XRD AND FTIR INVESTIGATION OF THE STRUCTURAL CHANGES OF THE HUMAN TOOTH INDUCED BY CITRIC ACID

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Abstract. The structural stability of the enamel and dentine of human tooth exposed to the action of citric acid during different time intervals was analyzed by *X-ray diffraction* (XRD) and infrared spectroscopy (FTIR) methods. Before any degradation with the acid, the XRD spectra of the enamel and the dentine show an ordered structure determined mainly by the hydroxyapatite, as well as an amorphous phase determined by other components of the enamel. This structure remains almost unaffected after 4 days of immersion in the citric acid. After a total period of 8 days of exposure to the acid, the ordered structure of the enamel and dentine is affected, but the degradation is more obvious for the dentine. This behavior is confirmed by the results obtained by FTIR investigation. It was observed that the molecular bonds of the enamel become to be affected by the acid after 4 days of immersion, a process that is amplified after 8 days. The dentine is more affected by the acid, fact confirmed by the major modification of the FTIR spectrum after 8 days of degradation. That indicates penetration of the acid into the structure of the dentine. Our study shows an acceptable protection offered by the enamel to the inner part of the tooth for short periods of time of exposure to the citric acid.

Key words: Enamel, dentine, citric acid, degradation, XRD, FTIR.

1. INTRODUCTION

In the actual context of diversification of human alimentation and continuously changes of the alimentary habitudes of the peoples, a special care must be accorded to the impact of the food composition on the structure of the tooth. The human tooth has complex structure, but for the sake of simplicity we describe this structure being composed of two parts: the inner part, named dentine and the outer part, the enamel. The main component of the enamel and dentine is the natural *hydroxyapatite* (HA), which represents 96% of the volume of the enamel and 74% of the dentine [1]. The water represents 1–6% wt of the enamel and 15–30% wt of the dentine [2]. Apart from the carbonated apatite the enamel and dentine contain

organic material, up to 4% wt for the enamel and up to 74% wt for the dentine, and other inorganic impurities as carbonate ions CO_3^{2-}. These ions are located in the PO_4^{3-} tetrahedron and represent up to 3.5% wt of the enamel and up to 5.6 % wt of the dentine [3, 4]. If the dentine is associated with the vital part of the tooth, the enamel plays the role of protection against the negative action of foods, liquids, or other substances presents in the oral cavity during the nutrition. Frequently such aliments have an aggressive action on the structure of the tooth. We can mention here the citric and acetic acids contained in the fruits or other eatable. Apart from the foods, sometimes the products for the whitening of the tooth can have aggressive effects. Usually the enamel ensures enough protection of the dentine at low concentration and short time of exposure to such substances, but this protection can be strongly reduced by high concentration or the long exposure to such eatables. Moreover, these substances can be in direct contact with the dentine, if the enamel is fissured, or missing, for instance in the cases of periodontal affections or caries. The degradation caused by the exposure of the tooth to chemicals from drinks, food, microorganisms and saliva can undergo softening and roughening of the enamel and dentine making their surfaces more susceptible to the physical forces occurring during the mastication [5]. Then it is important to know the effect of such substances on the structure of the tooth. The clinical examination can detect only visual deterioration of the tooth but cannot reveal the structural modification. Such changes can be put on evidence only by microscopic methods of analysis as *X-ray diffraction* (XRD) and *Fourier Transform Infrared Spectroscopy* (FTIR). By XRD it is highlighted the crystal structure of the samples, whereas by the FTIR analysis are identified the vibration modes of the molecular bonds. Previous studies reported in the literature aims with this subject. For instance, the investigation of the whitening process with carbamide peroxide solution on the enamel and dentine structure was studied by IR method [6]. IR and Raman techniques were used for the study of the surface structure of the dental cavities after mechanical excavation of caries and etching with different acids [7]. FTIR spectroscopy was used to investigate the changes of the enamel structure of some fossilized tooth [8]. XRD and FTIR were used for the investigation of the crystallinity indices of human tooth enamel and synthetic hydroxyapatite [9]. XRD and Raman were used for the investigation of the effect of some acids on the structure of dental material Herculite [10]. However, few studies were reported on the literature concerning the XRD and FTIR analysis of the effect of alimentary citric acid on the structure of the tooth. For this reason, in the present report, the behavior of the teeth in extreme conditions has been studied, by maintaining them in a high concentration of citric acid for 4 and 8 days respectively.

