Effect of white tea and xylitol on structure and properties of demineralized enamel and jawbone

EI Auerkari1, R Kiranahayu1, D Emerita1, P Sumariningsih1, D Sarita1, MS Adiwirya1, AW Suhartono1

1Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta
2Department of Prosthodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta
3Center for Ageing Studies, Universitas Indonesia, Jakarta, Indonesia
4Department of Genetics, University of Leicester, Jakarta, Indonesia

Jl Salemba Raya 4, DKI Jakarta, 10430, Indonesia

*Email: eiauerkari@yahoo.com

Abstract. White tea and xylitol have been suggested as potential agents to combat dental caries and osteoporosis through enhanced remineralization. This investigation aimed to determine the effects of exposure to white tea with and without xylitol on the structure, composition and hardness of demineralized human dental enamel. For control, samples of untreated and demineralized enamel and samples of untreated rat jawbone were subjected to similar measurements. For demineralization, the enamel samples were immersed for two days at 50°C in an acetate solution (pH 4.0). All samples were then soaked for two weeks at 37°C in a solution containing three different concentrations of white tea, xylitol or both, and an optional addition of the remineralization ingredients including Ca, P and F. For enamel samples without preceding demineralization and without added remineralization ingredients, the results showed highest mean hardness after immersion in a solution containing both white tea and xylitol, practically independently of their applied concentration level. However, for demineralized enamel samples with added remineralization ingredients, the resulting mean hardness was also dependent on concentration of white tea and xylitol. With sufficient concentration, hardness was again higher for combined white tea and xylitol than for either of these used alone.

Keywords: dental enamel, jawbone, white tea, xylitol, hardness

1. Introduction

In Indonesia, about 70% of the teenage and adult population suffers from dental caries, which remains one of the most common infective diseases in humans [1]. The principal cariogenic bacteria are the mutans Streptococci that promote caries damage by producing lactic acid and extracellular polysaccharides of the plaque [2-5]. The resulting decay of the enamel and dentin occurs by tooth demineralization by acids from bacterial by-products. On the other hand, demineralization of bone or osteoporosis is a condition that is increasingly affecting elderly people and those with unfavourable lifestyle. Although caries and osteoporosis emerge through different mechanisms, effective prevention or repair would in both cases require a remineralization process for balance [6-8].

Xylitol is a five-carbon non-toxic sugar alcohol sweetener that is resistant to fermentation by the caries-inducing mutans Streptococci of the plaque [2,4]. Sufficient oral exposure to xylitol can reduce the acidogenic bacterial activity and plaque formation on dental surfaces [6-10]. It has also been suggested that by forming complexes with calcium and phosphate ions to prevent general calcium...
phosphate precipitation, xylitol can facilitate transport of the essential ions for remineralization of demineralized enamel [7,8]. The multifunctional effects of xylitol against caries (Fig 1) are mostly acknowledged at present. However, the relative importance the contributing factors is not well established due to combined involvement of physical and biological mechanisms.

Figure 1. Principal remineralization mechanisms in exposure to xylitol

White tea is the least processed of tea, and consists of highest fraction of young buds that give the freshly picked tea a silvery or whitish colour. Of all qualities of tea, white tea is said to contain the highest content of cathekins (catechins) and flavonoids that have been suggested as the active ingredients in the observed or claimed beneficial effects of consuming tea. As an example of such benefits, tea drinking has been reported to be related with significant improvement in bone density [11]. This investigation aimed to determine in vitro the effects of white tea and xylitol on the surface structure, composition and hardness of human dental enamel and rat jawbone. The study was designed to look at the potential for independent remineralization in absence of contributing biological mechanisms.

2. Materials and Methods

White tea base solution was prepared by mixing 150 g of white tea to 1500 cc of distilled water and heating the mixture for 15 min in a water bath at 90°C. This tea was further concentrated by steaming to a volume of about 120 cc. The resulting solution is called below 100% white tea, of which 1:2 (50%) and 1:4 (25%) diluted solutions by volume were also prepared. The xylitol base solution (called 100% solution below) was made by mixing 10 g xylitol powder (from PT Lotte Indonesia) and 10 ml distilled water until homogeneous. Of this base solution, 1:2 (50%) and 1:4 (25%) diluted xylitol solutions (by volume) were prepared by adding distilled water. For combined white tea and xylitol treatment, equal amounts of the above solutions were mixed to 100%-100%, 50%-50% and 25%-25% mixtures.

