

COMPARISON OF THE EFFECTS OF SUGAR ALCOHOLS AT DIFFERENT CONCENTRATIONS ON THE GROWTH OF CARIOGENIC BACTERIA

SAMYELI SARIHAN^{1,3}, NURSEN TOPCUOGLU², FIGEN EREN³

¹*Department of Pediatric Dentistry, Institute of Health Sciences, Marmara University, 34854 Istanbul, Turkiye*

²*Department of Basic Medical Science, Faculty of Dentistry, Istanbul University, 34134 Istanbul, Turkiye*

³*Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, 34854 Istanbul, Turkiye*

ABSTRACT

This study aimed to evaluate the effects of various concentrations of xylitol and erythritol on the growth of cariogenic bacteria, specifically *Streptococcus mutans*, *Streptococcus sobrinus*, and *Scardovia wiggisiae*. Type strains of *S. mutans* (ATCC 25175), *S. sobrinus* (ATCC 33478), and *S. wiggisiae* (DSM 22547) were cultured and exposed to different concentrations of xylitol, erythritol, and their combinations. The concentrations tested included 10% xylitol, 10% erythritol, 5% xylitol + 5% erythritol, 2.5% xylitol + 7.5% erythritol, 2.5% erythritol + 7.5% xylitol and a control group with 5% sucrose. Xylitol and erythritol significantly inhibited the growth of *S. mutans*, *S. sobrinus* and *S. wiggisiae*. The combination of 5% xylitol + 5% erythritol also demonstrated substantial inhibitory effects. Statistical analyses indicated significant differences in absorbance changes over time, with the 5% sucrose group consistently showing higher growth rates compared to other groups. Both xylitol and erythritol, particularly at higher concentrations, effectively inhibit the growth of key cariogenic bacteria. The combination of these sugar alcohols exhibits potential synergistic effects, suggesting their utility in dental care products to prevent dental caries. Further research is needed to optimize the formulations and concentrations for maximum therapeutic benefit.

KEYWORDS: xylitol; erythritol, *Streptococcus mutans*, *Streptococcus sobrinus*, *Scardovia wiggisiae*, sugar alcohols.

INTRODUCTION

Xylitol and erythritol are sugar alcohols commonly used as sucrose substitutes ^[1]. Both have been tested for fermentation by oral microorganisms and are considered hypoacidogenic or non-acidogenic ^[2]. Studies show they have minimal or no cariogenic effects ^[3].

Xylitol, a five-carbon sugar alcohol, is as sweet as sucrose and naturally found in many fruits and vegetables [4]. It was first reported to inhibit *Streptococcus mutans* in 1975 [5]. Clinical studies have shown that xylitol reduces *Streptococcus mutans* counts, plaque accumulation, and caries in children [6].

Erythritol (1,2,3,4-butanetetrol) is a tetraol compound (C₄H₁₀O₄) [7]. It is absorbed and eliminated unmetabolized in humans, minimally impacting glucose and insulin levels [8]. Its higher tolerance level for gastrointestinal effects makes it suitable for diabetic and obesity-friendly products. Erythritol inhibits *mutans streptococci* similarly to xylitol [9] and enhances the fungicidal activity of benzethonium chloride against candidal biofilm [10].

Caries in humans are primarily caused by *mutans streptococci*, especially *S. mutans* and *S. sobrinus* [11]. Both erythritol and xylitol inhibit *mutans streptococci* in vitro [11]. It is suggested that combinations of xylitol and erythritol might reduce caries more effectively than either alone, though this requires confirmation [2]. Additionally, there is limited data on the effect of polyols on *Scardovia wiggisiae*, a pathogen linked to early childhood caries. Furthermore, *Scardovia wiggisiae* is recognized as a cariogenic bacterium responsible for early childhood caries, especially in pediatric populations, yet there is a significant lack of research focusing on this pathogen [12]. Previous studies examining the combined use of erythritol and xylitol, particularly those investigating their synergistic effects, are limited. This in vitro study examined the effects of various concentrations of erythritol and xylitol on the growth rates of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Scardovia wiggisiae*.

MATERIALS AND METHODS

Preparation of sugar alcohols

To assess the effects of various sugar alcohol concentrations on cariogenic bacteria, five experimental groups were established with the following compositions: 10% xylitol (Sigma, St. Louis, MO, USA), 10% erythritol (Sigma, St. Louis, MO, USA), 5% xylitol + 5% erythritol mixture, 2.5% xylitol + 7.5% erythritol mixture and 7.5% xylitol + 2.5% erythritol mixture and a control group containing 5% sucrose was also included.

Stock concentrations of these sugar alcohols were prepared at the Department of Basic Sciences, Faculty of Dentistry, Marmara University. The sugar alcohols were sterilized by filtration at the Microbiology Research Laboratory, Faculty of Dentistry, Istanbul University and added to brain-heart infusion (BHI) broth.

