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Research Article

Effect of Exposure to Acidic Food Items on Dentin Characteristics: An ATR-FTIR Study

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ATR-FTIR spectroscopy is a powerful technique for unraveling the fine features of dentin; however, its use in the analysis of eroded dentin remains underexplored. Herein, the impact of different acidic food products (a soft drink, vinegar, and yogurt) on dentin was investigated using ATR-FTIR spectroscopy. The durations of exposure were one, three, and seven days. One-way and two-way analyses of variance were employed to analyze differences in mean values among groups, followed by Dunnett's and Tukey's tests. After exposure, the mineral-to-matrix ratio in the three media was significantly lower than that in the control group. The carbonate-to-phosphate ratio and extent of crosslinking increased considerably after exposure to all media; however, the crystallinity index values decreased relative to that in the control group. These findings imply that ATR-FTIR may be an effective noninvasive technique for detecting erosion-induced changes in dentin, having a significant clinical impact on dental care.

1. Introduction

Dental erosion (DE) is the depletion of the structure over time that results in the demineralization of the tooth [1, 2]. It is an irreversible chemical process that causes permanent damage to the surfaces of teeth. Consumption of acidic liquids and food is among the main causes of dental erosion [3]. The global prevalence of DE is reported to be 30% in permanent teeth. Tooth erosion is widespread and continues to increase across the population [4]. According to a recent study, the prevalence ranged from 36.6% in the 15–18 years age group to 61.9% in the 55–60 years age group [5]. Due to a shift in food habits, the consumption of acidic food and beverages has grown; therefore, it is necessary to prioritize studies that further the understanding of the genesis and treatment of tooth erosion [6, 7].

One reason that complicates DE is the fact that the tissues involved in DE, enamel, and dentin are quite different with respect to composition and biomechanical attributes [8]. Dentin is largely organic, as opposed to enamel, which is primarily mineral with traces of collagen, organic material, and water [9]. Despite the fact that the same processes are at work on dentin, the variation in composition causes it to react to erosive activity and wear differently than enamel [10]. Most importantly, when erosive acids attack dentin, a demineralized organic matrix emerges. The softening and liquefaction of dentin significantly influence the amount, extent, and pace of loss of the tooth surface. These changes can also expose the dentin tubules, resulting in tooth sensitivity [11, 12].

To date, numerous qualitative and quantitative approaches have been explored in vivo, in situ, and in vitro to examine the erosion of enamel and dentin [13, 14]. Gastric acids, fruit juices, and soft drinks are risk factors for erosive wear [15]. This was corroborated by a meta-analysis conducted by Hi et al. who discovered that soft drinks had a statistically significant odds ratio for developing DE [15]. Crystallinity, collagen crosslinking, and mineral content are some of the main characteristics that determine the functional quality of dentin [16]. However, estimating these

characteristics poses several technical challenges [16]. New techniques are required to better study structural changes in dentin, especially in DE [17]. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy is a promising nondestructive tool for in vitro assessment of DE [18]. To investigate dentin erosion, ATR-FTIR has also been reported to be a fast and reliable technique for calculating vital parameters, such as the amide: phosphate ratio and the carbonate: phosphate ratio [19–21].

Cola beverages, vinegar, and yogurt are frequently consumed; however, their erosive effects on dentin have not been investigated in detail. Considering the above-described needs, this study employed ATR-FTIR to investigate the effect of these food items on dentin after exposure for various durations. Crystallinity, carbon-mineral ratio, and collagen crosslinking were investigated before and after exposure.

2. Methodology

2.1. Specimen Preparation. Ten human third molars were obtained from the Faculty of Dentistry Clinics at Jordan University of Science and Technology. Defective teeth were excluded from the study. Samples were stored in a 0.1% thymol solution to inhibit microbial growth [22]. The samples were embedded in Kallocryl-CPGH. Teeth slices of 1 mm thickness were sectioned using a water-cooled diamond blade (Isomet; Buehler Ltd., Lake Bluff, NY, USA) mounted on an IsoMet 1000 precision saw machine (Buehler, Lake Bluff, IL, USA). The tooth slices were randomized into four groups ($n = 10$ each). The sample size and the power of the study were calculated using G*Power 3.1.9.2 (https://www.gpower.hhu.de), using a sample size of 10 in each group with a significance level of 0.05, to detect any significant differences between the study groups and the control group. The specimens were manually polished with 1200-grit silicon carbide paper (English Abrasives Ltd., London, UK) before the measurements were performed.

