The effect of different deproteinization agents on microleakage and penetration depth of fissure sealants in permanent molars: An in vitro study

Kubra Yaman Sisman¹, Ezgi Baltaci² ✉, Neslihan Ozveren³

Abstract

**Aim:** Acid etching alone is insufficient to remove organic residues from enamel, which can compromise the microretention of fissure sealants and lead to microleakage and failure. This study evaluated the impact of four different deproteinization agents on the microleakage and penetration depth of fissure sealants in human molars. **Methods:** A total of 170 caries-free mandibular third molars were randomly assigned to five groups (n=34 per group): (1) The Control group (acid etching only), (2) The NaOCl group (sodium hypochlorite + acid etching), (3) The Papacarie Duo group, (4) The Brix 3000 group (both papain enzyme + acid etching), and (5) The Bromelain group (10% bromelain + acid etching). Fissure sealants were applied after etching and deproteinization. Microleakage and penetration depth were evaluated after an aging process involving 1000 thermal cycles between 5-55ºC, using a dye penetration method under a stereomicroscope. **Results:** Within the groups, the Brix 3000 group demonstrated the most favorable microleakage scores, while the Papacarie Duo group exhibited the least favorable scores with regard to microleakage. Furthermore, a penetration depth score of 3 was observed in 29.4%, 29.4%, 26.5%, 11.8%, and 8.8% of the teeth in the Brix, Papacarie Duo, Control, Bromelain, and NaOCl groups, respectively. **Conclusions:** Deproteinization with Brix 3000 gel prior to acid etching in fissure sealant applications resulted in lower microleakage and greater penetration depth compared to other deproteinization agents and acid-etching alone. Deproteinization agents can be a promising way to yield favorable results in fissure sealant applications in terms of microleakage and penetration depth.

**Keywords:** Bromelains; Dental Enamel; Papain; Pit and Fissure Sealants; Phosphoric Acid
INTRODUCTION
Pits and fissures on occlusal surfaces are prone to caries due to their immature enamel during the first four years following eruption and difficulty in cleaning. Approximately 80% of caries in children and adolescents begin in pits and fissures.1 Fissure sealants are a proven caries prevention strategy, forming a physical barrier that protects tooth surfaces from acid attacks, facilitates cleaning, and prevents bacterial access to nutrients.2 However, their clinical success depends on pretreatment, retention, and marginal sealing quality.3

Phosphoric acid (H3PO4) etching is the gold standard for fissure sealant pretreatment4, but it cannot dissolve organic matter.5 Various surface pretreatment methods, including enamel deproteinization with 5.25% sodium hypochlorite (NaOCl), have been shown to improve fissure sealant adhesion and retention.5 However, NaOCl is a solid oxidizing agent with potential side effects.6

Papain (Papacarie Duo) and bromelain are biocompatible proteolytic enzymes that have been shown to improve fissure sealant adhesion and retention by removing the smear layer, breaking down proteins, and increasing the surface energy.7 Brix 3000 is a commercially available chemical caries removal product that contains papain enzyme, but it has different properties and working time than Papacarie Duo. However, there is limited research on its effects as a pretreatment before acid etching in fissure sealant applications. Despite the promising potential of papain and bromelain enzyme complexes as deproteinizing agents, there is a scarcity of research on their effects on the performance of fissure sealants. To the best of our knowledge, no previous studies have compared Brix 3000, bromelain enzyme complex, H3PO4, NaOCl, and Papacarie Duo for deproteinizing the enamel. The rationale for using four different deproteinizing agents was to explore a range of available options and to identify the most effective agent for fissure sealant applications.

This study aimed to evaluate the effects of four different deproteinizing agents on the microleakage and penetration performance of fissure sealant applications in permanent human molars. The null hypothesis was that there were no differences in microleakage and penetration scores between fissure sealant groups pretreated with different deproteinizing agents.

METHODS
Ethical approval was obtained from Trakya University, Institutional Ethics Committee with protocol number 2021/30-03/36.

Inclusion and exclusion criteria
Mandibular third molars extracted for non-study reasons, with ICDAS II scores of 0 and 1, and without hypomineralization, fractures, or extraction-related defects were included in the study.8 Teeth were excluded from the study if they were carious, had restorations on the occlusal surface, had cracked enamel, or had developmental defects.

Sample size calculation
To determine the minimum sample size in the study, a study with similar phases was used.9 It was calculated that at least 33 teeth should be in each group with a control: experimental group ratio of 1:1 to evaluate the relationship between the control group and other methods in terms of microleakage scores and penetration capability scores.

