ORIGINAL RESEARCH

Invitro Evaluation of the Inhibitory Effect of Probiotic Enriched and Traditional Yogurt Extracts on Dental Enamel Demineralization- Comparative Study

Chanchal Singh[1], Sulekha Doley[2]

1-Professor &Head .Department of Pedodontics &Preventive Dentistry, K.D Dental & Hospital, Mathura, U.P. 2-Post Graduate Student, Department of Pedodontics & Preventive Dentistry, K.D Dental College & Hospital, Mathura, U.P.

Correspondence to:
Dr. Dr.Sulekha Doley, Post Graduate Student, Department of Pedodontics &Preventive Dentistry, K.D Dental College & Hospital, Mathura, U.P.
Contact Us: www.ijohmr.com

ABSTRACT

Background: Dairy products were recognised in the late 1950s as food group exhibiting most anticaries activities. Yogurt, a dairy product, almost as commonly consumed as milk has been subjected to less research in the context of its effect on oral health, especially on dental enamel. **AIM:** To compare invitro the effectiveness of probiotic enriched yogurt extract and traditional yogurt extract in inhibiting dental enamel demineralization and promoting its remineralisation. **Materials& methodology:** Sixty intact caries free human premolars were selected and divided into three groups comprising 20 teeth in each group. Invitro demineralization procedures were performed at pH 4.8 on these three groups of teeth but with the addition of probiotic enriched yogurt extracts and traditional yogurt extracts on group1 and group2 respectively. The effects of these procedure were evaluated quantitatively by Atomic absorption spectrophotometer, (AAS) and qualitatively by confocal laser scanning microscopy, (CLSM). Statistical analysis of the results was performed using one way ANOVA. **Results**: One way Anova showed statistically significant difference between the three groups .CLSM and AAS analysis demonstrated more inhibitory effect of the traditional yogurt group compared to the probiotic enriched group. **Conclusion:** Traditional yogurt has more inhibitory effect than probiotic enriched yogurt on dental enamel demineralization.

KEYWORDS: CPP-ACP, Demineralization, Probiotics, Remineralization, Yogurt

INTRODUCTION

Dental caries is an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissues. However dental caries can be prevented, intervened and reversed by either reducing the pathological factors that lead to demineralization or by enhancing the protective factors that lead to remineralisation. Dairy products were recognised in the late 1950s as food group exhibiting most anticaries activities.[1]The cariostatic effect of milk and milk products has been attributed to their high content of Ca and P ions, the buffering capacity, and the presence of casein phosphopeptides (CPPs).[2] The CPPs are tryptic digestion products of casein, a bovine milk protein[3]. In yogurt, it is produced partly by the proteolytic activity of the Lactic Acid Bacteria (LAB) present in the yogurt. [4]

The CPPs have a remarkable ability to stabilize calcium and phosphate, preserving them in a soluble form as amorphous calcium phosphate (ACP).[5]The ACPs being highly soluble are readily converted to hydroxyapatite making them a suitable remineralising agent .[6] The CPP-ACPcomplex bind to the surface of the teeth as well to dental plaque and deposits high concentration of ACP in close proximity to the tooth surface. This localized CPP-ACP buffers the free calcium and phosphate ions under acidic condition, substantially increasing the level of calcium phosphate in plaque and thereby maintain a state of supersaturation that inhibits enamel demineralization and enhance remineralisation .[7,8,9] A human in situ caries study model demonstrated that two exposures of CPP-ACP solution per day to one side of enamel slabs produced 51±19% reduction in enamel mineral loss caused by frequent sugar solution exposure , compared to control side .[9]The remineralising potential of CPP-ACP has also been proved to be more effective than 500 ppm of NaF.[10]

Yogurt, a dairy product, almost as commonly consumed as milk and exhibiting higher natural CPPs content than that in milk[11] has been subjected to less research in the context of its effect on oral health, especially on dental enamel. Although studies have demonstrated the protective effect of probiotic enriched yogurt(due to high CPPs content) on dental enamel demineralization,[12,13] no studies till now have been conducted comparing the inhibitory effect of probiotic enriched yogurt extract and traditional(non probiotic) yogurt extract on dental enamel demineralization. The aim of the present study is to test

How to cite this article:
Singh C, Doley S. Invitro Evaluation of the Inhibitory Effect of Probiotic Enriched and Traditional Yogurt Extracts on Dental Enamel Demineralization-Comparative Study. Int J Oral Health Med Res 2016;3(1):31-35.

in vitro the effectiveness of probiotic enriched yogurt extract and traditional yogurt(non probiotic enriched) extract in preventing dental enamel demineralization. and promoting its remineralization.