2. EXPERIMENTAL

For our study we used a molar human tooth, provided by a dental care office, without any previous caries or excavation. The tooth was longitudinally cut parallel

with its side face, resulting samples with two parts, an external enameled face (FS) and an inner face constituted from pure dentine (FD). The samples were immersed in citric acid of 96% concentration for four days (96 hours). We have chosen this high concentration to check the behavior of samples in extreme conditions. The samples were analyzed with a diffractometer equipped with an X-ray tube with CuKα (λ = 1.54056 Å) radiation, in 2θ geometry, with a scanning speed of 2°/min and a step size of 0.02 in a 2θ range of 10 to 80°.

Infrared absorption spectra were obtained using a Fourier Transform-Infrared spectrometer in the spectral domain 400–2000 cm^{-1}, with a resolution of 4 cm^{-1} and 256 scans. For the FTIR investigations about 1.8 mg from each sample was mixed with 150 mg KBr powder and compressed in pellets by pressed at 5 tones. The background spectrum of the KBr pellet was recorded under the same instrumental conditions and automatically subtracted from each sample spectrum. Data analysis was performed using Spectra Analysis software.

3. RESULTS AND DISCUSSION

The main constituent of the bones and tooth is the hydroxyapatite, a complex compound containing calcium, phosphorous, oxygen and hydrogen atoms with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ [11, 12]. This compound appears in the human tooth in both amorphous and crystalline phases. The ratio of the two phases and the size of the crystalline grains can change in function of the action of different external aggressive agents. It was found that thermal treatment of the natural hydroxyapatite leads to modification of the percentage of different types of crystalline phases [13].

To highlight the changes that occur in the present study we compared the diffraction patterns of enamel and dentine before and after the immersion in citric acid. We used the following abbreviations: FS and FD for the initial samples before any immersion in the acid, FS-4dCI and FD-4dCI for the samples immersed in citric acid for 4 days, CI for the citric acid and HA for the hydroxyapatite.

The diffractogram of the enamel, before immersion, contains a broad part between 10º and 40º determined by the non-crystalline part of the hydroxyapatite [14]. Superimposed on this part we can see distinctive peaks at 2θ = 26.6º, 32.8º, 34.7º, (Fig. 1 FS). They are associated to the diffraction plans (002), (211) and (300) respectively of the crystalline part of the hydroxyapatite, (according to the X-ray Powder Diffraction File (PDF) card number 024–0033 and Hydroxyapatite PDF number 86–1200 of the Joint Committee on Powder Diffraction Standards (JCPDS)) [15]. Other distinctive peaks with high intensities are observed at 2θ = 50.1º, 53.9º, 62.4º, 64.6º, 76.2º, 77.4º. The peaks at 50.1º and 53.9º are assigned to planes (213) and (004) of the hydroxyapatite, [9].