Specimen preparation
A total of 170 teeth were cleaned from soft and hard tissue attachments using a periodontal curette.
under running water. They were then stored in a saline solution containing 0.1% thymol in the refrigerator at +4°C until the experiment.

Prior to pretreatment, pits and fissures were cleaned using sodium bicarbonate powder (Air-flow classic prophylaxis powder, EMS, Nyon, Switzerland) and an airflow device (Air-flow® handy 2+, EMS, Nyon, Switzerland). The powder was sprayed laterally at a distance of 2 mm from the enamel surface, while horizontal movements were made for 5 seconds. The occlusal planes were measured in width and length and categorized into small, medium, and large sizes. They were then randomly assigned into five groups, each consisting of 34 teeth (Figure 1).

**Enamel pretreatment and fissure sealant application**

**Control group:** The enamel surface was etched with 37% H3PO4 gel (Jade Acid Gel, Dharma Research, Romania) for 20 seconds, then rinsed with distilled water, and dried.

**NaOCl group:** 5.25% NaOCl (Cloraxid, CERKAMED, Poland) was applied for 60 seconds with a sterile cotton pellet, then rinsed, dried, and etched as in the control group.

**Papacarie Duo group:** Papain gel (Papacarie Duo®, F&A Pharmaceutical Laboratory Ltd, Sao Paulo, Brazil) was applied for 60 seconds according to the manufacturer’s instructions. It was then removed with an excavator, followed by rinsing, drying, and etching as in the control group.

**Brix 3000 group:** Papain gel (Brix 3000, Brix Medical Science, Carcarana, Argentina) was applied for 2 minutes according to the manufacturer’s instructions. It was then removed with an excavator, and the enamel surface was rinsed, dried, and etched as in the control group.

**Bromelain group:** A 10% bromelain solution (prepared by dissolving 10 g of bromelain powder in 100 ml of distilled water) was applied to the enamel surface for 60 seconds using a sterile cotton pellet. It was then rinsed, dried, and etched as in the control group.

After the etching and demineralization+etching stage, fissure sealants (Clinpro Sealant, 3M Espe, St Paul, MN, USA) were applied to each group and polymerized with the LED light device (Elipar S10, 3M ESPE, St Paul, MN, USA) for 20 seconds, following the manufacturer's instructions (Table 1).

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**Figure 1. Flowchart of the study**

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Table 1. Materials and contents used in the study

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M-Clinpro™ Sealant</td>
<td>3M St. Paul, Minnesota, U.S.A.</td>
<td>Bis-GMA, TEGDMA, Silane, Tetrabutyrammoniumtetrafluoroborate, DiphenylHexafluorophosphate, EDMAB,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Titanium Hydroxide, Hydroquinone</td>
</tr>
<tr>
<td>37% Phosphoric Acid Gel (Jade)</td>
<td>DHARMA, Romania</td>
<td>37% orthophosphoric acid</td>
</tr>
<tr>
<td>Chloraxid 5.25% Sodium Hypochlorite Solution</td>
<td>CERKAMED, Poland</td>
<td>5.25% NaOCl solution</td>
</tr>
<tr>
<td>Brix 3000 gel</td>
<td>Brix Medical Science Carcarana, Argentina</td>
<td>Papain 30.000 U / mg 10 g, Propylene Glycol, Citric Pectin, Triethanolamine, Sorbitan Monolaurate, Disodium Phosphate, Monopotassic Phosphate, Toluidine Blue, Distilled Water</td>
</tr>
<tr>
<td>Papacarie Duo gel</td>
<td>F&amp;A, Pharmaceutical Laboratory Ltd, Sao Paulo, Brazil</td>
<td>Papain 6000 U/mg, Chloramine T, Polyethylene Glycol, Propylene Glycol, Toluidine blue, distilled water</td>
</tr>
<tr>
<td>Bromelain powder extract</td>
<td>Nurbal Şifa Aktar Dogal Gida Sanayi Ltd, Istanbul, Turkey</td>
<td>50 g bromelain powder extract</td>
</tr>
<tr>
<td>Basic Fuchsin solution</td>
<td>Mediko Kimya, Istanbul, Turkey</td>
<td>500 ml 0.5% basic fuchsin solution</td>
</tr>
</tbody>
</table>

Microleakage and penetration depth evaluation

Prior to the staining method for microleakage and penetration depth evaluation, the teeth underwent 1000 thermal cycles between 5-55 ºC for the aging process using the SD Mechatronik Thermocycler (SD Mechatronik GmbH, Feldkirchen-Westerham, Germany). Subsequently, the apices of all teeth were covered with wax and embedded in acrylic blocks, leaving the crowns exposed.