Preparation of bacterial suspensions

Type strains of *Streptococcus mutans* (ATCC 25175), *Streptococcus sobrinus* (ATCC 33478), and *Scardovia wiggisiae* (DSM 22547) were used. These strains were obtained from the Microbiology Research Laboratory, Faculty of Dentistry, Istanbul University. *S. wiggisiae* was cultured on Brucella Agar (Merck KGaA, DE) with 5% sheep blood, 5 mg/mL hemin and 5 mg/mL Vitamin K. *S. mutans* and *S. sobrinus* were cultured on Brain-Heart Infusion (BHI) Agar (Merck) in an anaerobic jar with an anaerobic atmosphere pouch (BD GasPak EZ, US), maintaining an anaerobic environment of 80% N₂, 10% CO₂, and 10% H₂ at 37°C for 48 hours.

Bacterial suspensions were prepared in BHI broth medium supplemented with yeast extract, hemin, and Vitamin K, adjusted to a turbidity of 0.5 McFarland standard (108 CFU/ml) and subsequently diluted 100-fold (final concentration: 106 CFU/ml).

Evaluation of bacterial growth with different polyol concentrations

Sterile 96-well plates (Greiner, Sigma–Aldrich) were used for each bacterial species. Of the 12 rows of wells, 11 were used for each solution group, and 1 was left empty. In the first 10 wells, 180 µl of the sugar alcohol solution and 20 µl of the bacterial suspension were added. The 11th well was left empty and the 12th well contained only the sugar alcohol solution as a control for sterility. Absorbance values at 630 nm were measured using a Microplate Elisa Absorbance Reader (MS4-MaxiRead96, MaxiLab Biotechnology) at 0, 1, 2, 4, 6, and 24 hours.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 22. The normality of parameters was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. Since absorbance levels showed a normal distribution, a two-way repeated measures ANOVA, followed by post hoc Bonferroni and Tukey tests, was used. For absorbance changes relative to the 0-hour time point, which did not show a normal distribution, the Kruskal-Wallis test and post hoc Dunn's test were employed. Statistical significance was set at $p < 0.05$.

RESULTS

Table 1 (Evaluation of the joint effect of bacteria type, concentration and time on absorbance values.)

	Type III Sum of Squares	df	Mean Square	F	p
Time	5,485	5	1,097	8973,854	0,001*
Time * Bacteria	2,432	10	0,243	1989,242	0,001*
Time * Concentration	1,359	25	0,054	444,616	0,001*
Time * Bacteria * Concentration	0,842	50	0,017	137,73	0,001*

Two-way Repeated Measures ANOVA Test * $p < 0.05$

The absorbance values were analyzed using a two-way repeated measures ANOVA. Changes in absorbance over time were statistically significant ($p = 0.001$; $p < 0.05$). The interaction between bacteria and time, as well as between concentration and time, showed significant effects on absorbance ($p = 0.001$; $p < 0.05$). Additionally, the combined interaction of bacteria, concentration and time was also significant ($p = 0.001$; $p < 0.05$) (Table 1).

Table 2 (Evaluation of absorbance levels in concentration groups of different bacterial species relative to baseline (0 hours))

Bacteria	Absorbance	10% xylitol	10% erythritol	5% xylitol+	2.5% xylitol+	2.5% erythritol+	5% sucrose
		(Mean±SD)	(Mean±SD)	(Mean±SD)	(Mean±SD)	(Mean±SD)	(Mean±SD)
<i>Streptococcus mutans</i> (ATCC 25175)	0 hours	0,107±0,003	0,110±0,003	0,112±0,006	0,110±0,006	0,109±0,004	0,103±0,008
	1 hour	0,107±0,003	0,108±0,003	0,113±0,005	0,109±0,006	0,109±0,004	0,104±0,008
	2 hours	0,109±0,003	0,112±0,003	0,114±0,005	0,111±0,006	0,110±0,004	0,109±0,007
	4 hours	0,117±0,003	0,121±0,004	0,121±0,006	0,122±0,007	0,117±0,004	0,120±0,007
	6 hours	0,133±0,004	0,137±0,004	0,137±0,007	0,145±0,008	0,134±0,006	0,144±0,008
	24 hours	0,294±0,005	0,283±0,010	0,295±0,008	0,316±0,007	0,293±0,006	0,630±0,021
	0-1-hour <i>p</i>	1,000	0,336	1,000	1,000	0,993	0,001*
	0-2 hours <i>p</i>	0,016*	1,000	1,000	0,014*	1,000	0,001*
	0-4 hours <i>p</i>	0,001*	0,001*	0,001*	0,001*	0,001*	0,001*
	0-6. hours <i>p</i>	0,001*	0,001*	0,001*	0,001*	0,001*	0,001*
	0-24 hours <i>p</i>	0,001*	0,001*	0,001*	0,001*	0,001*	0,001*
<i>Streptococcus sobrinus</i> (ATCC 33478)	0 hours	0,111±0,003	0,130±0,006	0,120±0,007	0,117±0,004	0,118±0,003	0,108±0,005
	1 hour	0,114±0,004	0,200±0,042	0,214±0,038	0,234±0,047	0,220±0,045	0,119±0,009

