Influence of Natural Fruit Juices in Removing the Smear Layer from Root Surfaces — An In Vitro Study

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Abstract

Certain elements of a patient's diet may be associated with dentin hypersensitivity. The intent of this study was to evaluate the degree of removal of the smear layer from dentin surfaces by various fruit juices. A smear layer was created on extracted human teeth by manual scaling. The roots were reduced and distributed into 8 experimental groups. Distilled water was the negative control. The juices were applied by 2 methods: topical application and topical application with friction. Specimens were photomicrographed and graded according to an index of smear layer removal. With topical application, all but 2 of the tested substances resulted in significantly greater removal of the smear layer and opening of dentinal tubules than was the case with the negative control ($p = 0.05$); the exceptions were Gala apple and Italian grape juices, which were no different from the control. For the active application (with friction), most substances removed more smear layer than the control ($p < 0.05$); Gala apple, Italian grape and orange juices were similar to the control. For each of the tested substances, removal of the smear layer did not differ with the method of application (topical vs. friction; $p > 0.05$). It is concluded that natural fruit juices can remove the smear layer from dentin surfaces, and the efficacy of this removal varies with the type of juice.

MeSH Key Words: *dentin hypersensitivity; diet/adverse effects; smear layer*

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Patients often seek professional help for acute tooth pain of sudden onset and short duration that arises when dentin is exposed to various stimuli, typically thermal, evaporative, tactile, osmotic or chemical, and that cannot be ascribed to any other dental defect or disease.[1] The pain occurs during the ingestion of food or beverages that are cold or sweet, during breathing, while the patient is brushing his or her teeth or even while he or she is simply chewing food.[2,3]

This painful situation is known as dentin hypersensitivity[1,2] and has been reported to affect up to 35% of the population.[4] Chabanski and others[5] reported that between 72.5% and 98% of various groups of periodontal patients were affected by root sensitivity.

The most widely accepted theory explaining the pain originating in hypersensitive teeth is the hydrodynamic theory, proposed by Brannstrom.[6] According to this theory, thermal, osmotic or tactile stimuli on the exposed dentinal tubules provoke a rapid flow of dentin fluid toward the oral cavity, which leads to shrinkage of odontoblastic extensions within the dentin tubules and activation of the sensorial units in the dental pulp.

The dentin hypersensitivity could arise from a loss of the dental structure in the cervical region (enamel, dentin or cement), from denuding of the root surface (gingival recession) or after periodontal treatment.[1]

The cement in the cervical region is very thin, ranging from 20 to 50 mm, even when intact and histologically normal. This layer offers very little protection against thermal shock or any other irritant. This cement can be easily removed by the action of tooth scrapers, periodontal curette, abrasive toothpaste, toothbrushes and even food.[7]

Conversely, O'Leary and Kafrawy[8] found residual cementum on the root surface of periodontally involved teeth even after 50 root-planing strokes, which indicated that total removal of the cementum is not a realistic clinical objective with hand instruments.

Once exposed, the dentin may exhibit hypersensitivity, a condition that appears to share many of the etiological factors associated with tooth wear. Tooth wear is rarely attributable to the action of a single physical or chemical agent but arises from interactions between 2 or more of the putative etiological agents.[9,10]

Dentin hypersensitivity may be attributed to various indigenous factors (e.g., defects related to the formation of teeth, defects at the cementoenamel junction, periodontal disease, or systemic disease such as bulimia, nervous anorexia, hyperthyroidism or gastric disturbances), but exogenous factors are also important (incorrect tooth-brushing habits, inappropriate dietary habits, occlusal trauma, clinical procedures like periodontal surgery, other forms of dental treatment, retraction of the gums and wear of the dental structure).

