AN ASSESSMENT OF THE XYLITOL INFLUENCE ON THE NATURAL REMINERALIZATION PROCESS

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ABSTRACT

Homeostasis in the oral medium represents the balance between demineralization and remineralization processes.

The positive results from the conducted studies evaluating the influence of the sucrose substance (xylitol) on the bacterial colonization of *Streptococcus mutans* on dental plaque, motivated us to check its influence on the natural remineralization process.

The study was conducted in vitro, on healthy teeth, with daily submergence in fresh saliva enriched with 10% xylitol solution over two weeks period. The enamel was pulverized to dust and further analyzed with Fourier Transform Infrared Spectroscopy (FTIR).

Analysis demonstrated no significant difference in the molecular structure of the crystal bond after the treatment of hydroxyapatite with xylitol. Thus, it cannot directly affect the natural remineralization process.

Xylitol has an indirect positive effect on the natural remineralization by "conditioning" of the oral medium. The quality of remineralization improves due to its non-fermentable and non-acidic characteristics, accompanied with drastic reduction in *S. mutans* colonization being the crucial factor for initiation of dental caries.

Keywords: xylitol; remineralization; caries prevention

Introduction

The perfection of human body is evident as the presence and functioning of the complex compensatory mechanisms sustain the natural balance. These mechanisms are inherited protecting the overall health. From a perspective of dental medicine, the maintenance of dental health depends on the equilibrium between the processes of demineralization and remineralization of dentition.

According to The World Health Organization (WHO) recommendations, the evaluation of the DMFT – index in The Republic of Macedonia was made by calibrated teams at 12 years old children. The value of the index in 2008 was 6, 88 representing a high value indicating that the natural process of remineralization is often insufficient and inadequate.[1] Demineralization process rapidly accelerates and overcomes the remineralization because of the bad oral-hygienic habits. This includes inadequate and insufficient oral hygiene and uncontrolled intake of easily fermentable carbohydrates in daily diet. These habits create unfavorable conditions in the oral medium thus helping the process of demineralization to become more frequent not allowing remineralization to overcome. The result is high dental caries score in young patients (children to adolescents). [2]

Dental scientific research, especially the one that deals with prevention, usually strives to find means and ways to prevent the caries causal chain and to improve the natural remineralization process. In the last few years, several studies researched the xylitol as a sucrose substitute. They showed a significant influence of xylitol on the colonization of *S. mutans* in dental plaque. It considerably decreased he colonization and the risk of caries process initiation.

Xylitol, is a 5-carbonate sucrose alcohol and belongs to sweetener category. It is obtained from cellulose-xylan cellulose through different ways. The fact that xylitol is a product of human carbohydrate metabolism, as a normal intermediary, is interesting to be mentioned. In the human body, on a daily basis, 5-15 grams of xylitol are formed, and this process takes place in the liver cells. The chemical properties of xylitol allow its use in diet without any significant changes in taste, apart from so-called cooling effect, which is a result of negative heat effect in dissolution reaction. This cooling effect is the initiating factor for increased salivary secretion, a process which has multiple benefits in the oral medium: improved self-cleaning of the dental tissues and increased puffer capacity at a salivary level. Furthermore, xylitol is a hypo-acidic and non-fermentable sucrose substitute by the microorganisms from the *S. mutans* species. They barely or never metabolize xylitol, omitting the acid production effect and the creation of acido-genic episodes inside the dental plaque. Thus, a decrease in plaque pH values does not happen, and pH does not reach critical values which may allow initiation of demineralization process. With a drastic decrease in *S. mutans* colonization, restructuring of the bacterial flora in the plaque happens and commensal bacterial species become prevalent. Xylitol is low in calories and is suitable for use by individuals with diabetes.

The maintenance of the oral health can be observed from different aspects, and the aim of this study was to determine the oral health status through the xylitol influence on the natural remineralization process.

Materials and method

We evaluated the reparative action of xylitol on the tooth enamel, or its probably positive effect on the remineralization processes. The study was performed in vitro, and healthy teeth with indication for extraction caused by impaction were analyzed. Over a two weeks period, the teeth were submerged in fresh saliva enriched with a 10% xylitol solution on a daily basis. After this period, the teeth were pulverized to dust.

The analysis was performed with Fourier Transform Infrared Spectroscopy (FTIR). The preparation of the enamel from the extracted teeth for FTIR was performed after the removal of the dentin and the pulp. The collected specimens were further processed and sifted through a 150 mM sieve and dried at lyophilization (Labconco, FreeZone 2.5 L Feeze Dry System, USA) at a temperature of -40^{0}C and a pressure of 2 mbar to a constant mass. The values were compared with the hydroxylapatite (type A and type B).