	2 hours	0,118±0,004	0,137±0,004	0,153±0,027	0,218±0,059	0,170±0,044	0,123±0,006
	4 hours	0,126±0,004	0,146±0,005	0,132±0,009	0,135±0,005	0,129±0,003	0,172±0,007
	6 hours	0,135±0,004	0,156±0,005	0,138±0,009	0,151±0,005	0,137±0,003	0,230±0,008
	24 hours	0,304±0,009	0,518±0,018	0,421±0,018	0,48±0,023	0,300±0,010	0,768±0,015
	0-1-hour <i>p</i>	0,059	0,008*	0,001*	0,001*	0,001*	0,042*
	0-2 hours <i>p</i>	0,001*	0,001*	0,150	0,005*	0,067	0,001*
	0-4 hours <i>p</i>	0,001*	0,001*	0,001*	0,001*	0,001*	0,001*
	0-6. hours <i>p</i>	0,001*	0,001*	0,001*	0,001*	0,001*	0,001*
	0-24 hours <i>p</i>	0,001*	0,001*	0,001*	0,001*	0,001*	0,001*
<i>Scardovia wiggisiae</i> (DSM 22547)	0 hours	0,106±0,004	0,127±0,009	0,116±0,006	0,112±0,005	0,115±0,004	0,103±0,003
	1 hour	0,109±0,004	0,129±0,010	0,119±0,007	0,114±0,006	0,118±0,005	0,104±0,003
	2 hours	0,110±0,004	0,132±0,011	0,119±0,008	0,114±0,007	0,118±0,005	0,105±0,002
	4 hours	0,111±0,005	0,132±0,011	0,121±0,009	0,116±0,007	0,119±0,006	0,107±0,002
	6 hours	0,111±0,005	0,132±0,010	0,120±0,009	0,113±0,008	0,118±0,007	0,108±0,003
	24 hours	0,133±0,016	0,146±0,011	0,136±0,018	0,128±0,010	0,133±0,015	0,141±0,004
	0-1-hour <i>p</i>	0,022*	0,072	0,013*	0,484	0,732	0,272
	0-2 hours <i>p</i>	0,005*	0,024*	0,015*	0,434	1,000	0,081

0-4 hours p	0,003*	0,010*	0,007*	0,143	1,000	0,001*
0-6. hours p	0,009*	0,005*	0,033*	1,000	1,000	0,001*
0-24 hours p	0,007*	0,001*	0,003*	0,001*	0,048*	0,001*

Bonferroni Test * $p < 0.05$

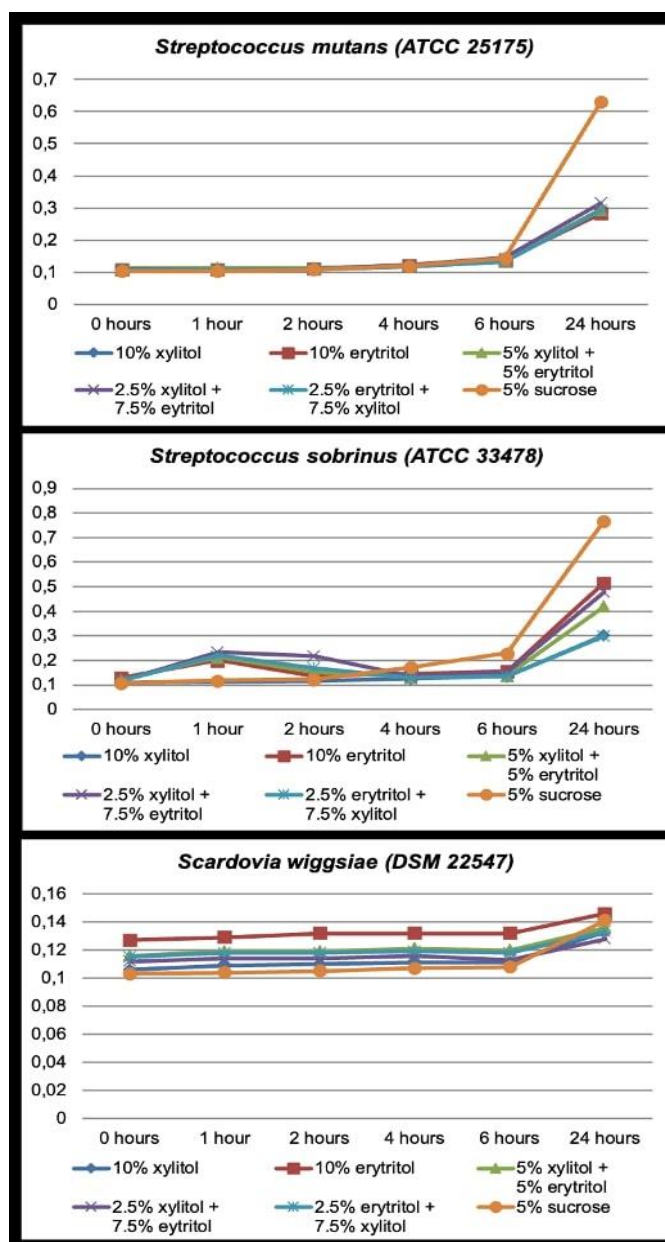


Figure 1 (This figure illustrates the growth patterns of three bacterial species—*Streptococcus mutans* (ATCC 25175), *Streptococcus sobrinus* (ATCC 33478), and *Scardovia wiggsiae* (DSM 22547)—when exposed to different concentrations and combinations of xylitol, erythritol, and sucrose. Growth was monitored at 0, 1, 2, 4, 6, and 24 hours.)

