The aim of this study was to assess in vitro the surface roughness (Ra) of human enamel exposed or not exposed to the action of a bleaching agent containing 10% carbamide peroxide (CP) after brushing with different dentifrices. Ninety-six human enamel specimens were divided into 2 groups: GI – exposed to the action of 10% CP; GII – not exposed. These were subdivided into 4 brushing subgroups: (CEW) Close-Up Extra Whitening, (CUB) Colgate Ultra Branco, (CCP) Crest Cavity Protection and (DW) Deionized Water. The specimens from Group GI were exposed to 10% CP for 6 hours/14 days and those from Group GII were stored in artificial saliva for 14 days. Then they were submitted to 35,600 brushing cycles. Ra was measured before and after brushing. Ra difference was compared by two-way ANOVA. Ra was compared between subgroups using ANOVA and Tukey’s test. Ra was compared between groups using T-test ($\alpha = 0.05$). Final and initial Ra were compared by Paired t-test; using SPSS (15.0). Two-way ANOVA difference in the outcome revealed that the use of bleaching agent did not affect the difference in Ra ($p = 0.45$). Brushing significantly influenced the difference in Ra ($p <0.001$), but the interaction between the two factors was not significant ($p = 0.20$). Among the brushing subgroups, a significant increase in Ra was observed for Subgroup CEW – GI: Rai 0.691 (0.112)a, Raf 0.993 (0.264)a; Raf-Rai: 0.303a(43.7%) – G2: Rai 0.794(0.167)a, Raf 1.006(0.488)a; Raf-Rai: 0.212a (26.7%) with a statistical difference for Subgroup CUB – GI: Rai 0.639 (0.163)a, Raf 0.506 (0.113)b; Raf-Rai: -0.133b(-20.8%) – GI: Rai 0.647(0.166)a, Raf 0.472b(0.260); Raf-Rai: -0.134b(-27%). Regardless of whether or not the enamel had been exposed to 10% CP, Ra values varied according to the abrasives in the composition of the different dentifrices.

Key words: dental enamel, bleaching agent, dentifrices.
patients. As a result, new products with alleged bleaching action are constantly released on the market, claiming to improve the appearance of the smile when it has color alteration.

Tooth color may be altered by the combination of extrinsic and intrinsic staining substances that come into contact with the tooth structure. Since tooth bleaching is a conservative treatment, it is considered as a first choice among alternative treatments in aesthetic dentistry. Extrinsic stains are usually the result of surface precipitation of coloring agents and pigments in the diet (black tea, coffee, red wine) or habits (smoking) on the acquired film of enamel \(^1\)\(^-\)\(^3\), whereas intrinsic stains are determined by the layer of dentin underlying the enamel surface, which becomes discolored as a result of fluorosis, trauma, use of antibiotics, systemic conditions and natural aging of teeth \(^4\)\(^-\)\(^4\). To remove these stains, teeth can be bleached with bleaching agents and/or bleaching dentifrices, which have different action mechanisms.

The most popular dental bleaching method is the supervised home technique which uses 10% carbamide peroxide as a bleaching agent to remove both intrinsic and extrinsic stains. This bleaching agent is very unstable, and when it comes into contact with the tissues and saliva, it dissociates into 3% hydrogen peroxide and 7% urea. Urea degrades to ammonia and carbon dioxide, while hydrogen peroxide breaks down easily into water and oxygen, penetrating into the enamel and dentin, promoting dental bleaching \(^5\). However, the effects of bleaching agents on dental structures are still controversial because some studies have shown no significant change \(^6\)\(^-\)\(^12\), while others conclude that bleaching agents cause significant morphological changes, which range from changes in the mineral content to changes in surface roughness and micro hardness of the dental structure \(^13\)\(^-\)\(^17\). Despite these controversies, it is known that if changes occur in the surface roughness of the structure, they may contribute to the appearance of extrinsic stains and plaque accumulation, which is reflected by mineral loss and inflammation of the gingival tissues \(^18\).

Another option that has become popular is the use of dentifrices with supposed bleaching action, which may be purchased at supermarkets and drugstores. It is known that these bleaching dentifrices in some way promote dental bleaching by removing and/or controlling extrinsic stains on the tooth surface through the abrasion process \(^1\)\(^-\)\(^2\). The following abrasive agents are typically found in these bleaching dentifrices: hydrated silica, calcium carbonate, dicalcium phosphate dihydrate (DCPD), calcium pyrophosphate, alumina, sodium bicarbonate and perlite \(^3\)\(^-\)\(^4\),\(^19\). The abrasiveness of dentifrices depends on particle hardness, shape, size, distribution range and concentration \(^20\)\(^-\)\(^23\). However, this abrasiveness needs to be moderate in order not to cause damage to hard and soft tissues \(^24\).

