

Research Article

## Remineralization Efficacy of Grape Seed Oil Paste on Demineralized Enamel after Bracket Debonding Compared to Casein Phosphopeptide-Amorphous Calcium Fluoride Phosphate Paste: An In Vitro Study

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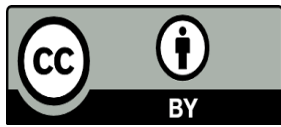
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### Abstract

**Aims:** The present study aimed to evaluate the remineralization potential of grape seed (GS) oil incorporated into a prepared paste with different concentrations and compare it with casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACPF) on enamel caries induced on debonded enamel surfaces.

**Materials and Methods:** Forty-four sound permanent premolars were gathered for this study. After mounting, bracket bonding, and then debonding, the teeth were arbitrarily divided into four groups. The artificial enamel caries was created by immersing the samples in a demineralization solution. The enamel surface treated with: Group I (n=11), Control negative (artificial saliva), Group II (n=11), CPP-ACFP paste. Group III (n=11) 5% GS (paste1). Group IV (n=11) 10 % GS (paste2). 8 specimens were exposed to Surface Micro Hardness test (SMH), and 3 were exposed to Scanning Electron Microscope with Energy Dispersive X-ray Spectroscopy (SEM-EDX) after a period of 30 days of treatment.

**Results:** The results of SMH exhibited a highly statistically significant difference in surface micro-hardness after treatment between the study groups, and the greatest mean value of surface microhardness was seen in 5 % GS (paste1). EDX results exhibited no statistically significant difference after treatment among the study groups. SEM observations showed that a protective layer was formed on the surface of the treated groups.

**Conclusion:** The grape seed oil used in this study displayed a potential remineralization effect in artificial carious lesions on enamel

**Keywords:** Grape seed oil, CPP-ACPF, Debonding, Demineralization, Remineralization.

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## INTRODUCTION

Dental enamel is the hardest, most mineralized structure in the human body. It is comprised of 96 wt.% of mineral content as hydroxyapatite crystals combined with 4 wt.% of organic material and water. Enamel is not an inert system; it is a unique self-assembly in the mineral world (a cellular, vascular, and non-innervated), contributes in many processes such as ion transport, from saliva to dentine, or ion exchange reactions, such as phenomena of demineralization and remineralization (1).

Loss of this hardest tissue may occur, especially during orthodontic treatment in the addition to enamel cracks, scratches, and scarring that also may happen, and all orthodontic treatment stages are associated with potential damage to dental enamel such as polishing with abrasives, the bonding procedure, acid etching, removal of bracket, composite remnants removal by rotary instruments and the rebonding of failed brackets. In addition to all, enamel demineralization and white spot lesions (WSLs) formation are encountered as an undesirable complication and a common one that could occur during or after the completion of fixed orthodontic treatment (2).

WSLs may appear clinically on the 4th week after the beginning of fixed orthodontic treatment, and may rapidly develop if the oral hygiene is poor, with their prevalence ranging from 2% to 97% (3).

According to the minimal invasive dentistry strategy, incipient carious lesions of enamel must be treated through non-invasive approaches of remineralization. For this persistence, dentists turned to use dentifrices, mouthwashes, topical gels, and varnishes that contain fluoride to treat WSLs (4).

In recent years, there are a number of the remineralization systems that developed beyond fluoride remineralization and these systems divided into as called intrinsic and extrinsic systems, intrinsic approach such as proteins, peptides and dendrimers which are able to adsorb to specific sites and to accumulate the calcium ions and phosphate ions in the saliva and this accumulation lead to mineralization, while extrinsic approach such as nanocrystalline and amorphous calcium phosphate which

are provided calcium and phosphate from external sources to the sites to be mineralized(5).

Casein phospho-peptide amorphous calcium phosphate (CPP-ACP) is an example of an extrinsic remineralization system, a bioactive agent derivative from casein, which is a bovine milk protein, and many studies demonstrated its anticariogenic activity, which can prevent enamel demineralization and promote the enamel lesion remineralization by maintaining a state of mineral supersaturation at the tooth surface. CPP-ACP interacted with fluoride ion to produce ACPF, which is a complex that is stabilized by CPP at the tooth surface and forms fluorapatite that is more resistant to acidic attack, and so promotes enamel remineralization (6).

In addition to these systems, there are some natural products, such as plant products, that have the ability to promote enamel remineralization such as *Galla chinensis*, Hesperidin, Gum Arabic, Propolis, and Grape seed extract (GSE) have been found to suppress the demineralization and promote the remineralization (5).

The grape seed oil has been documented in the literature since the 14th century in Spain, and the king decided to protect its composition and termed it “oil of the throne” or “royal oil” because of its therapeutic properties (7). It contains a big number of phenolic compounds, including flavonoids, phenolic acids, carotenoids, stilbenes, and tannins; the main polyphenols called Proanthocyanidins (PACs) are catechins, epicatechins, procyanidin B1, and trans-resveratrol, which are biologically active constituents of GS. In addition, it contains gallic acid(8). Also, GS oil presents a wide variety of macro-elements and micro-elements, including phosphorus, potassium, and calcium(9).

In dentistry, Grape seed extract has been used in many fields, but in the preventive field, GSE is used for the prevention of dental caries, either by acting on the prevention of biofilm adhesion as antiplaque agents or acting against cariogenic bacteria as antibacterial agents or acting on prevention of collagen matrix degradation as bio modifier agents or enhancing the natural remineralization progression as remineralizing agents (10).