Absorbance Changes in *Streptococcus mutans* (ATCC 25175) Across Different Sweetener Concentrations

Statistically significant differences in absorbance changes were observed at 1, 2, 4, 6 and 24 hours compared to the 0-hour across the concentration groups ($p < 0.05$). The 5% sucrose group showed significantly higher absorbance changes than other groups at all times ($p < 0.05$). At 4 hours, the 2.5% xylitol + 7.5% erythritol group had a significantly higher absorbance change than the 2.5% erythritol + 7.5% xylitol group ($p = 0.018$). At 6 hours, this group also showed higher absorbance changes than the 5% erythritol + 5% xylitol group ($p = 0.002$) and the 2.5% erythritol + 7.5% xylitol group ($p = 0.007$). At 24 hours, the absorbance changes in the 2.5% xylitol + 7.5% erythritol group was higher than in the 10% erythritol ($p = 0.001$) and the 5% erythritol + 5% xylitol groups ($p = 0.024$). No significant differences were found among the other groups ($p > 0.05$). (Table 2, Fig 1.)

Absorbance Changes in *Streptococcus sobrinus* (ATCC 33478) Across Different Sweetener Concentrations

Statistically significant differences in absorbance changes were observed at 1, 2, 4, 6 and 24 hours compared to the 0-hour across the concentration groups ($p < 0.05$). The 5% sucrose group showed significantly higher absorbance changes than other groups at all times ($p < 0.05$). At 1 hour, the 10% xylitol group had lower absorbance changes than the 5% xylitol + 5% erythritol ($p = 0.002$), 2.5% xylitol + 7.5% erythritol ($p = 0.001$), and 2.5% erythritol + 7.5% xylitol groups ($p = 0.001$). At 2 hours, the 2.5% xylitol + 7.5% erythritol group showed higher absorbance changes than the 10% xylitol ($p = 0.001$) and the 10% erythritol groups ($p = 0.001$). At 4 and 6 hours, this group had higher absorbance changes than the 5% erythritol + 5% xylitol and 2.5% erythritol + 7.5% xylitol groups ($p < 0.05$). At 24 hours, the absorbance changes in the 10% erythritol group was higher than in the 10% xylitol ($p = 0.003$) and 2.5% erythritol + 7.5% xylitol groups ($p = 0.001$). The 2.5% xylitol + 7.5% erythritol group showed higher absorbance changes than the 2.5% erythritol + 7.5% xylitol group ($p = 0.001$). (Table 2, Fig 1.)

Absorbance Changes in *Scardovia wiggsiae* (DSM 22547) Across Different Sweetener Concentrations

No statistically significant differences in absorbance changes were observed at 1, 2, 4 and 6 hours compared to the 0-hour across the concentration groups ($p > 0.05$). However, significant differences were noted at 24 hours ($p < 0.05$). The 5% sucrose group had significantly higher absorbance changes than the other groups at 24 hours ($p < 0.05$). No significant differences were found among the other concentration groups ($p > 0.05$). (Table 2, Fig 1.)

Table 3 (Evaluation of absorbance changes at 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours compared to 0 hours across different concentrations for bacterial species.)

	Absorba nce	<i>Streptococcus</i> <i>mutans</i>	<i>Streptococcus</i> <i>sobrinus</i>	<i>Scardovia</i> <i>wiggsiae</i>
Concentration	Change	(ATCC 25175)	(ATCC 33478)	(DSM 22547)

		Mean±SD (Median)	Mean±SD (Median)	Mean±SD (Median)	<i>p</i>
10% xylitol	1 hour	0±0.001 (0)	0.004±0.003 (0.003)	0.003±0.002 (0.003)	0,001*
	2 hours	0.002±0.001 (0.002)	0.007±0.002 (0.007)	0.004±0.003 (0.004)	0,001*
	4 hours	0.01±0.001 (0.01)	0.015±0.002 (0.015)	0.005±0.003 (0.005)	0,001*
	6 hours	0.026±0.002 (0.025)	0.025±0.003 (0.024)	0.005±0.003 (0.005)	0,001*
	24 hours	0.187±0.005 (0.187)	0.194±0.009 (0.194)	0.027±0.016 (0.022)	0,001*
10% erythritol	1 hour	-0.002±0.002 (- 0.002)	0.07±0.042 (0.089)	0.002±0.002 (0.002)	0,001*
	2 hours	0.001±0.002 (0.002)	0.007±0.003 (0.007)	0.004±0.003 (0.005)	0,001*
	4 hours	0.01±0.003 (0.01)	0.016±0.004 (0.015)	0.005±0.003 (0.005)	0,001*
	6 hours	0.027±0.003 (0.027)	0.026±0.004 (0.026)	0.005±0.003 (0.005)	0,001*
	24 hours	0.173±0.008 (0.171)	0.387±0.017 (0.388)	0.018±0.004 (0.02)	0,001*
5% erythritol + 5% xylitol	1 hour	0.001±0.003 (0.001)	0.094±0.04 (0.111)	0.003±0.002 (0.003)	0,001*
	2 hours	0.001±0.002 (0)	0.033±0.032 (0.022)	0.003±0.003 (0.003)	0,001*

