Effect of catfish (*Clarias batrachus*) bone paste application to changes in enamel surface roughness

Pengaruh aplikasi pasta tulang ikan lele (*Clarias batrachus*) terhadap perubahan kekasaran permukaan email

Juni Jekti Nugroho
Department of Dental Conservation
Faculty of Dentistry, Hasanuddin University
Makassar Indonesia
Corresponding author: **Juni Jekti Nugroho,** email: **jektijuni@yahoo.co.id**

ABSTRACT

Background: Demineralization is a condition where the tooth surface experiences mineral loss by releasing the bonds of calcium, hydroxyapatite and phosphate compounds thereby increasing the surface roughness of the enamel. Calcium and phosphate can help improve the remineralization process by rebuilding hydroxyapatite crystals. Catfish (*Clarias batrachus*) bones contain 5.68% calcium and 3.78% phosphate which potentially remineralize tooth enamel. **Objective**: To determine the effect of catfish bone paste application on enamel surface roughness. **Method**: This experimental laboratory research used 32 samples of maxillary first premolar which were divided into 2 groups. All samples were immersed in cola type carbonated drinks for 2 minutes. Samples in each group were treated (6 minutes and 30 minutes) with catfish bone paste application in group I and CPP-ACP application in group II. The roughness test was carried out using a surface roughness tester on the surface of the initial tooth enamel, after soaking it in a cola type carbonated drink, and after application of the remineralization material for 6 minutes and 30 minutes. **Results**: There was a decrease in enamel surface roughness after application of catfish bone paste and CPP-ACP paste. **Conclusion:** The application of catfish bone paste significantly decreased enamel surface roughness.
Keywords: Enamel surface roughness, Cola-type carbonated drinks, Catfish bone paste, CPP-ACP paste

ABSTRAK

Latar belakang: Demineralisasi adalah suatu kondisi permukaan gigi mengalami kehilangan mineral dengan melepaskan ikatan senyawa kalsium, hidroksiapatit dan fosfat sehingga meningkatkan kekasaran permukaan email. Kalsium dan fosfat dapat membantu meningkatkan proses remineralisasi dengan membangun kembali kristal hidroksiapatit. Tulang ikan lele (*Clarias batrachus*) mengandung 5,68% kalsium dan 3,78% fosfat yang berpotensi meremineralisasi email gigi. **Tujuan**: Mengetahui pengaruh aplikasi pasta tulang ikan lele terhadap kekasaran permukaan email. **Metode**: Penelitian laboratorium eksperimental ini menggunakan 32 sampel gigi premolar pertama rahang atas yang dibagi menjadi 2 kelompok. Semua sampel direndam dalam minuman berkarbonasi jenis cola selama 2 menit. Sampel pada masing-masing kelompok diberi perlakuan (6 menit dan 30 menit) dengan aplikasi pasta tulang lele pada kelompok I dan aplikasi CPP-ACP pada kelompok II. Uji kekasaran dilakukan dengan menggunakan *surface roughness tester* (µm) pada permukaan email gigi awal, setelah direndam dalam minuman berkarbonasi jenis cola, dan setelah aplikasi bahan remineralisasi selama 6 menit dan 30 menit. **Hasil**: Terjadi penurunan kekasaran permukaan email setelah aplikasi pasta tulang ikan lele dan pasta CPP-ACP. **Simpulan**: Aplikasi pasta tulang ikan lele dapat menurunkan kekasaran permukaan email secara signifikan.
Kata kunci: kekasaran permukaan email, minuman berkarbonasi tipe cola, pasta tulang ikan lele, pasta CPP-ACP
Received: 1 September 2020 Accepted:1 December 2020 Published: 1April 2021