From the above information and consultation of current scientific literature, it can be seen that there is little information about the effect of dentifrices on the surface roughness of human enamel, exposed or not to the action of home-use bleaching agents. Thus, this study aimed to evaluate in vitro the surface roughness (Ra) of human enamel exposed or not the action of the bleaching agent carbamide peroxide (CP) 10%, after brushing with different dentifrices.

**MATERIAL AND METHODS**

**Experimental Design** (Table 1): The factor under study was the action of two bleaching dentifrices - Close-Up Extra Whitening (CEW) and Colgate Ultra Branco (CUB), a conventional dentifrice - Crest Cavity Protection (CCP) - positive control, and Deionized Water (DW) - negative control – on the average surface roughness of human enamel either exposed to the action of a bleaching agent containing 10% carbamide peroxide (CP) – Opalescence (Ultradent Product Inc, Salt Lake City, Lot:C129), or not. We used 96 human enamel specimens from 48 healthy third molars, recently extracted for orthodontic reasons, showing no surface changes due to trauma during the extraction, obtained from the Human Permanent Tooth Bank at UFSM.

They were divided randomly into 2 groups: Group I – exposed to the action of a bleaching agent with 10% CP and Group II – not exposed. Each of these groups were subdivided into 4 brushing subgroups.

<table>
<thead>
<tr>
<th>Table 1: Dentifrices assessed and their abrasive systems.</th>
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<tbody>
<tr>
<td><strong>Dentifrices</strong></td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Close-up extra whitening</td>
</tr>
<tr>
<td>Colgate ultra branco</td>
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<tr>
<td>Crest cavity protection</td>
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</table>

according to the dentifrice or deionized water used (control) (Table 2). The response variable was Ra, determined through a readout made with a roughness meter.

### Selection and preparation of the enamel specimens

Forty-eight extracted healthy human third molars were selected, from which 96 dental enamel specimens were obtained. The teeth were cleaned with Gracey type curettes (Newmar Surgical Instruments- São Paulo - SP - Brazil), pumice stone and water, applied with a Robinson brush (Microdont - São Paulo - SP - Brazil). After cleaning, the teeth were submitted to a sterilization process in a humid medium. The teeth were stored in saline solution at 5°C until the beginning of the study. To prepare the specimens, the sites for the longitudinal and cross sections were marked with graphite on the vestibular and/or lingual surfaces of the crowns. Sections measuring 5 x 5 x 2mm were cut from the flattest area of the crown (middle third), using a double-faced diamond disk (KG Sorensen- Cotia - SP - Brazil) driven by a handpiece at low speed with water irrigation. After this, the specimens were flattened with 600-grit abrasive paper on the side composed of dentin so that all the specimens had the same thickness. After preparation, the specimens were measured using a digital pachymeter. They were polished with 6-8 µm extra-thin polishing paste (Diamond- FGM - Joinvile - SC - Brazil), applied with a sandpaper disk and stored in deionized water up to the time they would or would not be bleached.

### Exposure to a bleaching agent, or not

The specimens in Group I (n= 48) were exposed to the action of 10% CP gel (Opalescence). A template corresponding to one drop of the bleaching gel was made so that all the specimens received the same amount of bleaching agent. The gel was applied on a glass slide, superimposed on the template. The specimens were placed on the bleaching gel and stored in plastic containers, covered with gauze dampened in deionized water, and remained in an oven at 37°C for 6 hours, for 14 days. Then they were washed with deionized water for 10 seconds and stored in artificial saliva at 37°C, simulating a complete home-bleaching treatment.

The specimens from Group II (n = 48) were stored in individual containers, duly identified, in artificial saliva at 37°C for 14 days during bleaching treatment.

### Brushing procedure

To perform brushing, a brushing device was devised by the Department of Operative Dentistry of the Dentistry Course at UFSM (Fig, 1) and designed and developed at the Mechanical Engineering course at UFSM. The machine consisted of a motor that produced back-and-forth movements of 10 arms by means of pulleys, onto which the toothbrushes were fixed. Oral-B Indicator Plus 40 (Gillette do Brasil Ltda, Manaus-AM) soft-bristle toothbrushes were used. The machine was set up to run a 3.8 cm horizontal course on the tooth, applying a 200g axial load. A cycle was understood to be a complete back-and-forth movement of the toothbrush. In each brushing procedure, 10 toothbrushes were used, which were changed halfway through the complete brushing cycle in order to avoid the influence of toothbrush bristle wear on the result. For brushing, the enamel specimens were fixed in acrylic resin at the base of the brushing machine, so that they would be prominent, allowing better action of the toothbrush bristles.
The base where the specimens were fixed to the machine was turned 90° in the middle of the cycle so that brushing could be performed in two directions. The application of the Dentifrice was applied in the form of a suspension of toothpaste in deionized water in the proportion of 1:125. The paste formed by toothpaste diluted in deionized water was injected manually every 1 minute. After the tests were concluded, the specimens were removed from the brushing machine and immediately washed with jets of deionized water and stored in artificial saliva at 37°C.