To ensure isolation, two layers of nail polish were applied, covering 1 mm around the fissure sealant margins on all exposed tooth surfaces. The specimens were then placed in a 0.5% basic fuchsin solution (Mediko Kimya, Istanbul, Turkey) and incubated at 37ºC for 24 hours, followed by rinsing with distilled water.

Using a micro-sectioning device (Mecatome T180, PRESI-Métallographie, Eybens, France), the teeth were separated in the buccolingual direction into three sections, resulting in four surfaces per specimen. These surfaces were evaluated using a stereomicroscope (Leica M205C, Leica Microsystems GmbH, Wetzlar, Germany) at magnifications of 25X, 50X, and 100X.

Microleakage evaluation was performed using the criteria proposed by Pardi et al.: 0 represented no dye penetration, 1 indicated dye penetration limited to the outer half of the sealant, 2 denoted dye penetration extending to the inner half of the sealant, and 3 indicated dye penetration extending to the underlying fissure. Penetration depth was evaluated based on the criteria by Kane et al11: 1 represented sealant penetration of 1/3 of the total length of the fissure, 2 denoted sealant penetration of 1/2 of the total length of the fissure, and 3 indicated sealant penetration of the entire length of the fissure. The highest microleakage score and the
lowest penetration depth score among the four surfaces were determined and recorded as the final score for each specimen.

**Statistical analysis**

IBM SPSS Statistics for Windows v.21.0 (IBM Corp, Armonk, NY, USA) was used for the statistical analyses. The data were categorical and expressed as numbers and percentages. Chi-square and Fisher’s exact tests were used for comparisons. The effect size was expressed as the odds ratio with a 95% confidence interval. Bonferroni corrections were applied for multiple comparisons. The Type I error rate limit was set at 5%.

**RESULTS**

Table 2 presents the comparison and distribution of microleakage scores among the groups. The Brix 3000 group had the most favorable microleakage scores, while the Papacarie Duo group had the least favorable scores. A microleakage score of 0 was observed in 17 (50%), 11 (32.4%), 8 (23.5%), 7 (20.6%), and 6 (17.6%) teeth in the Brix 3000, NaOCl, Bromelain, Control, and Papacarie Duo groups, respectively (p=0.02). The odds ratios (95% confidence intervals) for a microleakage score of 1 or higher were 3.25 (1.15-9.19) for Bromelain vs. Brix 3000, 3.86 (1.32-11.24) for control vs. Brix 3000, and 4.67 (1.54-14.14) for Papacarie Duo vs. Brix groups, respectively. Figure 2 shows sample stereomicroscope images of the microleakage scoring.

Table 2. Comparison and distribution of microleakage scores between groups

<table>
<thead>
<tr>
<th>Microleakage score</th>
<th>Control (n=34)</th>
<th>NaOCl (n=34)</th>
<th>Papacarie Duo (n=34)</th>
<th>Brix 3000 (n=34)</th>
<th>Bromelain (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7 (20.6%)a</td>
<td>11 (32.4%)</td>
<td>6 (17.6%)b</td>
<td>17 (50%)c,s</td>
<td>8 (23.5%)c</td>
</tr>
<tr>
<td>1</td>
<td>15 (44.1%)</td>
<td>13 (38.2%)</td>
<td>13 (38.2%)</td>
<td>12 (35.3%)</td>
<td>17 (50%)</td>
</tr>
<tr>
<td>2</td>
<td>3 (8.8%)</td>
<td>2 (5.9%)</td>
<td>5 (14.7%)</td>
<td>1 (2.9%)</td>
<td>4 (11.8%)</td>
</tr>
<tr>
<td>3</td>
<td>9 (26.5%)</td>
<td>8 (23.5%)</td>
<td>10 (29.4%)</td>
<td>4 (11.8%)</td>
<td>5 (14.7%)</td>
</tr>
</tbody>
</table>

Pairs of superscript letters (a,b), (b,c) and (c,d) on the same line represent a statistically significant (post hoc adjusted p<0.05) difference.

Figure 2. Representative stereomicroscope images of samples showing microleakage scores; score 0 (a): no dye penetration, score 1 (b): dye penetration limited to the outer half of the sealant, score 2 (c): dye penetration extending to the inner half of the sealant, and score 3 (d): dye penetration extending to the underlying fissure
Table 3 presents the comparison of penetration depth scores among the groups. The Brix group exhibited the best penetration depth score profile, while the worst score profile was observed in the Bromelain group. Figure 3 shows sample stereomicroscope images of the penetration depth scoring.

