Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2024 June; 57(2): 131–138

Original article

Tegillarca granosa shell combination with *Vitis vinifera* and fluoride in decreasing enamel microporosity

Grace Caroline Setiawan, Adelia Tinisia, Muhammad Galang Adhinata Abdul Rahim, Fitria Rahmitasari, Widyasri Prananingrum
Department of Dental Materials, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia

ABSTRACT

Background: *White spot lesion is a demineralization process indicated by the increased of enamel microporosity. A tegillarca granosa shell contains 98.7% calcium and Vitis vinifera contains phytochemical compounds with fluoride, which has a potential to stimulate remineralization.* ***Purpose:*** *To analyze the Tegillarca granosa shell combination with Vitis vinifera and fluoride in decreasing enamel microporosity.* ***Methods:*** *The cream was prepared by combining 10% and 20% Tegillarca granosa shell with 10 grams of Vitis vinifera extract and 100 mg of fluoride. The cream was tested beforehand for viscocity and pH. Furthermore, 16 premolars were etched and divided into four groups. Group 1 was smeared with placebo (negative control) and Group 2 was smeared with casein phosphopeptide-amorphous calcium phosphate (positive control). The other groups were smeared with cream 10% (Group 3) and 20% (Group 4) Tegillarca granosa shell combination with Vitis vinifera and fluoride. Teeth were treated three times a day for 30 minutes and soaked in artificial saliva. After 14 days, the enamel microporosity was carried out using a scanning electron microscope. The data was analyzed with one-way analysis of variance (ANOVA) test followed by post-hoc least significant difference (LSD).* ***Results:*** *The enamel microporosity showed significant difference between Group 1 and the other groups. There was no significant difference between Groups 2, 3, and 4 ($p<0.05$). Although there was no significant difference between Group 3 and 4, the lowest one was in Group 4 ($p>0.05$).* ***Conclusion:*** *The cream, prepared by combining Tegillarca granosa shell with Vitis vinifera and fluoride, is effective in decreasing the enamel microporosity.*

Keywords: *enamel microporosity; fluoride; medicine; Vitis vinifera; Tegillarca granosa shell*
Article history: *Received 18 January 2023; Revised 1 September 2023; Accepted 11 October 2023; Published 1 June 2024*

Correspondence: Widyasri Prananingrum, Department of Dental Materials, Faculty of Dentistry, Universitas Hang Tuah. Jl. Arif Rahman Hakim 150 Surabaya, Indonesia. Email: widyasri.prananingrum@hangtuah.ac.id

INTRODUCTION

Oral disease is one of the most prevalent public health problems, which have undergone significant changes in epidemiology, especially dental caries. Dental caries is a chronic and preventable oral disease that has profound effects such as destruction of hard tissue and in some cases, also results in tooth loss.[1] In 2020, The World Health Organization estimated that 2.3 billion people in the world were diagnosed with caries and claimed that poor oral health has a profound significant effect on general health as well as virtue of life. This disease is most prevalent in Latin American countries and South Asia. Indonesia also has significantly higher prevalence in dental caries; it is encountered 88.8% and >70% in all age groups. Dental caries is caused by the interaction between the host and substrate such as carbohydrates and cariogenic microorganism for a certain time period, which forms an acidic product that effects a decrease in pH. When the pH falls below the threshold value, it can initiate the demineralization process, which is characterized by the formation of early stage of dental caries white spot lesion.[2,3] Demineralization is also a process involving the formation of enamel microporosity due to mineral loss and dissolution, for instance calcium, phosphate, and crystal apatite in particular and hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ and collagen from the enamel prism.[4,5]

Dynamic equilibrium happens within the process of demineralization and remineralization. Remineralization is a natural cycle process of forming apatite crystals, hydroxyapatite, and fluorapatite from mineral ions such as calcium and phosphate that were lost and dissolved during

DOI: 10.20473/j.djmkg.v57.i2.p131–138

the demineralization process.[6] The remineralization process is supported by remineralization materials and containing agents. One of the important material properties is viscosity; the viscosity value of a remineralizing material affects the ability of important ingredients such as calcium ions obtained from *Tegillarca granosa* and phosphate shells, which can in turn diffuse into the enamel microporosity and increase hydroxyapatite saturation so that the process of closing the enamel microporosity occurs as a result of successful remineralization.[7]