In total, 20 human enamel blocks were obtained by sectioning 11 teeth that had been extracted due to orthodontic treatment of 20-40 years old dental patients. For demineralization, nine sample blocks were immersed at 50°C for 2 days in 0.01M acetate buffer solution (pH 4.0) that was replaced daily, and then washed with distilled water and dried in air. No demineralizing treatment was applied to 11 enamel block samples. An aqueous remineralizing solution (without xylitol) was prepared with 0.1M CaCl₂, 0.01M KH₂PO₄, 0.005M NaF and 2.9M NaCl, adjusted to pH of 7.3 by adding 50 mM KOH to a volume of 100 ml.

One human enamel sample without demineralization was immersed into each of the aqueous test solutions with 100%, 50% and 25% white tea and/or xylitol (in total 9 samples) at 37°C for two weeks. For control, one sample was immersed in distilled water. In addition, similar immersion solutions and periods were applied for rat jawbone samples. In parallel experiments with added remineralization solution, one human enamel sample after demineralization was immersed into each of the aqueous test solutions as above (9 samples) but diluted to half by volume through the added remineralizing solution. For control, one sample was similarly immersed in the remineralization solution only. Again, parallel immersion solutions and periods were applied for rat jawbone samples. The remineralizing solution was replaced weekly.
After immersion treatments, the blocks were washed with distilled water, dried in air and the enamel subjected to electron diffraction X-ray (EDX) and X-ray diffraction (XRD) analysis, enamel surface inspection in scanning electron microscopy (SEM, in CMPFA laboratory of UI), and Vickers microhardness (HV0.05) testing. The test locations were selected to avoid regions with initially poor or lacking enamel. Differences in observations between the test groups were statistically analyzed using t-testing.

3. Results
The observed microhardness (HV0.05) of the enamel block samples immersed to the solutions containing white tea, xylitol, and both are shown in Fig 2a and in Table 1. Here for each type of immersion solution, the results from three different concentration levels (25, 50 and 100%) are combined together, as the scatter according to concentration was much smaller than that related to type of solution. The control group results are those from immersion in distilled water. The corresponding results for enamel samples after demineralization and remineralization treatments, and for rat jawbone samples without demineralization treatment are shown in Tables 2 and 3 (and Figs 2b and 3a), respectively.

The results for the enamel samples with demineralization and treatment with added remineralization solution showed high scatter and no significant difference between the treatment groups when grouped as shown in Fig 2b. This is apparently because now the results also depend strongly on the concentration of white tea and xylitol (Fig 3b). The highest mean hardness is associated with the highest (100%) concentrations of white tea and xylitol, and the lowest hardness with the 25% immersion solutions.

Fig 2. Enamel microhardness (mean and SD) after immersion in white tea (WT), xylitol (XYL) and combined (WT+XYL) solutions, a) combined groups without preceding demineralization; b) with demineralization and added remineralization ingredients; CG = control group

Table 1. Enamel microhardness after immersion in white tea (WT), xylitol (XYL) and combined (WT+XYL) solutions, without preceding demineralization; CG = control group, immersion in distilled water

| Group      | 100%  | 50%   | 25%   | All %  |
|------------|-------|-------|-------|--------|
| CG (all)   | -     | -     | -     | 287 ± 61 |
| WT         | 150 ± 29 | 105 ± 6 | 149 ± 8 | 135 ± 27 |
| XYL        | 349 ± 36 | 390 ± 25 | 361 ± 19 | 367 ± 30 |
| WT+XYL     | 565 ± 77 | 367 ± 42 | 502 ± 59 | 509 ± 92 |
Table 2. Enamel microhardness after immersion of all test groups with preceding demineralization; abbreviations as in Table 1

| Group     | Microhardness (HV0.05, mean ± SD), VHN |
|-----------|----------------------------------------|
|           | 100%        | 50%         | 25%         | All %       |
| CG (all)  | -           | -           | -           | 341 ± 5    |
| WT        | 495 ± 117   | 146 ± 13    | 222 ± 10    | 288 ± 170  |
| XYL       | 349 ± 46    | 163 ± 9     | 142 ± 13    | 218 ± 102  |
| WT+XYL    | 466 ± 18    | 403 ± 87    | 163 ± 21    | 344 ± 146  |