A change in the redistribution of carbonates in the crystal structure of the apatite in caries samples (white spots lesions) could be expected as a consequence of demineralization. This kind of picture can be obtained from the FTIR spectroscopy of the enamel in healthy teeth and carious teeth (**Table 1**). The changes in the carbonate

Table 1. Ascension of carbonate absorption peaks

Carbonate type	Vibration type	Frequency (cm^{-1})
A	Asymmetric stretching	1545
		1450
	Asymmetric scroll	880
B	Asymmetric tension	1465
		1412
	Asymmetric bending	872

positions presented in the crystal structure were accompanied by an increased presence of acid calcium phosphate (CHPO42H2O). Subtle changes in the phosphate region of 1 200 - 900 cm^{-1} could be traced to the second derivative of the spectrum in the healthy and carious tooth.

In the healthy enamel, peaks of absorption at 985, 1030, 1975, 1096, 1116, 1145 cm^{-1} were observed. The obtained absorption peaks at the waves number 1025, 1036, 110 cm^{-1} in carious teeth speaks for the presence and/or increased amount of acid calcium phosphate. The absorption peaks of 520-530 and 540-550 cm^{-1} are characteristic for acid phosphate. The study included 70 teeth, from which 20 healthy teeth, 30 teeth with caries and 20 teeth treated with xylitol.

The analysis of the specimens was conducted using FTIR (Fourier Transform Infrared Spectroscopy).[2] Speed and sensitivity of this method make it ideal for micro-analysis. According to literature data, FTIR uses interferometry to record information about material placed in the IR beam. The Fourier Transform results in spectra that analysts can use to identify or quantify the material. Analysis of the vibration spectrum can help to perceive the molecular structure of the mineral phases of the calcified tissue. Moreover, it allows demineralization and remineralization processes to be followed.

Results

The natural dental tissue remineralization process is very important for maintenance of the oral medium equilibrium. The possibility for reverting the lost minerals from the hydroxyapatite crystal bond during demineralization is responsible for achieving restitutio ad integrum. Our analysis showed that there are no significant changes in the mineral phases of the apatite structure. Therefore, xylitol has no significant direct influence on the natural remineralization process.

The carbonate ion is known to occupy two different positions in the hydroxylapatite structure of the enamel: position A (hydroxyl position) and position B (phosphate position). The hydroxyl position is more prevalent in enamel development. There is a proportionate dependence between the quantity and the position of the carbonates from one side and the solubility of the enamel from the other side. It is logical to assume that there is dependence between the quantity and the position of the carbonates and the progression of caries.

During demineralization and remineralization processes, the quantitative change and redistribution of the carbonates in the hydroxyl-apatite occur. The compositions of carbonates in healthy teeth and in the teeth subjected to xylitol action in biological saliva did not differ significantly. The mean values in healthy teeth were 0, 26 ± 0, 02 for carbonate type A, and 1,95 ± 0,01 for carbonate type B, while after xylitol application 0,24 ± 0,03 for type A and 1,93 ± 0,01 for type B (**Table 2**).

Table 2. Contain of carbonate in dental enamel

Carbonate type	Healthy teeth (20) Mean ± SD	Carious teeth (30) Mean ± SD	Xylitol treating teeth (20) Mean ± SD
A	0,26 ± 0,02	0,21 ± 0,01	0,24 ± 0,03
B	1,95 ± 0,01	1,71 ± 0,01	1,93 ± 0,01

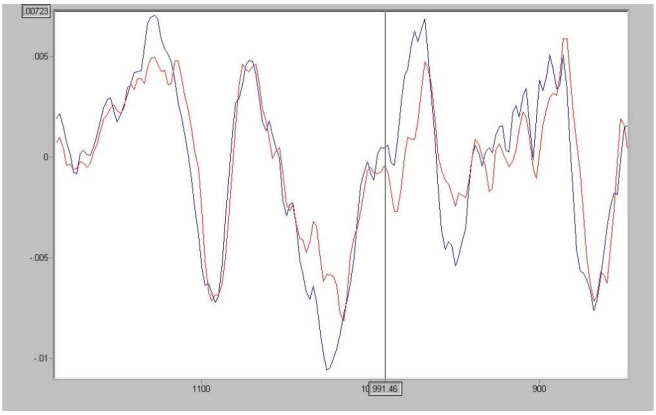
A change in redistribution of carbonates in the crystal structure of the apatite in caries samples (white spots lesions) could be expected as a consequence of demineralization. Redistribution can be observed by FTIR spectroscopy of the enamel in healthy and carious teeth. The changes in the carbonate positions present in the crystal structure were accompanied by an increased presence of acid calcium phosphate ($CHPO_4 2H_2O$). Subtle changes in the phosphate region of 1200-900 cm^{-1} could be traced in the second derivative of the spectrum in both healthy and carious tooth.