Remineralization agents include calcium, fluoride, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and others. The first and primary remineralization agent is calcium, a mineral ion that can rebuild and improve enamel structure that is lost in demineralization through diffusion process in enamel microporosity.[8] The calcium ions that are released are also affected by the acidity level from the tooth enamel.[9] A study conducted by Abdelnabi showed that 10% to 20% of calcium is effective to prompt remineralization process.[10] Along with calcium, there is fluoride, a mineral ion that is a gold standard for remineralization, which can prevent demineralization by increasing the acid resistance of the enamel and triggering ion precipitation from saliva to the tooth structure, setting off remineralization by forming fluorapatite, and becoming an antibacterial agent, which inhibits the production of glucosyltransferase enzyme.[11] One of the sources of fluoride that used in dentistry to prevent secondary caries is calcium fluoride.[12] Finally, there is a cream, CPP-ACP, which is used in public as a remineralization agent. It is a cream containing casein from milk's protein, which perchance maintains a neutral condition in the enamel by binding and stabilizing the calcium and phosphate ions from saliva.[13,14] In spite of the advantage of casein, there are more areas that could be looked at, which could potentially be a disadvantage to patients. CPP-ACP, for middle to lower classes of society, is less affordable and uneconomical, which leads to a limitation in its use as well.[15] In a study conducted by Matsui, casein in CPP-ACP showed the epitome of allergy among patients.[16] The prevalence of casein allergy, estimated as ranging from 0.5% to 3%, begins at the age of 1.[17] CPP inhibited casein binds with IgE in a concentration-dependent manner and will contribute to the limitations of using CPP-ACP only in non-allergic patients.[16] The insufficiency and disadvantages that were found in CPP-ACP can be overcome by using natural sources of calcium that are affordable and not from milk products and their derivatives, one of which is *T. granosa* shell. It is supported by the prevalence of shellfish allergy, found to be 0-0.9%.[18]

T. granosa shell is one of the natural calcium sources that is widely found in Indonesia, a maritime country, which potentially produces abundant marine commodity. The abundant *T. granosa* was evidenced by the Indonesian Directorate General of Capture Fisheries in 2012, which estimated that *T. granosa* shell production reached 48.994 tons. Though there is sufficient production of *T. granosa* shell, its function has been limited only to its meat for consumption, which results in an extensive amount of *T. granosa* shell waste, estimated to reach 2-2.4 tons every week.[19] The repercussion of *T. granosa* wastage allows negative concussion such as disturbance and impairment to the coastal and aquatic environment, disrupting and damaging the quality of Indonesian waters, and creating an environment that is not conducive.[20] On the other hand, *Tegillarca granosa* shell plays a quiescent role in remineralization, because it is formed by 98.7% calcium carbonate and other minerals such as 0.05% Mg, 0.9% Na, 0.02% P, which can be enforced in the remineralization process.[21] Differing from CPP-ACP, *T. granosa* shell was chosen to overcome casein allergy in some patients. Furthermore, it has potential as a natural ingredient containing 95% calcium carbonate that was proved by the study conducted by Sari et al.,[22] Lopata et al.,[23] and Zailatul et al.[24] and did not have any interaction or effect allergy on patients. This was supported by the study on shellfish allergy that is mainly caused by proteins, specifically tropomyosin, which are present in the gastrointestinal regions of the shellfish.[22–24]

T. granosa shell waste, due to its high calcium content, could be used as the fundamental ingredient in making the cream that increases remineralization. *T. granosa* shell waste is a profound source of calcium combined with natural ingredients like grapes (*Vitis vinifera*) and plays the role of an antibacterial agent due to its phytochemical compounds, including polyphenol and flavonoid. Phytochemical compounds in polyphenol also include proanthocyanidins, which are used in the dentin remineralization process and provide protection for collagen matrix.[6] Grapes were chosen based on the research conducted by Rachmawati et al.[13] and Busman et al.,[25] which proved that grapes can be antibacterial, especially against cariogenic bacteria such as *Streptococcus mutans*. Another fundamental ingredient is fluoride that can prevent demineralization, enhance remineralization and act as a bacterial agent. These three essential ingredients can be potentially put together to invent a cream to prompt tooth remineralization and treat white spot lesions.[25,26] Candidates for the remineralization cream should accomplish good quality and be applicable; hence, they must run several tests to achieve the standards so that it can be used in society. The remineralization cream underwent several tests such as a pH test, where a result of 4.5-10.5 was the standard for a favorable and intact product and a viscosity test, where the result would determine how substantial and adequate important ingredients of the material could diffuse into the microporosity of the enamel, which had an impact of triggering remineralization with the ideal number between 20 and 500 dPa.s.[27–29] Based on these, further research is needed about the effectiveness of the cream prepared using 10% and 20% *T. granosa* shell combination with *V. vinifera* and fluoride to decrease the enamel microporosity as a remineralization agent.

DOI: 10.20473/j.djmkg.v57.i2.p131–138

MATERIALS AND METHODS

Preparation of components for creating the cream began with *Tegillarca granosa* shells. The sludge was cleaned off them by immersing them in water for 24 hours and brushing with a steel brush. Then, they were preserved in the sun until the shell became dry. Next, the shells were crushed with a mortar and pestle and sieved with 100 mesh to get a finer and preferable powder. The extract *V. vinifera* was prepared using the freeze-dry method. The quantity of *V. vinifera* needed for making the cream is 800 grams; the grapes were then juiced using a blender after the freeze-dry stage, which was carried out by freezing the grape juice in a round bottom boiling flask at low temperature in a freeze dryer and then dried.[30] This was followed by increasing the temperature from -40°C up to 0°C until 95% grape juice lost the water and lowering the pressure to less than 4.58 mmHg. Final step of the drying process was continued by increasing the temperature to 35°C and the pressure. The results of the freeze-dry processing of grapes were brownish in color and had a sticky consistency.

