Original paper

Effects of different concentrations of bromelain and papain enzymes on shear bond strength of composite resin to deep dentin using an etch-and-rinse adhesive system

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D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(1):85–91

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Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors would like to thank the Vice-Chancellor of Shiraz University of Medical Sciences for supporting the research. This manuscript is relevant to thesis No. 1396-01-03-14585, and is based on the thesis of Dr. Mina Safari. The authors would also like to thank Dr. Mehrdad Vossoughi for statistical analysis of the data, Research Editor Company for editing this article and the Biomaterials Research Center for testing the specimens.

Received on October 16, 2020
Reviewed on February 11, 2021
Accepted on February 16, 2021

Published online on February 29, 2024

Cite as
Sharafeddin F, Jowkar Z, Safari M. Effects of different concentrations of bromelain and papain enzymes on shear bond strength of composite resin to deep dentin using an etch-and-rinse adhesive system. *Dent Med Probl.* 2024;61(1):85–91. doi:10.17219/dmp/133404

DOI
10.17219/dmp/133404

Abstract

Background. The dentin substrate can be modified by proteolytic agents, which may affect the bonding strength of adhesive systems to the treated dentin surface. Papain, a cysteine protease enzyme with antibacterial and anti-inflammatory properties, can be used for deproteinization of dentin. An alternative deproteinizing enzyme is bromelain.

Objectives. This study aimed to evaluate the impact of deproteinization on the shear bond strength (SBS) of composite resin to deep dentin using different concentrations of bromelain and papain.

Material and methods. Sixty upper premolars were extracted and randomly divided into 5 groups ($n = 12$ per group). In all groups, the dentin surface was etched with 37% phosphoric acid. Group 1 did not receive any enzyme treatment, group 2 was treated with a 10% papain solution, group 3 was treated with a 15% papain solution, group 4 was treated with a 6% bromelain solution, and group 5 was treated with a 10% bromelain solution. After applying an etch-and-rinse adhesive system, the specimens were restored with composite resin and the SBS was measured.

Results. Statistically significant differences were found between groups 2 and 3 (10% papain and 15% papain, $p = 0.004$), groups 2 and 4 (10% papain and 6% bromelain, $p = 0.017$), groups 4 and 5 (6% bromelain and 10% bromelain, $p = 0.021$), and groups 3 and 5 (15% papain and 10% bromelain, $p = 0.005$).

Conclusions. Deproteinization with papain and bromelain at different concentrations after acid etching did not affect the SBS of composite resin to deep dentin when using an etch-and-rinse adhesive system. However, the group deproteinized with 15% papain demonstrated a higher SBS than the group deproteinized with 10% papain, and the group deproteinized with 6% bromelain showed a higher SBS compared to the group deproteinized with 10% bromelain.

Keywords: shear bond strength, papain, bromelain, dentin

Introduction

The mechanism of bonding to dentin is based on the hybridization concept. In etch-and-rinse adhesive systems, acid etching agents are applied to the dentin surfaces to remove the smear layer, demineralize the dentin and expose the collagen fibril network. Adhesion occurs through the diffusion of adhesive monomers into the exposed collagen layer and subsequent formation of the hybrid layer.[1–4] However, excessive dehydration can cause the collapse of the collagen fibril network, reducing the infiltration of monomers into deeper areas and increasing the risk of adhesive failure. On the other hand, excess water prevents penetration and polymerization of the bonding systems.[5,6]

Improving the physical properties of the bonding agent or modifying the dentin substrate itself can enhance dentin bonding.[7–10] In a process known as dentin deproteinization, proteolytic agents are used to modify acid-etched dentin and eliminate the organic content of the dentin substrate. Deproteinizing solutions modify the dentin surface by dissolving exposed collagen fibrils, leading to greater exposure of the dentinal tubules. This results in dentin that is more similar to etched enamel, which has promising characteristics for promoting adhesion. This type of surface has shown multiple irregularities, with good mechanical retention of the adhesive in the modified dentin substrate.[11–13]