2.2. Acidic Food Items. A soft drink (Jordan Ice and Aerated Water Co.—Pepsi, Amman, Jordan; ingredients: carbonated natural flavor water, citric acid, caffeine, phosphoric acid, high-fructose corn syrup, caramel color, and sugar), vinegar (Apple Vinegar, Al Durra International for Food Products Co. Ltd., Amman, Jordan; ingredients: water, apple juice, cloudifier, caramel color [E 223 d], and sodium metabisulfite preservative [E 223]), and yogurt (Teeba Yogurt, Teeba Investment for Developed Food Processing Co., Amman, Jordan; ingredients: milk and bacteria) were investigated in this study.

2.3. Treatment Protocol. One group was used as a control, and the other three groups were immersed in different acidic food items (soft drinks, vinegar, and yogurt) obtained from local markets. All groups were subjected to ATR-IR under the same conditions. The control group was measured only once, whereas the specimens of the other three groups were tested three times after 1, 3, and 7 days of immersion.

2.4. ATR-FTIR Spectroscopy. An FTIR instrument (TENSOR II, platinum ATR, Bruker Optik GmbH) was used. The spectra ranged from 400 to 4000 cm^{-1} and the spectrum of each sample was collected as an average of 32 scans at a resolution of 4 cm^{-1}. The data were analyzed using Origin Pro 2020 software (OriginLab Corporation, Northampton, MA, USA).

The carbonate/phosphate ratio (C/P) was determined from the intensity of the band at 1414 cm^{-1} to the intensity of the band at 1030 cm^{-1}. The crystallinity index was measured using the intensity ratio of the sub-band at1030 cm^{-1} to the intensity of the sub-band at 1020 cm^{-1} as well as by measuring the full width at half maximum (FWHM) of the peak at 604 cm^{-1} due to ν_4(PO4). The sharper the peak at 604 cm^{-1}, the higher the crystallinity; therefore, FWHM is inversely related to crystallinity. The mineral-to-matrix ratio was estimated from the phosphate band area of 900–1200 cm^{-1} to the amide I band area (1590–1710 cm^{-1}). Collagen crosslinks were estimated from the intensity of the sub-band at 1660 cm^{-1} to the intensity of the sub-band at 1690 cm^{-1}.

2.5. Statistical Analysis. The data were found to be normally distributed. The investigated parameters were analyzed by ANOVA (analysis of variance), one way and two way, followed by Dunnett's and Tukey's tests to determine the effect of the study variables (media solution and exposure time) and to determine the differences between the groups and the control and between the groups of different media and time. A p value equal to or less than 0.05 was considered significant.

3. Results

3.1. pH of the Media. The pH of the medium before and after immersion of the specimen was different in the case of soft drink and vinegar at different time intervals (Table 1). The pH of the soft drink was 1.6 before immersion, which increased to 3.6 on day 7. The pH of vinegar followed a similar trend, being 1.5 before immersion, and increased to 4.1 on day 7. In contrast, the pH of yogurt was 4.0, which changed to only 4.1 on day 7.

3.2. ATR-FTIR Spectra. The ATR-FTIR spectra of dentin and band assignments are shown in Figure 1. Immersion in the media did not yield new infrared bands; only the intensity of the bands changed. There was a strong band at 560 cm^{-1} that was attributed to PO_4^{3-} $\upsilon 4$; another prominent band was at 1013 cm^{-1}, which was attributed to HPO_4^{2-}. Vibrational bands corresponding to the amide group and CO_3^{2-} were also evident. Dentin exhibited peaks associated with amides and an organic matrix (C–H bands). The deconvolution of the specific bands is shown in Figure 2.

3.3. Mineral-to-Matrix Ratio in Dentin. The mineral-matrix ratio was greatly decreased in the three media compared to that in the control (Table 2). One-way ANOVA revealed a significant difference between groups ($p < 0.001$), and the

Table 1: Changes in the pH of the medium before and after immersion of the dentin sample.