After the preparation of the sample material was completed, the cream was then prepared with a concentration of 10% and 20% *T. granosa* shell combination *V. vinifera* and fluoride. After making cream with a concentration of 10% using a ratio of 1: 1: 0.01, i.e., 10 grams of *T. granosa* shell, 10 grams of extract *Vitis vinifera* with a concentration of 50%, and 100 mg of fluoride 900 ppm, the process was continued by heating 50 ml of aquabidestilata until it reached a temperature of 80°C, then 100 mg of Na CMC was added and allowed to swell until it formed a clear gel mass, after which 30 ml of aquabidestilata was added at room temperature and stirred until it became homogeneous. This stage of making cream with a concentration of 20% using a ratio of 2: 1: 0.01 i.e. 20 grams of *T. granosa* shell, 10 grams of extract *V. vinifera* with a concentration of 50% and 100 mg of fluoride 900 ppm was continued by heating 50 ml aquabidestilata until it reached a temperature of 80°C , then 100 mg of Na CMC was added and allowed to swell until it formed a clear gel mass, after which 30 ml of aquabidestilata was added at room temperature and stirred until homogeneous.

The cream that had been made was then tested for its viscosity and pH. The viscosity test of cream *T. granosa* combination with *Vitis vinifera* and fluoride was conducted using a Viscotester VT-04E (Rion Viscotester VT-04E, China). The cream preparation sample was placed in the beaker; thereupon, rotor number 2 was used and lowered into the beaker to the specified limitation. The results were displayed by observing the movement of the needle on a scale indicating the level of solidity of the cream suspension in the form of dPa.s units. The pH test of cream, prepared using *T. granosa* combination with *V. vinifera* and fluoride, was conducted first by soaking the tooth sample in artificial saliva; then, the pH of the artificial saliva was checked periodically using a universal pH strip (Merck, Germany). If the pH of the artificial saliva is below 7, then 2-3 drops of 0.1 N NaOH solution are given, and if the pH of artificial saliva is above 7, then it is given 2-3 drops of 0.1N HCl solution until the pH reaches the optimum value again and is read after 14 days using a pH meter.

The cream was then be evaluated with teeth samples. 16 premolars had been extracted and selected with the criteria of a healthy tooth without any sign of caries, filling, and restoration. The sample preparation started by cleaning the teeth using a prophylactic brush, then soaking in saline water, and storing in the refrigerator. The teeth samples were divided into four groups (n=4), such as Group 1: smeared with placebo as a negative control, Group 2: smeared with CPP-ACP (GC Tooth Mousse Plus^{TM}, USA) as a positive control, Group 3: smeared with cream with 10% *T. granosa* shell combination *V. vinifera* and fluoride and Group 4: smeared with cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride. Each sample was etched using hexaetch etching (Hexaetch, Indonesia) with a concentration of 37% phosphoric acid on the buccal part of the tooth, was allowed to stand for 30 seconds, and then, rinsed with water. The application of 37% etching aims to form microporosity on the teeth. Next, the teeth samples were immersed in artificial saliva for 60 seconds. The material was applied to the buccal teeth and then, allowed to stand for 30 minutes; this was applied thrice a day and immersed in artificial saliva within 24 hours. The treatment was done every day for 14 days, after which the enamel microporosity was measured using scanning electron microscope (SEM) (Hitachi Type TM-3000, Japan). The SEM image was formed with magnifications 500x and 1500x.[6,31]

The statistical analysis of the enamel microporosity was comprised and analyzed using one-way ANOVA and Post Hoc Tukey HSD multiple comparison tests with SPSS software (Version 26.0; SPSS Japan Inc., Tokyo, Japan). The significant difference was accepted at $p < 0.05$.

RESULTS

The viscosity of the sample materials, as shown in Figure 1, was found to be in the range 300-400 dPa.s. The viscosity of CPP-ACP was 350 dPa.s. The viscosity of the cream with 10% *T. granosa* shell combination *Vitis vinifera* and fluoride was 300 dPa.s, while the viscosity of the cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride was 400 dPa.s.

The pH of each sample material, as shown in Figure 2, was in the range of 6.8–7.3. The pH of CPP-ACP was 6.8. The pH of the cream with 10% *T. granosa* shell combination *V. vinifera* and fluoride was 7, while the pH of the cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride was 7.3.