	4 hours	0.009±0.002 (0.009)	0.012±0.003 (0.011)	0.005±0.005 (0.005)	0,007*
	6 hours	0.025±0.004 (0.023)	0.018±0.004 (0.017)	0.004±0.005 (0.003)	0,001*
	24 hours				0,001*
		0.183±0.007 (0.182)	0.301±0.015 (0.303)	0.02±0.016 (0.015)	
2.5% xylitol + 7.5% erythritol	1 hour	-0.001±0.001 (- 0.001)	0.117±0.045 (0.133)	0.002±0.002 (0.001)	0,001*
	2 hours	0.001±0.001 (0.001)	0.102±0.057 (0.132)	0.002±0.003 (0.002)	0,001*
	4 hours	0.012±0.002 (0.011)	0.018±0.004 (0.018)	0.004±0.004 (0.004)	0,001*
	6 hours	0.035±0.004 (0.034)	0.035±0.004 (0.035)	0.001±0.005 (0.002)	0,001*
	24 hours	0.206±0.004 (0.206)	0.363±0.019 (0.365)	0.016±0.007 (0.015)	0,001*
2.5% erythritol + 7.5% xylitol	1 hour	-0.001±0.001 (- 0.001)	0.102±0.044 (0.115)	0.003±0.003 (0.002)	0,001*
	2 hours	0.001±0.001 (0)	0.052±0.043 (0.054)	0.003±0.005 (0.002)	0,001*
	4 hours	0.008±0.002 (0.008)	0.011±0.001 (0.011)	0.003±0.006 (0.002)	0,004*
	6 hours	0.025±0.004 (0.024)	0.019±0.001 (0.019)	0.003±0.006 (0)	0,001*

	24 hours	0.184±0.006 (0.182)	0.182±0.008 (0.185)	0.018±0.014 (0.012)	0,001*
5% sucrose	1 hour	0.002±0.001 (0.002)	0.011±0.009 (0.009)	0.001±0.001 (0.001)	0,001*
	2 hours	0.006±0.001 (0.006)	0.015±0.002 (0.015)	0.003±0.002 (0.003)	0,001*
	4 hours	0.018±0.002 (0.018)	0.063±0.003 (0.063)	0.004±0.002 (0.005)	0,001*
	6 hours	0.041±0.005 (0.041)	0.122±0.005 (0.122)	0.005±0.002 (0.005)	0,001*
	24 hours	0.528±0.019 (0.53)	0.66±0.013 (0.661)	0.038±0.004 (0.038)	0,001*

*Kruskal Wallis Test *p<0.05*

In all concentration groups, there were statistically significant differences in absorbance changes at 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours compared to the 0-hour among the bacteria ($p = 0.001$; $p < 0.05$).

In all concentration groups, the absorbance change of *Streptococcus sobrinus* was found to be higher than that of *Streptococcus mutans* and *Scardovia wiggsiae* at 1 and 2 hours ($p < 0.05$).

At 4 hours, the absorbance change of *Streptococcus sobrinus* was higher than that of *Streptococcus mutans* in the 10% xylitol group and the 2.5% xylitol + 7.5% erythritol group. In the other groups, there was no significant difference in the absorbance change between *Streptococcus sobrinus* and *Streptococcus mutans* ($p > 0.05$). At 4 hours, the absorbance change of *Streptococcus sobrinus* was found to be higher than that of *Scardovia wiggsiae* in all concentration groups ($p < 0.05$).

At 6 hours, no significant difference was found in the absorbance change between *Streptococcus mutans* and *Streptococcus sobrinus* in all concentration groups. The absorbance change of *Scardovia wiggsiae* at 6 hours was found to be statistically significantly lower than that of *Streptococcus mutans* and *Streptococcus sobrinus* in all concentration groups ($p < 0.05$). At 24 hours, the absorbance change of *Streptococcus mutans* was found to be statistically significantly lower than that of *Streptococcus sobrinus* in the 10% erythritol, 5% xylitol + 5% erythritol, and 2.5% xylitol + 7.5% erythritol groups ($p < 0.05$). The absorbance change of *Scardovia wiggsiae* was found to be statistically significantly lower than that of *Streptococcus mutans* and *Streptococcus sobrinus* in all concentration groups. ($p < 0.05$) (Table 3, Fig 2.)