Table 3. Rat jawbone microhardness after immersion of all test groups without preceding demineralization; abbreviations as in Table 1

| Group     | Microhardness (HV0.05, mean ± SD), VHN |
|-----------|----------------------------------------|
|           | 100%        | 50%         | 25%         | All %       |
| CG (all)  | -           | -           | -           | 21.9 ± 2.2  |
| WT        | 24.0 ± 2.9  | 25.4 ± 3.7  | 24.0 ± 4.0  | 24.5 ± 3.2  |
| XYL       | 23.5 ± 1.4  | 30.4 ± 3.0  | 22.3 ± 6.0  | 25.4 ± 5.1  |
| WT+XYL    | 19.3 ± 1.2  | 20.8 ± 4.5  | 24.3 ± 1.7  | 21.0 ± 3.3  |

**Fig 3.** Microhardness of a) the rat jawbone samples after remineralization treatment; and b) demineralized enamel samples grouped according to concentration of the remineralization solution

Nevertheless, there is still considerable scatter in Fig 3b, and both concentrations and solution types need to be considered. Fig 6 shows the microhardness after immersion in the different 25% solutions. Fig 7 presents the corresponding results for the 50% solutions. Here the immersion solutions containing 50% white tea or 50% xylitol only were again insufficient to increase hardness to the initial (control) level, unlike combined white tea and xylitol. Fig 8 shows the results for the enamel samples immersed in the 100% remineralizing solutions after demineralization. In this case all immersion solutions, including white tea, xylitol and both combined, were sufficient to repair the hardness at least to the control level.

In the case of rat jawbone samples (Table 3 and Fig 4), no significant differences were observed between samples grouped as in Fig 2. As expected, the absolute hardness levels for bone are much
lower than those for dental enamel. However, also the relative scatter was much less than for demineralized and remineralized enamel samples, and no regrouping was found to indicate significant differences between groups. It therefore seems that none of the applied treatments showed benefits in terms of hardness (strength) for the bone samples.

The enamel sample surfaces show even after the white tea and xylitol treatments variable damage as pores and pits with a typical size up to about 6 µm, and also as cracks (Figs 9-12).

![Graph](image1.png)  ![Graph](image2.png)

**Fig 4.** Microhardness of demineralized enamel samples immersed in a) 25% and b) 50% remineralization solutions

![Image](image3.png)

**Fig 5.** a) Microhardness of demineralized enamel immersed in 100% remineralization solutions; b) surface appearance of an untreated enamel sample (control)

![Image](image4.png)

**Fig 6.** Surface appearance of demineralized enamel after exposure to remineralization solution with a) 100% and b) 25% white tea solution
Fig 7. Surface appearance of demineralized enamel after exposure to remineralization solution with a) 100% and b) 25% xylitol

Fig 8. Surface appearance of demineralized enamel after exposure to remineralization solution with a) 100% and b) 25% white tea + xylitol

4. Discussion
For enamel samples without preceding demineralization and without added remineralization ingredients (Fig 2), exposure to white tea appears to be of no particular benefit regardless of concentration. The effect of xylitol also appears to be practically independent of concentration. However, although considerably higher hardness is indicated than for samples immersed in white tea, the xylitol-exposed samples do not show significantly different hardness from the control samples. A significantly higher mean hardness was observed from samples immersed in solutions containing both white tea and xylitol. Hence although white tea alone reduced mean hardness from the level of control samples, combined white tea and xylitol clearly increased hardness.

