

Effect of Probiotic Yogurt and Xylitol-Containing Chewing Gums on Salivary *S Mutans* Count

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Background and Aims: In addition to improving gastrointestinal health and intestinal microflora, probiotic bacteria have been recently suggested to decrease cariogenic agents in the oral cavity. The aim of this study was to investigate the effects of probiotic yogurt and xylitol-containing chewing gums on reducing salivary *Streptococcus mutans* levels. **Study design:** This randomized clinical trial recruited 50 female students with over 10^5 colony forming units *S. mutans* per milliliter of their saliva. The participants were randomly allocated to two equal groups to receive either probiotic yogurt containing *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 29521 (200 g daily) or xylitol-containing chewing gums (two gums three times daily after each meal; total xylitol content: 5.58 g daily) for three weeks. At baseline and one day, two weeks, and four weeks after the interventions, saliva samples were cultured on mitis-salivarius-bacitracin agar and salivary *S. mutans* counts were determined. Data were analyzed with independent t-tests, analysis of variance, and Fisher's least significant difference test. **Results:** In both groups, *S. mutans* counts on the first day, second week, and fourth weeks after the intervention were significantly lower than baseline values ($P < 0.05$). The greatest level of reduction in both groups was observed in the second week after the intervention. Moreover, although the reduction was greater in probiotic yogurt consumers, the difference between the two groups was not statistically significant. **Conclusion:** Probiotic yogurt and xylitol-containing chewing gums seem to be as effective in reduction of salivary *S. mutans* levels. Their constant long-term consumption is thus recommended to prevent caries.

Key words: Probiotics, yogurt, xylitol, chewing gum, saliva, *Streptococcus mutans*.

INTRODUCTION

Dental caries are the most prevalent chronic disease throughout the world¹. They are caused by decalcification of the inorganic portion and destruction of the organic matrix of the teeth in the presence of three major factors, i.e. host, fermentable carbohydrates, and acid-producing bacteria^{1,2}. Therefore, efforts to prevent dental caries have often focused on methods to control the activity of oral bacteria. *Streptococcus mutans* is one of the most important bacterial species involved in the demineralization of tooth enamel and initiation of dental caries^{1,2}. Various methods including the application of toothpastes, antibacterial compounds (e.g. chlorhexidine), and fluoride-containing mouthwashes along

with the use of non-fermentable sweeteners such as xylitol have been suggested to reduce the activity of these bacteria.

Xylitol, a five-carbon sugar alcohol, naturally occurs (of course in low concentrations) in many types of fruits and vegetables. It is widely used as an added sweetener in sugar-free products, especially chewing gums, and some mouthwashes and toothpastes^{3,4}. Xylitol has been found to prevent dental caries through reducing dental plaque and limiting salivary *S. mutans* counts and their produced levels of lactic acid⁵. It not only decreases biofilm development by interfering with bacterial adhesion, but also acts as a calcium transporter and remineralizes hard tissue. The effects of xylitol-containing chewing gums on decreasing *S. mutans* counts in both dental plaques and saliva have been well documented⁵⁻⁸. While some researchers have reported these effects to be dose-dependent⁶, others have rejected any associations between the beneficial effects of xylitol and its consumed dose⁵. Nevertheless, like other interventions, this polyol has been found ineffective on bacterial counts in the oral cavity of individuals with limited caries⁹.

Food and dental products containing probiotic bacteria, particularly *Lactobacilli* and *Bifidobacteria*¹⁰, are receiving increasing attention in caries prevention. According to the World Health Organization (WHO) and US Food and Drug Administration (FDA), ingestion of adequate levels of probiotics leads to beneficial health effects in the hosts of these microorganisms¹¹. Although probiotic bacteria are also marketed as supplementary pills, dairy

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products, including different types of yoghurt, are also known as carriers of probiotics^{12,13}.