Several studies have encouraged the usage of grape seed extract (GSE) as a remineralizing efficiency for dentin lesions, but there is a deficiency of data about the effect of GS oil on the remineralization of WSLs (10). So, this study may be of worth, that intended to determine the effect of GS oil in two different concentrations (5% and 10%) incorporated in a paste on the demineralized enamel after bracket debonding and compared with CPP-ACPF. The null hypothesis verified that there were no statistically significant differences detected in the SMH test and SEM-EDX among the study groups.

## **MATERIALS AND METHODS**

### **Teeth selection**

Forty-four permanent sound premolar teeth were selected from patients undergoing orthodontic treatment, by means of an ethical protocol that was approved by the ethical committee at College of Dentistry, University of Mosul (approval no. **UoM.Dent 23/63**, November 15, 2023).

The collected teeth were rinsed with tap water, cleaned using a soft toothbrush to remove any tissue debris and blood, 0.1 % thymol solution was used for the storage of these collected teeth at room temperature until use. The teeth were excluded from the study if any enamel cracks, defects, WSLs, and fluorosis were observed on the buccal tooth surface that checked and examined by an LED light (11).

### **Sample preparation**

The teeth were mounted in plastic rings; first, the ring was filled to a half by dental stone, after stone setting; the tooth positioned perpendicular to the center of the ring base using sticky wax, after that the ring was adjusted on the surveyor's base as the surveyor's analyzing rod should be parallel with tooth's long axis then the ring was filled until cemento-enamel junction with auto polymerized acrylic resin, all the samples were polished with non-fluoridated pumice using polishing rubber cup, then the samples were kept at room temperature in distilled water until bonding procedure (12). Phosphoric acid 37% gel (Maquira/ Brazil) was used to etch the buccal enamel

surface for 30 sec., then washed away with water spray for 15 sec., and dried by compressed air for 10 sec. Premolar standard edgewise 0.022 brackets (Dentaurum/ Germany) were bonded with the fluorescent orthodontic adhesive (FIX/ Maquira/ Brazil) and light-cured ( Woodpecker/ Jiangsu/ China) for 10 seconds on each side (total 40 seconds) (13). After the bonding procedure, all specimens were kept at room temperature in distilled water for 24 hour and then, the bracket was removed by a universal testing machine (GESTER/ Fujian/ China) as shown in (Figure 1 a).

The remaining adhesive was removed from the buccal surface using truncated cone rounded-end, carbide bur (Ortho Technology/ England) as shown in (Figure.1 b) in a high-speed handpiece with water cooling (300,000 rpm) according to the manufacture instruction of the debonding bur, for 20 seconds, until no residue remained that can be visible by the fluorescent tracker of the adhesive system that used, then using a sof-lex polishing disc (Tor VM / Russia) in a low-speed handpiece(13).

The samples were arbitrarily separated into the following 4 groups (n = 11) based on the treatment of the enamel surface:

**Group I:** Control negative (demineralized only and stored in artificial saliva)

**Group II:** demineralized, followed by the application of CPP-ACFP paste

**Group III:** demineralized, followed by the application of GS paste 1

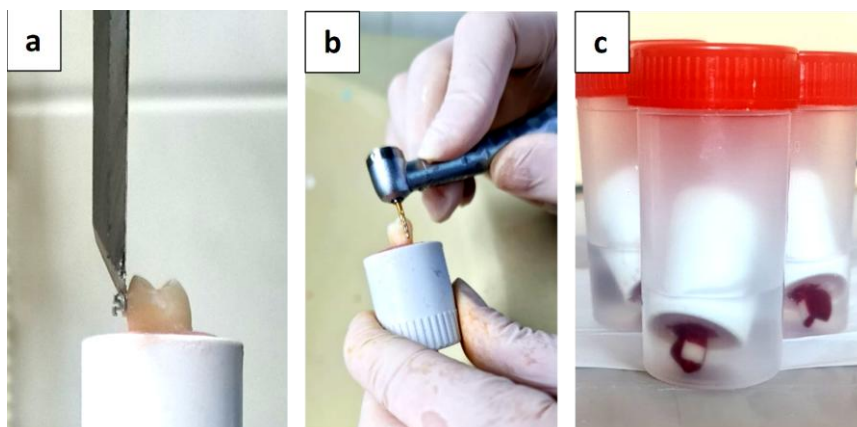
**Group IV:** demineralized, followed by application of GS paste 2.

All the specimens were kept in artificial saliva at room temperature, and after 30 days, they were subjected to surface microhardness using a Vickers microhardness tester (SMH) and Scan electron microscope with energy dispersive X-ray spectroscopy (SEM-EDX).

#### **Artificial carious lesions preparation**

The middle third, 5\*5 mm in size, of the buccal surface was wrapped by adhesive tape, and the entire tooth surface was varnished by using acid-resistant nail paint. to protect it. The adhesive tape was detached, leaving a 5\*5 mm buccal window. Every tooth was individually immersed in 15 ml of demineralizing solution for 96 h at 37°C.

as shown in (Figure.1 c). The demineralizing solution consisted of 2.2mM CaCl<sub>2</sub>, 2.2mM Na<sub>2</sub>HPO<sub>4</sub>, and 0.05 M CH<sub>3</sub>COOH with 1M KOH to obtain pH= 4.4 (14), that adjusted by using a pH meter (PH-009(I) A, China). The demineralizing solution was replaced with a fresher one every 24 h to maintain the pH constant.