The morphological patterns of the enamel microporosity after treatment was shown in Figure 3, and the number of enamel microporosities were measured for each group. The red arrows indicate the enamel microporosity.

DOI: 10.20473/j.djmkg.v57.i2.p131–138

Furthermore, Figure 4 shows that there was a significant difference between Group 1 compared with the other groups (p=0.000). Although there was no significant difference between Group 2 and Group 3 (p=0.994), Group 2 and Group 4 (p=0.635), Group 3 and 4 (p=0.778), and Group 4, the lowest number of enamel microporosities was found in Group 4 with an average value of 4.

DISCUSSION

A successful remineralization is characterized by lesser enamel microporosity. Microporosity refers to the presence of open cavities, and the greater the microporosity, the lower the strength and resistance of a material. The size of the microporosity voids and the degree of microporosity can be reduced or increased using materials containing calcium and phosphate.[32] The remineralization process can occur if the salivary pH is normal, and there is a sufficient amount of Ca^{2+} and PO_4^{3-} ; the hydroxyapatite dissolution can become neutral in the presence of buffering and rebuild the apatite crystals that have dissolved and filled in the enamel microporosity.[32] Fluoroapatite, which is also formed during remineralization, plays a role in the increased resistance to acid dissolution that potentially affects the remineralization.[31] The viscosity of the remineralization agent also plays an important role in facilitating remineralization by closing the enamel microporosity initiated by ions and minerals containing substances that diffuse and penetrate the enamel crystals, particularly the hypomineralized or demineralized enamel. The continuous process of ion precipitation, especially calcium and phosphate, also enables the remineralization process. The viscosity of the remineralized cream has a standard value of > 50 dPa.s, as set by the Indonesian National Standard. Meanwhile, the viscosity of an ideal cream ranges between 20 and 500 dPa.s. The gaps in the values obtained by the cream with 10% *T. granosa* shell combination *Vitis vinifera* and fluoride and the cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride showed that different concentrations of the ingredients affect the viscosity. Different calcium carbonate contents in groups with 10% *Tegillarca granosa* shell combination *V. vinifera* and fluoride and cream with 20% *T. granosa*

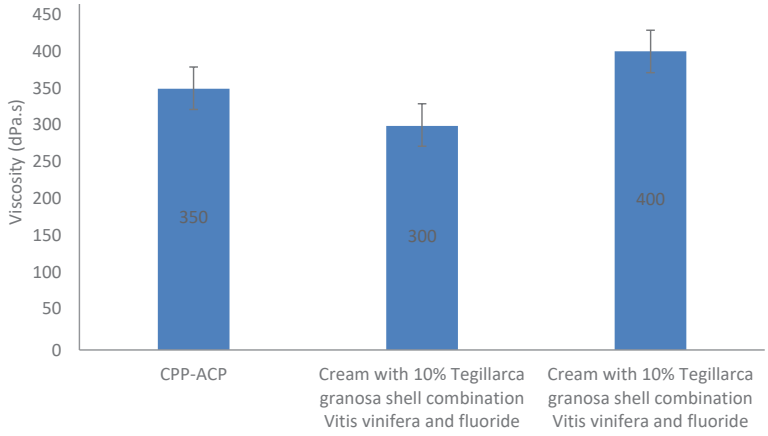

Figure 1. Viscosity of CPP-ACP, cream with 10% *Tegillarca granosa* shell combination *Vitis vinifera* and fluoride and cream with 20% *Tegillarca granosa* shell combination *Vitis vinifera* and fluoride.

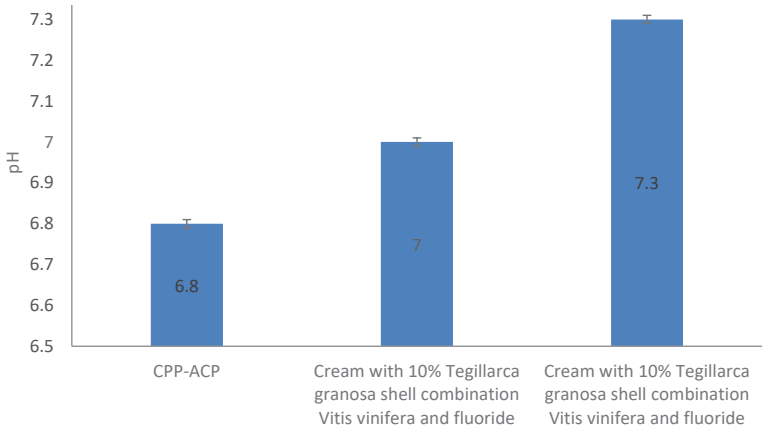

Figure 2. pH of CPP-ACP, cream with 10% *Tegillarca granosa* shell combination *Vitis vinifera* and fluoride and cream with 20% *Tegillarca granosa* shell combination *Vitis vinifera* and fluoride.