In healthy enamel, peaks of absorption were observed at 985, 1030, 1975, 1096, 1116, 1145 cm^{-1}. The obtained absorption peaks at the waves number 1025, 1036, 1100 cm^{-1} in carious teeth speaks for the presence and/or increased amount of acid calcium phosphate.

The absorption peaks by 520, 530, и 540 и 550 cm^{-1} are specific for acid phosphate and FTIR peaks for healthy teeth are shown in **Fig. 1**.

Healthy teeth treatment in biological saliva with xylitol did not show significant changes compared to the primary situation. The changes of the carbonate composition of the teeth enamel did not show significant changes of tested parameters compared to healthy teeth too.

Figure 1. FTIR specters of healthy and xylitol treating teeth.



Discussion

The results from the studies conducted by Soderling [3] and Makinen [4] are important to be pointed out. They advocate the opinion that the xylitol plays a role of a mediator in maintaining the natural balance in the oral medium with its non-acidic and non-fermentable characteristics as one of the most favorable arguments. These findings are completely in accordance with ours. According to Bar [5], the anti-caries effect of xylitol can be attributed to the mechanism of its action, where the xylitol interferes with the enamel demineralization. It enables remineralization growth, resulting in formation of a calcium complex. It is hypothesized that this complex leads to a decrease in the transport of dissolved apatite out of the demineralized side as a barrier, with this favoring remineralization. Xylitol, consumed through chewing gums, can lead to increased remineralization because of its effect on mastication and increased salivary flow. Salivary flow increases because of the simulative cooling effect of xylitol. [6,7]

In this context, it is very important to mention the finding of Moss [8] in his critical review of the xylitol effect to remineralization. He estimates that salivary stimulation, combined with its concomitant effect on the buffer capacity, pH values and the concentration of Ca and phosphates, improves the remineralization effects on the initial caries lesions (caries incipient). Xylitol molecules can form complexes with Ca because of the hydrophilic system presence into the molecule. Ca^{2+} is primarily accepted by the saliva, suggesting that xylitol can stabilize the CaP dissolution in saliva.

The recent findings of Shen [9] from their study conducted in vitro, showed that polyols in a physiological concentration did not improve the remineralization of the enamel surface lesion by facilitating of the Ca intake in the lesion.

The positive results from the previous studies related to the bacteriological response, i.e. *S. mutans* plaque colonization with a drastic decline in habitual xylitol use, favor the caries protective effect of this polyol. [8, 10, 11] This is true particularly because of the fact that the oral medium secures quality natural remineralization, especially because it is necessary to secure its "conditioning" with commensal, non-cariogenic bacterial flora, an increased salivary flow and normal pH values both at salivary and plaque levels. The xylitol allows these aspects to be secured at maximum. We cannot ignore the efficient oral hygiene and controlled intake of carbohydrates - segments which are individually dependent.

In the context of our standpoint, which is conclusive and clear, an effective natural remineralization of the dental tissue may occur only in optimal conditions in the oral medium and in absence of frequent demineralization and removal of caries causes.

The latest study of Cardoso [12], related to the xylitol remineralization potential suggests that the 20% xylitol varnish for topical use may be a promising alternative to fluorides for increasing of remineralization on the surface and undersurface zone of an artificial carious lesion in situ. Nevertheless, further research is needed to confirm this standpoint.

The findings by Souza [13] who analyzed the effects of 10% and 20% xylitol varnish solutions on enamel erosive changes are also interesting. They noted a significant reduction in the enamel erosions. However, a 10% xylitol solution produced a soft coating on the eroded enamel surface and reduced the erosion. This way, xylitol is indicated as a viable solution for the partial reduction in the enamel erosion.

Our standpoint on these essential questions is in accordance with Makinen [4] and Soderling [14] standpoints. They state that the enriched remineralization observed after habitual xylitol consumption is a part of multiple effects such as a considerably decrease of the *S. mutans* colonization on the salivary and plaque levels, stimulation of the salivary secretion, maintenance of normal pH values, and its non-acidic and non-fermentable characteristics.

In this context, we point out that the use of xylitol as an active agent in the preventive programs may be compared to the programs for immunization of the children for polio, smallpox and tetanus. [4]

If we note the facts which support oral cavity "conditioning" with xylitol and the better conditions that secure effective natural remineralization, even indirectly, the thesis gains its confirmation.

Our findings for the possible remineralization potentials are derived from the results obtained by the FTIR spectroscopy being appropriate for evaluation of the remineralization level after the treatment with xylitol. It is also important to note that the comparison of the spectra in our study was performed with hydroxylapatite (type A and B).