Media	pH before immersion	pH after immersion		
		Day 1	Day 3	Day 7
Soft drink	1.6	1.8	1.9	3.6
Vinegar	1.5	1.6	1.7	3.0
Yogurt	4.0	4.0	4.0	4.1

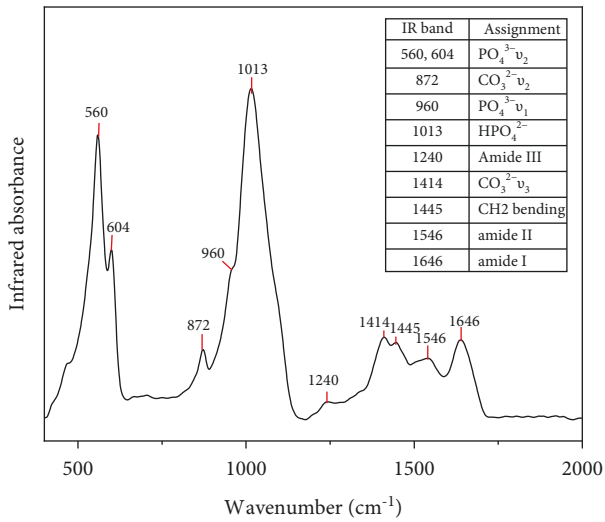

Figure 1: Absorbance spectrum of typical dentin with absorption peaks of the main dentin components and related band assignments.

Dunnett's test at 95% confidence intervals showed that all three media groups at the three exposure times had significantly lower mineral-to-matrix ratios than the control group. Further analyses by two-way ANOVA revealed that both media and time had a significant effect ($p = 0.005$ and $p < 0.001$, respectively), with significant interaction ($p = 0.001$). Analysis of each study variable using a one-way analysis of variance followed by Tukey's pairwise comparison test at 95% confidence intervals showed that, for each media group, exposure time had a highly significant effect in the soft drink and yogurt groups ($p < 0.001$ and $p = 0.005$, respectively), but not in the vinegar groups ($p = 0.328$). Tukey's test showed that in the soft drink groups, a significant difference was observed three times, and the longer the exposure time, the lower the mineral-matrix ratio. The effect of the media at different times of exposure was significant only in the 1-day group ($p = 0.003$) but not in the 3- and 7-day groups ($p = 0.219$ and $p = 0.381$, respectively). Follow-up by Tukey's test for the 1-day showed that the difference was only between the soft drink and vinegar ($p < 0.05$), whereas the difference with yogurt was insignificant ($p > 0.05$).

3.4. Collagen Crosslinking. The mean values of collagen crosslinking in the dentin of the tooth samples are listed in Table 3. The mean collagen crosslinking varied among the three media over time. In vinegar and yogurt, the mean values increased with time; while in soft drinks, they were higher on day 1, then decreased on day 3, and then increased again on day 7. Statistical analysis of all groups, including the control, by one-way ANOVA, showed a significant difference between the groups ($p < 0.001$), and the Dunnett test at 95% confidence intervals revealed that the values at 3 days for soft drink, yogurt, and vinegar, as well as the 1-day vinegar, were not significantly different from the control, but the values at 7 days for the three media and the 1-day soft drink were significantly higher than the control. In contrast, collagen crosslinking after the 1-day of exposure to yogurt was significantly lower. Further analyses using a two-way ANOVA revealed that both media and time had a highly significant effect ($p < 0.001$), with a significant interaction ($p < 0.001$). The follow-up analysis for each study variable using a one-way analysis of variance followed by a pairwise Tukey's comparison test at 95% confidence intervals showed that for each media group, and the exposure time had a significant effect on the soft drink groups ($p = 0.002$) and was highly significant in the vinegar and yogurt groups ($p < 0.001$). Tukey's test showed that in the soft drink groups, the significant difference was between the 1-day and 3-day values only, while in the vinegar groups, the 7-day value was significantly higher than the 3-day and 1-day values, which were not significantly different from each other. However, in the yogurt groups, the 7 and 3 days were not significantly different from each other, but both had significantly higher values than the 1 day, which was extremely low. The effect of the media at different times of exposure was significant in the 1-day and 7-day groups ($p < 0.001$ and $p = 0.023$, respectively), but not in the 3-day group ($p = 0.818$). Follow-up by the Tukey's test for the 1-day groups showed that the significant differences were between all three media ($p < 0.05$), while in the 7-day group, the significant difference was only between the vinegar and yogurt groups ($p < 0.05$) but not with soft drink group ($p < 0.05$).