MATERIALS AND METHODS

Preparation of the enamel specime: Sixty intact caries free human premolars extracted for orthodontic purpose were selected. The crowns were polished with pumice dust non –fluoride toothpaste .They were then rinsed in distilled water. The roots were cut at the level of CEJ by a diamond disk mounted on a hand piece. Pulp tissue, if present, was removed using a spoon excavator and pulp chambers were sealed using cyanoacrylate glue. Standardized 5x3mm² enamel windows were isolated on the buccal and lingual surface of each tooth using acid resistant nail varnish.(Fig.1) The 60 specimens were divided into three different groups, which subsequently underwent three different chemical treatments . (table1)

Fig. 1 Preparation of the Enamel specimens

Groups and treatments			
Treatment group	Group 1	Group 2	Group 3
Sample number	1-20	21-40	41-60
Treatment solution	Solution 1	Solution 2	Solution 3
pH of the solution	4.8	4.8	4.8
Demineralising agent	Lactic acid (LA)	LA	LA
Protective agent	Probiotic yogurt supernatant (YS)	Non probiotic YS	None

Table 1. Groups and treatments

Demineralising System: A standardized demineralization procedure for dental enamel at a pH 4.8 was established. Demineralising solution was prepared to have the following composition: 6%wt carboxymethy lcelluose gel, 0.1 M lactic acid (LA) & 1M KOH solution for titration to the desired pH.

Preparation of yogurt extracts: Probiotic yogurt (Mother Dairy B-Active) and traditional non-probiotic enriched yogurts(Plain, Mother Dairy) made from toned milk (Mother Dairy, Pilkuwas Dairy, Ghaziabad-Haryana road) were subjected to centrifugation(in the Department of Biotech, GLA Univ. Mathura) at 4000 rpm at 25ºC. After three rounds of centrifugation at specific settings, the supernatant or the soluble fraction was collected. (Fig.2)

Fig .2 Preparation of yogurt extracts

Teeth from the three treatment groups were immersed in the respective treatment solutions for 96hrs at 37°C.(Fig.3)

Fig. 3 Incubation of the treatment groups for 96 hours.

Assessment methods: At the end of 96 hours all the specimens were rinsed, air dried and evaluated by quantitative and qualitative analysis. The aim of the quantitative analysis was to measure calcium content in treatment solutions where calcium content of each solution (both pretreatment and post-treatment) was determined using atomic absorption spectrophotometer (AAS) (Fig.4)

Fig. 4 Atomic Absorption Spectrophotometer (AAS)

Qualitative analysis involved Measurement of demineralized area (fluorescent area) under confocal laser scanning microscope. Ten samples from each group were randomly chosen and sectioned buccolingually through the treatment windows. 0.1mM of Rhodamine B solution was prepared by adding 23.95mg of rhodamine B dye to 500 ml of distilled water. The sectioned specimens were stored in 0.1mM rhodamine B for 24 hrs in order to stain them and then placed in distilled water for further use. The cross sectioned specimens were viewed using Leica tcs sp5 confocal microscope.

Intensities of fluorescent area (demineralised area) from the images of three treatment groups were Quantified with the help of LAS AF viewers software by Leica Microsystems, Germany.(Fig .5) The statistical analysis was done using one way Anova. P≤0.05 was considered statistically significant for all tests.