**Surface Roughness Analysis**

Average surface roughness (Ra) of each enamel specimen was analyzed using a digital roughness meter (Mitutoyo SurfTest SJ-201P). To perform the roughness readout, the diamond point of the roughness meter would run on the specimens at a constant speed of 0.25mm/s and force of 4mN. The cut-off value was adjusted to act at 0.25 µm and surface roughness was characterized by the arithmetic average of surface peak and valley heights found within a central line along the area assessed (Ra), in micrometers (µm). Five readings were performed on each specimen in different directions. The average of these readings was used for the statistical analysis.

The initial Ra reading (Rai) was performed 24 hours after exposure (Group I) to the bleaching agent, or not (Group II). 24 hours after the Rai reading, the brushing procedures began and at the end of this stage, the specimens were stored for 24 hours in artificial saliva and the final Ra reading (Raf) was performed.

**Statistical Analysis**

Ra difference was compared by two-way ANOVA. Subgroup Ra was compared by ANOVA and Tukey’s test. Group Ra was compared by T-test ($\alpha=0.05$), Comparison between final versus initial Ra was done by Paired t-test

**Scanning Electronic Microscopy (SEM)**

With the purpose of visualizing and illustrating the results, a SEM of the specimens of each subgroup chosen randomly after brushing was performed. The microscopies that were most representative of the results were selected, since it was not the aim of this study to perform SEM analysis. To perform SEM, the selected enamel specimens were dehydrated and submitted to the metallization process with gold-palladium alloy. The images were captured at 500X magnification and observed under a Scanning Electronic Microscope JEOL A110 (Figs. 2-9).

**RESULTS**

Two-way ANOVA difference in the outcome revealed that the Ra factor Group (exposure or not to bleaching agent) did not affect the difference in Ra ($F= 0.57; p = 0.45$). The subgroup factor (brushing) significantly influenced the difference in Ra ($F= 12.37; p <0.001$), but the interaction between the two factors was not significant ($F=1.54; p = 0.20$).

Table 3 shows the differences in Rai and Raf in Groups I and II for each brushing subgroup. In both groups, there was a statistically significant increase in Ra for the CEW dentifrice subgroup. For the
Surface roughness after different treatments

Fig. 4: Image obtained by SEM of the surface micromorphology of enamel exposed (E) to the action of the bleaching agent and brushed with CCP dentifrice.

Fig. 5: Image obtained by SEM of the surface micromorphology of enamel exposed (E) to the action of the bleaching agent and brushed with DW.

Fig. 6: Image obtained by SEM of the surface micromorphology of enamel not exposed (NE) to the action of the bleaching agent and brushed with CEW dentifrice.

Fig. 7: Image obtained by SEM of the surface micromorphology of enamel not exposed (NE) to the action of the bleaching agent and brushed with CUB dentifrice.

Fig. 8: Image obtained by SEM of the surface micromorphology of enamel not exposed (NE) to the action of the bleaching agent and brushed with CCP dentifrice.

Fig. 9: Image obtained by SEM of the surface micromorphology of enamel not exposed (NE) to the action of the bleaching agent and brushed with DW.
other brushing subgroups, no statistically significant alteration in Ra was observed. For Groups I and II, within each brushing subgroup, no statistically significant difference in Ra was found (capital letters on the horizontal line). No statistically significant difference in Rai was found among the brushing subgroups. Similar results were observed both for Raf and difference in Ra (lowercase letters in the vertical column). CEW is statistically different from CUB; in turn, CCP and DW did not differ statistically from the other brushing subgroups.

In the images obtained by SEM (Figs. 2-9) it was possible to observe alterations in surface micromorphology of enamel exposed, or not, to the action of the bleaching agent and brushed with different dentifrices and deionized water, which were consistent with the results of this study. The results can be observed in differences of the surface micromorphology of the specimens shown in Figs. 2 and 3 of GI (rough appearance) and in Figs. 6 and 7 of GII (smoothness / polishing characteristics).

**DISCUSSION**

This study tested the effect of brushing with different dentifrices on average surface roughness of human enamel either exposed or not exposed to the action of a bleaching agent with 10% carbamide peroxide. Statistical analysis of the values obtained for Ra showed that the behavior of the different brushing subgroups was the same. Whether or not the enamel had been exposed to the bleaching agent did not influence the difference in Ra obtained after the action of the different dentifrices. Therefore, the performance of each brushing subgroup will be discussed separately.