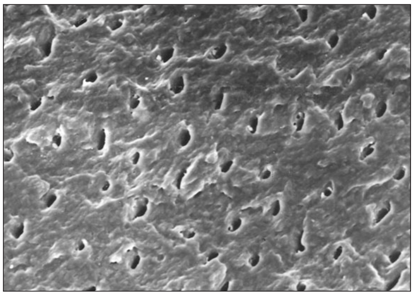

Figure 1: *Photomicrograph of sample treated by topical application of acerola fruit juice shows grade 1 removal of smear layer, characterized by opened dentin tubules (1500×).*

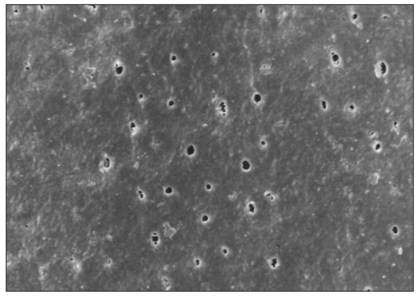

Figure 2: *Photomicrograph of sample treated by friction application of acerola fruit juice shows grade 1 removal of smear layer, characterized by opened dentin tubules (1500×).*

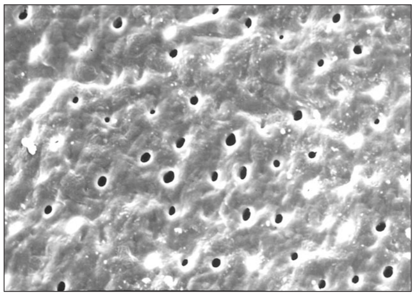

Figure 3: *Photomicrograph of sample treated by topical application of lemon fruit juice shows grade 1 removal of smear layer, characterized by opened dentin tubules (1500×).*

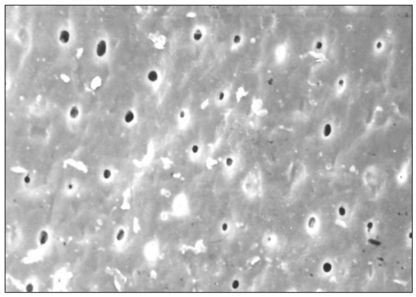

Figure 4: *Photomicrograph of sample treated by friction application of lemon fruit juice shows grade 1 removal of smear layer, characterized by opened dentin tubules (1500×).*

The objective of the present study was to evaluate, using a scanning electron microscope (SEM), the degree of removal of the smear layer present on dentin surfaces associated with various natural fruit juices.

Materials and Methods

The study protocol was approved by the Research Ethics Committee of the School of Dentistry at Araraquara, State University of São Paulo.

A total of 32 recently extracted human teeth were used for the study, without any selection related to tooth type or the patient's sex or age. The teeth had been extracted either because of severe periodontal disease or for orthodontic reasons. From these teeth, a total of 90 dentin samples were prepared. A single operator (FOBC) used high-speed diamond-coated burs to remove the cementum from the cervical portion of the roots. The burs were introduced until the middle of their thickness to obtain standardized samples. Subsequently, the same operator created a smear layer by applying 40 working strokes to each surface using Gracey's curettes 5-6. The roots were then reduced with a diamond disk to obtain dentin samples of 3 mm^2, and the samples were divided randomly into 8 experimental groups, one for each of the 8 natural fruit juices (2 varieties of apple [Gala and green], acerola, kiwi, lemon, orange, pear and Italian grape). Negative control samples were treated with distilled water. The fruit juices were prepared by the investigators immediately before the experiment, and the pH of each was determined at that time. The juices were not buffered, since pH may be a factor in smear layer removal and, consequently, may influence dentinal sensitivity.

Each experimental group and the control group consisted of 10 samples, subdivided into 2 groups of 5 samples each. Fruit juice was applied to the samples by one of the following methods:

- Topical: Samples were immersed in the juice for 5 minutes and washed with a stream of tap water for 15 seconds.
- Topical with friction: Samples were immersed in the juice for 5 minutes, brushed with a soft toothbrush for 30 seconds and washed with a stream of tap water for 15 seconds.