Pre-treatment with proteolytic enzymes has been recommended to achieve better adhesion to dentin.[8,11] Papain is a proteolytic enzyme that removes caries without damaging surrounding tissues. It is extracted from the ripe fruit of *Carica papaya*, a member of the Caricaceae family. The enzyme is a cysteine protease that has demonstrated antibacterial and anti-inflammatory properties.[8,14] It has been reported that the use of 10% papain as a deproteinizing agent before acid etching increases subsequent bond strength by removing organic elements.[15] Another related study showed that the highest bond strength values of orthodontic brackets bonded with resin-modified glass ionomer cement (RMGIC) were attained after enamel deproteinization with 8% and 10% papain, which were more effective than lower concentrations of the enzyme (2%, 4% and 6%).[14]

Bromelain is a deproteinizing enzyme commercially extracted from the fruit or stem of the pineapple.[16] It has been shown to improve the bond strength when applied after acid etching of dentin.[11] However, no study has compared the effects of different concentrations of bromelain and papain enzymes on the shear bond strength (SBS) of composite resin to deep dentin. The aim of this investigation was to evaluate the effects of treatment with different concentrations of enzymes on the SBS of composite resin to deep dentin using an etch-and-rinse adhesive system. The null hypothesis states that there is no correlation between the application of different concentrations of bromelain and papain enzymes as dentin pre-treatments and the SBS of subsequent composite bonding to deep dentin.

Material and methods

Specimen preparation

The Ethics Committee of Shiraz University of Medical Sciences approved the research protocol (approval No. IR.SUMS.REC.1396.S509). For this in vitro experimental study, we collected 60 extracted human upper premolars that were free of caries, restoration or cracks. The teeth were washed under running water to remove residual debris and tissue, and stored in a 0.1% thymol solution at 4°C for 1 week. Afterwards, the teeth were thoroughly washed with tap water and embedded in self-cure acrylic resin (Acropars; Marlik Co., Tehran, Iran) up to the cement–enamel junction. The occlusal surface was positioned parallel to the acrylic resin surface, making it ready for experimental surface preparation. The occlusal thirds of the crowns were sectioned perpendicular to the long axis of the tooth using a water-cooled, low-speed cutting machine (Mecatome T201 A; Presi, Grenoble, France) to remove the occlusal enamel and superficial dentin, and obtain flat, deep dentin surfaces. To polish the superficial dentin and create a uniform smear layer, we applied 600 grit silicon carbide paper to the prepared surfaces. Papain powder (Organika, Vancouver, Canada) and bromelain enzyme powder (Biozym Scientific GmbH, Olendorf, Germany) were weighed using a balance with an accuracy of ±0.1 mg (GR-300; A&D Company Ltd., Tokyo, Japan), and added to distilled water to achieve different concentrations of these enzymes. Specifically, 10 g and 15 g of papain powder were added to 100 mL of distilled water to prepare 10% and 15% papain solutions, respectively. Additionally, 6 g and 10 g of bromelain powder were added to 100 mL of distilled water to achieve concentrations of 6% and 10%, respectively. The 60 teeth were randomized into 5 groups (n = 12 per group). Each group was assigned to a different method of dentin pre-treatment.

For group 1, the dentin surface was etched with 37% phosphoric acid (DenFil; VERICOM Co., Ltd., Chuncheon, Korea) for 15 s, then rinsed with distilled water for 10 s and blot dried. For groups 2 and 3, the dentin surface was etched with 37% phosphoric acid for 15 s, rinsed with distilled water for 15 s, and blot dried with a cotton pellet to remove excess water. The surface was then treated with 10% papain (group 2) or 15% papain (group 3) for 60 s, washed with distilled water for 15 s and blot dried. For groups 4 and 5, the dentin surface was etched with 37% phosphoric acid for 15 s, rinsed with distilled water for 10 s and blot dried. Subsequently, the dentin surface was treated with 6% bromelain (group 4) or 10% bromelain (group 5) for 60 s, washed with distilled water for 15 s and blot dried.

After preparing the dentin surface, composite resin was bonded to 10 specimens in each group for SBS testing. The remaining 2 specimens in each group were prepared for evaluation using scanning electron microscopy (SEM), as described below.