Probiotics inhibit the implantation and growth of pathogenic bacteria by adhering to gastrointestinal mucosa and covering the adhesion sites of these microorganisms. Probiotics such as *Lactobacilli para-casei* have also been reported to co-aggregate with *S. mutans*. They consume the existing nutrients before pathogenic microorganisms find the opportunity to use them. Probiotics can eliminate toxins, produce antimicrobial compounds, change pH, and regulate local and systemic immune responses¹⁴⁻¹⁶. *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, the first identified probiotic bacteria, have been shown to have potential health benefits in both children and adults. They are known to shorten the duration of acute infectious diarrhea and decrease susceptibility to infection, allergic reactions, and eczema in infants, relieve colic symptoms in children, and generally regulate blood pressure and serum cholesterol levels and facilitate the absorption of vitamins and minerals by the body^{15,17-19}. Studies on oral ecology by Caglar *et al*^{14,20}, Chuang *et al*²¹, and Sudhir *et al*²² have confirmed the efficacy of probiotic bacteria in reducing salivary *S. mutans* counts. Nevertheless, Lesan *et al*¹⁶ and Montalto *et al*²³ detected unchanged *S. mutans* counts subsequent to the consumption of probiotic products. Apparently, the role of probiotic bacteria in defining oral microflora is still a controversial issue. Since very few studies have compared the effects of available probiotic and xylitol-containing products on the number of cariogenic bacteria, the current research sought to clarify the benefits of probiotic yogurt and xylitol-containing chewing gums in decreasing salivary *S. mutans* counts.

MATERIALS AND METHOD

This clinical trial (registered in the Iranian Registry of Clinical Trials; ID: IRCT2014091319131N1) was conducted on female dormitory residents in Islamic Azad University (Isfahan Branch, Khorasgan, Isfahan, Iran). The students were explained about the study objectives and procedure and asked their willingness to participate. Finally, 60 volunteers (age: 19-27 years, mean age: 23 years) were recruited and randomly allocated to two equal groups. However, only 50 individuals (25 in each group) completed the study. Individuals with *S. mutans* counts $\geq 10^5$ colony forming units (CFU) per milliliter of saliva who had no systemic diseases, no orthodontic appliances, and no history of consuming probiotic products or xylitol-containing chewing gums during the past month, fluoride therapy or use of antibacterial and fluoride mouthwashes over the past two weeks, and antibiotic treatment over the past four weeks were included. According to previous studies, *S. mutans* counts of about 10^5 - 10^6 CFU per ml of stimulated saliva would be sufficient to increase the risk for caries in adults²⁴. Students were not included if they had smoking habits or were using oral contraceptives. The subjects were excluded if they underwent any dentistry procedures, developed any systemic diseases, or had to use antibiotics, antihistamines, steroids, antibacterial mouthwashes, and topical fluoride (except fluoride toothpaste) during the course of the study. All participants were asked to continue their routine tooth brushing and flossing habits during the study period.

After receiving written consent from all volunteers, a trained researcher collected saliva samples and cultured the samples on

the mitis-salivarius-bacitracin agar (Quelab, Canada) media to calculate salivary *S. mutans* counts. Eligible students (those with *S. mutans* counts $\geq 10^5$ CFU/ml) were then identified and randomly allocated to two equal groups to receive either probiotic yogurt or xylitol-containing chewing gums for three weeks. At the beginning of each week, the first group was provided with fourteen 100 g servings of probiotic yogurt (1400 g in total). The yogurt, produced by Kalleh Dairy Company (Amol, Iran), contained 1.5×10^8 CFU/g *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 29521. All yogurt packs were purchased from an authorized distributor of the company and had similar production dates. The subjects were asked to eat two servings (200 g) of the provided yogurt 10 minutes after dinner every night and not to brush their teeth until one hour later. The second group was asked to chew two xylitol-containing chewing gums (Orion Dr. Xylitol, Orion Food Vina Co., Vietnam) for at least five minutes three times a day (after each meal). Since each chewing gum contained 0.93 g xylitol, the participants received 5.58 g xylitol daily^{5,25}.

In order to determine *S. mutans* counts, saliva samples were collected and cultured on the mentioned media before the interventions and one day, two weeks, and four weeks after the end of the intervention. The students were instructed to have breakfast but not to brush their teeth on sampling days. Sampling was performed at least two hours after breakfast. The participants were requested to rinse their mouths and to pour their paraffin-stimulated saliva directly into sterile microtubes. The samples were maintained in ice containers and transferred to the laboratory within two hours. Upon their delivery, the samples underwent microbiological tests by an expert examiner.