**Figure (1):** (a) bracket removal by universal testing machine. (b) remanent adhesive removal, (c) samples in demineralizing solution.

- **GS paste1 preparation.** 1% (w/v) Carboxy methylcellulose (CMC) dissolved in hot distilled water to prepare CMC solution by using a magnetic stirrer (hot plate and magnetic stirrer/labnet/ Mexico) at 500 rpm with 40°C for about 30 min until complete dissolution. Then, 1% (w/v) Sodium lauryl sulfate (SLS) was dissolved in distilled water in another beaker to form an SLS aqueous solution. 30% (w/v) sorbitol with SLS solution was added gradually to the CMC solution under continuous stirring at 300 rpm at 40°C for 15 min until complete polymerization and a homogeneous mixture was obtained. A 5 % grape seed oil (Sigma-Aldrich, USA) was added dropwise and very slowly to the mixture under continuous stirring, then addition of 0.5% (w/v) titanium dioxide and 0.1% (w/v) of methylparaben for opacification and preservation with gentle stirring at room temperature until paste like formula was obtained(15). The prepared paste was then filled in dark and closed container labeled as 5 % GS and stored in the refrigerator until used.

- **GS paste2 preparation.** The same procedure of GS paste1 preparation, except that 10 % grape seed oil (Sigma-Aldrich, USA) was used, and the prepared paste was labeled as 10 % GS.

### **Grouping and Surface Treatment**

Group I (11 teeth): the samples were subjected to demineralization solution only and then kept in artificial saliva.

Group II, III and IV (33 teeth): after demineralization, the buccal surface through the window of the samples were varnished by a micro brush applicator with CPP-ACFP (GC America Inc. / USA), 5 % GS (paste 1) and 10 % GS (paste 2) respectively according to the instructions of manufacturer for CPP-ACPF (twice daily), and left undispensed for 3 min at that time spread in a circular motion over the surface for 1 min and then after 5min washed away with deionized water for 30s and kept in artificial saliva(16).

All the samples in all groups were stored in artificial saliva during the period (30 days) at 37 °C. The composition of artificial saliva according to Taqa *et al.* was as follows: 0.4g KCL, 0.4g NaCL, 0.780g NaH<sub>2</sub>PO<sub>4</sub> · 2H<sub>2</sub>O, 0.795g CaCL<sub>2</sub> · 2H<sub>2</sub>O, 0.005g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> · H<sub>2</sub>O and 1g CO(NH<sub>2</sub>)<sub>2</sub> dissolved in 1L deionized water and adjusted to pH=7 by KOH (17), and was replaced every 24 h to keep the pH constant.

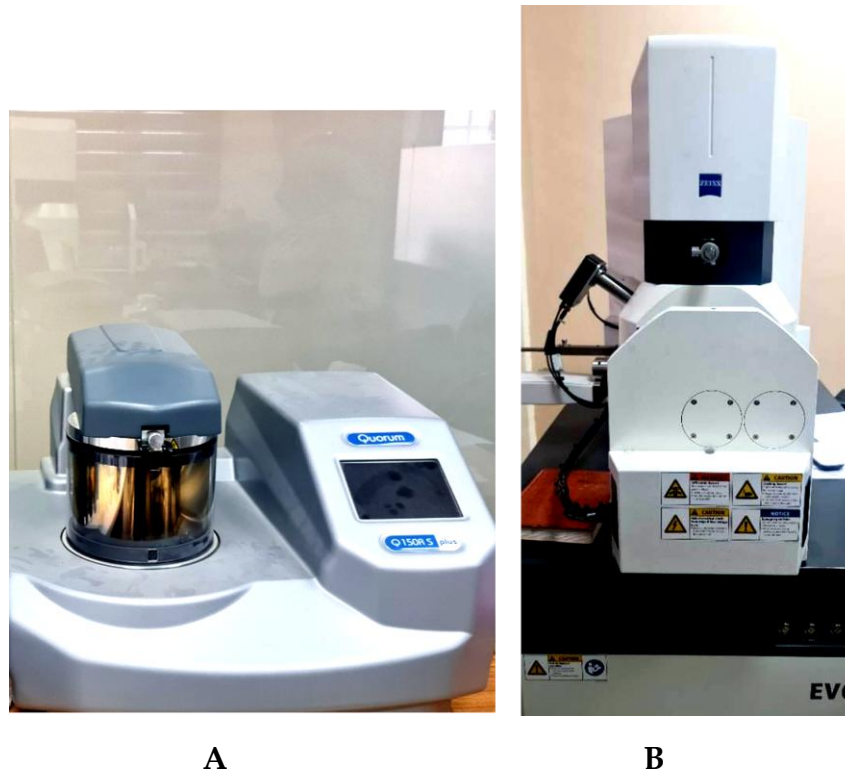
### **Surface Microhardness measurement (SMH)**

Surface microhardness test was carried out by using a Vickers microhardness tester (HVS 1000, Lian gong Co. Ltd, China) under 500 g load for 15 sec., which was constant throughout the entire study for all the samples (18). Three indentations were measured on the exposed enamel window of each sample, and the mean value of microhardness readings was calculated and considered.

### **Surface morphology and Elemental measurement (SEM-EDX)**

Three samples from each group were employed for this analysis, the specimens were decoronated from the mold and 8 mm in height rubber rings poured with auto polymerized acrylic resin used for holding the coronal portions of the teeth with the labial surface facing upward then dehydrated by ethanol, dried in air and gold coated by sputter coater (Quorum, Q300T T, USA) as shown in (Figure 2 a). SEM (Zeiss/

version 7.01/ Germany) as in (Figure .2 b) investigated the surface morphology of an enamel sample with 13 kV accelerating voltage. The elemental analysis of the enamel lesion surface was examined by EDS.



