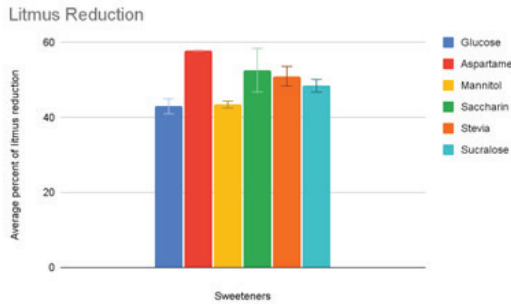
(A) Litmus reduction  
Each bar represents the average percent of litmus in the litmus milk media that has been reduced after 24 hours. The error bars represent the standard deviation.



(B) Lactose fermentation  
Each bar represents the average percent of fermented lactose in the media after 24 hours. The error bars represent the standard deviation.

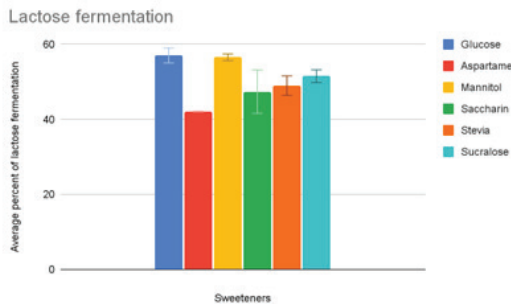
**Figure 5.**

*Average percent growth rate of Streptococcus stearothermophilus with different sweeteners after 48 hours.*



(A) Litmus reduction

*Each bar represents the average percent of litmus in the litmus milk media that has been reduced after 48 hours. The error bars represent the standard deviation.*



(B) Lactose fermentation

*Each bar represents the average percent of fermented lactose in the media after 48 hours. The error bars represent the standard deviation.*

## Discussion

Studies show that diets alter the structure and function of the gut microbiome, including its ability to ferment fiber (13). Non nutritive sweeteners are linked to metabolic dysfunctions, but the exact mechanism is unknown (2). Although humans consume a large quantity of non nutritive sweetener, the effects they have on the gut are still unclear. Because the gut microbiome accounts for roughly 10% of our daily caloric intake, the metabolism of sugar substitutes could significantly impact on caloric intake. This study evaluated the effects five sweeteners (aspartame, mannitol, saccharin, stevia, and sucralose) had on *Streptococcus stearothermophilus* and *Bifidobacteria sp.* We grew both microbes in litmus milk media containing each sweetener (glucose was the control), and recorded the metabolic activity (Table 1,2,3). When compared to the control, the result showed that *Streptococcus stearothermophilus* and *Bifidobacteria sp.* grown with

non nutritive sweeteners can have an effect on the metabolism, except for *Bifidobacteria sp.* grown with sucralose, which had no effect. (Figure 2,3,4,5).

In *Streptococcus stearothermophilus* grown with mannitol, the microorganism's metabolism was similar to that of the control. However, *Streptococcus stearothermophilus* grown with other sugar substitutes, i.e., aspartame, stevia, saccharine, and sucralose, experienced a decrease in metabolism (Figure 5). *Bifidobacteria sp.* grown with aspartame, saccharin, and stevia showed a decrease in metabolism compared to the control. *Bifidobacteria sp.* grown with mannitol experienced an increased metabolism, while *Bifidobacteria sp.* grown with saccharin had similar metabolic activity compared to the control. Some of the trends that were observed in the results were different from those in other studies. An earlier study found that aspartame increased the quantity of *Bifidobacteria* present in the gut and sucralose decreased *Streptococcus* (5). *Bifidobacteria sp.* grown with stevia has faster metabolism compared to other sugar substitutes, while *S. stearothermophilus* was able to metabolize aspartame and stevia faster than other sugar substitutes (Figure 2,4). Aspartame hydrolysis occurs quickly, which might have affected the microbe's metabolism (1). Although *Bifidobacteria sp.* and *S. stearothermophilus* cannot hydrolyze stevia, it can be used as a substrate for their growth.

Saccharin is one of the most tested sweeteners on the gut microbiome (9). Although it is one of the most researched sweeteners, the results from different research are inconsistent. When rats were fed 90 mg of saccharin over a ten-day period, the researchers found the overall number of anaerobic microbes was not affected by saccharin(14). In another study, saccharin suppressed six bacterial species' growth(15). In the case of sucralose, when several doses were administered to rats, sucralose consumption decreased in *Bifidobacteria* (16). Also, Bian tested the effect of 0.1 mg/ml of sucralose on mice. After 3 to 6 months, there was a significant change in the *Streptococcus* (12). In *in vitro* studies where stevia was tested against different bacteria, there was no significant growth of any major gut microbe(16). Stevia does not affect the fecal composition and fermentation capacity of the fecal microbiome.

In this study, *Bifidobacteria sp.* and *Streptococcus stearothermophilus* grown with stevia had an increase in metabolism (Table 1). Under anaerobic conditions, there was a 3% increase in the metabolism of *Bifidobacteria* in comparison to glucose (Table 2B). Although it was reported that aspartame increased the growth of bacteria in the gut microbe, the effect of aspartame is very difficult to understand

since it rapidly hydrolyzes in the upper GI tract and does not reach the lower intestine (1). This study provides more information and understanding of the effect non nutritive sweeteners have on the metabolism of gut microbes.

### Conclusion

Non nutritive sweeteners are used to replace regular sugar because of their health benefits (manages diabetics), but it also has its negative side. Although non nutritive sweeteners have different calorie content and metabolism pathways, they have the ability to alter the makeup of microbes present in the gut(1). Thus far, saccharin, sucralose, and stevia have been extensively studied and are known to affect the composition and diversity of the gut microbiota (1). The alteration of the gut community could either increase or decrease the metabolism of the microbe. Although NNSs are advertised as a healthy tool for weight loss, there is no solid evidence that supports that. This is because NNSs affect the microbes in the gut community differently, which depends on the amount and type that is consumed.

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