DOI: 10.20473/j.djmkg.v57.i2.p131–138

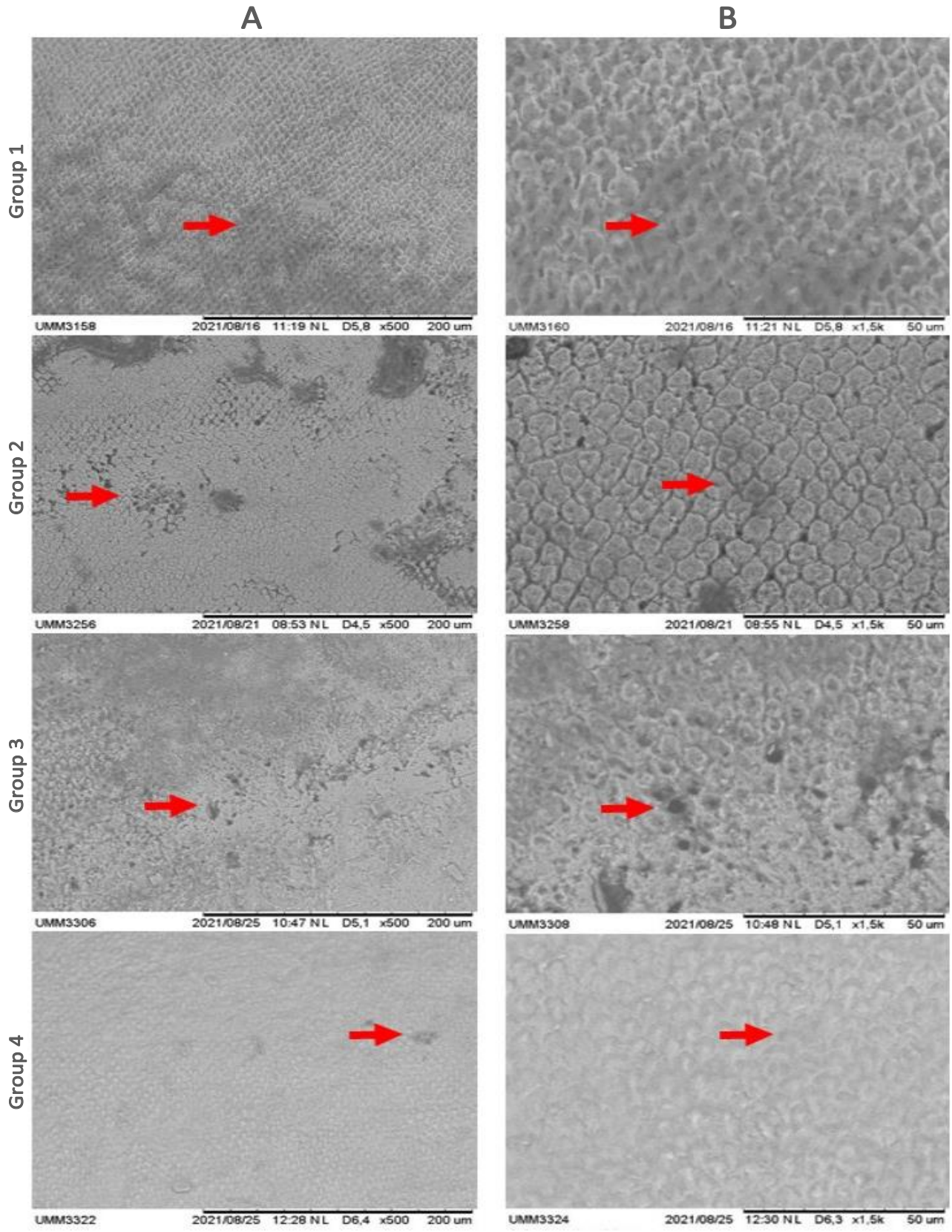

Figure 3. The SEM images of enamel microporosity after treatment for 14 days, in 500x magnification (A) and 1500x magnification (B). The red arrows show the enamel microporosity. Group 1: smeared with placebo as a negative control; Group 2: smeared with CPP-ACP as a positive control; Group 3: smeared with the cream with 10% *Tegillarca granosa* shell combination *Vitis vinifera* and fluoride; Group 4: smeared with the cream with 20% *Tegillarca granosa* shell combination *Vitis vinifera* and fluoride.