**Figure (2):** (a) gold coater sputter. (b) scanning electron microscope.

### Statistical analysis

The sample size for each test was computed regarding 80 % power with a statistical significance level determined at  $p < 0.05$ . The SPSS version 26 software program (SPSS IBM Statistics, USA) was used for statistical analysis. Data was explored the normality distribution using the Shapiro-Wilk test, and conducting parametric tests for SMH and non-parametric tests for EDX. The statistically significant level is set at ( $p < 0.05$ ).

## RESULTS

### Surface-Microhardness

According to the Shapiro-Wilk test, all the data were distributed normally. The mean value  $\pm$  standard deviation (SD) of the specimens in the all groups are shown in Table1. that revealed the 5 % GS group had the highest surface microhardness mean

value, One-way ANOVA and Tukey's post hoc test multiple comparisons are shown in Table 2 and Table 3 respectively, One-way ANOVA revealed a significant difference (0.004) among all study groups ( $p < 0.05$ ). Tukey's post hoc test showed that there was a significant difference (0.003) between 5 % GS group and the control negative group, but there were no significant differences among the samples treated with CPP-ACFP, 5 % GS, and 10 % GS at  $p < 0.05$ .

**Table (1):** Mean  $\pm$  Standard Deviation of Surface Microhardness

| Groups           | N | Mean $\pm$ SD      |
|------------------|---|--------------------|
| Control negative | 8 | 130.15 $\pm$ 39.89 |
| CPP-ACFP         | 8 | 211.12 $\pm$ 91.13 |
| 5 % GS           | 8 | 249.21 $\pm$ 19.75 |
| 10 % GS          | 8 | 181.15 $\pm$ 13.69 |

**Table (2):** One-way ANOVA of SMH

|                | Sum of Squares | df | Mean Square | F     | P-value |
|----------------|----------------|----|-------------|-------|---------|
| Between Groups | 59187.581      | 3  | 19729.194   | 5.441 | 0.004   |
| Within Groups  | 101520.744     | 28 | 3625.741    |       |         |
| Total          | 160708.325     | 31 |             |       |         |

Significant at  $p$  value  $< 0.05$

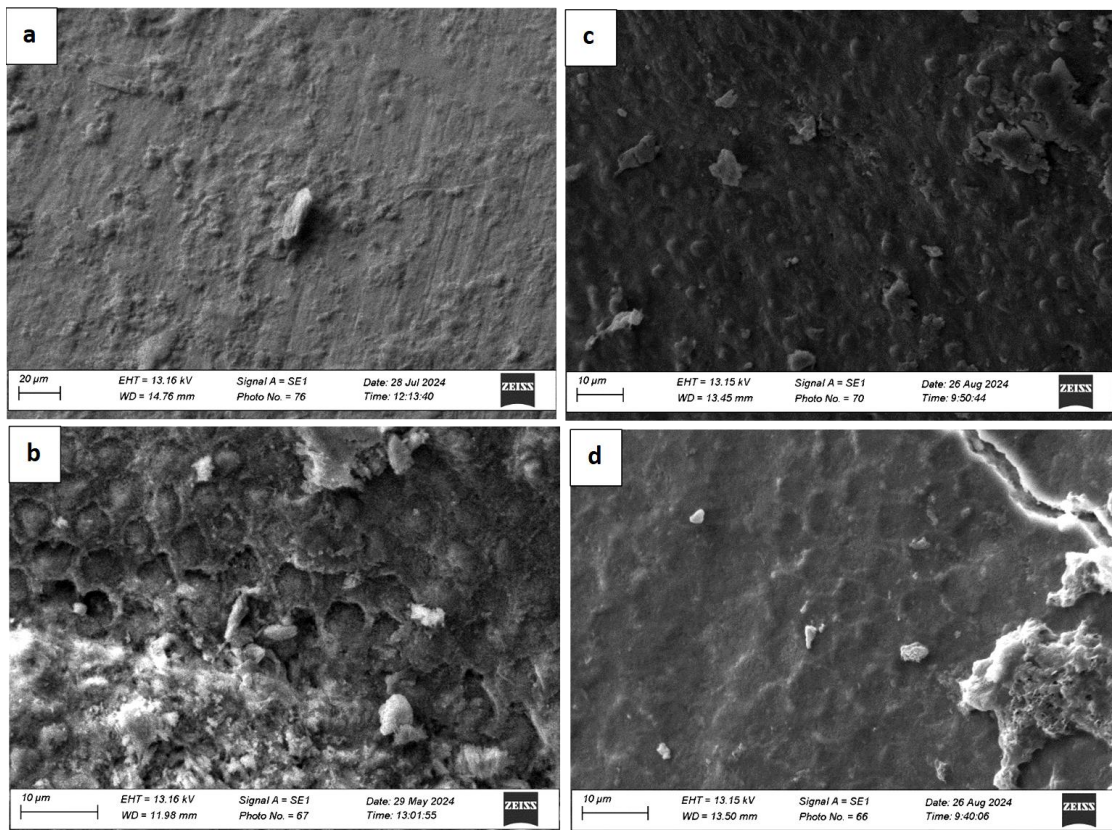
**Table (3):** Tukey post hoc test of SMH Multiple Comparisons

| Groups           |          | Mean Differences | Std. Error | P-value |
|------------------|----------|------------------|------------|---------|
| Control negative | CPP-ACPF | -79.52500        | 30.10706   | 0.061   |
|                  | 5 % GS   | -117.61250*      | 30.10706   | 0.003*  |
|                  | 10 % GS  | -49.55000        | 30.10706   | 0.370   |
| CPP-ACPF         | 5 % GS   | -38.08750        | 30.10706   | 0.592   |
|                  | 10 % GS  | 29.97500         | 30.10706   | 0.753   |
| 5 % GS           | 10 % GS  | 68.06250         | 30.10706   | 0.132   |