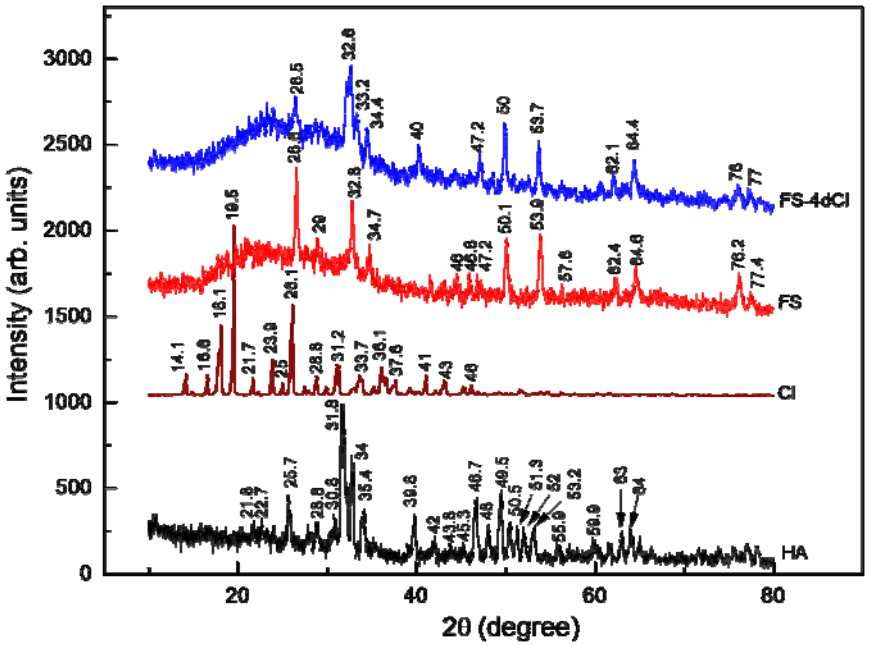

Fig. 1 – XRD patterns of hydroxyapatite (HA), pure citric acid (CI), enamel before immersion in the acetic acid (FS) and enamel after 4 days of immersion (FS-4dCI).

The sample was immersed in citric acid and then investigated again. After 4 days of immersion the XRD spectrum of the enamel is very similar to the spectrum before immersion. It contains all the diffraction peaks of the initial enamel with similar intensities. Only the peaks at 2θ = 26.5º and 47.2º have smaller intensities, (Fig. 1, FS-4dCI). In addition, in this diffractogram we can observe a new peak at 2θ = 40º close to the peak 2θ = 39.8º of the hydroxyapatite. The peak 26.5º is close to the peak 26.1º of the citric acid. Apart this peak none other lines of the acid can be seen in the spectrum of the enamel after treatment. That means very weak inclusion of the acid into the structure of the enamel and a good stability of the enamel against this acid at moderate time exposure.

Similar investigations were done on the dentine. The XRD patterns of hydroxyapatite (HA), pure citric acid (CI), dentine before immersion in the acetic acid (FD) and dentine after 4 days of immersion (FD-4dCI) are presented in Figure 2. The XRD diffractogram of the dentine, before the treatment with the acid, contains a broad part between 10º and 40º, and some distinctive peaks at 2θ = 22.7º, 31.8º, 33º, 49.6º, 53.4º, (Fig. 2 FD). These peaks can be seen in the spectrum of the enamel, being assigned to the similar diffraction planes. Some of these peaks are very close to those of the hydroxyapatite 2θ =31.8º, 32.8º, 49.5º, 53.2º. This behavior is obvious because the hydroxyapatite is the main component of the enamel and dentine. However, the peaks of the dentine are larger than these of the

enamel. This is explained on the basis of the size of the crystals, which is larger in the enamel (50 nm on average) than in the dentine (10 nm on average) [16].