INTRODUCTION

The tooth structure consists of enamel, dentin, pulp and cementum. Part of the enamel includes an enamel prism formed of hydroxyapatite crystals and rod sheath consisting of an organic fibrous substance. Enamel can undergo demineralization and remineralization processes.[1-3] The demineralization process is the dissolution of part and even all of the mineral ions from the hydroxyapatite crystals in tooth enamel, thereby increasing the surface roughness of the enamel.[4] The demineralization process can be in the form of erosion caused by the habit of consuming acidic drinks such as carbonated drinks. Acid exposure on the enamel surface causes the release of hydroxyapatite minerals.[5] Hydroxyapatite crystals can return to their initial conditions if the pH in the oral cavity is neutral and there is sufficient calcium ion to support remineralization. Calcium and phosphate can help improve the remineralization process by rebuilding hydroxyapatite crystals.[6]

Sources of calcium can be found in several food ingredients and remineralization materials in dosage forms such as *casein-phosphopeptide-amorphous calcium phosphate* (CPP-ACP). CPP-ACP preparations are derived from casein milk protein and contain more complex remineralization materials to replenish lost minerals such as calcium and phosphate into the tooth structure.[7] These materials from an economic perspective are quite expensive and not widely known to the public. Consuming fish is a good source of calcium because fish is a calcium rich food. Calcium in fish is not

DOI 10.35856/mdj.v10i1.384

only found in the meat, but also in the bones. The type of fish that can be used for bones is catfish (*Clarias batrachus*), but the bones are wasted as waste from their processing.[8] In fact, catfish bones contain 5.68% calcium and 3.78% phosphate, which are essential minerals for bone growth and have the potential as an alternative to enamel remineralization.
Research by Gunawan, regarding the application of anchovy paste (*Stolephorus* sp) can improve enamel surface remineralization in terms of surface roughness and hardness, however, there has been no research on catfish bone paste on changes in enamel surface roughness.[9]

Therefore, this study aims to determine the potential of catfish bone paste to reduce enamel surface roughness. The change in roughness on the enamel surface can be seen through a surface roughness tester.

METHODS

This is a laboratory experimental research. The making of catfish bone paste begins with boiling, drying and grinding the bones, followed by calcination to obtain catfish bone sediment which will be dried into powder. The next step is heating distilled water, adding nipagin and 0.2 g cellulose. The results of calcined catfish bones are crushed until smooth and moistened with 1 g of glycerol, mixed with 0.05 g of menthol and alcohol until dissolved, then crushed into a homogeneous paste.

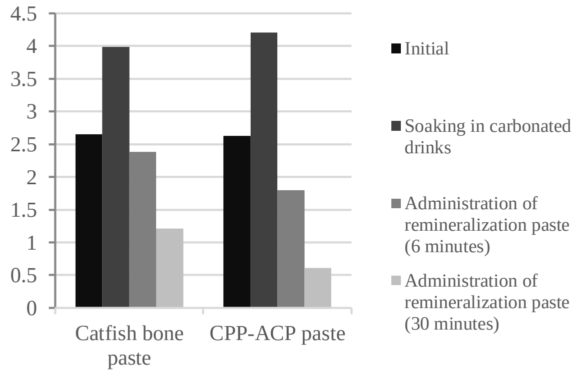

Figure 1 Graph of mean initial enamel roughness values, after immersion of carbonated drinks, and after application of enamel surface remineralization paste

This study used 32 samples of the maxillary first premolar and cut the roots of the teeth to the extent of the CEJ using a carborundum disc bur. The samples were divided into 2 groups, namely group I and group II, taking the initial roughness measurement using a surface roughness tester (SRT) and soaking the sample in a cola type carbonated drink for 2 minutes, remeasuring the roughness of the enamel, followed by the application of catfish bone paste in group I and CPP-ACP paste in group II for 6 minutes each, measuring the roughness of the enamel, reapplying catfish bone paste and CPP-ACP paste for 30 minutes each group, then a final roughness measurement was taken. The data obtained were analyzed using the one-way Anova and Shapiro Wilk test with a confidence level of 95% ($p < 0.05$).