Significant at  $p$  value  $< 0.05$

**-Micro morphological observation**

SEM of the demineralized enamel surface revealed an irregular surface with discontinuity, scratches, and roughness consequential from the enamel crystals dissolution by the demineralizing process (Figure .3 a). In the samples treated with CPP-ACFP, SEM showed the deposition or formation of disaggregated nanoclusters with a globular structure like  $\text{CaF}_2$  deposits, and the honeycomb pattern can be observed (Figure .3 b). SEM of the samples treated with 5 % GS (paste 1) showed the normal surface with elevated humps of enamel rods without the honeycomb pattern (restoring the normal structure of enamel that appears as convexities) (Figure .3 c). SEM of the samples treated with 10 % GS (paste 2) revealed the enamel surface with erosion of the humps (concavities) of the enamel prisms with more density, and despite the honeycomb pattern being observed, the surface appeared as a dense, compact layer with less irregularity (Figure 3 d).



**Figure (3):** SEM of: (a) control negative group. (b) CPP-ACFP group. (c) 5% GS group. (d) 10 % GS group.

**-EDX**

The mean  $\pm$  SD of the Ca, P, and F elements in weight percentage, in addition to the Ca/P ratio of all groups, are shown in Table 4. There were no statistically significant differences according to the Kruskal-Wallis test between Ca, P, and F elements, in addition to the Ca/P ratio among the study groups, as shown in Table 5. But 5 % GS had the highest mean value of Calcium in weight percentage, followed by CPP-ACPF.

**Table (4):** Mean  $\pm$  standard deviation of Calcium, Phosphorus, and Fluoride

| Groups           | N | Mean $\pm$ SD    |                 |               |                |
|------------------|---|------------------|-----------------|---------------|----------------|
|                  |   | Ca               | P               | F             | Ca/P           |
| Control negative | 3 | 28.96 $\pm$ 4.0  | 17.70 $\pm$ 2.4 | 1.2 $\pm$ 0.3 | 1.64 $\pm$ .18 |
| CPP-ACFP         | 3 | 32.03 $\pm$ 10.6 | 16.70 $\pm$ 2.5 | 1.5 $\pm$ 0.4 | 1.88 $\pm$ .41 |
| 5 % GS           | 3 | 33.70 $\pm$ 0.5  | 16.40 $\pm$ 0.3 | 1.0 $\pm$ 0.3 | 2.05 $\pm$ .01 |
| 10 % GS          | 3 | 27.93 $\pm$ 3.8  | 11.50 $\pm$ 1.0 | 1.6 $\pm$ 0.3 | 2.41 $\pm$ .12 |

**Table (5):** Kruskal-Wallis test of Calcium, Phosphorus and Fluoride

| EDX         | Kruskal-Wallis | df | P-value |
|-------------|----------------|----|---------|
| Calcium     | 3.923          | 3  | 0.270   |
| Phosphorus  | 6.590          | 3  | 0.086   |
| Fluoride    | 4.751          | 3  | 0.191   |
| Ca/ P ratio | 7.964          | 3  | 0.05    |

Significant at *p-value* < 0.05

**DISCUSSION**

The removal of residual adhesive after orthodontic treatment is critical to avoid potential damage to the enamel surface. No technique has been established to efficiently and thoroughly remove the residual adhesive without producing minor damage to the enamel surface, such as roughness, cracking, wear, overheating of the teeth, and damage to the pulp (19). It is valued that (5–20)  $\mu\text{m}$  of the enamel is lost. Koprowski *et al.* evaluated the enamel quality after treatment and the cleaning-up procedure of bracket debonding through the use of computed tomography. The results revealed that the thickness of enamel after orthodontic treatment had reduced by around 125 $\mu\text{m}$  (20).

Many studies recommended different methods for the resin remnants removal, such as manual removal, rotary removal, ultrasonic devices, hydro abrasion, and laser. In the current study using 24-blade rounded-end carbide bur was used for removing a thick excess of adhesive if present, according to the concept of the ultraviolet fluorescent tracker that is present in orthodontic adhesive which enables the selective removal of the adhesive from the tooth sample followed by sof-lex polishing disc as it has better polishing of the enamel surface dependent on the D. Greenhalgh *et al.* (13)

A newer approach for the remineralization process of dental hard tissues is the use of natural products or nutraceuticals that may be considered the best choice for their biocompatibility, safety, shelf life, availability, efficacy, and low toxicity with low cost (10). Because of all previous studies conducted on grape seed extract efficacy, and there were no available data about the grape seed oil on the tooth surface, this study tested the potential effect of GS oil incorporated in paste with two different concentrations on the demineralized enamel surface.

In the current study, enamel surface micro-hardness and surface morphological analysis with elemental assessments were tested to study the effect of nutraceuticals' remineralization potential that exists in GS oil with two different concentrations (5% and 10%) compared to CPP-ACPF on demineralized enamel.

