Ex Vivo Evaluating Xylitol's Effects on Plaque Acid Production

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ABSTRACT

Sugar alcohols such as xylitol and sorbitol are effective as non-cariogenic substitutes when used in place of glucose or sucrose. Chewing gums containing xylitol have been shown to affect acid production by dental plaque (Söderling, *et al*, Caries Research, 23:378-384, 1989). The production of plaque acid is known to effect hard and soft tissue. An easy to use and reproducible methodology to evaluate the effects of sugar alcohols on plaque acid production is needed. The objective of this study was to determine whether changes in plaque acid production could be detected following exposure to xylitol solutions , utilizing a modification of the Plaque Glycolysis and Regrowth Model (PGRM) - [White, *et al*., J. Clin Dent., VI:59-70, 1995]. This study evaluated the changes in plaque acid production of the following variables: 1) xylitol levels (0-100 mM); 2)xylitol exposure times (0-240 min.); 3) type of sugar and concentrations (sorbitol/glucose/etc. 0-100mM); 4) incubation media of the human plaque (0.03%-0.3% TSB with & without dextrose). In this modification of the PGRM model, on the morning of each test, subjects presented to the site having refrained from oral hygiene the previous 12 hours. A resting plaque sample was collected from the entire dentition of 4-8 subjects. Plaque samples suspended into a trypticase soy broth solution were pooled together to give a uniform sample. The biomass was normalized according to the PGRM methodology and divided into multiple samples. The plaque samples were treated to assess the variables described above, and incubated in TSB buffer at 37º C with gentle agitation for 4 hours. The pH of the samples was monitored. Plaque acid production was determined by measuring the pH at different time points during the 4 hours incubation. Profiles of the plaque pH curves were obtained for each set of conditions. Plaque samples exposed to xylitol and given a sugar challenge had different pH profiles than the control plaque samples. **These results show that harvested plaque samples exposed to xyltiol have an altered pH profile and produce less acid than the control samples (xylitol AUC 827.475 vs sucrose AUC 746.78). This suggests that this new methodology for assessing plaque acid production can be used to monitor acid production following exposure to xylitol containing oral care formulations .**

INTRODUCTION

Test methods that predict the effects of oral care products on plaque, tartar, gingivitis and caries are very important to dental researchers and oral product manufacturers. Sugar alcohols are being extensively researched for their effects on plaque, plaque acid production, and caries. A simple and reproducible testing method needs to be developed to determine the effects of oral product formulations containing alternative nonsweetners such as xylitol.

OBJECTIVE

The purpose of this study was to develop a model to evaluate the effects of xylitol on plaque acid production *ex vivo*.

MATERIALS AND METHODS

A modification of the PGRM methodology (White, *et al*., J. Clin Dent., VI:59-70, 1995) was developed where treatments were conducted *ex vivo*.

A. Having refrained from oral hygiene the previous 12 hours, a resting plaque sample was collected from the entire dentition of 4-8 subjects utilizing a sterile cotton tipped swab.

B. Plaque samples were suspended into a trypticase soy broth solution (TSB, 0.03 or 0.3%), pooled together to give a uniform plaque sample, normalized according to the PGRM methodlogy, and divided into multiple samples.

C. Plaque samples were treated to assess the variables described below and incubated in TSB buffer at 37°C with gentle agitation for up to 4 hours. The pH of the treated samples was monitored to provide a pH profile curve for each set of conditions.

Treatment Variables

- Varying conditions of plaque incubation media (TSB) were evaluated at 2 different concentrations (0.03% and 0.3% with and without added dextrose).
- Several sugars were evaluated including sucrose, glucose, sorbitol, and xylitol at concentrations ranging from 0 to 100 mM. For 180 minutes post-treatment, pH values were monitored.
- Treatment with varied xylitol concentrations (0-100 mM) prior to a sugar challenge were monitored for their effects on plaque pH.
- In addition, exposure of plaque to xylitol prior to a sugar challenge (sorbitol) was evaluated over time (0-240 minutes) for its effects on plaque pH.