Shear bond strength testing

An etch-and-rinse system (Adper Single Bond 2; 3M ESPE, St. Paul, USA) was applied to the treated dentin surfaces, according to the manufacturer's instructions. A light-emitting diode (LED) polymerizing unit (Bluelex GT-1200; MONITEX Industrial Co., Ltd., New Taipei City, Taiwan) with a wavelength of 470 nm and a light intensity of 1,200 mW/cm^2 was used for curing. Subsequently, a plastic mold with a height of 2 mm and an internal diameter of 3 mm was placed over the prepared dentin surface. A 2-mm thick increment of composite resin (Filtek™ Z350; 3M ESPE) was inserted into the mold and light cured for 40 s from the occlusal direction. The mold was removed and the specimens were stored in distilled water at 37°C for 24 h in an incubator (ES 252; NÜVE, Ankara, Turkey) before testing. The specimens were individually transferred to the universal testing machine (Z020; ZwickRoell, Ulm, Germany) and subjected to SBS analysis at a crosshead speed of 1 mm/min. The experimental design used in this study is presented in Fig. 1. Figure 2 shows a prepared specimen transferred to the universal testing machine.

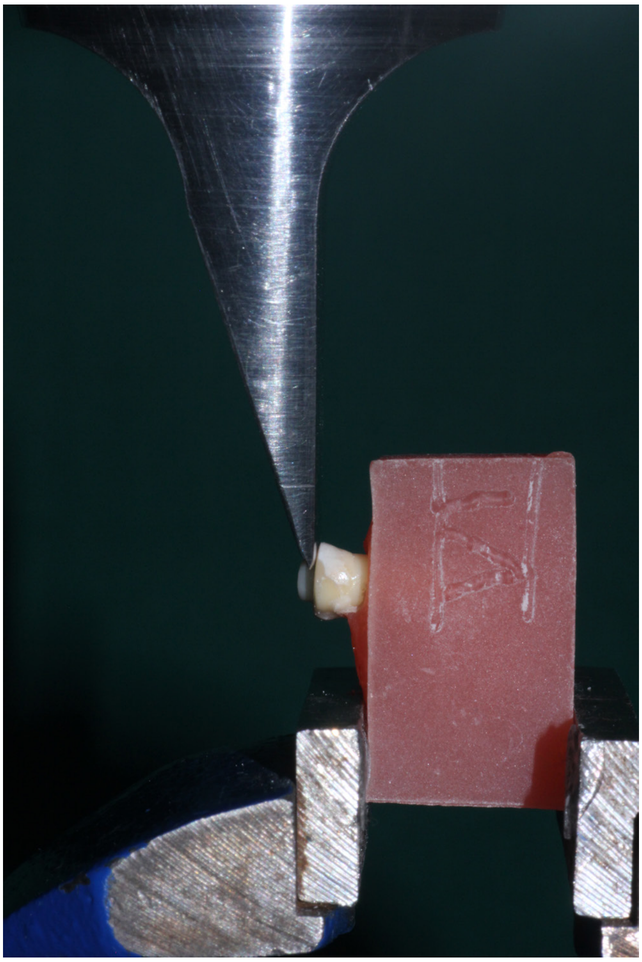

Fig. 2. Sample prepared and transferred to the universal testing machine for measuring the shear bond strength