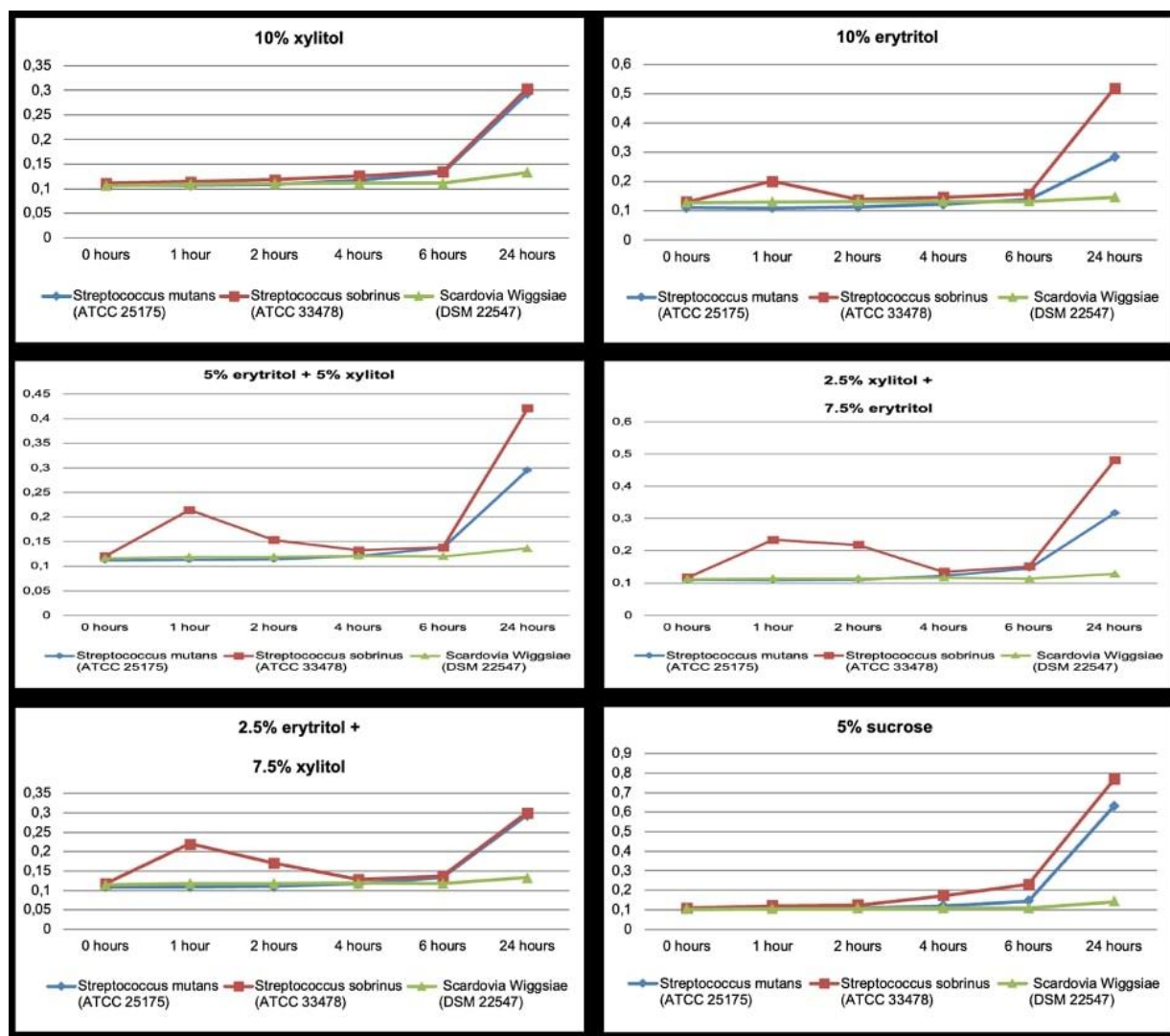


Figure 2 (These graphs depict the 24-hour growth trends of *Streptococcus mutans* (ATCC 25175), *Streptococcus sobrinus* (ATCC 33478), and *Scardovia wiggsiae* (DSM 22547) in the presence of 10% xylitol, 10% erythritol, 5% erythritol + 5% xylitol, 2.5% xylitol + 7.5% erythritol, 2.5% erythritol + 7.5% xylitol, and 5% sucrose. The graphs provide a comparative analysis of the effects of these sweeteners on bacterial growth.)

DISCUSSION

In our study, we aimed to determine the effect of erythritol and xylitol prepared in various concentrations using *S. mutans*, *S. sobrinus* and *S. wiggsiae*, which are the main cariogenic bacteria in the etiology of dental caries, on the growth of these bacteria for 24 hours. We found that the 5% sucrose group, which we used as a control group, caused the highest growth compared to the other groups. This result coincides with the conclusion that sucrose promotes the production of extracellular polysaccharides that facilitate the adhesion of bacteria and the formation of biofilms [18].

In 2009, Söderling et al. aimed to correlate the effects of polysaccharide production on the adhesion of oral streptococci to glass surfaces and to correlate these effects with growth inhibition. As a result of this study, it was found that erythritol and xylitol significantly reduced the adhesion of these streptococci to glass surfaces and our study is consistent with these findings. In this study, it was

reported that the adhesion of *S. mutans* Ingbritt strain decreased in the presence of xylitol, while no decrease was observed in the presence of erythritol. Similarly, in our study, the growth of *S. mutans* and *S. sobrinus* was significantly inhibited by xylitol at 10% concentration. The consistent result of xylitol strengthens its anticariogenic property.