Each dentin slice was considered a sample unit. The samples were submitted to routine processing for SEM analysis and 2 photomicrographs were obtained from the centre of each sample, with magnifications of 750× and 1500×, respectively; these images were intended to be representative of the most common features observed on each

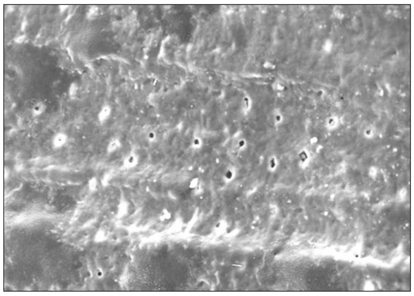

Figure 5: *Photomicrograph of sample treated by topical application of orange fruit juice showing partial removal of smear layer and partial opening of dentin tubules (grade 2) (1500×).*

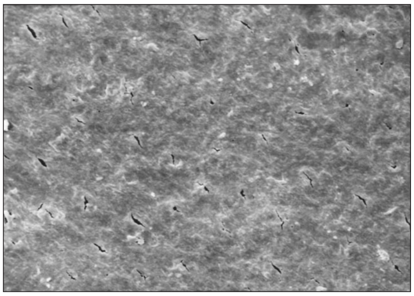

Figure 6: *Photomicrograph of sample treated by topical application of Italian grape fruit juice shows the smear layer is still present on the dentin surface and there are indications of dentinal tubule openings (grade 2) (1500×).*

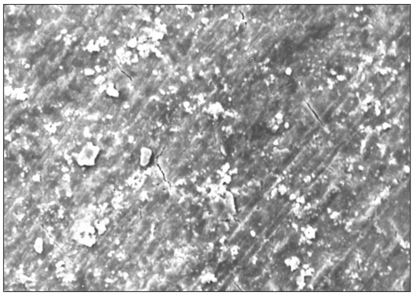

Figure 7: *Photomicrograph of sample treated by topical application of negative control (water) shows the root surface covered by the smear layer (grade 4) (1500×).*

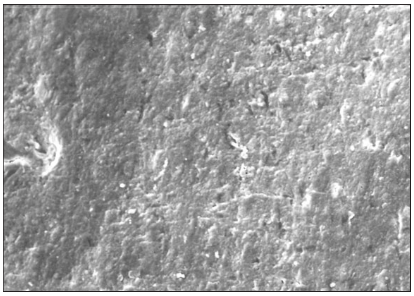

Figure 8: *Photomicrograph of sample treated by friction application of orange juice shows the smear layer present on the dentin surface and obliteration of the dentinal tubules (grade 4) (1500×).*

dentin slice. These photomicrographs were subsequently assessed by one examiner (JECS) who had been previously calibrated and who was blind to the experimental groups, using the following index of smear layer removal:

Grade 1: Complete removal of smear layer, dentinal tubules open (**Figs. 1–4**).

Grade 2: Partial removal of smear layer, dentinal tubules partially open (**Fig. 5**).

Grade 3: Smear layer present on the dentin surface, indication of opening of dentinal tubules (**Fig. 6**).

Grade 4: Smear layer present on the dentin surface, total obliteration of dentinal tubules (**Figs. 7, 8**).

Because the data were based on a graded index, nonparametric analyses were applied. For comparisons among the experimental groups, the different natural fruit juices were considered independent with respect to the method of application (topical vs. friction). Thus, nonparametric analysis of variance was used to compare the groups within each method of application. Then, the Mann–Whitney test was used to compare the 2 methods of application for each natural fruit juice tested. A 5% confidence level was used, and the calculations were performed with the software Statistica, version 5.1 (StatSoft Inc, Tulsa, Okla.).

Results

Figure 9 represents the frequency distribution of grades within each group of samples treated by topical application. There was a significant difference among the substances (Kruskal–Wallis test, $H = 20{,}281$, $p = 0.005$), and post hoc paired comparisons demonstrated that most of the test substances were significantly different from the negative control ($p < 0.05$). The exceptions were Gala apple juice and Italian grape juice, which had grades of predominantly 3 and 4, similar to the control group (grades of 4 for all samples). All of the other groups had an average rank lower than that of the negative control group, which indicates greater removal of the smear layer and greater opening of the dentin tubules.

The results for the application of fruit juices with friction were similar to those for topical application. Again, there was a significant difference among groups (Kruskal–Wallis test, $H = 24{,}553$, $p = 0.009$), and post hoc paired comparisons showed that most of the natural fruit juices were significantly different from the negative control ($p < 0.05$). The exceptions were Gala apple juice, Italian grape juice and orange juice, which had a higher frequency of samples with grade 4 (**Fig. 10**).