For demineralized enamel samples with added remineralization ingredients, the resulting mean hardness was dependent on concentration of white tea and xylitol (Figs 6-8), and scatter is generally much reduced when compared to that in Fig 5. For 25% concentration level (Fig 6), hardness remains well below that of the control samples, implying that the remineralization treatment was insufficient to repair the demineralization damage. For 50% concentration, the same applies for treatment with white tea or xylitol solutions alone, but combined white tea and xylitol solution repaired the hardness level to at least the same level as in the control samples (Fig 7). In case of 100% concentration (Fig 8), all white tea, xylitol and combined solutions repaired the enamel hardness at least to the same level as in the control samples. However, in case of combined white tea and xylitol, the increase in hardness was not as much as in Fig 2, i.e. without demineralization and without added ingredients such as Ca and P in the solutions. For xylitol alone, the hardness results shown in Fig 2 (all concentrations) and in Fig 8 (100% only) do not significantly differ from each other or from the
corresponding control levels. For white alone, hardness levels in Fig 2 are very much lower than control, while in Fig 8 white tea alone was sufficient for the required improvement. The tooth block samples were apparently somewhat variable in terms of the enamel surface condition already in the initial state.

Through enhanced remineralization, white tea and xylitol have been suggested to be useful in in combating dental caries and osteoporosis. The present study aimed to assess the impact of exposure to white tea with and without xylitol on the structure, composition and hardness of demineralized human dental enamel. Similar measurements were used for the control samples of untreated and demineralized enamel and untreated rat jawbone. Demineralization treatment was conducted by immersing the enamel samples for two days at 50°C in an acetate solution with pH of 4.0. Then all samples were soaked for two weeks at 37°C in a solution containing different concentrations of white tea, xylitol or both, and optional remineralization additives including Ca, P and F.

When the enamel samples were not subjected to preceding demineralization and remineralization additives, highest hardness resulted from an immersion in a solution containing both white tea and xylitol, regardless of their concentration. However, for demineralized enamel samples with remineralization additives, the resulting hardness also depended on the concentration of white tea and xylitol. Higher hardness was reached with combined white tea and xylitol than with either white tea or xylitol alone, provided that the concentration was sufficiently high.

5. Conclusion
In conclusion, enamel samples not subjected to preceding demineralization and remineralization additives showed highest hardness after immersing the samples in a solution containing both white tea and xylitol, regardless of their concentration. However, for demineralized enamel samples with remineralization additives, the resulting hardness also depended on the concentration of white tea and xylitol. Higher hardness was reached with combined white tea and xylitol than with either white tea or xylitol alone, when the concentration was sufficiently high.

References
[1] Maharani DA, Adiatman M, Rahardjo A, Burnside G and Pine C 2017 An assessment of the impacts of child oral health in Indonesia and associations with self-esteem, school performance and perceived employability BMC Oral Health 17 65
[2] Söderling E and Scheinin A 1991 Perspectives on xylitol-induced oral effects Proc. Finn. Dent. Soc. 87 217-29
[3] Forssten SD, Björklund M and Ouwehand AC 2010 Streptococcus mutans, caries and simulation models Nutrients 2 290-8
[4] Trahan L 1995 Xylitol: a review of its action on mutans streptococci and dental plaque - its significance Int. Dent. J. 48 87-92
[5] Ibrahim-Auerkari E, Soufyan A, Alkatiri F, Verisqa F, Megantoro A, Sumawinata N and Mangundjaja S 2010 Effect of xylitol on remineralization of demineralized dental enamel Int. J. Clin. Prev. Dent. 6 73-7
[6] Auerkari E I and Auerkari P 1997 Caries control by using xylitol as a dietary sugar substitute J. Dent. Indonesia 4 633-40
[7] Sano H, Nakashima S, Songpaisan Y and Phantomvanit P 2007 Effect of a xylitol and fluoride containing toothpaste on the remineralization of human enamel in vitro J. Oral Sci. 49 67-73
[8] Miake Y, Saeki Y, Takahashi M and Yanagisawa T 2003 Remineralization effects of xylitol on demineralized enamel J. Electron Microsc. 52 471-6
[9] Vissink A, s-Gravenmade E J, Gelhard T B F M, Panders A K and Franken M H 1985 Rehardening properties of mucin- or CMC-containing saliva substitutes on softened human enamel Caries Res. 19 212-8
[10] McIntyre J M 2005 Dental caries – the major cause of tooth damages *Preservation and Restoration of Tooth Structure*, 2nd ed ed G J Mount and W R Mount (Queensland: Knowledge Books and Software) pp 21-33

[11] Devine E, Hodgson JM, Dick IM and Prince RL 2007 Tea drinking is associated with benefits on bone density in older women *Am. J. Clin. Nutr.* 86 1243-7