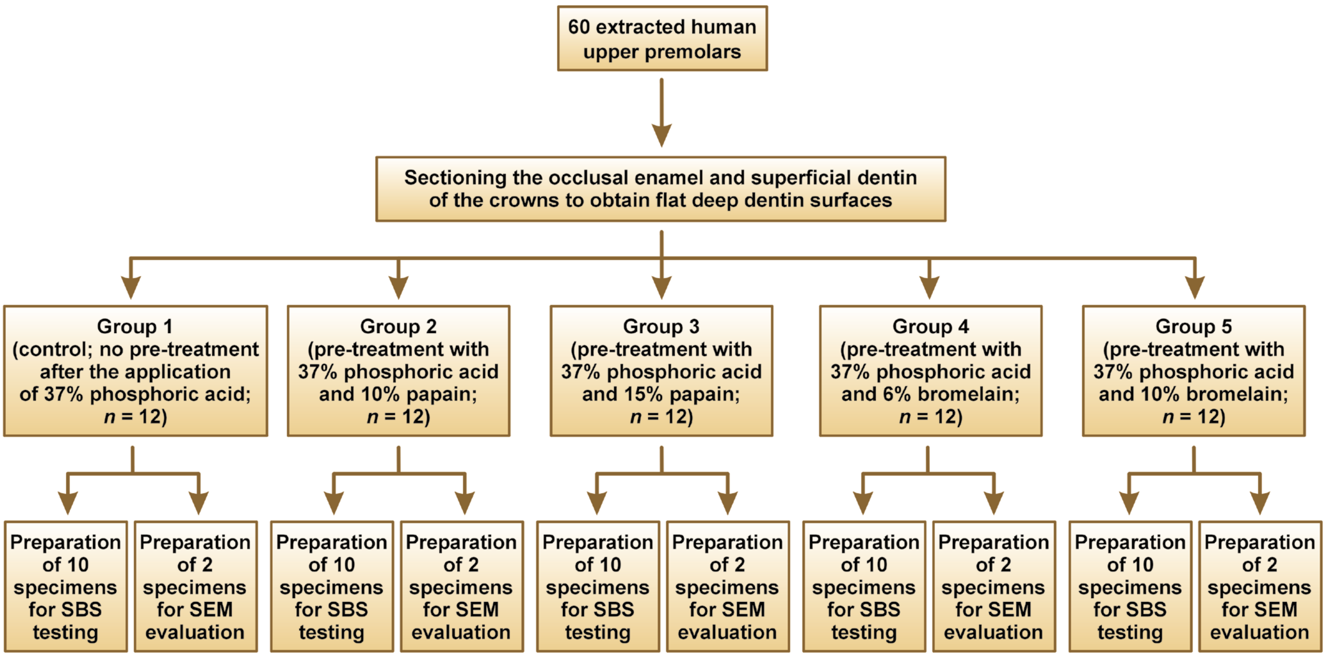

Fig. 1. Schematic diagram of the study design

SBS – shear bond strength; SEM – scanning electron microscopy.

Scanning electron microscopy evaluation

Two specimens from each group were evaluated using a scanning electron microscope (KYKY-EM3200; KYKY Technology Co. Ltd., China). The specimens were initially sectioned using a diamond disc to obtain dentin samples with a thickness of 2 mm. These samples were then dried in a desiccator for 24 h, sputter-coated with gold and examined under the microscope at 2 different magnifications (×3,000 and ×4,000).

Statistical analysis

Statistical analysis was conducted using SPSS for Windows software, v. 16 (SPSS Inc., Chicago, USA), with data analysis performed using one-way analysis of variance (ANOVA) followed by Tukey's test. The significance level set for the study was $p < 0.05$.

Results

The descriptive statistics of the experimental SBS values for all groups, including the mean (*M*), standard deviation (*SD*), and minimum and maximum values, are presented in Table 1. Moreover, the *M* and *SD* values of the SBS for all groups are shown in Fig. 3. One-way ANOVA revealed statistically significant relationships between the experimental groups ($p < 0.05$). The study results indicate that group 3 (37% phosphoric acid + 15% papain) and group 4 (37% phosphoric acid + 6% bromelain) had the highest mean SBS values compared to the other experimental groups.

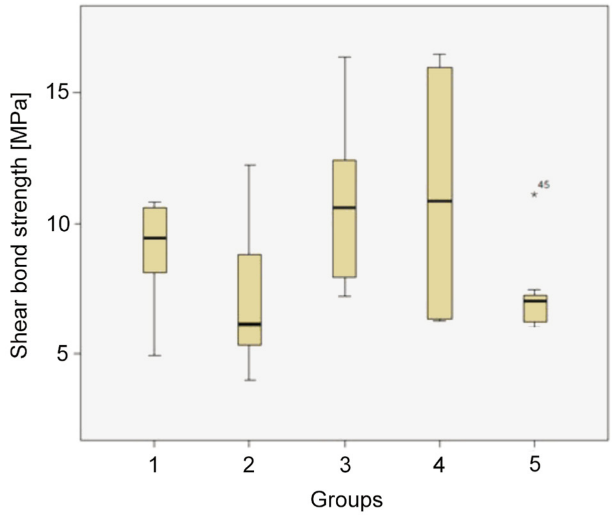

Fig. 3. Shear bond strength values in the experimental groups

group 1 – the control group etched with 37% phosphoric acid; group 2 – treated with 37% phosphoric acid and 10% papain; group 3 – treated with 37% phosphoric acid and 15% papain; group 4 – treated with 37% phosphoric acid and 6% bromelain; group 5 – treated with 37% phosphoric acid and 10% bromelain.