Based on table 1, in group I with the application of catfish bone paste, the mean initial roughness value was 2.65 µm, then there was an increase in roughness after soaking with carbonated drinks by 3.99 µm and there was a decrease in roughness after application of catfish bone paste for 6 minutes to 2.38 µm, then decreased to 1.21 µm after 30 minutes of catfish bone paste application. Whereas in group II using CPP-ACP, the mean initial roughness was 2.63 µm, then there was an increase in roughness after soaking with carbonated drinks by 4.21 µm and a decrease in roughness after CPP-ACP application for 6 minutes to 1.80 µm and decreased to 0.61 µm in the CPP-ACP application for 30 minutes.

Observations in the catfish bone paste and CPP-ACP paste groups used the Shaphiro-Wilk statistical test to determine the normality value and obtained a p-value is more than 0.05, which means that the data is normally distributed so the test is continued with the parametric test, namely one-way Anova. Based on the one-way Anova statistical test showed a significance value of 0.000 ($p < 0.05$) in the catfish bone paste and CPP-ACP paste groups, which means that there were significant differences between observation times in each group.

DISCUSSION

Enamel is a network that undergoes high mineralization processes such as remineralization and demineralization which are susceptible to acids. The decrease in pH and the length of exposure to the enamel surface with acidic food and drinks affects the mineral ion density on the enamel surface and allows microporosity to occur. This demineralization can be followed by a decrease in hardness and an increase in microroughness on the enamel surface.[2,10-12]

This study uses a cola type carbonated drink because of its low pH, namely 2.4-2.74 and the content of strong acids such as phosphoric acid and citric acid which can bind calcium. This is because the more acidic the drink is the more hydrogen ions can increase the potential for the release of calcium ions on the enamel surface.[13-16] The immersion time of the sample was chosen for 2 minutes, based on the results of preliminary research conducted by the author which proved to significantly increase enamel surface roughness.

In group I, application of catfish bone paste for 6 minutes and 30 minutes significantly reduced enamel surface roughness after immersion with carbonated

DOI 10.35856/mdj.v10i1.384

RESULTS

Table 1 The mean value of initial enamel roughness and after treatment (µm)

Observation time	Catfish bone paste		CPP-ACP paste	
	X	SD	X	SD
Initial measurements	2.65	0.45	2.63	0.38
Demineralization	3.99	0.62	4.21	0.58
Remineralized paste application (6 minutes)	2.38	0.45	1.80	0.56
Remineralized paste application (30 minutes)	1.21	0.45	0.61	0.67

Notes: X = average; SD = Standard Deviation

Table 2 The results of the enamel roughness value test in each treatment group

Observation time	**Catfish Bone Paste**		**CPP-ACP Paste**	
	*Normality test**	*Comparison test***	*Normality test**	*Comparison test***
Initial	0.185*		0.190*	
Soaking in carbonated drinks	0.120*	0.000**	0.168*	0.000**
Remineralized paste application (6 minutes)	0.831*		0.567*	
Remineralized paste application (30 minutes)	0.064*		0.408*	

*Shapiro Wilk test: $p>0.05$; data distribution normal

**One-Way Anova: $p<0.05$; significant

drinks. This occured due to the calcium content of 5.68% and 3.78% phosphate contained in catfish bone paste.[20] The mineral content of calcium comes from the collagen matrix in fish bones. The process of synthesizing calcium from fish bones is carried out with the aim of changing the particle size of fish bones to maximize the process of calcium absorption on the enamel surface.[21] Sufficient calcium and phosphate concentrations will penetrate into interprismatic enamel which will inhibit the process of decomposing hydroxyapatite and allow rebuilding or partial reconstruction soluble hydroxyapatite crystals.[22,23]

The mineral apatite formation process occurs as soon as the calcium, phosphate, and fluorine ions come into contact with the enamel. The duration of exposure to the remineralization material with the enamel surface has an effect on reducing the surface roughness of the enamel.[17,24] The application time of the remineralization material for 30 minutes is an efficient time to reduce the surface roughness of the enamel. This can be seen in the preliminary research author where the value of the reduction in roughness was high occurs in the application for 30 minutes. The remineralization process is influenced by the amount of calcium and phosphate ions and neutral pH. When the pH is at a value of 5.5 or neutral, a pH balancing process will occur to prevent the dissolution of apatite crystals on the enamel surface.[18,25] The formation of apatite crystals begins with the deposition of small crystals; over time the small crystals melt and solidify to form larger crystals to reach their maximum size.[7,24]