DOI: 10.20473/j.djmkg.v57.i2.p131–138

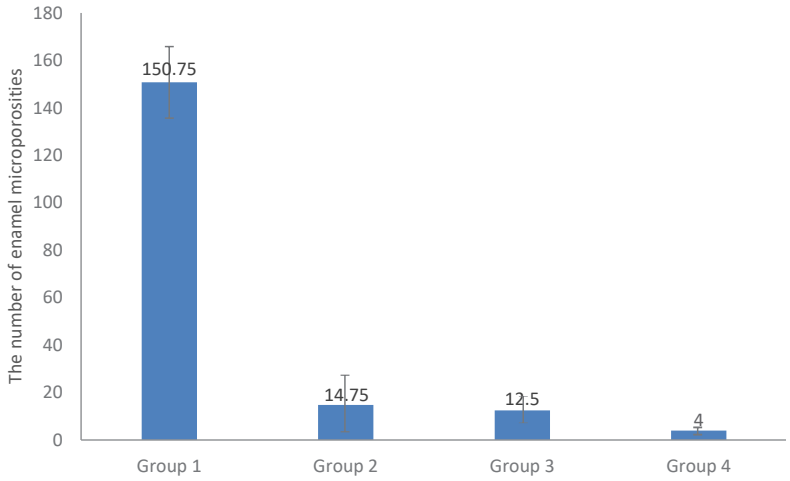

Figure 4. The number of enamel microporosities in all groups after treatment for 14 days. *significance *p* value (< 0.05)

shell combination *V. vinifera* and fluoride affect the concentration of the solution and the viscosity value. Variations in the concentration of calcium in groups 3 and 4 affect the solution concentration and the resulting viscosity values. The concentration of the solution increases if there are many particles of dissolved substance; this is caused by friction between the particles due to smaller distance between them, which creates a cohesive force or attraction between the particles that will be higher and directly proportional to the resulting viscosity value.[33,34] Based on the results of the study, the cream with 10% *T. granosa* shell combination *Vitis vinifera* and fluoride and the cream with 20% *T. granosa* shell combination *Vitis vinifera* and fluoride met the applicable cream standards, while the group with 10% *T. granosa shell* combination *V. vinifera* and fluoride had a viscosity of 300 dPa.s lower than CPP-ACP. The cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride with a viscosity of 400 dPa.s was an ideal remineralization material that met the criteria.

Referring to the SNI standards for remineralization creams, the normal pH ranges between 5.5-10.5. The optimal pH oral cavity to support the remineralization process is greater than 5.5.[27] While a low pH (acid) leads to demineralization, a pH > 5.5 can trigger remineralization.[35] The acids can cause the local pH to fall below a critical value, resulting in demineralization of the tooth tissue.[36] The pH of the cream supports the remineralization process by conditioning the oral cavity to neutralize the pH, since demineralization occurs when the oral cavity is acidic. Thus, to support the remineralization, the pH of the remineralization cream should be neutral in the range 5.5-10.5.[34] The pH in each cream group was still greater than 5.5-10.5, indicating that the creams were safe to use and support the remineralization process. Based on the results of the study, the cream with 10% *T. granosa* shell combination *V. vinifera* and fluoride and the cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride met the SNI standards for remineralization. Nonetheless, the cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride has the highest pH value of 7.3, which also has a greater impact on enhancing remineralization.

Changes in enamel microporosity related to the success of remineralization can be measured using SEM at 1500 times magnification. The results of this study showed that negative control had the highest average number of microporosities, because no remineralization agent was added and the artificial saliva immersion caused the demineralization process to occur continuously; however, the concentration of calcium in the artificial saliva could not significantly increase remineralization. As a result, the enamel microporosities were not closed. Group 2 that was smeared with CPP-ACP as a positive control had a lower number of microporosities than the negative control group; however, the average enamel porosity was higher than Group 3, which was smeared with the cream with 10% *T. granosa* shell combination *V. vinifera* and fluoride and Group 4, which was smeared with the cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride. CPP-ACP is reviewed as a gold-standard cream and is a source of calcium and phosphate ions, which support the formation of hydroxyapatite. However, the solubility of CPP-ACP in an unfavorable acidic pH and penetration on the enamel surface with low erosion resulted in lower hydroxyapatite crystals from the calcium and phosphate bonds produced by CPP-ACP, which are less adhesive to the enamel surface that underwent the demineralization process. In addition, it also became less stable and was easily released when it came in contact with water during the rinsing, causing the remineralization process to be disrupted.

Group 4, which was smeared with cream with 20% *T. granosa* shell combination *V. vinifera* and fluoride showed a lower number of microporosities than Group 3, which was smeared with cream with 10% *T. granosa* shell combination *V. vinifera* and fluoride. This was because Group 3 had 10 grams of the active ingredient in *T. granosa* shells, while Group 4 consisted of 20 grams of the active ingredient in *T. granosa* shells. Although there was no significant difference between Group 3