Tukey's test was used to compare the mean SBS values among all experimental groups (Table 2). The results showed statistically significant differences between the mean SBS values of group 2 (37% phosphoric acid + 10% papain) and group 3 (37% phosphoric acid + 15% papain), as well as between the mean SBS values of group 2 (37% phosphoric acid + 10% papain) and group 4 (37% phosphoric acid + 6% bromelain), with *p*-values of 0.004 and 0.017, respectively. The differences between the mean SBS values of group 4 (37% phosphoric acid + 6% bromelain)

Table 1. Shear bond strength values of the study groups

Study group	Shear bond strength [MPa]		
	M ±*SD*	minimum	maximum
Group 1 (37% phosphoric acid)	8.919 ±2.01	4.91	10.80
Group 2 (37% phosphoric acid and 10% papain)	6.98 ±2.59	3.99	12.20
Group 3 (37% phosphoric acid and 15% papain)	10.90 ±3.44	7.20	16.40
Group 4 (37% phosphoric acid and 6% bromelain)	10.83 ±4.40	6.28	16.50
Group 5 (37% phosphoric acid and 10% bromelain)	6.77 ±0.54	6.04	7.47

M – mean; *SD* – standard deviation.

Table 2. Pairwise comparison of mean shear bond strength values between all groups using Tukey's test

Study group	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1 (37% phosphoric acid)	–	0.063	0.309	0.606	0.072
Group 2 (37% phosphoric acid and 10% papain)	0.063	–	0.004*	0.017*	0.991
Group 3 (37% phosphoric acid and 15% papain)	0.309	0.004*	–	0.616	0.005*
Group 4 (37% phosphoric acid and 6% bromelain)	0.606	0.017*	0.616	–	0.021*
Group 5 (37% phosphoric acid and 10% bromelain)	0.072	0.991	0.005*	0.021*	–

* statistically significant ($p < 0.05$; Tukey's post hoc test).

and group 5 (37% phosphoric acid + 10% bromelain), and between the mean SBS values of group 3 (37% phosphoric acid + 15% papain) and group 5 (37% phosphoric acid + 10% bromelain), were also statistically significant, with p-values of 0.021 and 0.005, respectively. However, no statistically significant differences were found between group 1 (control) and the other experimental groups (all p-values > 0.05).

Figure 4 presents the SEM images of the dentin surfaces in the various experimental groups, displaying the surface topography of the dentin substrate after different treatments. There was no collagen network covering the peritubular dentin in group 2 (37% phosphoric acid + 10% papain), group 3 (37% phosphoric acid + 15% papain) or group 4 (37% phosphoric acid + 6% bromelain). The orifices of the dentinal tubules in these groups were wider than those in the control group.