Carbonate apatite constitutes the inorganic component of the enamel, dentine and bone having a concentration of carbonate of several mass percent. The incorporation of carbonate into the apatite grid affects the structure and morphology of the apatite and the biological reactivity. Dental enamel represents a crystal structure of various minerals but the base is composed of the complex mineral calcium, phosphates and carbonates. The initial demineralization of dental caries takes place under the surface of the enamel, during the first few hours, in various speed. Later on it continues with a constant velocity of dissolution which is in direct dependence on the internal structure of the enamel. The loss of minerals is primarily in

direct dependence and controlled through the processes occurring inside the enamel areas where the dissolution begins. The transport of acids and products of dissolution through the enamel is also of a great importance.

The incorporation of carbonates in the enamel-mineral stream makes it susceptible to acidic solution. The loss of carbonate from the apatite in the carious enamel represents the most significant change in the chemical composition. However, this loss and its relation to the crystalline structure of the apatite have not been fully clarified. Thus, the unchanged structure of the crystalline apatite aperture is not a proof for chemical changes absence.

Studies have shown that a large number of ions can be removed from the hydroxylapatite crystal without altering its structural integrity. Such an enamel is able to transmit the sense of warm, cold, pain and pressure more easily than normal.

Due to the complexity of the problem and the attempts to obtain a precise image of all changes occuring during the dissolution of the enamel in the caries development, it is useful to use a number of methods, both qualitative and quantitative. Also, the possibility of structural characterization of the examined material (diffraction with X rays, IR spectroscopy, Atomic force microscopy etc.) should be taken into consideration.

Conclusion

From the results of the study it can be concluded that xylitol indirectly and positively influences the natural remineralization process by conditioning the oral medium with a non-pathogenic macrobiotic flora, increasing salivary secretion and maintaining the normal pH values at plaque and salivary levels. The habitual use of xylitol in different forms may significantly contribute to effective caries prevention.

Caries protective effects of xylitol and the subtle mechanisms of its action should be subject to further research.

References

1. Sarakinova O, Carcev M, Getova B, Carceva Salja S, Janevska S. National strategy for prevention of oral diseases in children from 0 to 14 years old age in the Republic of Macedonia for the period 2008-2018. Prilozi Makedon. Acad Nauk Umetn, Odd Med Nauki 2013; 34(2):129-34.
2. Bohic S, Heyman D, Pouezat JA, Gauthier O, Daculsi G. Transmission FTIR microspectroscopy of mineral phases in calcified tissues. Comp Ren Acad Sci 1998; 321(10): 865-876.
3. Soderling E, Talonpoika J, Makinen KK. Effects of xylitol containing carbohydrate mixtures on acid and ammonia production in suspension of salivary sediment. Caries Res 1997; 21:109-116.
4. Makinen KK, Makinen PL, Pape HR, Peldyak J, Hujoel P, Isotupa K, Soderling E, Isokangas PJ, Allen P, Bennett C. Conclusion and review of the Michigan Xylitol Programme (1986-1995) for the prevention of dental caries. International Dental Journal 1996; 46(1):22-34.
5. Bar A. Caries prevention with xylitol, a review of the scientific evidence. World Rev Nutr Diet 1988; 55:183-209.
6. Edgar WM, O'Mullane DM. Saliva and oral health. British Dental Association 1996; pp 140.
7. Manning RH, Edgar WM, Agalamanyi EA. Effects of chewing gums sweetened with sorbitol or a sorbitol/xylitol mixture on the remineralisation of human enamel lesions in situ. Caries Res 1992; 26:104-9.
8. Moss SJ. Xylitol-an evaluation. Int Dent J 1999; 49:00-00.
9. Shen P, Walker GD, Yuan Y, Reynolds C, Reynolds E. Polyols and remineralisation of enamel surface lesions. Journal of Dentistry 2017; 66:71-75.
10. Roberts M, Riedy C, Coldwell S, Nagahama S, Judge K, Lam M, Kaakko T, Castillo J, Milgrom P. How xylitol-containing products affect cariogenic bacteria. JADA 2002; 133(4):435-440.
11. Rolla G, Oppremann RV, Waaler SM, Assev S. Effect of aques solutions of sorbitol/xylitol on plaque metabolism and on growth of Str.mutans. Scand Dent Res 1981; 89:249-50.
12. Cardoso CA, Cassiano LP, Costa EN, Souza-E-Silva CM, Magalhaes AC, Gizzo LT, Caldana ML, Bastos JR, Buzalaf MA. Effect of xylitol varnishes on REM of artificial enamel caries lesions in situ. J Dent 2016; 50:74-78.
13. Souza JG, Rochell ID, Pereira AF, Silva TC, Rios D, Machado MA, Buzalaf MA, Magalhaes AC. Effects of experimental xylitol varnishes and solutions on bovine enamel erosion in vitro. J Oral Sci 2010; 52(4):553-9.
14. Soderling E, Trahan L, Tamniala-Salonem T., Hakkinen L. Effects of xylitol/sorbitol and placebo chewing gums on the plaque of habitual xylitol consumers. Eur J Oral Sci 1997; 105:170-177.