The collected samples were diluted with sterile normal saline solution. The 10^{-3} to 10^{-6} dilutions were plated on mitis-salivarius-bacitracin agar (Quelab, Canada) media, the plates were incubated in a CO₂ incubator (Memmert, Germany) at 37°C and 5% CO₂ for 24-48 hours. The colonies on each plate were then carefully inspected. In macroscopic evaluations, colonies appeared as light blue, small to medium-sized granules with convex sides and irregular shapes and margins. In microscopic evaluations, the bacteria formed Gram-positive cocci chains which were not only resistant to bacitracin, but also able to ferment mannitol. Moreover, negative catalase test and positive Voges-Proskauer test results confirmed the grown bacteria to be *S. mutans*^{26,27}. The number of colonies in each sample was calculated and reported as CFU per ml saliva (CFU/ml).

The collected data were recorded in specific forms and analyzed with independent t-tests, analysis of variance (ANOVA), and Fisher's least significant difference (LSD) test. All analyses were conducted with SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

According to independent t-test results, the two groups had no significant differences in the mean salivary *S. mutans* counts either at baseline or on the first day, second week, and fourth week after the interventions (Table 1). Nevertheless, intragroup comparisons using repeated measures ANOVA revealed that the mean salivary *S. mutans* counts in both groups were significantly different at different times (Tables 2 and 3).

Table 1. The mean salivary *Streptococcus mutans* counts ($\times 10^5$ colony forming units per ml) in the first and second groups (receiving probiotic yogurt and xylitol-containing chewing gums, respectively) at different times

Time	<i>S. mutans</i> counts		P value
	First group	Second group	
Baseline	23.62 \pm 4.38	22.28 \pm 5.97	0.86
One day after the intervention	7.67 \pm 2.64	8.41 \pm 2.93	0.85
Two weeks after the intervention	4.03 \pm 1.37	5.26 \pm 2.01	0.62
Four weeks after the intervention	7.86 \pm 4.30	9.04 \pm 3.00	0.82
P value	0.004	0.03	-

Table 2. Pairwise comparisons between the mean salivary *Streptococcus mutans* counts in students receiving probiotic yogurt at different times (based on Fisher's least significant difference test results)

	P values		
	One day after the intervention	Two weeks after the intervention	Four weeks after the intervention
Baseline	0.001	\leq 0.001	0.01
One day after the intervention	-	0.04	0.95
Two weeks after the intervention	-	-	0.045

Table 3. Pairwise comparisons between the mean salivary *Streptococcus mutans* counts in students receiving xylitol-containing chewing gums at different times (based on Fisher's least significant difference test results)

	P values		
	One day after the intervention	Two weeks after the intervention	Four weeks after the intervention
Baseline	0.01	0.005	0.02
One day after the intervention	-	0.02	0.77
Two weeks after the intervention	-	-	0.04

Based on Fisher's LSD test results, salivary *S. mutans* counts in both groups significantly decreased from baseline to the first day after the intervention and from the first day to the second week after the intervention. However, the values significantly increased on the fourth week after the intervention (compared to the second week). In fact, the numbers of colonies were not significantly different on the fourth week and the first day after the intervention. However, *S. mutans* counts on the fourth week were still significantly lower than baseline values.

Finally, although percentage reduction in salivary *S. mutans* counts was slightly higher in the first group compared to the second group, this difference was not statistically significant according to independent t-test results (Table 4).

Figure 1. The mean salivary *Streptococcus mutans* counts ($\times 10^5$ colony forming units per ml) at different time intervals in the two groups receiving probiotic yogurt and xylitol-containing chewing gums