RESULTS

Effects of Incubation Media								
1) 0.03% TSB w/o dextrose								
Time	**0**	**15**	**30**	**60**	**90**	**120**	**180**	**240**
pH	7.22	6.98	6.90	6.82	6.82	6.82	6.80	6.82
2) 0.03% w/dextrose								
Time	**0**	**15**	**30**	**60**	**90**	**120**	**180**	**240**
pH	7.15	6.83	6.62	6.42	6.45	6.46	6.42	6.43
3) 0.3% TSB w/o dextrose								
Time	**0**	**15**	**30**	**60**	**90**	**120**	**180**	**240**
pH	7.41	7.36	7.30	7.11	7.00	6.90	6.74	6.82
4) 0.3% TSB w/dextrose								
Time	**0**	**15**	**30**	**60**	**90**	**120**	**180**	**240**
pH	7.41	7.33	7.28	7.06	6.96	6.81	6.66	6.84

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Results indicate that the greatest effect on plaque pH is seen from the 0.03% TSB w/dextrose. To minimize the effects of the incubation media on plaque pH, 0.03% TSB w/o dextrose was chosen for addition work.

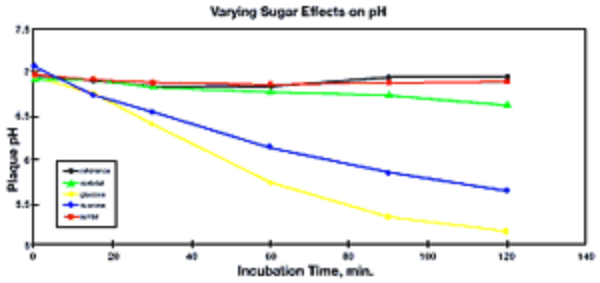
Effects of Varying Exposure Times of 100 mM Xylitol								
Exposure Time	**0**	**15**	**30**	**60**	**90**	**120**	**180**	**240**
0 min.	7.41	7.32	7.26	7.08	6.97	6.8	6.12	5.60
30 min.	7.43	7.19	7.09	6.97	6.81	6.73	6.79	6.96
120 min.	7.43	6.80	6.73	6.83	6.89	6.87	6.87	6.93
240 min	7.44	6.92	6.97	6.92	6.95	6.99	6.97	*nm*
nm=not measured								

Plaque initially treated with xylitol and followed immediately with a sugar challenge (time= 0 min.) had a much lower plaque pH profile than plaque treated with xylitol for extended periods of time before a sugar challenge. This suggests that xylitol may effect the acid production of plaque bacteria.

Effects of Varying Xylitol concentrations							
Time	**0**	**15**	**30**	**60**	**90**	**120**	**180**
0 mM	6.97	6.89	6.84	6.80	6.80	6.78	6.71
1 mM	7.01	6.94	6.90	6.88	6.89	6.91	6.85
10 mM	6.98	6.92	6.89	6.87	6.89	6.90	6.84
100 mM	7.01	6.91	6.90	6.88	6.91	6.90	6.85

Increasing the level of xylitol immediately before a sugar challenge had no effect on the pH profile of exposed plaque.

Effects of Different Sugars Area Under the pH Profile Curve (AUC):
10 mM Sugar (AUC): Reference (833.25), Xylitol (827.48), Sorbitol (815.63), Sucrose (746.78), Glucose (724.20).



Plaque exposed to xylitol has a higher plaque pH profile curve than any of the other 3 sugars. Sorbitol also exhibited a higher pH profile than the other sugars, but was slightly lower than xylitol.

CONCLUSION

These results show that harvested plaque samples exposed to xylitol have an altered pH profile and produce less acid than the control samples. This suggests that this methodology for assessing plaque acid production can be used to monitor acid production following exposure to xylitol containing oral care formulations.

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