Söderling et al. also reported that the adhesion of *S. mutans* strain 10449 and *S. sobrinus* strain OMZ 176 was affected by neither xylitol nor erythritol ^[13]. In contrast, our study found that erythritol had a significant inhibitory effect on both *S. mutans* and *S. sobrinus* at higher concentrations, suggesting that erythritol may be more effective for certain strains or under specific conditions. This discrepancy could be due to differences in experimental design, bacterial strains or environmental conditions and suggests that the efficacy of sugar alcohols may vary depending on these factors.

Our findings are also consistent with those reported by Mäkinen et al., who demonstrated that xylitol and erythritol reduced plaque formation and levels of mutans streptococci in saliva. In clinical trials, erythritol was demonstrated to be well tolerated and to have minimal gastrointestinal side effects, thereby establishing its suitability for long-term use in dental care products ^[14]. Our study builds upon these findings by showing that erythritol inhibits the growth of *S. mutans* and *S. sobrinus* in vitro, even at lower concentrations.

One of the innovative aspects of our study was investigating the combined effects of xylitol and erythritol on cariogenic bacteria. The combination of 7.5% xylitol + 2.5% erythritol and 5% xylitol + 5% erythritol significantly inhibited the growth of *S. mutans* compared to a 10% xylitol solution alone. This finding suggests a potential synergistic effect, which is particularly promising for dental health applications. Cannon et al. hypothesized that combinations of sugar alcohols might reduce caries more effectively than single agents, and our results empirically support this hypothesis ^[15].

Interestingly, at various time points, combinations of xylitol and erythritol (especially 2.5% xylitol + 7.5% erythritol) showed different growth patterns among the tested bacteria. For instance, after 4 hours, this combination showed higher absorbance changes compared to the reverse ratio (2.5% erythritol + 7.5% xylitol), suggesting a potential synergistic effect of xylitol and erythritol at specific ratios. This finding supports the potential to optimize polyol mixtures to maximize their inhibitory effects on oral pathogens.

In addition to synergistic effects, the additive effects of different concentrations of xylitol and erythritol combinations were also noteworthy. The combination of 5% xylitol + 5% erythritol resulted in substantial inhibition of both *S. mutans* and *S. sobrinus*. This suggests that even at lower concentrations, the combined use of these sugar alcohols can be more effective than higher concentrations of a single agent. This finding has practical implications, as using lower concentrations could reduce potential side effects and lower the costs associated with these compounds in dental products.

Runnel et al. investigated the effects of xylitol and erythritol on dental plaque. Their findings indicated that erythritol demonstrated a more pronounced efficacy in reducing plaque accumulation compared to xylitol. Furthermore, it was demonstrated to effectively reduce the rate of *S. mutans* in both saliva and dental plaque ^[16]. Our findings lend further support to the conclusions of the aforementioned study. The use of erythritol alone or in combination with xylitol may prove to be a more effective method for the prevention of dental caries.

In our study, we observed that the growth pattern of *Scardovia wiggisiae* was different from other caries-causing bacteria. Our findings suggest that although xylitol and erythritol are effective against other caries-causing bacteria, their effects on *S. wiggisiae* may be more complex. In particular, the increase in biofilm formation observed by Siiri Kõljalg et al. suggests that *S. wiggisiae* may have different adaptive mechanisms to polyol exposure [17]. Therefore, further research is required to optimize the use of polyols in dental care products by targeting this bacterium. Understanding these interactions is crucial for developing effective strategies for the prevention of early childhood caries, especially given the important role of *S. wiggisiae* in this condition.

The inhibitory effects of xylitol on *S. mutans* have been well-documented. Xylitol disrupts glycolysis by entering the bacterial cell and being phosphorylated to xylitol-5-phosphate, which cannot be further metabolized. This futile cycle depletes the bacterial cell's energy resources, leading to growth inhibition [19]. Our study confirms this mechanism, as xylitol effectively inhibited the growth of *S. mutans*, particularly at a 10% concentration. This aligns with previous studies [13, 20], reinforcing the understanding of xylitol's mode of action.

The mechanism of erythritol's inhibitory action, however, is less understood. Some studies, such as those by Mäkinen et al., have suggested that erythritol may interfere with bacterial adhesion and biofilm formation [21]. Our results support this, as erythritol significantly reduced biofilm production, especially at higher concentrations. Unlike xylitol, erythritol is not phosphorylated by bacterial cells, which might suggest a different mechanism of action, possibly related to the disruption of cell surface interactions or biofilm matrix integrity. Further research is needed to elucidate the exact pathways through which erythritol exerts its inhibitory effects.

The clinical implications of our findings are substantial. Dental caries remains a widespread health problem worldwide and effective preventive measures for caries are crucial. The significant inhibition effect of erythritol on *S. mutans* and *S. sobrinus*, especially when combined with xylitol, suggests that these sugar alcohols can be effectively used together in oral hygiene products to prevent dental caries.

Erythritol is more readily tolerated by the gastrointestinal tract than xylitol and is non-cariogenic, rendering it a suitable alternative or complement to xylitol in dental care products. The results of our investigation indicate that products comprising both sugar alcohols may confer enhanced protection against bacteria that are responsible for dental caries. This could lead to the development of new dental care products, such as toothpaste, mouthwash, and chewing gum, specifically formulated to maximize the synergistic effects of these compounds.