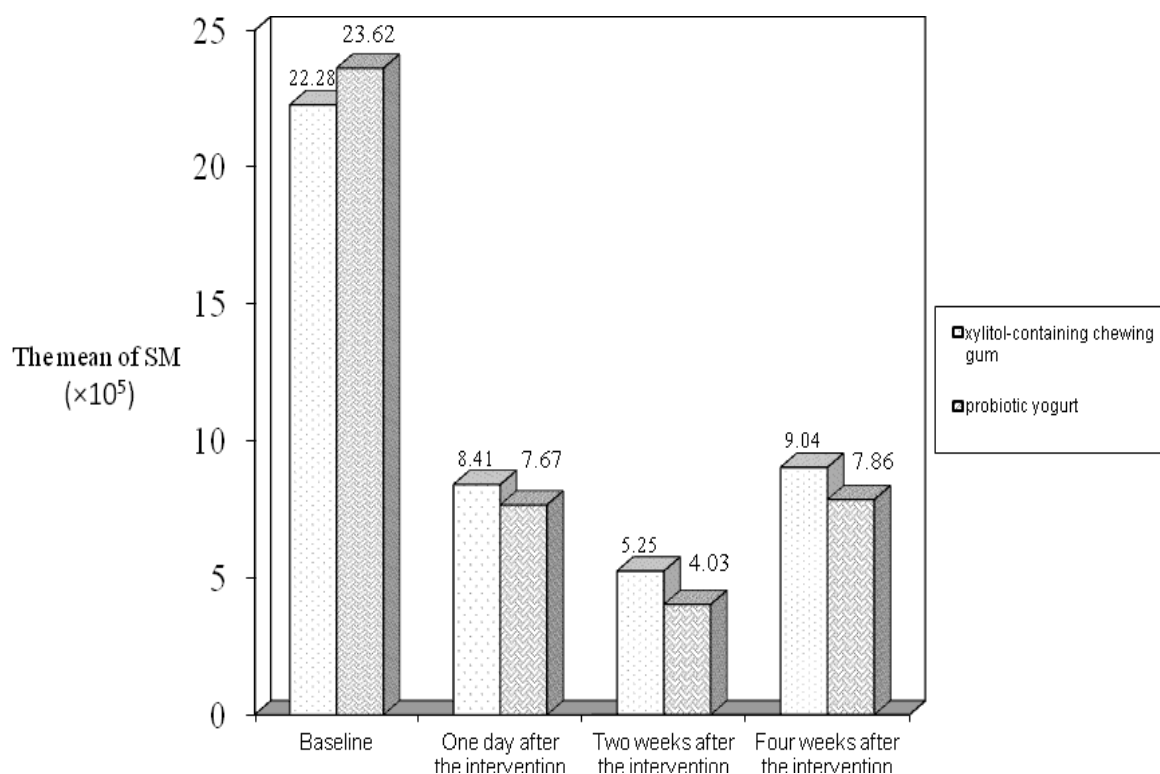


Table 4. Percentage reduction in salivary *S. mutans* counts at different post-intervention time intervals compared to baseline in the two groups receiving probiotic yogurt (the first group) and xylitol-containing chewing gums (the second group)

Time	Percentage reduction in <i>S. mutans</i> counts (%)		P value
	First group	Second group	
First day after the intervention	67.5 ± 10.0	62.2 ± 13.0	0.62
Two weeks after the intervention	82.9 ± 11.0	76.4 ± 11.0	0.65
Four weeks after the intervention	66.7 ± 42.0	59.4 ± 37.0	0.59

DISCUSSION

Based on our findings, both probiotic yogurt and xylitol-containing chewing gums could significantly reduce salivary *S. mutans* counts. The greatest level of reduction in both groups was observed two weeks after the intervention. Although the number of bacteria increased during the fourth week after the intervention, the values still remained lower than baseline counts.

The effectiveness of xylitol on decreasing salivary *S. mutans* counts has also been reported by several other studies^{6,8,14,28-31}. Rather than being a cariogenic agent, which is metabolized by oral bacteria and reduces biofilm pH by acid production, xylitol is regarded as an anti-cariogenic polyalcohol derived from D-xylose. By penetrating into bacterial cytoplasm and accumulating in bacterial cells as xylitol-5-phosphate, xylitol interferes with glycolysis and adenosine triphosphate (ATP) and acid production, inhibits bacterial growth, and ultimately causes bacterial cell death^{8,32}. This polyol has also been found to decrease the adhesion of *S. mutans* to tooth surfaces^{8,25}. In addition to its established role in reducing salivary and plaque *S. mutans* counts, regular and long-term consumption of xylitol has been shown to promote the emergence of xylitol-tolerant strains with lower pathogenic potential and adhesion capability compared to xylitol-sensitive strains^{5,8,33-36}.