DOI: 10.20473/j.djmkg.v57.i2.p131–138

and Group 4, Group 4 showed a lower average number of enamel microporosities compared to Group 3. The different calcium concentrations of *T. granosa* shells resulted in different viscosity values, which affected the ease of application to tooth enamel. The calcium content in the *T. granosa* shell can diffuse and penetrate the enamel microporosity, because enamel microporosity can only be filled with mineral ions that have the same ionic radius.[36,37] The fluoride content in grapes, i.e. sodium fluoride of 900 ppm, also affected the remineralization through the fluoroapatite formation mechanism. The fluoride increases the acidity resistance, which affects the precipitation of calcium and phosphate ions into the tooth structure.[11] The mixture was administered three times a day and left for 30 minutes. After 14 days, the calcium and fluoride could diffuse between the enamel and were absorbed into hypomineralized enamel, increasing hydroxyapatite saturation, which then closed the enamel microporosity.[36] This study on the enamel microporosity reducing cream has been proven from in vitro research, and whatever the results may be, it still cannot be concluded that it is the superior method for getting the best results. Therefore, it is presumed that doing an in vivo research can be used to conclude the effectiveness of this cream in reducing enamel microporosity.

With regards to the results and discussions of this study, it can be concluded that the cream containing 20% *T. granosa* shell and a combination of grape and fluoride is the most effective agent in remineralizing the demineralized enamel and white spot lesion. It is suggested to study further regarding the bond or adhesion between materials, side effects, and long-term effects of this research.

REFERENCES

1. Haikal M, Adhani R, Wardani I. Hubungan laju aliran saliva terhadap kejadian karies gigi pada penderita hipertensi yang mengonsumsi obat antihipertensi. Dentin J Kedokt Gigi. 2020; 4(2): 39–42.
2. Kunarti S, Saraswati W, Lashari DM, Salma N, Nafatila T. Enamel remineralisation-inducing materials for caries prevention. Dent J. 2021; 54(3): 165–8.
3. Triwardhani A, Budipramana M, Sjamsudin J. Effect of different white-spot lesion treatment on orthodontic shear strength and enamel morphology: In vitro study. J Int Oral Heal. 2020; 12(2): 120–8.
4. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: A review of literature. Int J Clin Pediatr Dent. 2019; 12(2): 139–44.
5. Carabelly AN, Puspitasari D, Syahrina F, Wahyudi MD, Salsabila Karno DA, Mohd-Said S. Demineralized dentin characteristics after application of Mauli banana stem gel. Dent J. 2024; 57(1): 33–7.
6. Metly A, Sumantri D, Oenzil F. The effect of pasteurized milk and pure soy milk on enamel remineralization. Padjadjaran J Dent. 2019; 31(3): 202.
7. Rahayu YC. Peran agen remineralisasi pada lesi karies dini. Stomatognatic (JKG Unej). 2013; 10(1): 25–30.
8. Dianti F, Triaminingsih S, Irawan B. Effects of miswak and nano calcium carbonate toothpastes on the hardness of demineralized human tooth surfaces. J Phys Conf Ser. 2018; 1073(3): 032008.
9. Adiba SH, Effendy R, Zubaidah N. Fluoride varnish effect on dental erosion immersed with carbonated beverages. J Int Dent Med Res. 2018; 11(1): 299–302.
10. Abdelnabi A, Hamza NK, El-Borady OM, Hamdy TM. Effect of different formulations and application methods of coral calcium on its remineralization ability on carious enamel. Open Access Maced J Med Sci. 2020; 8(D): 94–9.
11. Ritter A V, Boushell LW, Walter R. Sturdevant's art and science of operative dentistry. 2019. p. 417–18.
12. Apriani A, Naliani S, Djuanda R, Teanindar SH, Florenthe JQ, Baharudin F. Surface roughness assessment with fluoride varnish application: An in vitro study. Dent J. 2023; 56(3): 154–9.
13. Rachmawati D, Kurniawati C, Hakim L, Roeswahjuni N. Efek remineralisasi casein phospopeptide-amorphous calcium phospate (CPP-ACP) terhadap enamel gigi sulung. E-Prodenta J Dent. 2019; 3(2): 257–62.
14. Prananingrum W, Prabowo PB. The increasing of enamel calcium level after casein phosphopeptideamorphous calcium phosphate covering. Dent J. 2012; 45(2): 93–6.
15. Yuanita T, Zubaidah N, A R MI. Enamel hardness differences after topical application of theobromine gel and casein phosphopeptide-amorphous calcium phosphate. Conserv Dent J. 2020; 10(1): 5–8.
16. Matsui T, Naito M, Kitamura K, Makino A, Sugiura S, Izumi H, Ito K. Casein phosphopeptide in cow's milk is strongly allergenic. Authorea. 2021; : 1–4.
17. Flom JD, Sicherer SH. Epidemiology of cow's milk allergy. Nutrients. 2019; 11(5): 1051.
18. Moonesinghe H, Mackenzie H, Venter C, Kilburn S, Turner P, Weir K, Dean T. Prevalence of fish and shellfish allergy. Ann Allergy, Asthma Immunol. 2016; 117(3): 264-272.e4.
19. Sawiji A, Perdanawati RA. Pemetaan pemanfaatan limbah kerang dengan pendekatan masyarakat berbasis aset (Studi kasus: Desa Nambangan Cumpat, Surabaya). Mar J. 2017; 03(01): 10–9.
20. Ma'ruf A, Hartati S. Production and characterization of nano-chitosan from blood clamshell (Anadara granosa) by ionic gelation. Nat Environ Pollut Technol. 2022; 21(4): 1761–6.
21. Nastiti AD, Widyastuti W, Laihad FM. Bioviabilitas hidroksiapatit ekstrak cangkang kerang darah (Anadara granosa) terhadap sel punca mesenkimal sebagai bahan graft Tulang. Dent J Kedokt Gigi. 2015; 9(2): 122–8.
22. Sari RP, Sudjarwo SA, Rahayu RP, Prananingrum W, Revianti S, Kurniawan H, Bachmid AF. The effects of Anadara granosa shell-Stichopus hermanni on bFGF expressions and blood vessel counts in the bone defect healing process of Wistar rats. Dent J. 2017; 50(4): 194–8.
23. Lopata AL, Kleine-Tebbe J, Kamath SD. Allergens and molecular diagnostics of shellfish allergy. Allergo J Int. 2016; 25(7): 210–8.
24. Zailatul HMY, Rosmilah M, Faizal B, Noormalin A, Shahnaz M. Malaysian cockle (Anadara granosa) allergy: Identification of IgE-binding proteins and effects of different cooking methods. Trop Biomed. 2015; 32(2): 323–34.
25. Busman B, Edrizal E, Utami DWP. Uji efektivitas ekstrak buah anggur hijau (Vitis Vinivera L) terhadap daya hambat laju pertumbuhan bakteri Streptococcus mutans dan Lactobacillus acidophilus. Ensiklopedia Sos Rev. 2021; 2(3): 325–32.
26. Jawale KD, Kamat SB, Patil JA, Nanjannawar GS, Chopade RV. Grape seed extract: An innovation in remineralization. J Conserv Dent. 2017; 20(6): 415–8.
27. Ahmad I. Pemanfaatan limbah cangkang kerang darah (Anadara granosa) sebagai bahan abrasif dalam pasta gigi. J Galung Trop. 2017; 6: 49–59.
28. Maulana F, Mulawarmanti D, Laihad FM. Combination administration of Stichopus hermanii gel and hyperbaric oxygen therapy the number of fibroblasts in diabetes mellitus rats with periodontitis. Dent J Kedokt Gigi. 2017; 11(2): 9–17.
29. Makmur SA, Utomo RB. Pengaruh aplikasi gel Theobromine terhadap kekasaran permukaan email gigi desidui pasca demineralisasi. ODONTO Dent J. 2019; 6(2): 95–8.
30. Ari DPS, Yonatasya FD, Saftiarini G, Prananingrum W. Variations of gelatin percentages in HA-TCP scaffolds as the result of 6- and 12-hour sintering processes of blood cockle (Anadara granosa) shells against porosity. Dent J. 2018; 51(4): 158–63.