In group II, application of CPP-ACP for 6 minutes and 30 minutes, there was a decrease in enamel surface roughness which was descriptively higher than that in group I. This was because CPP-ACP had the ability to suffice and stabilize calcium and phosphate ions on the enamel surface. Calcium and phosphate levels in CPP-ACP will prevent the release of hydroxyapatite crystals on the enamel surface, stop demineralization and can reduce acid conditions to allow the remineralization process on the enamel surface, assisted by the content of casein phosphopeptide.[17,19,27] Casein phosphopeptide can quickly deliver calcium phosphate and also helps ACP bind to enamel. In acidic conditions, casein will settle because it has low solubility and can function as a reservoir for storing calcium and phosphate. In the oral cavity the casein content of CPP-ACP will help increase the resistance of the enamel surface to bacteria and can reduce the number of caries-causing bacteria.[26,27] In addition, the paste texture of CPP-ACP is smoother when compared to catfish bone paste. A smoother preparation and lower viscosity will make it easier for the diffusion process of calcium ions to be absorbed by hypomineralized enamel and help remineralization.[23,29]

High calcium levels and complex CPP-ACP content can guarantee the need for calcium in the remineralization process that occurs in the oral cavity and can stimulate remineralization after the demineralization due to soaking cola type carbonated drinks.[13,26-28]

It was concluded that application of catfish bone paste for 6 minutes and 30 minutes can cause enamel surface changes in the form of a significant reduction in enamel surface roughness and has the potential to increase the enamel remineralization process.

Acknowledgement

We are grateful to all parties involved in this research.

Conflict of interest

The authors report no conflicts of interest.