3.5. Crystallinity. The mean values of the crystallinity index intensity ratio at 1030/1020 cm^{-1} are presented in Table 4. It can be seen that all the groups in the three media at the three exposure times had less crystallinity than the control group, and the time of exposure did not have much effect, except in the vinegar group at 7 days only, where the crystallinity was much lower than in the 1-day and 3-day groups. One-way ANOVA revealed a significant difference between groups ($p < 0.001$), followed by Dunnett's test at 95% confidence intervals, which revealed that all groups had significantly lower crystallinity than the control. Analysis of the data of the samples exposed to different media at different times using two-way ANOVA showed that the media and time of both variables had a significant effect ($p = 0.001$), with a significant interaction ($p = 0.04$). In general, the follow-up analysis for each study variable using one-way ANOVA showed that the time variable did not have a significant effect in the soft drink or yogurt groups ($p = 0.814$ and $p = 0.602$, respectively). In contrast, in the vinegar group, a significant effect was found ($p < 0.001$), and Tukey's test showed a significant difference only after seven days of exposure. When

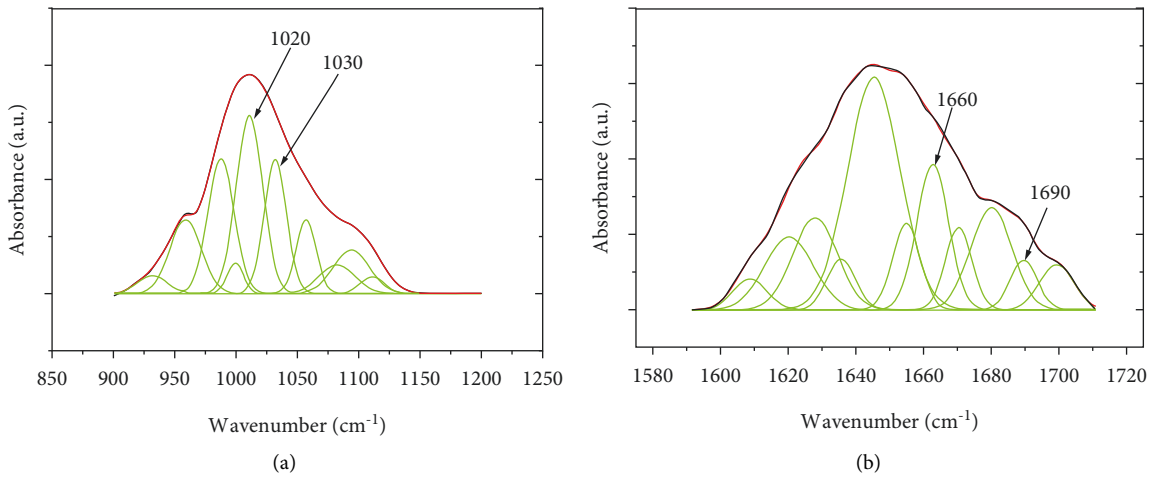

Figure 2: Curve-fitting analysis of the phosphate band (a) and amide I profile (b) of the IR dentin spectra illustrated the sub-bands. The experimental spectrum (black) is overlaid with the reconstructed envelope (red), which is composed of the fitted curves (green).

Table 2: Mineral-to-matrix ratio (mean ± SD) in the dentin of tooth samples after exposure to the different media at variable times, including the control group.

Exposure time	Media			Control
	Soft drink	Vinegar	Yogurt	
1 day	2.601 ± 0.990	1.148 ± 0.648	1.729 ± 0.879	
3 days	1.393 ± 0.578	1.144 ± 0.300	1.029 ± 0.473	8.075 ± 1.695
7 days	0.622 ± 0.289	0.823 ± 0.421	0.795 ± 0.307	

Table 3: Collagen crosslinking (mean ± SD) in the dentin of tooth samples after exposure to different media at variable times, including the control group.

Exposure time	Media			Control
	Soft drink	Vinegar	Yogurt	
1 day	3.932 ± 0.691	2.045 ± 0.394	0.835 ± 0.054	
3 days	2.734 ± 0.721	2.644 ± 0.665	2.536 ± 0.693	2.102 ± 0.820
7 days	3.298 ± 0.647	3.834 ± 0.743	3.025 ± 0.441	

comparing the effect of the media types at the three-time points, one-way ANOVA did not show significant differences between the three media at 1 day and 3 days ($p = 0.454$ and $p = 0.234$, respectively). However, at 7 days, a significant difference was observed ($p < 0.001$). A follow-up Tukey's test showed that only vinegar was significantly different from the other media. However, the difference between soft drinks and yogurt was not significant ($p < 0.05$). Crystallinity measurements were performed using FWHM of the peak at 604 cm^{-1} (Table 5). These results were in agreement with those obtained by estimating the crystallinity index intensity ratio at 1030/1020 cm^{-1}.