DOI: 10.20473/j.djmkg.v57.i2.p131–138

31. Pamungkas GB, Karunia D, Suparwitri S. Desensitizing agents' post-bleaching effect on orthodontic bracket bond strength. Dent J. 2024; 57(1): 45–9.
32. Wulandari E, Wardani FRA, Fatimattuzahro N, Dewanti IDAR. Addition of gourami (Osphronemus goramy) fish scale powder on porosity of glass ionomer cement. Dent J. 2022; 55(1): 33–7.
33. Mailana D, Nuryanti, Harwoko. Formulasi sediaan krim antioksidan ekstrak etanolik daun alpukat (Persea americana Mill.). Acta J Indones. 2016; 4(2): 7–15.
34. Nurjannah W, Yusriadi, Nugrahani AW. Uji aktivitas antibakteri formula pasta gigi ekstrak batang karui (Harrisonia Perforata Merr.) terhadap bakteri Streptococcus mutans. Biocelebes. 2018; 12(2): 52–61.
35. Dianawati N, Setyarini W, Widjiastuti I, Ridwan RD, Kuntaman K. The distribution of Streptococcus mutans and Streptococcus sobrinus in children with dental caries severity level. Dent J. 2020; 53(1): 36–9.
36. Hediana VAK, Probosari N, Setyorini D. Lama perendaman gigi di dalam air perasan jeruk nipis (Citrus aurantifolia Swingle) mempengaruhi kedalaman porositas mikro email (Duration of immersing teeth in lime (Citrus aurantifolia Swingle) juice affects on microporosity depth of enamel). J Dentomaxillofacial Sci. 2015; 14(1): 45–9.
37. Junaidi, Sinala S. Farmasi fisik: Bahan ajar farmasi. Jakarta: Kementerian Kesehatan Republik Indonesia; 2017. p. 1–51.

DOI: 10.20473/j.djmkg.v57.i2.p131–138