A comparison of the test substances, without regard to application method, indicated that lemon juice, green apple juice and acerola juice were associated with lower average rank, indicating a greater loss of the smear layer. For samples treated by topical application, the only significant differences were between Gala apple juice and acerola juice and between acerola juice and Italian grape juice. For samples treated with friction, Gala apple juice yielded results that were significantly different from those for lemon juice, green apple juice, acerola juice and kiwi juice (**Table 1**); green apple juice also yielded results that were significantly different from orange juice and Italian grape juice. The grades from all samples were ranked in ascending order, and the average rank of each group was calculated.

The Mann–Whitney test revealed no significant differences between application methods for each juice (topical vs. friction).

Discussion

In the present work, a smear layer (as shown in **Fig. 7**) was induced by instrumentation with hand curettes, for

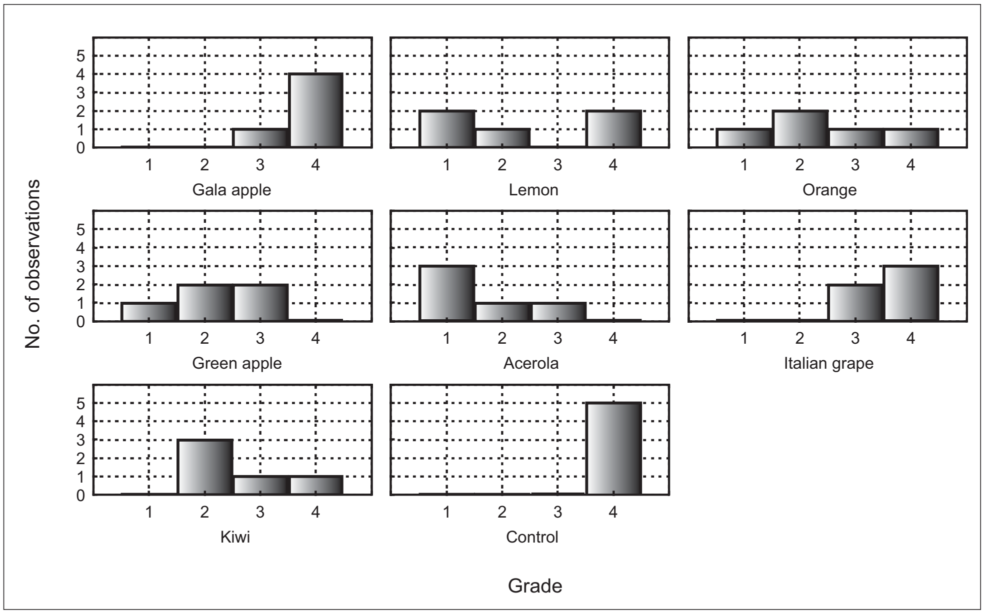

Figure 9: *Frequency distribution of scores for topically applied samples of each substance tested.*