DOI 10.35856/mdj.v10i1.384

REFERENCES

1. Kunin AA, Evdokimova AY, Moiseeva NS. Age-related differences of tooth enamel morphochemistry in health and dental caries. EPMA J 2015; 6 (3): 2-3
2. Nugroho JJ, Hafsari WR. The effective of betel leaf (*Piper betle Linn*) exctract gel and cocoa bean (*Theobroma cacao L*) extract gel application against the hardness of enamel surface in vitro. J Dentomaxillofac Sci 2017; 2(1): 23
3. Wu YQ, Arsecularatne JA, Hoffman M. Attrition-corrosion of human dental enamel: A review. Biosurface and Biotribology 2017; 3(1): 197-8
4. Syafira G, Permatasari R, Wardani N. Theobromine effects on enamel surface microhardness: in vitro. J Dent Indonesia 2012; 19: 32-6
5. Hayashi O, Chiba T, Shimoda S, Momoi Y. Demineralization and remineralization phenomena of human enamel in acid erosion model. J Hard Tissue Biol 2016; 25(1): 27-8
6. Neel EAA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, et al. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomed 2016; 11: 4748-54
7. Divyaapriya GK, Yavagal PC, Veeresh DJ. Casein phosphopeptide-amorphous calcium phosphate in dentistry: An update. Int J Oral Health Sci 2019; 6(1): 18-23
8. Ratnawati SE, Ekantari N, Pradipta RW, Paramita BL. Aplikasi *respons surface methodology* (RSM) pada optimasi ekstraksi kalsium tulang lele. Jurnal Perikanan Universitas Gadjah Mada 2018; 20(1): 41-2
9. Gunawan HA. Pengaruh aplikasi substrat ikan teri pada permukaan email terhadap remineralisasi email. Jurnal Kedokteran Gigi Universitas Indonesia 2003; 10: 128-30
10. Meyer F, Amaechi BT, Fabritius HO, Enax J. Overview of calcium phosphates used in biomimetic oral care. Open Dent J 2018; 12: 414
11. Sabal N. Enamel of primary teeth morphological and chemical aspects. Swedish Dent J Suplement 2012: 1-4
12. Fraunhofer JA, Rogers MM. Dissolution of dental enamel in soft drinks. Gen Dent 2004; 52: 308
13. Lussi A. Dental erosion from diagnosis to therapy. Monogr Oral SCI 2008; 20: 94-143
14. Barac R, Gasic J, Trutic N, Sunaric S, Popovic J, Djekic P, dkk. Erosive effect of different soft drinks on enamel surface in vitro: Application Of Stylus Profilometry. Med Princ Pract 2015; 24: 453-457
15. Panigoro S, Pangemanan DHC, Juliatri. Kadar kalsium gigi yang terlarut pada perendaman minuman isotonik. Jurnal e-GiGi (eG) 2015; 3(2): 357
16. Wegehaupt FJ, Taubock TT, Stillhard A, Patrick R, Schmidlin, Attin T. Influence of extra-and intra-oral application of CPP-ACP and fluoride on rehardening of eroded enamel. Acta Odontol Scand 2012; 70: 177-83
17. Karda B, Jindal R, Mahajan S, Sandhu S, Sharma S, Kaur R. To Analyse the erosive potential of commercially available drinks on dental enamel and various tooth coloured restorative materials-An in-vitro study. J Clin Diagn Res 2016; 10(5): 117-21
18. Nugroho JJ, Natsir N, Trilaksana AC, Rovani CA, Atlanta MM. The Increase of tooth enamel surface hardness after application blood cockle Shells (*Anadara granosa*) paste as remineralization agent. Int J Appl Pharm 2019; 11(4): 26-9
19. Hikmah N, Nugroho JJ, Rovani CA, Mooduto L. Enamel remineraliation after extracoronal bleaching using nano-hydroxyapatite (nHA) from synthesis result of blood clam (*Anadara granosa*) shells. J Dentomaxillofac Sci 2019; 4(1): 28
20. Zakariah M, Yahya A, Mshelia PA, Gazali Y, Kwari HD. Gross morphometry of the heart of farmed African catfish (*Clarias gariepinus*) in Maiduguri Nigeria. Int J Fisheries Aquatic Studies 2017; 5(2): 687-9
21. Yin T, Park JW, Xiong S. Effect of micron fish bone with different particle size on the properties of silver carp (*Hypophthalmichthys molitrix*) surimi gels. Journal Food Quality 2017; 1(2): 7-8
22. Walsh LJ. Contemporary technologies for remineralization therapies: A review. International Dentistry. 11(6): 6-7
23. Puspitasari A, Adi P, Devinta F, Rubai. Pemanfaatan cangkang kerang darah *(Anadara granosa)* dalam remineralisasi gigi sulung. Journal of Indonesian Dental Association 2018; 1(1): 42-5
24. Wiryani M, Sujatmiko B, Bikarindrasari R. Pengaruh lama aplikasi bahan remineralisasi casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) terhadap kekerasan email. Majalah Kedokteran Gigi Indonesia 2016; 2(3):141-6
25. Rahayu YC. Peran agen remineralisasi pada lesi karies dini. Stomatognatic (J.K.G) 2013; 10(1): 25-30
26. Soares R, Ataide IDN, Fernandes M, Lambor R. Assessment of enamel remineralization after treatment with four different remineralising agents: A Scanning Electron Microscopy (SEM) study. J Clin Diagn Res 2017; 11(4): 136-7
27. Reynolds EC, Cai F, Cochrane NJ, P Shen, Walker GD, Morgan MV, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2008, 87(4): 344-8
28. Zhang Q, 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: 375
29. Liwang B, Irmawati, Budipramana E. Kekerasan mikro enamel gigi permanen muda setelah aplikasi bahan pemutih gigi dan pasta remineralisasi. Dent J 2014; 47(4): 206-10.

DOI 10.35856/mdj.v10i1.384