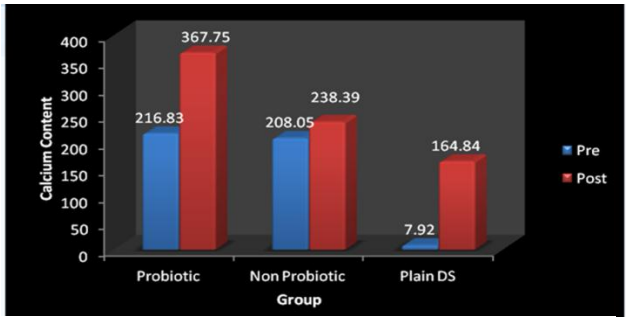
Fig. 4 Atomic Absorption Spectrophotometer (AAS)

MATERIALS AND METHODS

1)Calculation of calcium content through Atomic Absorption spectrophotometer (AAS).

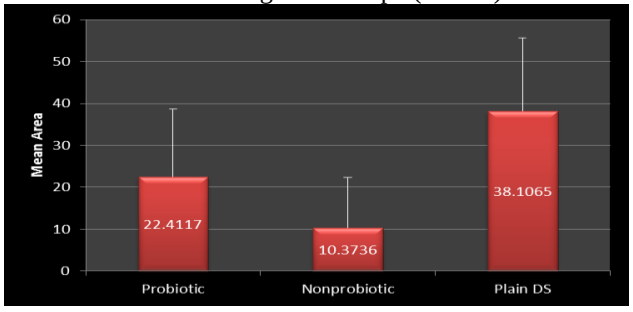
- The amount of calcium released into the solutions was calculated by subtracting the pre treatment from the post treatment calcium content of the three solutions.
- The difference was least for traditional Non-probiotic group and greatest for Plain Demineralising solution. (Graph1)

Quantitative analysis with AAS shows that artificial demineralization in the presence of natural protective factors from traditional non probiotic enriched yogurt supernatant provides significantly lower calcium loss than the probiotic enriched yogurt supernatant.



Graph 1. Calcium content of the treatment solutions

2)Calculation of Demineralised (fluorescent) area using Confocal Laser Scanning Microscope (CLSM).



Graph2. Mean of the demineralised (fluorescent) area in three treatment groups.

One way ANOVA showed highly significant difference between the three treatment groups. (table.2, graph 2 &Fig 6 a, b, c).

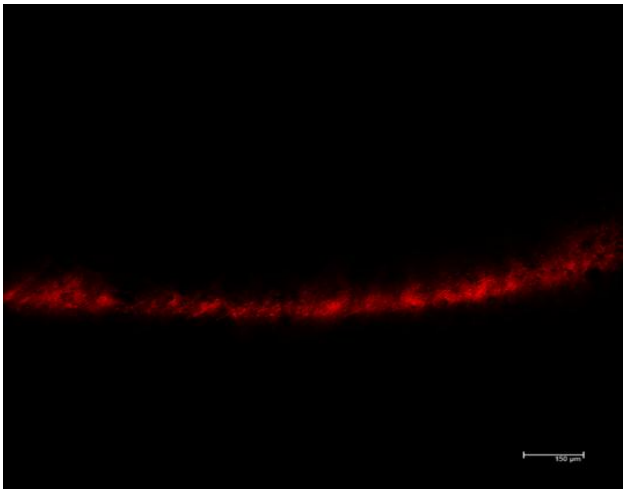


Fig .6a Representative confocal image for the Probiotic enriched Group.