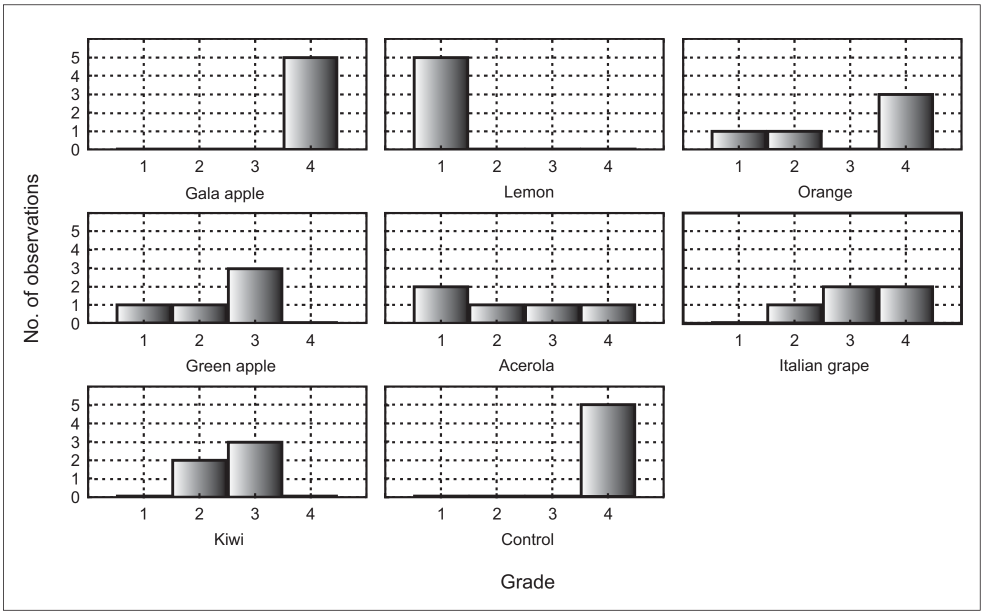

Figure 10*: Frequency distribution of scores for samples of each substance applied with friction.*

potential removal by fruit juices and exposure of dentin tubules (as shown in **Figs. 1–4**).

Dentin hypersensitivity does not occur immediately after scaling and root planing. The smear layer formed on the root surface after hand instrumentation covers the dentin and completely obliterates the dentin tubules (as observed in the present study), which may be why dentin hypersensitivity typically occurs a few days later, when the smear layer has been removed by tooth-brushing, dietary substances, saliva or other endogenous factors.[11]

The pH of a substance is one factor affecting the opening of dentin tubules. Clark and others[12] compared the acidity of common dietary substances, including lemon juice (pH 2.2 to 2.4), orange juice (pH 2.8 to 4.0) and grape juice (pH 3.3 to 4.5). These pH values are in agreement with the results obtained in the present study (**Table 2**).

Some previous studies have indicated that the critical pH for enamel dissolution is 5.5, and any solution with a lower pH may cause erosion.[13,14] Vanuspong and others[15] investigated erosion of dentin by citric acid at different pH values and exposure times to determine whether surface softening or demineralization of dentin occurs and whether the zone can be remineralized by artificial saliva. They concluded that erosion of dentin depends on both the pH value of the acid and the contact time.[15,16] In that study, the critical pH for dentin dissolution was at least as high as pH 6.0 and erosion was considerable even at pH levels well above those observed for most fruits, acidic drinks and some mouth rinses.[15]

In the present study, test substances with lower pH were associated with greater opening of the dentin tubules. Lemon juice (pH 2.2 [**Table 2**]) had the lowest average rank for the active application method (friction) (**Table 1**), whereas Gala apple juice (pH 4.2) had the highest average rank with the same application method; these 2 juices represented the highest and lowest effectiveness, respectively, in terms of removal of the smear layer and exposure of dentin tubules.

Nonparametric analysis of variance (Kruskal–Wallis) showed that all but 2 of the fruit juices tested (the exceptions being Gala apple juice and Italian grape juice) resulted in more effective removal of the smear layer and opening of dentin tubules (**Figs. 1** to **4**) than with the negative control (**Fig.** 7). When the substances were actively applied to the dentin samples (friction method), orange juice (**Fig. 8**), as well as Gala apple juice and Italian grape juice, yielded similar results to the negative control. The Mann–Whitney

Table 1 **Average rank[a] of experimental groups, according to tested substance and application method**

Fruit juice	Topical application	Application with friction
Acerola juice	8.9	15.0
Control (water)	32.5	32.5
Gala apple juice	30.1	32.5
Green apple juice	13.8	15.5
Italian grape juice	27.7	23.5
Kiwi juice	17.8	17.0
Lemon juice	17.0	5.0
Orange juice	16.2	23.0

[a]For each group, the grades from all samples were ranked in ascending order, and the average rank was calculated.

Table 2 **Acidity of fruit juices immediately after preparation of juice but before application to dentin samples**

Substance	pH
Lemon juice	2.2
Acerola juice	2.8
Kiwi juice	3.2
Green apple juice	3.6
Italian grape juice	3.7
Orange juice	3.8
Gala apple juice	4.2
Control (water)	5.9

test did not reveal any significant differences between application methods (topical vs. friction) for any of the tested substances. Nevertheless, there were some differences in average ranks between the methods of application for the various fruit juices (**Table 1**).