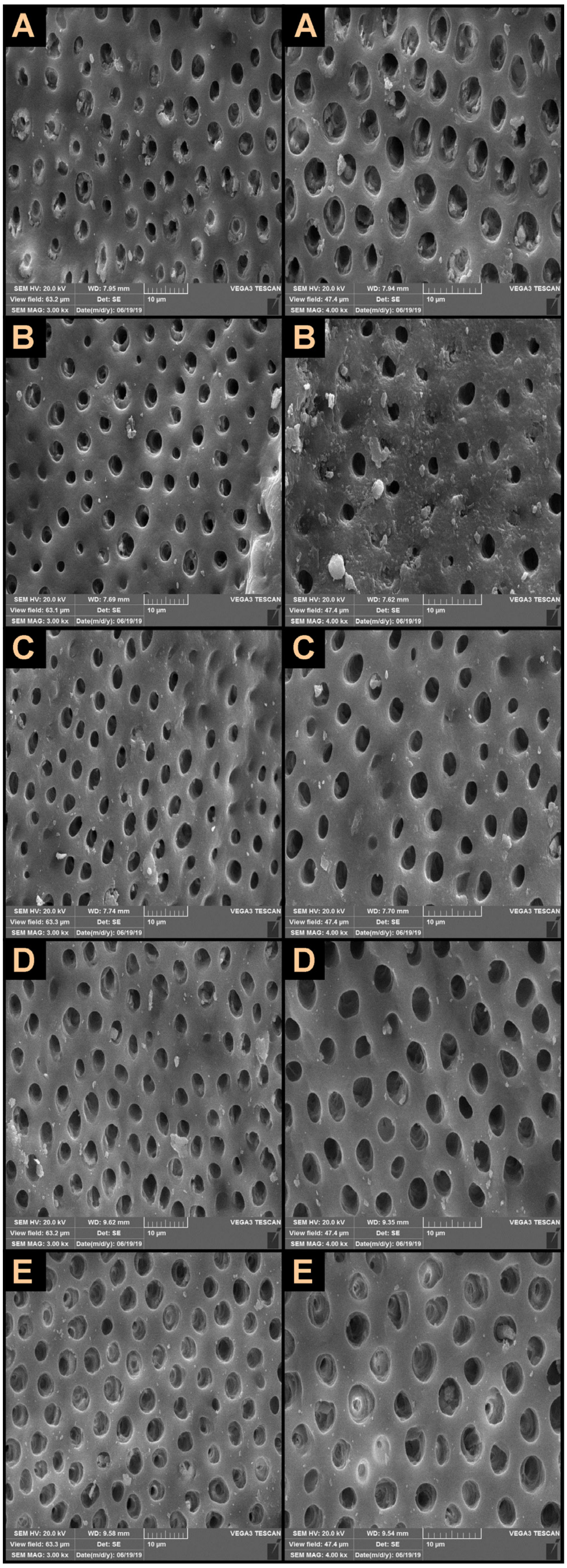

Fig. 4. Scanning electron micrographs of all groups taken at ×3,000 (left) and ×4,000 (right) magnification

A. Control surface etched with 37% phosphoric acid; B. Surface treatment with 37% phosphoric acid and 10% papain; C. Surface treatment with 37% phosphoric acid and 15% papain; D. Surface treatment with 37% phosphoric acid and 6% bromelain; E. Surface treatment with 37% phosphoric acid and 10% bromelain.

Discussion

The present study found that the application of bromelain and papain to dentin did not result in a statistically significant increase in SBS values. This observation contradicts the results of some previous studies.[11,16] Chauhan et al. reported that the deproteinization of dentin and removal of unsupported collagen fibers with bromelain treatment after acid etching could statistically improve the SBS of the adhesive system to dentin.[11] The application of the deproteinizing agent was reported to increase the permeability of the dentin substrate due to the reduction of collagen on the acid-etched surface and the widening of dentinal tubules on the outer surface of the exposed dentin.[11,17] Moreover, treatment with bromelain can increase the surface energy of dentin and the infiltration of monomers. Due to the high surface energy of hydroxyapatite and the low surface energy of collagen, the removal of the latter from etched dentin results in a reduction of organic content, an increase in surface energy and altered hydrophilic properties of the dentin, leading to better penetration of adhesive monomers.[18,19] In this study, group 3 (etched with 37% phosphoric acid and deproteinized with 15% papain) and group 4 (etched with 37% phosphoric acid and deproteinized with 6% bromelain) demonstrated higher SBS values compared to the other experimental groups. However, no significant difference in the SBS was observed between the control group and any of the groups deproteinized with bromelain or papain.

In agreement with the present findings, Hasija et al.[20] and Agarwal et al.[21] reported that the application of 10% papain after acid etching did not affect the SBS to enamel. The similarity between these results may be attributed to the use of papain and bromelain enzymes after the acid etching process. The results suggest that the use of deproteinization agents with lower acidity, such as papain or bromelain enzymes, after the acid etching process with phosphoric acid does not significantly increase the SBS. The present results are consistent with previous reports on the subject. However, it should be noted that the specimens in the current study were restored using a two-step, etch-and-rinse adhesive system and 1 type of composite resin. Other materials may perform differently, as observed in a previous investigation that used 10% papain before restoring enamel with RMGIC.[15]