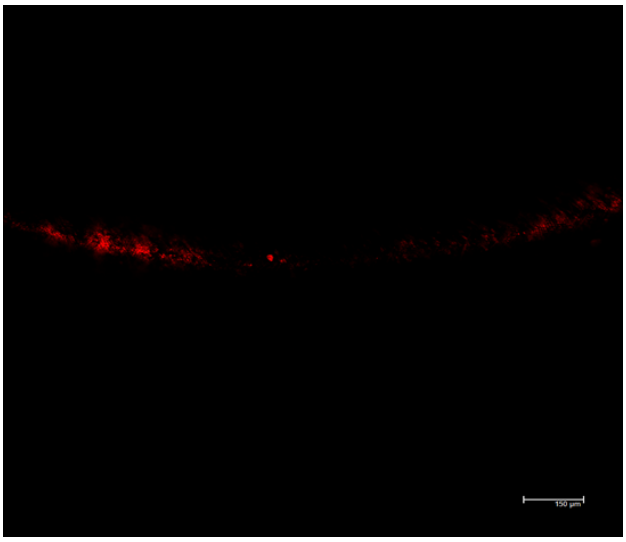


Fig .6b Representative confocal image for the Traditional Non probiotic Group

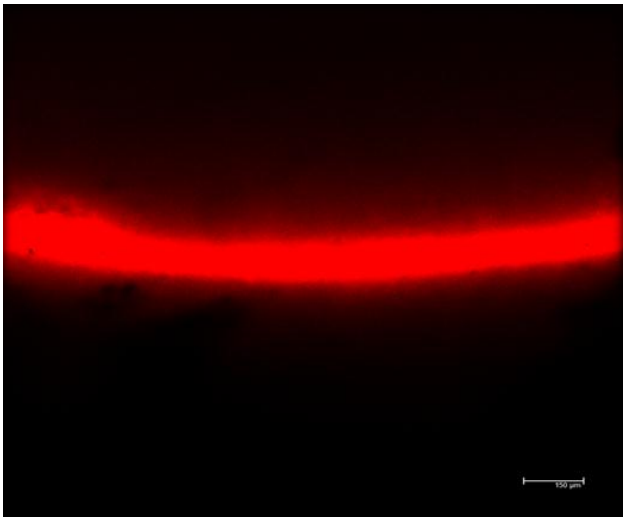


Fig .6c Representtive confocal image for thePlain demineralizing Group.

Confocal images from group1 (control) show substantial lesions where surface morphology looks fluorescent red. Comparatively group 1 and 2 shows lesser lesion area than group 3 .Also, the lesion area of group 2 was less compared to 1 and did not show much erosive lesion. When the images from the three groups were compared it was clear that CPP present in yogurt played a protective role and CPP from non probiotic enriched traditional yogurt appeared to be more protective .The quantitative and qualitative analysis of the results obtained through artificial demineralization ,in the presence of a dairy derived Remineralizing factor clearly demonstrates the protective effects of natural CPP'S on early dental enamel caries .The encouraging results obtained in group 2 could pave the way for development of preventive measures against dental caries with the use of simple traditional yogurt rather than the probiotic enriched yogurt that offer the advantage of being completely biocompatible ,easy to obtain ,no side effects and inexpensive.

DISCUSSION

Yogurt, one of the most widely distributed dairy products has been used in the present study. It is a coagulated milk product that results from the fermentation of milk by Lactobacillus bulgaricus and streptococcus thermophillus producing lactic acid.[14] Yogurt is also an excellent source of calcium and phosphorous and has a higher protein content.[12] Due to the proteolytic activity of microorganisms(LAB activity) contained in the yogurt the natural CPPs content in yogurt is higher than that in milk .[11] Moreover the fermentation process has little effect on the mineral content of the milk and therefore the total mineral content remain unaltered in the yogurt.[12] It is one of the readily available, inexpensive common dietary constituent of the Indian population that can be used in several different combinations acceptable to the paediatric age groups.

In recent years, there has been a lot of interest in the use of probiotics in maintaining good oral health and treating oral infections including dental caries and periodontal diseases. Probiotics have are live micro-organisms which confer a health benefit on the host when administered in adequate amounts.[15] For some decades now, bacteria known as probiotics have been added to various foods because of their beneficial effects . In our present study probiotic enriched yogurt has been used. Probiotic bacterial strains generally belong to the genera *Lactobacillus* and *Bifidobacterium.*[16] These bacterial genera are regarded as a part of the normal human microbiota. It has been shown lactobacilli and bifidobacterium may exert beneficial effects in the oral cavity by inhibiting cariogenic streptococci and candidal species.[17] There are studies demonstrating the enamel protective and anticaries effect of yogurt. A cross sectional study conducted among 2058 Japanese children aged 3 years provided a clear dose response relationship of yogurt intake and prevalence of dental caries.Yogurt intake more than four times per week (≥4 times/week) was significantly associated with lower prevalence of caries compared to those consumed less than equal to one time a week.(≤1 time/week).[18] Yogurt without any added sugar are found to be noncariogenic and to some extent cariostatic as they increase calcium and phosphorous concentration in dental plaque.[19] It has been demonstrated that CPP contained in yogurt have an inhibitory effect on dental enamel demineralization.[12] In another study it was shown that probiotic yogurt extract is effective in reducing demineralization of enamel under experimental conditions.[13]