It was found that the different dentifrice formulations had different effects on the surface roughness of enamel. This could be related to the different abrasives present in their compositions, which is supported by the study by Pickles22, who reported that abrasiveness of the dentifrice depends on particle hardness, shape, size, distribution range and concentration. Camargo et al. demonstrated that the larger the size of the abrasive particles, the greater is the abrasiveness of the dentifrice. However, different types of abrasives with similar particle sizes present different abrasiveness values. According to these authors, this difference in abrasiveness may be attributed to the difference in hardness of the abrasive particles. With regard to the shape of the abrasive particles, Ashmore et al. observed that dentifrices that contain calcium carbonate in their composition, in more regular oval or rhombohedral shape, were less abrasive than those with more irregular aragonite particles. Davis and Winter showed that dentifrices that contain fine particles, such as calcium carbonate and silica, are less abrasive than those with rougher particles.

Two dentifrices with alleged bleaching action, Close-up Extra Whitening (CEW) and Colgate Ultra Branco (CUB) and one regular dentifrice, Crest Cavity Protection (CCP) were assessed. The regular dentifrice CCP has only silica as an abrasive component, while the other dentifrices contain different abrasives in their compositions. The dentifrice CUB has calcium carbonate, aluminum, bicarbonate of soda and sodium silicate as abrasives, and in its composition the dentifrice CEW has abrasives of the calcium carbonate, perlite and silica type. Perlite is a natural volcanic glass with flat glass-shaped particles and sharp cutting edges. While in use under load, the abrasive particles are broken down and the cutting edges become rounded and rhombohedral. The perlite particles thus remain parallel to the tooth surface, reducing the potential for scratches on the surface and increasing their polishing capacity. The use of perlite as an abrasive is common in prophylactic pastes, which are excellent stain removers, combined with good polishing properties and low abrasiveness. Table 3 shows that there was a statistically significant increase in enamel Ra only for the brushing subgroup CEW, possibly due to the presence of the perlite abra-

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**Table 3: Mean surface roughness values (Ra) for Groups I and II, before (Rai) and after (Raf) brushing with each subgroup.**

<table>
<thead>
<tr>
<th>Brushing subgroups</th>
<th>Group I</th>
<th></th>
<th></th>
<th>Group II</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rai (±dp)</td>
<td>Raf (±dp)</td>
<td>%</td>
<td>Raia (±dp)</td>
<td>Raf (±dp)</td>
<td>%</td>
</tr>
<tr>
<td>CEW</td>
<td>0.691(0.112)b</td>
<td>0.993(0.264)a</td>
<td>43.7</td>
<td>0.794(0.167)b</td>
<td>1.006(0.488)a</td>
<td>26.7</td>
</tr>
<tr>
<td>CUB</td>
<td>0.639(0.163)a</td>
<td>0.506(0.113)a</td>
<td>-20.8</td>
<td>0.647(0.166)a</td>
<td>0.472(0.260)a</td>
<td>-37.7</td>
</tr>
<tr>
<td>CCP</td>
<td>0.735(0.170)a</td>
<td>0.764(0.224)a</td>
<td>3.9</td>
<td>0.724(0.303)a</td>
<td>0.771(0.165)a</td>
<td>6.5</td>
</tr>
<tr>
<td>DW</td>
<td>0.789(0.201)a</td>
<td>0.814(0.419)a</td>
<td>3.2</td>
<td>0.684(0.217)a</td>
<td>0.616(0.164)a</td>
<td>-9.9</td>
</tr>
</tbody>
</table>

The means followed by the same lowercase letter do not significantly differ according to Tukey’s test (p<0.05).
Table 4: Comparison of the values of Rai, Raf and respective Ra differences between each brushing subgroup in Groups I and II.

<table>
<thead>
<tr>
<th>Brushing subgroups</th>
<th>Group I (bleached)</th>
<th>Group II (not bleached)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rai (±sd)</td>
<td>Raf (±sd)</td>
</tr>
<tr>
<td>CEW</td>
<td>0.691(0.112)a</td>
<td>0.993(0.264)a</td>
</tr>
<tr>
<td>CUB</td>
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</tr>
<tr>
<td>DW</td>
<td>0.789(0.201)a</td>
<td>0.814(0.419)ab</td>
</tr>
</tbody>
</table>

The means with the same lower case letters in the vertical and capital letters in the horizontal do not significantly differ according to Tukey's test (p < 0.05).
ness of the abrasive particles are fundamental for the correct choice of dentifrice, but the information present on the packages of these products indicate only the abrasive present in the formula. This rein-

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