The inhibitory effects of xylitol on bacterial growth depend on its method of administration. While substituting sugar with xylitol in food preparation cannot decrease salivary *S. mutans* counts, constant direct contact between xylitol and tooth surfaces, such as that provided through chewing gums and candies, can ensure the desired benefits^{7,8,28}. In other words, inconsistencies in the findings of different studies can be justified by the role of duration, frequency, and concentration of xylitol administration in its beneficial effects on bacterial counts^{1,8,21,37,38}.

While previous studies have suggested different dosages of xylitol (6.0-11.6 g daily) to be effective on reducing oral bacteria^{2,5,14,34,39}, we found a lower dosage of this polyol (5.58 g daily) to have caries prevention effects which lasted for as long as four weeks. According to the American Academy of Pediatric Dentistry (AAPD) guideline, using 3-8 g xylitol twice daily would be required to observe its clinical benefits^{8,25,40}. Apparently, the applied dosage in the current research fell within the recommended range.

Furthermore, since similar benefits have been documented following the use of other sugar substitutes (e.g. sorbitol) and even placebo chewing gums^{8,40}, the effects of xylitol on *S. mutans* counts may not be the only factor responsible for its caries prevention

properties. In other words, regular use of chewing gums may stimulate and increase salivary flow rate, improve the buffer capacity of saliva, and ultimately facilitate both substrate removal and the remineralization process^{34,35,41-43}. Nevertheless, Holgerson *et al* concluded that xylitol's mechanism of action was not limited to the above-mentioned procedure. They reported both xylitol- and sorbitol/mannitol-containing chewing gums to decrease dental plaque formation and acid production. However, only xylitol-containing chewing gums were able to alter oral bacterial composition and reduce salivary *S. mutans* counts⁴⁴. Other studies have also confirmed the efficacy of xylitol, not other sugar substitutes or placebo chewing gums, in decreasing salivary *S. mutans* counts^{1,5,8,14,31,45}.

Similar to our findings, Fraga *et al* reported salivary *S. mutans* counts to be significantly lower than baseline values even one month after using xylitol-containing chewing gums⁸. Such a finding highlights the fact that the effects of xylitol can remain persistent for certain periods of time. In the present study, however, the lowest number of salivary *S. mutans* was observed two weeks after the intervention (the bacterial counts in the fourth week were higher than those in the second week). Since *S. mutans* reside and colonize in the oral cavity, in the presence of stable dietary and brushing habits, *S. mutans* counts are expected to increase and reach their baseline levels after discontinuing the use of xylitol-containing chewing gums⁸. Therefore, regular chewing of such gums can be recommended as a safe and side effect-free method to prevent caries.

Probiotic bacteria and their beneficial effects on gastrointestinal health and intestinal microflora have received increasing attention during the recent years. In order to evaluate the effects of probiotics on reducing salivary *S. mutans* counts and potential caries prevention, we asked our participants to eat probiotic yogurt (containing 1.5×10^8 CFU/g *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) daily for three weeks. This intervention could significantly reduce salivary *S. mutans* counts (the highest reduction was observed at the second week after the end of the intervention). Moeiny *et al*¹⁰, Caglar *et al*^{14,20}, Chuang *et al*²¹, Sudhir *et al*²², Poureslami *et al*⁴⁶, and Soderling *et al*⁴⁷ have also reported similar reductions in *S. mutans* counts following the consumption of probiotic food products.

Although the exact mechanism through which probiotic food products affect *S. mutans* is not known, it is believed to resemble the process involved in the gastrointestinal tract^{11,20}. In fact, as probiotics adhere to oral mucosa and surface of teeth as a part of biofilm (plaque), they prevent the adhesion, colonization, and proliferation of cariogenic bacteria, such as *S. mutans*, and periodontal pathogens. Therefore, both direct and indirect mechanisms, i.e. competing with cariogenic bacteria for adhesion sites and neutralizing free electrons, are adopted by probiotic bacteria to inhibit the formation of pathogenic plaques. Moreover, these agents reduce the number of oral *S. mutans* strains by producing various cell adhesion inhibitors and antibacterial compounds such as organic acids, hydrogen peroxide, carbon oxide, diacetyl, and bacteriocins. The acid produced by probiotic bacteria can also reduce pH and limit the survival of oral pathogens. Since these bacteria suppress the inflammatory response in the mouth, they use a combination of local and systemic immune response and non-immunological defense mechanisms to combat pathogenic agents in the oral cavity^{14-16,20,48-50}.