3.6. Carbonate Phosphate Ratio. The mean values of the carbonate phosphate ratios in the dentin are presented in Table 6. It can be seen that the ratio of carbonate to phosphate increased for the three media by approximately 3 to 4 times compared to the control, with the greatest increase after 7 days of exposure, particularly in the yogurt group. One-way ANOVA showed a significant difference between the groups ($p < 0.001$), followed by Dunnett's test at the 95% confidence interval, which showed that all three media groups at the three exposure times had a significantly higher carbonate phosphate ratio than the control group.

Further analyses using two-way ANOVA revealed that both media and time variables had a highly significant effect ($p < 0.001$), with a significant interaction between them ($p = 0.001$). Follow-up analysis for each study variable using one-way analysis of variance followed by pairwise comparison Tukey's test showed that for each media group, the exposure time had a highly significant effect ($p < 0.001$). Tukey's test showed that the significant difference was only at 7 days, while the difference between 1 and 3 days was not significant in the three media groups ($p > 0.05$). The effect of the medium at different exposure times was not significant at 1 day ($p = 0.330$) or 3 days ($p = 0.642$); however, at 7 days, the medium had a highly significant effect ($p < 0.001$).

Table 4: Crystallinity index intensity ratio at 1030/1020 (mean ± SD) for dentin of tooth samples after exposure to different media at variable times, including the control group.

Exposure time	Media			Control
	Soft drink	Vinegar	Yogurt	
1 day	1.560 ± 0.242	1.602 ± 0.395	1.787 ± 0.567	
3 days	1.539 ± 0.215	1.551 ± 0.237	1.795 ± 0.552	2.431 ± 0.173
7 days	1.477 ± 0.407	0.905 ± 0.083	1.605 ± 0.211	

Table 5: Crystallinity index based on FWHM of 604 cm^{-1}(mean ± SD) for the dentin of tooth samples after exposure to different media at variable times, including the control group.

Exposure time	Media			Control
	Soft drink	Vinegar	Yogurt	
1 day	13.491 ± 2.769	15.500 ± 2.740	13.353 ± 1.731	
3 days	15.140 ± 3.670	16.640 ± 1.993	14.433 ± 2.957	11.506 ± 1.022
7 days	18.016 ± 2.371	15.963 ± 2.452	13.400 ± 3.640	

Table 6: Carbonate to phosphate (mean ± SD) in the dentin of tooth samples after exposure to different media at variable times, including the control group.

Exposure time	Media			Control
	Soft drink	Vinegar	Yogurt	
1 day	0.811 ± 0.065	0.824 ± 0.083	0.863 ± 0.088	
3 days	0.897 ± 0.044	0.876 ± 0.083	0.900 ± 0.053	0.248 ± 0.093
7 days	1.140 ± 0.066	1.081 ± 0.092	1.321 ± 0.171	

Follow-up by Tukey's test showed that in the 7-day exposure groups, yogurt had the highest mean ($p < 0.05$), while the difference between the soft drink and groups was not significant ($p > 0.05$).

4. Discussion

Recent studies have shown that acidic drinks and food significantly affect the hardness [23] and flexural strength [24] of dental restorative materials. Additionally, the research has been conducted on the impact of drinks or acidic foods on teeth; however, the predominant emphasis has been on the enamel, with the effects on the dentin being relatively underexplored [1, 12, 25–29]. Our in vitro study of dentin revealed that dentin characteristics were significantly altered depending on the nature of the medium and duration of contact. The soft drink and vinegar had higher pH values than yogurt and the pH of the medium remained almost constant for two days. Even on day 1, the mineral-to-matrix ratio decreased significantly in all three media. On day 7, there was no discernible difference among the three groups, indicating that the difference in pH between the soft drink, vinegar, and yogurt was not the only predictor of changes in dentin characteristics. It should be noted that previous studies also did not observe a correlation between enamel erosion and beverage pH [30–32]. However, it should be noted that host-based intrusion detection characteristics and personal food habits can substantially affect DE [28, 33]. Additionally, saliva plays an important role in controlling the pH of the oral cavity [26]. As a result, our study was not intended to mimic normal oral cavity conditions. Essentially, this study aimed to produce a relative value of changes in dentin characteristics when exposed to common acidic food items evaluated in terms of a material attribute.