DISCUSSION
This study aimed to assess the impact of four different deproteinizing agents (NaOCl, papain enzyme [Papacarie Duo® and Brix 3000], and bromelain enzyme complex) on microleakage and penetration performance of fissure sealant applications in permanent human molars, in comparison to acid etching gel alone. The null hypothesis, which proposed no differences in microleakage and penetration scores among fissure sealant groups pretreated with various demineralization agents, was rejected. The Brix 3000 group exhibited superior microleakage and penetration depth results compared to the other deproteinizing agents and the control group.

There are some limitations to this study. Firstly, it was an in vitro study, which means that the findings may not be directly generalizable to clinical settings. Secondly, the effects of different application times for deproteinization agents were not compared. Thirdly, only one form of the bromelain enzyme complex was evaluated.

All deproteinization agents were applied before acid etching in this study, because a previous meta-analysis reported an increase in bond strength of resin-based materials to enamel when sodium hypochlorite or papain-based agents were applied before phosphoric acid etching. This suggests that applying deproteinizing agents before acid etching may improve the adhesion and retention of fissure sealants, thereby reducing the risk of microleakage and recurrent caries.

In our study, no statistically significant difference in microleakage was observed among the Control, NaOCl, Papacarie Duo, and bromelain groups. However, the bromelain group had significantly lower penetration depth scores than the NaOCl, Papacárie Duo, and Brix 3000 groups. This finding is in contrast to the study by Bhatt et al., which found that the bromelain group had the lowest microleakage scores compared to NaOCl and Papacáric Duo. This outcome is believed to be due to the preparation of the bromelain solution in this study by dissolving 10 g of bromelain powder in 100 ml of distilled water, similar to the method used by Sharafeddin and Safari, as there were no commercially available solution or gel form of bromelain during the experiments.

Table 3. Comparison and distribution of penetration depth scores between groups

<table>
<thead>
<tr>
<th>Penetration depth score</th>
<th>Control (n=34)</th>
<th>NaOCl (n=34)</th>
<th>Papacarie Duo (n=34)</th>
<th>Brix 3000 (n=34)</th>
<th>Bromelain (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 (11.8%)a</td>
<td>1 (2.9%)b</td>
<td>2 (5.9%)c</td>
<td>0</td>
<td>8 (23.5%)b,c,d</td>
</tr>
<tr>
<td>2</td>
<td>26 (76.5%)</td>
<td>30 (88.2%)a,b</td>
<td>22 (64.7%)b</td>
<td>24 (70.6%)</td>
<td>22 (64.7%)a</td>
</tr>
<tr>
<td>3</td>
<td>4 (11.8%)</td>
<td>3 (8.8%)a</td>
<td>10 (29.4%)a</td>
<td>10 (29.4%)</td>
<td>4 (11.8%)</td>
</tr>
</tbody>
</table>

Pairs of superscript letters (a,b), (b,c) and (c,d) on the same line represent a statistically significant (post hoc adjusted p<0.05) difference.
Figure 3. Representative stereomicroscope images of samples showing penetration depth scores; score 1 (a): sealant penetration of 1/3 of the total length of the fissure, score 2 (b): sealant penetration of 1/2 of the total length of the fissure, and score 3 (c): sealant penetration of the entire length of the fissure

Therefore, further studies using different carriers and forms of bromelain are warranted to comprehensively investigate the effects of bromelain enamel deproteinization on microleakage and penetration depth in fissure sealant applications.

In this study, the Brix 3000 group exhibited statistically significantly more favorable microleakage results than the Control, Papacarie, and Bromelain groups. Its microleakage scores were also superior to those of the NaOCl group, but the difference was not statistically significant. The superior microleakage results of the Brix 3000 group may be attributed to the encapsulation of the papain enzyme, which enhances the enzyme's proteolytic activity, stabilizes the gel form, and reduces dissolution in oral fluids. Additionally, Brix 3000 has a recommended application time of 2 minutes, as compared to the 1-minute application time for Papacarie Duo, which may allow for more effective deproteinization of the enamel surface. Furthermore, Brix 3000 contains 3000 IU/mg papain enzyme (10%), while Papacarie Duo contains 600 IU/mg papain enzyme (10%).

CONCLUSIONS

In conclusion, Brix 3000 deproteinization agent was demonstrated to be effective in our study, as evidenced by superior microleakage and penetration depth results in fissure sealant applications. Therefore, it has the potential to improve clinical outcomes in caries prevention and oral health promotion.

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Declarations

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Author contributions: Conception and design: All Authors; Acquisition of data: KYS; Interpretation of data: KYS, EB; Drafting article: KYS, EB; Revision article: All Authors; Final approval: All Authors.

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Data Availability: The data used to support the findings of this study can be made available upon request to the corresponding author.

Peer-review: Externally double-blinded peer-reviewed.