Vickers Micro-hardness test and SEM-EDX were chosen to be tested in this study as micro hardness test is the most appropriate for the enamel, and it is a simple, rapid, and nondestructive measurement that is used in cariology studies. SEM analysis is extremely sensitive to changes in the hard tissue surface(21), and EDX is indicative of chemical changes that occur as the minerals may be dissolved or replacement of ions may occur(22).

CPP-ACPF is one of the gold standards of treatment for early carious lesions of enamel, which is categorized as a bioavailable calcium phosphate remineralizing agent, and has displayed a satisfactory remineralization effect in many studies (16,22). An increase in the mean value of micro-hardness and Ca/P ratio after remineralization by CPP-ACPF was revealed in this study. CPP-ACPF nano complex, when applied on

the tooth surface, firmly binds and affords a reservoir of bioavailable phosphate, calcium, and fluoride ions that enter the enamel rods and increase the mineral contents and hardness values of the enamel surface after the demineralization process (23).

This study revealed no statistically significant difference in enamel surface microhardness amongst GS oil with two concentrations and CPP-ACPF groups, but there is a significant increase in the mean value of microhardness value after remineralization with GS oil at 5 % concentrations compared to control negative group, this outcome is in agreement with results of Hameed *et al.*(10), Rao *et al.* (21), and Sebastian *et al.* (24), and this can be attributed to the GS oil constituents, especially proanthocyanidins (PACs) and gallic acid (GA); several studies exhibited that type I collagen and type X collagen are existing in enamel matrix, and these collagens are proline-rich proteins that interact with PAs and form proline-PA complex, increasing cross-links collagen, and this increase could occur by different interactions either ionic, covalent, hydrogen bonding or hydrophobic interaction (22,25,26).

Collagen cross—links have two terminal groups: carboxyl (-OH) and amine (NH<sub>3</sub><sup>+</sup>) groups, and through these groups, these collagen peptides combine with hydroxyapatite (HA) crystal ions that facilitate the absorption of collagen peptide into the surfaces of HA, and this existence boost the hydroxyapatite growth (10). In addition to the gallic acid (GA) that interacts with Ca<sup>++</sup> from the surrounding media, forming the GA-Ca<sup>+2</sup> complex, its insoluble compounds, thus GA was supposed to enable the deposition of mineral, especially on the surface (22). According to these results, it is predictable that exogenous collagen cross- links involves in the remineralization process of WSL by GS oil.

According to these findings SEM micrographs showed a remarkable variation in the enamel surface, the samples treated with 5% GS oil revealed that GS oil restoring the natural features of enamel surface that appear as humps of prisms (convex structures) when comparing with other study groups, while the enamel surface of samples treated with 10% GS oil showed erosion of the humps (concave) with deposition of few irregular nanoparticles and these outcomes agree with Nagi *et al.*

(22), findings as when used 6% GSE, enamel surface porosities occluded with precipitations whereas 10% GSE, deposition of insoluble complexes with globular agglomerates (22, 23).

These results were reinforced by EDX outcomes of 5 % GS oil samples that revealed the highest Calcium mean value and Ca/P ratio among the study groups, and there were no significant differences between the two concentrations of GS oil and CPP-ACFP, but the 10 % GS group showed a slight increase in Ca/ P ratio with control negative group.

As there was an increase in the surface micro hardness of demineralized enamel, improvement in enamel surface morphology, and an increase in Calcium and Ca/P ratio mean value after treatment with experimentally prepared GS oil paste and CPP-ACPF paste, the null hypothesis was rejected.

## CONCLUSIONS

Within the limitations of the current study, it is possible to conclude that: The prepared paste from grape seed oil has a positive effect on the remineralization of the demineralized de-bonded enamel, making the grape seed oil an effective remineralizing potential natural agent.

## Editorial Independence Statement

**Dr. Ali R. Al-Khatib** is a co-author of this article and serves as Editor-in-Chief of *Al-Rafidain Dental Journal*. In accordance with the journal's editorial conflict-of-interest and independence policies, he was fully excluded from all editorial and peer-review processes related to this manuscript. This submission was processed under the journal's standard peer-review procedures and was handled independently by Dr. Mohammed Abdullatif Abdulla (Editorial Manager), who declared no conflict of interest and had full responsibility for the editorial evaluation and the final publication decision.

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#### **Authors' Contribution**

Agha NN., Qasim AA., Al-Khatib AR., Hassan R. completed the conceptualization, methodology, project administration, data curation, formal analysis, finding acquisition, investigation, resources, software, validation, visualization, writing-original draft, writing- review, and editing. Supervised by Qasim AA., and Al-Khatib AR.

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**Ethical statement:** Ethical approval was obtained from the Research Ethics Committee of the College of Dentistry, the University of Mosul, in 2023 (Ref. No. UoM.Dent.23/31).

#### **Conflict of interest**

The author declares that there are no conflicts of interest regarding the publication of this manuscript

**Availability of data and materials:** All data generated or analyzed during this study are included in this published article and its supplementary information files.