As mentioned previously, tooth erosion caused by acidic beverages has been studied primarily in the context of enamel, as it is the outer layer of the tooth and the first surface exposed to acidic beverages or food. Dentin is the second layer of the tooth structure; it contains tubules and has less mineralization than enamel. Although less studied, dentin is more prone to erosion if exposed to an acidic environment, which affects peritubular and intertubular dentin, eventually leading to tooth hypersensitivity [34]. Scanning electron microscopy, atomic force microscopy, microhardness, nanoindentation, Raman spectroscopy, and ATR-FTIR are among the most common methods for examining dental characteristics. ATR-FTIR has the obvious advantages of being nondestructive and providing information related to the chemical characteristics of dentin. In our previous study, ATR-FTIR and Raman spectroscopy were used to study the mineral-to-matrix ratio of human permanent and primary teeth, crystallinity, and collagen crosslinks [35]. Notably, the infrared spectrum of complex biological systems, such as dentin, is the sum of contributions originating mostly from the collagen and apatite phases [36]. The intensities of the IR absorption bands provided quantifiable information about the specimen content, depending on the nature of the molecular bonds, their structure, and their surroundings. Several studies have used infrared spectroscopy to investigate mineralization, and

important spectroscopic indications have been reported to identify changes in the apatite/collagen ratio [37, 38].

Intra- and intermolecular crosslinking are the major parameters that affect the mechanical properties of polymer materials [39]. The higher the crosslinking density, the harder and stiffer the matrix. Covalent bonds represent collagen crosslinking from a chemical standpoint, providing stability to the collagen network. With age and collagen maturity in dentin, the peak constituent at 1690 cm^{-1} decreases, but that at 1660 cm^{-1} grows. In terms of the organic matrix, collagen type I accounts for 90% of the organic matrix and forms a 3D network, while collagen types III and IV account for approximately 3% of the collagen. In general, the breakdown of crosslinks under hydrolysis conditions was observed in an acidic environment. The deviation in our results arises primarily from the fact that, in addition to pH, variables such as acid type, resistance to collagenase, and calcium content play a role in the erosive qualities of food [25, 33]. Furthermore, the distribution of collagen crosslinking differs as a function of the anatomical position of dentin molecules [40]. Therefore, more studies are needed to examine these factors.

From day 1, the mineral-to-matrix ratio was considerably reduced in all three media. A decreased mineral-to-matrix ratio in dentistry can result in decreased hardness of the dentin. On the other hand, the carbonate phosphate ratio also increased substantially, further corroborating the decrease in mineral content [27]. It may be noted that the mineralization of calcium phosphate crystals reinforces collagen fibers and is also associated with overall crystallinity. Carbonate substitution with more extended crystals affected the intensity ratio at 1030/1020, which represents mineral crystallinity. For the three media used in the current study, crystallinity decreased substantially after exposure to different times, indicating the possible demineralization of the pH. This also corroborates the findings for the mineral-to-matrix ratio. Hypocalcified formations may have clinical implications because they enable caries proliferation. Further studies are needed to improve our understanding of the precise mechanisms involved in the progression of dentin erosion.

Our results underscore the significance of food habit modification in preventive and therapeutic dental care approaches [41, 42]. ATR-FTIR is an easy tool for dentists to rapidly diagnose the severity of dentin erosion. Dentists should be vigilant of the early signs of DE and take timely interventions to avoid major erosion of the dentin. This study had certain limitations. As this was an in vitro study, it cannot be extrapolated entirely to an in vivo context. It is possible that the characteristics of individual hosts, such as the composition and flow of saliva and individual drinking habits, may alter these results. Furthermore, the approach used in this study may have limitations because it only records abnormalities and not the amount of missing dentin.

5. Conclusions

ATR-FTIR proved useful in determining the carbonate-to-phosphate ratio, collagen crosslinking ratio, mineral-to-matrix ratio, and crystallinity after exposure to acidic media. The crystalline content decreased significantly after exposure; a similar decreasing trend was observed for the mineral-to-matrix ratio, whereas the carbonate-to-phosphate ratio increased. The degree of collagen crosslinking increased after exposure. Both the exposure medium and the duration of exposure affected the level of degradation of the property. The information provided in the present study can help dentists provide more effective diagnostic and preventive treatments for DE and tooth wear in clinical dental practice.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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