There have been no documented studies comparing the inhibitory effect of probiotic enriched yogurt extract and traditional yogurt extract on dental enamel demineralization. In our the study probiotic enriched yogurt -Mother Dairy –B-active and for the traditional yogurt, Mother dairy yogurt was used. The probiotic enriched yogurt contained Lactobacillus acidophilus and Bifidobacteria as probiotic bacterial strain whereas traditional yogurt preparation Mother dairy contained Lactobacillus acidophilus and S.Thermophillus. The present demonstrated that supernatant of the two of types yogurts inhibited dental enamel demineralization and promoted its remineralisation which is in agreement with other studies showing the protective nature of yogurt on dental enamel demineralization.[12,13] However our results also demonstrated that the traditional yogurt extract had better inhibitory effect than the probiotic enriched yogurt extract. This can be attributed to the aciduric nature of the probiotic Bifidobacterium and the various studies demonstrating the cariogenic nature of this bacteria. A study showed a positive association of salivary Bifidobacteria level with the number of decayed and filled tooth surfaces.[20] In another study, it was reported that Salivary levels of Bifidobacteria caused significant caries experience in children, hence may be a useful marker of caries risk. [21] It has been indicated in a study that there was undetectable levels of S. mutans in 10% of subjects with rampant caries in permanent teeth, although Streptococcus mutans has been implicated as a major etiological agent of dental caries, Bacterial species other than S. mutans that are likely to play important roles in caries progression includes species of the genera Veillonella, Lactobacillus, Bifidobacterium, and Propionibacterium, low-pH non-S. mutans streptococci, Actinomyces spp., and Atopobium spp.[22]

Confocal laser scanning microscope was used to qualitatively assess this inhibitory effect.The results were reported as fluorescence units demonstrating the demineralized area and showed a statistically significant difference between the two groups and the control group. Microradiography is known to be the most accurate means of assessing demineralization/ remineralization . Quantitatively the inhibitory effect was assessed by the amount of calcium ion leached into the treatment solution with the Atomic absorption Spectrophotometer (AAS). The result demonstrated that the calcium ion leached was highest for the control group (plain demineralising solution) followed by probiotic enriched group and least

for the non probiotic enriched group .This shows that non probiotic enriched has better ability to prevent dental enamel demineralization.

It was reported in a study that probiotic yogurt has slightly more pH than natural /non probiotic yogurt.[14] It has also been demonstrated that lower pH of yogurt as compared to milk account for the favourable ion form of calcium.[23] Ionic form of calcium maintains a balance between the calcium of the tooth structure and the surrounding fluids. Decrease in salivary pH causes an increase in Ionic calcium concentration in the saliva.[24]Therefore, as the pH of non probiotic yogurt is lower than that of probiotic yogurt, ionic calcium concentration in nonprobiotic yogurt is more and hence the protective effect which is in accordance with the present study.

CONCLUSION

From the present study it can be concluded that traditional Non Probiotic yogurt has more protective effect than Probiotic enriched Yogurt and is more effective in preventing dental enamel demineralization under experimental conditions . This difference can be attributed to the aciduric nature of the probiotic Bifidobacterium and the various studies demonstrating the cariogenic nature of this bacteria.However demineralization and re mineralization invitro may be quite different when compared to a complex biological system like the oral cavity. Further studies are necessary to establish the invivo re mineralizing potential of both the types of yogurts .