In contrast to our findings, Lesan *et al*¹⁶ and Montalto *et al*²³ reported probiotic food products to be ineffective in decreasing salivary *S. mutans* counts. Differences in the designed procedures (level, type, and duration of exposure to probiotic bacteria) and probiotic species used (e.g. *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, and *Lactobacillus reuteri*) can be accountable for such inconsistencies. Even differences between various strains of a particular probiotic species can be responsible for the observed discrepancies^{15,23}. Most commonly, either the amount of probiotic being administered is inadequate for biofilm change, or a combination of probiotic bacteria strains that actually inhibit each other is used.

In the present research, the greatest reduction in *S. mutans* counts was seen in the second week after the consumption of probiotic yogurt. Although *S. mutans* levels increased in the fourth week, they were still significantly lower than baseline values. Likewise, Moeiny *et al.* concluded that maximum effects of probiotic yogurt lasted until two weeks after discontinuing its consumption¹⁰. In a study by Meurman *et al.*, *Lactobacillus rhamnosus GG* was identifiable until two weeks after ingesting probiotic yogurt⁵¹. While implantation and colonization of probiotics in the oral cavity seems to be the first step in the prediction of their long-term effects, there is little evidence to support such a hypothesis. Despite the fact that probiotic bacteria have been found in most oral samples during the intervals after the consumption of probiotic products, their presence does not necessarily reinforce the chance of their permanent residence in the oral cavity. The question would thus be if permanent residence of probiotics can be achieved in individuals with already stable oral microflora. Since several factors (e.g. short contact between probiotic products and dental plaque) might prevent the long-term effects of probiotics, especially after discontinuation of their use, their constant daily consumption (with minimum possible intervals) would be required to ensure their potential activities^{15,51}. Another possibility would be utilizing a normal oral inhabitant with antagonistic action to *S. mutans* as a probiotic⁵². *S. oralis*, *S. uberis*, and *S. rattus* have been incorporated into a commercially available probiotic lozenge (ProBiora3™) with such a concept intended⁵³.

The current research found both xylitol-containing chewing gums and probiotic yogurt to be able to significantly reduce salivary *S. mutans*. Although the reduction was greater in probiotic yogurt consumers, the difference between the two groups was not statistically significant. Consistent with our findings, Caglar *et al.* reported both xylitol-containing and probiotic chewing gums to

have significant beneficial effects on decreasing salivary *S. mutans* counts. They, however, did not determine whether *S. mutans* levels were significantly different in the two groups¹⁴.

Based on Cannon *et al* study's results, two over the counter-OTC- probiotic supplements affected the Caries Risk Test- CRT- results by significantly decreasing the number of *S. mutans* and lactobacilli present in the salivary samples⁵⁴.

Nevertheless, in spite of their dissimilar mechanisms of action, long-term consumption (even if interrupted with short intervals) of xylitol-containing chewing gums and probiotic products can decrease salivary *S. mutans* levels, plaque formation, and demineralization. This is undoubtedly of critical significance, particularly in children.

Overall, probiotics have been proven to have beneficial effects on general health and intestinal microflora^{11,15,17}. They are also able to induce greater (though not significantly) reduction in salivary *S. mutans* compared to xylitol. Moreover, probiotic products such as yogurt, cheese, and milk are rich in various nutrients (e.g. casein, calcium, phosphorous, and some vitamins) essential for the growth of children and adolescents. Since these products can be easily integrated in daily meals, their consumption seems to be superior to using xylitol-containing chewing gums (which have to be used three times daily). Therefore, daily consumption of 1.5-2.0 dl of probiotic products (containing 10⁸ probiotic bacteria per g or ml) is recommended^{15,55}. Future research should evaluate any possible synergistic effects of simultaneous use of probiotic products and xylitol or even the inhibitory effects of xylitol on probiotics.

CONCLUSION

Xylitol-containing chewing gums and probiotic yogurt are both as effective in reducing salivary *S. mutans* counts. Their long-term consumption is thus recommended as a safe and effective measure for caries prevention.

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