The present study found that the group treated with 15% papain had a higher SBS than the group treated with 10% papain, which is consistent with a previous study.[14] The previous study demonstrated that enamel deproteinization with 8% or 10% papain gel increased the SBS of orthodontic brackets bonded with RMGIC, compared to deproteinization with lower concentrations of papain (2%, 4% and 6% gels).[14] However, in the present study, the mean SBS for the group deproteinized with 6% bromelain was higher than that of the group deproteinized with 10% bromelain. The decrease in bond strength that occurred following the application of the higher concentration of bromelain may be due to damage to the dentin organic matrix and collagen fibers at this concentration of the enzyme. Bromelain is a protease (proteolytic enzyme) that can catalyze the hydrolysis of dentin proteins and cleave their peptide chains. The increased proteolytic activity of bromelain at higher concentrations may negatively affect the mechanical properties of dentin by destroying its organic content.[22] Consequently, organic adhesive monomers may not be able to adequately infiltrate the demineralized dentin, resulting in reduced bond strength.

The SEM observations of the present study showed that there was no collagen network covering the peritubular dentin in the groups deproteinized with 10% papain, 15% papain and 6% bromelain. The orifices of the dentinal tubules appeared wider in these groups compared to the other groups. Our results are consistent with previous studies and may be explained by the depletion of collagen from the acid-etched dentin caused by the action of bromelain and papain.[16,23] However, the SEM observations in this study were inconsistent with the results of the SBS test, which showed that the same 3 groups – those deproteinized with 10% papain, 15% papain and 6% bromelain – had higher SBS values than the other groups, although no statistically significant difference was found between any of the treatment groups and the control group. This could be attributed to other factors, such as the type of adhesive system used. In agreement with the current findings, Kasraei et al. reported that the treatment of the acid-etched dentin surface with 5% bromelain before the application of the adhesive had no significant effect on marginal microleakage of Class V composite restorations.[23] The SEM micrographs of the resin–dentin interface after the application of bromelain showed that the hybrid layer and resin tags were thick, and the resin tags were conical in shape in the bromelain-treated group. Additionally, spherical residues were observed on resin tags due to the minor infiltration of resin into accessory canals in the dentin.[23]

In the present study, peritubular dentin and collagen fibers were observed in the control group, where the dentin surface had been treated with phosphoric acid. The possible explanation for this finding is that the action of phosphoric acid may be self-limited due to the buffering capacity of deep dentin.[24] Moreover, the average number of dentinal tubules is higher in the deep dentin than in the superficial dentin,[25] which suggests that the high surface moisture content of deep dentin might have affected the etching effectiveness of phosphoric acid during the removal of the smear layer.

The SEM micrographs obtained from the group deproteinized with 10% bromelain displayed similar characteristics to those of the control group. Some of the dentinal tubules were partially or completely obscured by smear plugs, and peritubular dentin was observed in both groups.

This study is the first to survey and evaluate the effects of different concentrations of bromelain and papain, 2 common proteolytic enzymes, on the SBS of composite resin to deep dentin using an etch-and-rinse adhesive system. However, there are some limitations to consider. This was an in vitro study and therefore could not precisely simulate oral conditions, such as water sorption, masticatory cycle, and pH and thermal changes. Therefore, some differences may be observed between the present results and clinical studies on vital teeth, which should be undertaken as a future extension of this work. Additional research with larger sample sizes and varying enzyme concentrations is necessary. It should be combined with other adhesive bonding systems, such as self-etch adhesive bonding systems, to develop the most appropriate method for increasing the bond strength of composite resin to deep dentin.

Conclusions

Deproteinization of deep dentin with various concentrations of papain and bromelain after acid etching did not significantly affect the SBS of composite resin to the treated dentin. However, the group deproteinized with 15% papain demonstrated a higher SBS, on average, than the group deproteinized with 10% papain. Similarly, the group deproteinized with 6% bromelain showed higher SBS values compared to the group deproteinized with 10% bromelain.

Ethics approval and consent to participate

The Ethics Committee of Shiraz University of Medical Sciences approved the research protocol (approval No. IR.SUMS.REC.1396.S509).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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