#### **Declaration of Generative AI and AI-assisted technologies**

During the preparation of this work, the authors used a grammar tool to improve the readability of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

#### **REFERENCES**

1. Berrada G. Dental Enamel: Integrating Mineral Chemistry, Biochemistry, Cell Biology and Genetics. Univ Eur Madrid [Internet]. 2021; Available from: <http://hdl.handle.net/20.500.12880/524>
2. Sarafopoulou S, Zafeiriadis A, Tsolakis A. Enamel defects during orthodontic treatment. *Balk J Dent Med* [Internet]. 2018;22(3):64–73.
3. Naveed N, Sabapathy K. Treatment Of

- White Spot Lesions Post Fixed Orthodontic Therapy. *Eur J Mol Clin Med.* 2020;7(8):1824–9. doi:[10.2478/bjdm-2018-0012](https://doi.org/10.2478/bjdm-2018-0012)
4. Gocmen GB, Yanikoglu F, Tagtekin D, Stookey GK, Schemehorn BR, Hayran O. Effectiveness of some herbals on initial enamel caries lesion. *Asian Pac J Trop Biomed* [Internet]. 2016;6(10):846–50. doi:[10.1016/j.apjtb.2016.08.005](https://doi.org/10.1016/j.apjtb.2016.08.005)
  5. Grohe B, Mittler S. Advanced non-fluoride approaches to dental enamel remineralization: The next level in enamel repair management. *Biomater Biosyst* [Internet]. 2021;4:100029. doi:[10.1016/j.bbiosy.2021.100029](https://doi.org/10.1016/j.bbiosy.2021.100029)
  6. Shuping I, Lei M, Burrow MF, Lo EC man, Chu C hung. Prevention of secondary caries using silver diamine fluoride treatment and casein phosphopeptide-amorphous calcium phosphate modified glass-ionomer cement. *J Dent* [Internet]. 2016;1–7. doi:[10.1016/j.jdent.2016.12.001](https://doi.org/10.1016/j.jdent.2016.12.001)
  7. Martin ME, Grao-cruces E, Millan-linares MC, Paz SM de. Grape ( *Vitis vinifera* L .) Seed Oil : A Functional Food from the Winemaking Industry. *Foods.* 2020;9, 1360:1–20. doi:[10.3390/foods9101360](https://doi.org/10.3390/foods9101360)
  8. Garavaglia J, Markoski MM, Oliveira A, Marcadenti A. Grape Seed Oil Compounds : Biological and Chemical Actions for Health. *Nutr Metab Insights.* 2016;9:59–64. doi:[10.4137/NMI.S32910](https://doi.org/10.4137/NMI.S32910)
  9. Pop A, Iurian SM, Benedec D, Moldovan ML. Research Advances in the Use of Bioactive Compounds from *Vitis vinifera* By-Products in Oral Care. *Antioxidants.* 2020;9,502:1–32. doi:[10.3390/antiox9060502](https://doi.org/10.3390/antiox9060502)
  10. Hameed HM, Adel A, Tahlawy E, Saniour SH. Assessment of the Remineralizing Efficacy of Grape Seed Extract vs Sodium Fluoride on Surface and Subsurface Enamel Lesions : An In Vitro Study. *J Contemp Dent Pr.* 2022;23(12):1237–44. doi:[10.5005/jp-journals-10024-3442](https://doi.org/10.5005/jp-journals-10024-3442)
  11. Nalbantgil D, Oztoprak MO, Cakan DG, Bozkurt K, Arun T. Prevention of demineralization around orthodontic brackets using two different fluoride varnishes. *Eur J Dent.* 2013;7(1):41–7. doi:[10.1055/s-0039-1698994](https://doi.org/10.1055/s-0039-1698994)

12. Yaseen SN, Taqa AA, Al-khatib AR. The effect of incorporation Nano Cinnamon powder on the shear bond of the orthodontic composite ( an in vitro study ). J Oral Biol Craniofacial Res [Internet]. 2020;10(2):128–34. Available from: [doi.org/10.1016/j.jobcr.2020.03.008](https://doi.org/10.1016/j.jobcr.2020.03.008)
13. Thys DG, Martins FRP, Cardinal L, Ribeiro GLU. In vitro enamel surface roughness analysis of 4 methods for removal of remaining orthodontic adhesive after bracket debonding. Angle Orthod. 2023 Mar 1;93(2):213–21. doi:[10.2319/031722-227.1](https://doi.org/10.2319/031722-227.1)
14. Altinisik H, Erten H. Evaluation of the activities of toothpastes with different contents in the prevention of enamel demineralization. Curr Res Dent Sci [Internet]. 2022 Mar 17;33(1):27–34. doi:[10.54614/CRDS.2022.6199](https://doi.org/10.54614/CRDS.2022.6199)
15. Hamdi K, Hamama HH, Motawea A, Fawzy A, Mahmoud SH. Remineralization of early enamel lesions with a novel prepared tricalcium silicate paste. Sci Rep [Internet]. 2022;12:9926:1–11. doi:[doi.org/10.1038/s41598-022-13608-0](https://doi.org/10.1038/s41598-022-13608-0)
16. Almarsomy DH, Al-khayat FA, Al-tae LA. The preventive / therapeutic effect of CO 2 laser and MI Paste Plus ® on intact and demineralized enamel against Streptococcus mutans ( In Vitro Study ). Heliyon [Internet]. 2023;9(10):e20310. Available from: [doi.org/10.1016/j.heliyon.2023.e20310](https://doi.org/10.1016/j.heliyon.2023.e20310)
17. Taqa AA, Al-sarraf HA. Artificial Saliva Sorption for Three Different Types of Dental Composite Resin (An In Vitro Study). EC Dent Sci. 2019;18(10):2339–44.
18. Rashid SM, Qasim AA. Remineralizing Effect of GC Tooth Mousse versus Acidulated Phosphate Fluoride on the Surface Microhardness of the Demineralized Enamel. Iraqi Dent J. 2023;45(2):1–8. doi:[10.46466/idj.v45i2.280](https://doi.org/10.46466/idj.v45i2.280)
19. Ghaleb L, Ali N, Worafi A, Thawaba A, Abdulqader AA, Alkamel A, et al. Evaluation of enamel surface integrity after orthodontic bracket debonding : comparison of three different system. BMC Oral Health [Internet]. 2024;24:358:1–11. Available from: [doi.org/10.1186/s12903-024-04138-4](https://doi.org/10.1186/s12903-024-04138-4)
20. Tarcísio J, Ferreira L, Borsatto MC, Torres CP, Romano FL, Conceição M, et al. Evaluation of Enamel Roughness in Vitro After Orthodontic Bracket Debonding Using