Additionally, the use of lower concentrations of these sugar alcohols in combination could reduce potential side effects and costs, making these products more accessible and acceptable to a broader population. This is particularly important for individuals with conditions like diabetes or obesity, who require sugar substitutes that do not affect blood glucose levels or contribute to weight gain.

CONCLUSION

In conclusion, our study demonstrates that erythritol, alone and in combination with xylitol, effectively inhibits the growth and biofilm formation of key cariogenic bacteria. These findings support the potential use of erythritol as a beneficial sugar substitute in dental care products aimed at caries prevention. The observed synergistic effects with the combination of xylitol and erythritol warrant further exploration to optimize formulations for maximum therapeutic benefit. As research progresses,

these sugar alcohols could play a crucial role in advancing oral health and preventing dental caries. The integration of xylitol and erythritol into everyday oral hygiene products could significantly enhance their efficacy in reducing the incidence of dental caries, thereby improving overall oral health outcomes.

Availability of Data and Materials

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

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no competing interest.

References

1. Monedero, V., G. Pérez-Martínez, and M.J. Yebra, Perspectives of engineering lactic acid bacteria for biotechnological polyol production. *Applied microbiology and biotechnology*, 2010. 86: p. 1003-1015.
2. Mäkinen, K.K., Sugar alcohol sweeteners as alternatives to sugar with special consideration of xylitol. *Medical Principles and Practice*, 2011. 20(4): p. 303-320.
3. Van Loveren, C., Sugar alcohols: what is the evidence for caries-preventive and caries-therapeutic effects? *Caries research*, 2004. 38(3): p. 286-293.
4. Pepper, T., Philip M. Olinger. *Eastern Hemisphere Distribution*, 2001: p. 335.
5. Knuuttila, M.L. and K.K. Mäkinen, Effect of xylitol on the growth and metabolism of *Streptococcus mutans*. *Caries research*, 1975. 9(3): p. 177-189.
6. Ly, K.A., P. Milgrom, and M. Rothen, Xylitol, sweeteners, and dental caries. *Pediatric dentistry*, 2006. 28(2): p. 154-163.
7. Moon, H.-J., et al., Biotechnological production of erythritol and its applications. *Applied microbiology and biotechnology*, 2010. 86: p. 1017-1025.
8. Munro, I., et al., Erythritol: an interpretive summary of biochemical, metabolic, toxicological and clinical data. *Food and chemical toxicology*, 1998. 36(12): p. 1139-1174.

9. Oku, T. and S. Nakamura, Threshold for transitory diarrhea induced by ingestion of xylitol and lactitol in young male and female adults. *Journal of nutritional science and vitaminology*, 2007. 53(1): p. 13-20.
10. Ichikawa, T., et al., The enhancement effect of three sugar alcohols on the fungicidal effect of benzethonium chloride toward *Candida albicans*. *Journal of dentistry*, 2008. 36(11): p. 965-968.
11. Banas, J.A. and D.R. Drake, Are the mutans streptococci still considered relevant to understanding the microbial etiology of dental caries? *BMC oral health*, 2018. 18: p. 1-8.
12. Tanner, A., et al., Cultivable anaerobic microbiota of severe early childhood caries. *Journal of clinical microbiology*, 2011. 49(4): p. 1464-1474.
13. Söderling, E.M. and A.-M. Hietala-Lenkkeri, Xylitol and erythritol decrease adherence of polysaccharide-producing oral streptococci. *Current microbiology*, 2010. 60: p. 25-29.
14. de Cock, P., et al., Erythritol is more effective than xylitol and sorbitol in managing oral health endpoints. *International journal of dentistry*, 2016. 2016(1): p. 9868421.
15. Cannon, M.I., et al., In vitro studies of xylitol and erythritol inhibition of *Streptococcus mutans* and *Streptococcus sobrinus* growth and biofilm production. *Journal of Clinical Pediatric Dentistry*, 2020. 44(5): p. 307-314.
16. Runnel, R., et al., Effect of three-year consumption of erythritol, xylitol and sorbitol candies on various plaque and salivary caries-related variables. *Journal of dentistry*, 2013. 41(12): p. 1236-1244.
17. Kõljalg, S., et al., Exploration of singular and synergistic effect of xylitol and erythritol on causative agents of dental caries. *Scientific Reports*, 2020. 10(1): p. 6297.
18. Bowen, W. and H. Koo, Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. *Caries research*, 2011. 45(1): p. 69-86.
19. Takahashi, N. and B. Nyvad, Caries ecology revisited: microbial dynamics and the caries process. *Caries research*, 2008. 42(6): p. 409-418.
20. Ghezlbash, G.R., I. Nahvi, and M. Rabbani, Comparative inhibitory effect of xylitol and erythritol on the growth and biofilm formation of oral *Streptococci*. *Afr J Microbiol Res*, 2012. 6(20): p. 4404-8.
21. Mäkinen, K., et al., Similarity of the effects of erythritol and xylitol on some risk factors of dental caries. *Caries Research*, 2005. 39(3): p. 207-215.