Prati and others[17] evaluated modifications in dentin permeability after application of various acidic drinks and the effect on dentin permeability of brushing procedures with and without toothpaste. They found that dentin permeability after brushing with toothpaste was significantly lower than after brushing without toothpaste, which was in turn lower than that observed with previous application of acid. Toothbrushing subsequent to application of beverages only partially occluded dentin tubules with a thin debris layer that was pulled inside the tubule orifices. In the study reported here, there was no significant difference between the application methods (topical vs. friction) for all but one of the tested substances, the exception being orange juice, which was significantly different from the control group with topical application only (**Fig. 5**). This result suggests that application of orange juice with friction may have induced partial occlusion of the dentin tubules (**Fig. 8**).

McAndrew and Kourkouta[3] assessed the effects of tooth-brushing alone, tooth-brushing followed by exposure to orange juice and tooth-brushing subsequent to orange juice exposure. Their results suggested that in cases of dentin hypersensitivity, tooth-brushing alone was most effective in occluding the tubules, followed by tooth-brushing subsequent to dietary acid application and then by tooth-brushing before dietary acid application. According to these results, tooth-brushing should not immediately precede or follow ingestion of acidic drinks but should be separated from mealtimes. However, the Canadian Advisory Board on Dentin Hypersensitivity[1] has recommended that tooth-brushing occur before ingestion of meals and drinks in patients at risk of erosion or abrasion.

Furthermore, Eisenburger and others[18] suggested that the combination of erosion and abrasion resulted in significantly greater wear than erosion alone, but found no significant differences in wear after brushing with or without abrasive. Simultaneous erosion and abrasion resulted in about 50% more wear than alternating erosion and abrasion.

Absi and others[19] studied the effect of acidic dietary substances on scaled root surfaces and established a relationship between the acid used and the degree of exposure of dentinal tubules. The results of that study may be summarized as follows: application of orange juice (pH 3.0), white wine (pH 2.3) and red wine (pH 2.6) yielded visible dentinal tubules, whereas other substances such as milk and coffee together (pH 6.2) and coke (pH 2.9) were not associated with visible dentinal tubules. Conversely, apple juice (pH 4.1), tannic acid (pH 3.3), citric acid (pH 1.5) and lactic acid (pH 2.0) were associated with opened dentin tubules.

According to the index used in the present study, which represented the degree of opening of the dentin tubules, within the subgroups that underwent active application (friction), there was a much higher proportion of samples with complete obliteration of dentinal tubules in the negative control group (distilled water, pH 5.9) (**Fig. 7**) and the groups treated with Gala apple juice (pH 4.2), Italian grape juice (pH 3.7) and orange juice (pH 3.8) (**Figs. 8**, **9** and **10**). Green apple juice (pH 3.6) and kiwi juice (pH 3.2) were associated with only indications of dentin tubule openings, and the substances with the lowest pH, including lemon juice (pH 2.2) (**Fig. 4**) and acerola juice (pH 2.8) (**Fig. 2**) resulted in complete opening of dentin tubules.

The results of the present study support the hypothesis proposed by many authors,[17–20] whereby the prevention and treatment of dentin hypersensitivity depend on control of the patient's dietary habits and tooth-brushing behaviour, an approach that is also supported by the Canadian Advisory Board on Dentin Hypersensitivity.[1]

Conclusions

This study has shown that the pH of substances applied to dentin samples influenced removal of the smear layer and opening of dentinal tubules. All of the tested fruit juices, except for Gala apple juice and Italian grape juice, promoted a higher degree of removal of the smear layer than the negative control, regardless of the type of application (topical vs. friction). However, with friction treatment, orange juice also showed no significant difference from the control. In terms of applying these results to dental practice, it may be recommended that, whenever possible, an attempt be made to remove or modify factors predisposing the patient to dentin hypersensitivity, e.g., an acidic diet. Fruit juices, particularly from acidic fruits like acerola, lemon and kiwi, can remove the smear layer, open dentin tubules and provoke dentin hypersensitivity. ◆

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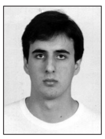

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