- Different Methods of Residual Adhesive Removal. Turk J Orthod. 2020;33(1):43–51. doi:10.5152/TurkJOrthod.2020.19016
21. Rao D, Panwar S, Kothari N. An in vitro comparative evaluation of casein phosphopeptide-amorphous calcium phosphate fluoride, tricalcium phosphate and grape seed extract on remineralization of artificial caries lesion in primary enamel. J Clin Pediatr Dent 2022. 2022;46(5):72–80. doi:10.22514/jocpd.2022.010
  22. Nagi SM, Hassan SN, El-alim SHA, Elmissiry MM. Remineralization potential of grape seed extract hydrogels on bleached enamel compared to fluoride gel : An in vitro study. J Clin Exp Dent. 2019;11(5):e401-7. doi:10.4317/jced.55556
  23. Reynolds EC. Casein Phosphopeptide-Amorphous Calcium Phosphate: The Scientific Evidence. Adv Dent Res [Internet]. 2009 Aug 7;21(1):25–9. doi:10.1177/0895937409335619
  24. Sebastian R, Paul ST, Azher U, Reddy D. Comparison of Remineralization Potential of Casein Phosphopeptide : Amorphous Calcium Phosphate , Nano- hydroxyapatite and Calcium Sucrose Phosphate on Artificial Enamel Lesions : An In Vitro Study. Int J Clin Pediatr Dent. 2022;15(1):69–73. doi:10.5005/jp-journals-10005-2339
  25. Mirkarimi M, Eskandarion S, Bargrizan M, Delazar A KM. Remineralization of artificial caries in primary teeth by grape seed extract: An in vitro study. J Dent Res Dent Clin Dent Prospect. 2013;7:206–10. doi:10.5681/joddd.2013.033
  26. Açıl Y, Mobasser AE, Warnke PH, Terheyden H, Wiltfang J SI. Detection of mature collagen in human dental enamel. Calcif Tissue Int. 2005;76:121–6. doi:10.1007/s00223-004-0122-0

فعالية معجون زيت بذور العنب في إعادة تمعدن المينا المنزوعة المعادن بعد إزالة التقويم مقارنةً بمعجون فوسفات الكازين وفوسفات فلوريد الكالسيوم غير المتبلور: دراسة مخبرية

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**المخلص**

**الأهداف:** هدفت الدراسة الى تقييم فعالية زيت بذور العنب المندمج في معجون بتراكيز مختلفة ومقارنته مع معجون الكازين فوسفو بيبنتيد الكالسيوم الغير متبلور المفلور على إعادة تمعدن الافة الصناعية على سطح مينا الاسنان بعد إزالة الحاصرة التقويمية. **المواد وطرائق العمل:** تم تجميع ٤٤ ضاحكا سليما لهذه الدراسة وبعد عميلة اسناد تثبيت الاسنان في قوالب خاصة وتثبيت الحواصر التقويمية ومن ثم ازالتها . تم تقسيم الاسنان الى اربع مجموعات بشكل عشوائي. تم انشاء الافة النخرية الصناعية عن طريق غمر العينات في محلول إزالة المعادن. ثم علاج سطح المينا عن طريق :- المجموعة ١: ( ١١ عينة) اللعاب الاصطناعي. المجموعة ٢:- ( ١١ عينة) معجون الكازين فوسفو بيبنتيد الكالسيوم الغير متبلور المفلور. المجموعة ٣ :- ( ١١ عينة) ٥٪ زيت بذور العنب (معجون ١). المجموعة ٤ :- ( ١١ عينة) ١٠٪ زيت بذور العنب (معجون ٢). تم تقييم سطح المينا بواسطة اختبار الصلادة الدقيقة للسطح والفحص المجهرى الالكتروني والمطياف بالأشعة السينية المشتت للطاقة بعد مدة ٣٠ يوما من العلاج. **النتائج:** أظهرت نتائج الصلادة السطحية وجود فرق ذو دلالة إحصائية عالية بين جميع المجموعات بعد العلاج , وشوهدت اكبر قيمة لمتوسط الصلادة السطحية في مجموعة ٥ ٪ زيت بذور العنب. بينما أظهرت نتائج المطياف بالأشعة السينية المشتت للطاقة لا يوجد فرق ذو دلالة إحصائية بين جميع المجموعات بعد العلاج. و مشاهدات الفحص المجهرى الالكتروني أظهرت وجود طبقة وقائية متكونة على السطح في المجاميع المعالجة. **الاستنتاجات:** زيت بذور العنب المستخدم في هذه الدراسة اظهر التأثير في إعادة التمعدن للافة النخرية الصناعية لمينا الاسنان.

**الكلمات المفتاحية:** زيت بذور العنب، CPP-ACPF، إزالة الروابط، إزالة المعادن، إعادة التمعدن