REFERENCES

1. Aimutis W.R .Bioactive Properties of Milk Proteins with Particular Focus on Anticariogenesis .Journal of Nutr 2004;134:989S-995S.
2. McDougall WA. Effect of milk on enamel demineralization and remineralization in vitro. Caries Res.1977; 11:166–72.
3. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aust Dent J 2003;48:240–43.
4. Pinto G, Caira S, Cuollo M, Fierro O, Nicolai MA, Chianese L, Addeo F. Bioactive Casein Phosphopeptides in Dairy Products as Nutraceuticals for Functional Foods. Walter L. Hurley (ed).*Milk Protein*. Croatia : InTech; 2012. pp. 3-44.
5. Cochrane NJ,Saranathan S,Cai F,Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium ,phosphate and fluoride .Caries Res 2008;42: 88-97.
6. Tung MS. Eichmiller FC.Dental applications of amorphous calcium phosphates. J Clin Dent 1999; 10:1-6.
7. Rose RK. Binding characteristics of S.mutans for calcium and casein phosphopeptide .Caries Res 2000; 34:427-31.
8. Reynolds EC,Cain CJ,Webber FL, et al .Anticariogenecity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat .J Dent Res 1995;74:1272-79
9. Reynolds EC, Black CL, Cai F .Advances in Enamel remineralization : anticariogenic casein phophopeptide – amorphous calcium phosphate .J Clin Dent 1999 ;10:86-88.
10. ZhangQ, Zou J,Yang R ,Zhou X .Remineralization effects of casein phosphopeptide amorphous calcium phosphate crème on artificial early enamel lesions of primary teeth .Int J Paediatr Dent 2011;21:374-81.
11. Rasic´ JL, Kurmann JA. Bifidobacteria and their role. Microbiological, nutritional-physiological, medical and technological aspects and bibliography. Experientia Suppl 1983;39:1–95.
12. Ferrazzano GF, Cantile T, Quarto M, Ingenito A,Chianese L, AddeoF. Protective effect of yogurt extract on dental enamel demineralization in vitro.Australian Dental Journal 2008;53:314-19.
13. Varghese L, Varughese JM , Varghese NO .Inhibitory Effect of yogurt Extract on Dental Enamel Demineralisation –An invitro study .Oral health and Preventive Dent 2013;11:369-74.
14. Hussain I, Rahman A , Atkinson A . Quality Comparison of Probiotic and Natural Yogurt. Pakistan Journal of Nutrition 2009; 8 : 9-12.
15. Boyle RJ, Robins –Browne RM ,Tang ML. Probiotic use in clinical practice .What are the risks ? Am J Clin Nutr 2006;83:1256 – 64
16. Rugg-Gunn A, Woodward M. Milk and oral health . http://www.borrowfoundation.org/assets/uploads/milk-and-oral-health.pdf(acessed 20 september 2015).
17. Caglar E,Kargul B,Tanboga I.Bacteriotheraphy and Probiotics role on oral health .Oral diseases 2005;11:131-7.
18. Tanaka K et al .Intake of dairy products and the prevalence of dental caries in young children .JDent 2010;38:579-83.
19. Ravishankar TL, Yadav V, Tangade P.S, Tirth A, Chaitra T.R. Effect of consuming different dairy products on calcium ,phosphorous and pH levels of human dental plaque :A comparative study .European Archieves of Pediatric Dentistry 2012;13:144-148.
20. Beighton D, Al-Haboubi M, Mantzourani M, Gilbert SC, Clark D, Zoitopoulos L, Gallagher JE Oral Bifidobacteria: caries-associated bacteria in older adults. J Dent Res. 2010;89:970-74
21. Kaur R, Gilbert SC, Sheehy EC, Beighton D.Salivary levels of Bifidobacteria in caries free and caries active children . Int J Paediatr Dent.2013 ;23:32-8
22. Aas JA, Griffen AL , Dardis SR, et al .Bacteria of dental caries in primary and permanent teeth in children and young adults .Journal of clinical microbiology 2008 ;46:1407-17.
23. Adolfsson O,Meydani SN,Russell RM.Yogurt and gut function .Am J Clin Nutr 2004;2:245-56.
24. Liena C , Forner L , Baca P . Anticariogenecity of Casein Phosphopeptide –amorphous Calcium Phosphate :A review of the Literature . The Journal of Contemporary Dental Practice 2009 ;10:1-9.

Source of Support: Nil
Conflict of Interest: Nil