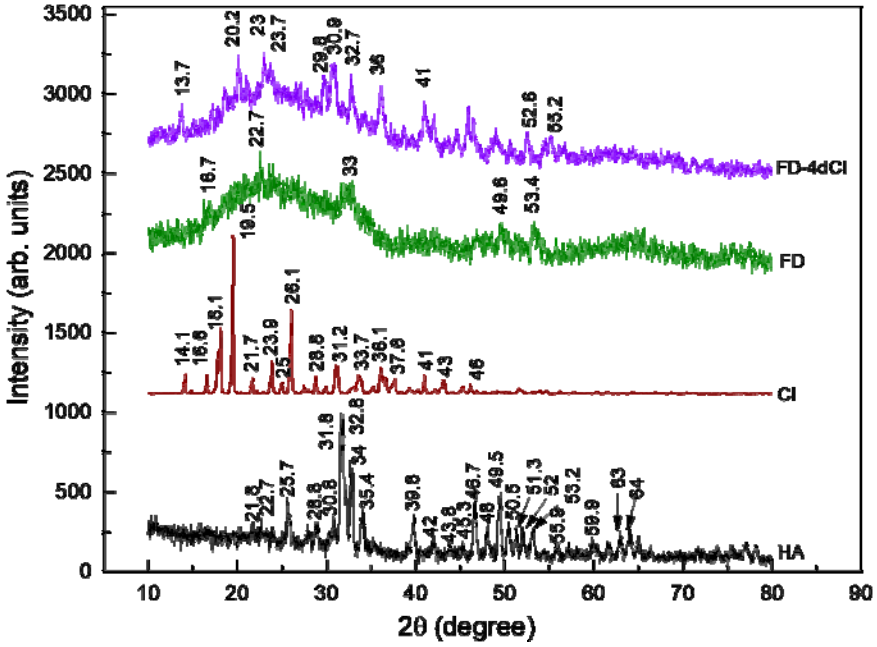

Fig. 2 – XRD patterns of HA, CI, FD and FD-4dCI.

Modification of the spectrum appears after immersion into the citric acid, (Fig. 2 FD-4dCI). It contains a broad part and many distinctive peaks located at 2θ = 13.7º, 20.2º, 23º, 23.7º, 29.8º, 30.9º, 32.7º, 36º, 41º, 42º, 42.2º, 52.6º, 55.2º. Some of these peaks are very close to those of the citric acid, 2θ = 23.9º, 31.2º, 36.1º, 41º. Without the protection of the enamel the acid penetrates the structure of the dentine that explains the apparition of these peaks. The spectrum contains also some peaks of the hydroxyapatite at 2θ = 30.8º and 32.8º. That fact indicates the persistence of the hydroxyapatite in the dentine, and only a partial destruction of the dentine by the acid.

After this treatment the samples were kept 30 days in dark, at room temperature, in closed containers and investigated again. We made this kind of investigation in order to observe the possibility of apparition of structural modifications after long time of conservation. As we can see from the Figure 3 the XRD spectrum of enamel immediately after the first degradation, (FS-4dCI) and the spectrum after 30 days of conservation (FS-4dCI+30dRT), are very similar. The main peaks appear at the same diffraction angle with similar amplitude. Similar behavior was observed for the dentine, (FD-4dCI and FD-4dCI+30dRT). That indicates good stability of the structure of the enamel and dentine during this period of conservation.

After this period, we continued the degradation with the citric acid up to 8 days, and the samples we measured again. The annotations of the samples are FS-8dCI, FD-8dCI. Some modification of the XRD pattern appears. The mains peaks, previously seen in the spectrum of the enamel, appear with smaller intensity after a total 8 days of degradation at 2θ =26.6º, 32.8º, 50.1º, 53.9º and 64.6º. The other peaks almost disappeared. More evident modifications can be seen in the spectrum of the dentine after 8 days of degradation. The spectrum is broad with a shoulder in the domain 2θ =26–34º. This corresponds to the peaks 2θ = 23.7º, 29.8º, 30.9º, 32.7º and 36º of the dentine observed after the first 4 days of degradation (FD-4dCI). Moreover, the diffractograms of the enamel (FS-8dCI) and dentine (FD-8dCI) after 8 days of degradation are almost similar. That suggests a massive destruction of the enamel after 8 days, so that the remaining part of the sample is constituted by the dentine. In these circumstances both spectra concern the structure of the dentine. The broadening of the spectra and the missing of the peaks indicate important destruction of the ordered structure after long exposure to the acetic acid.

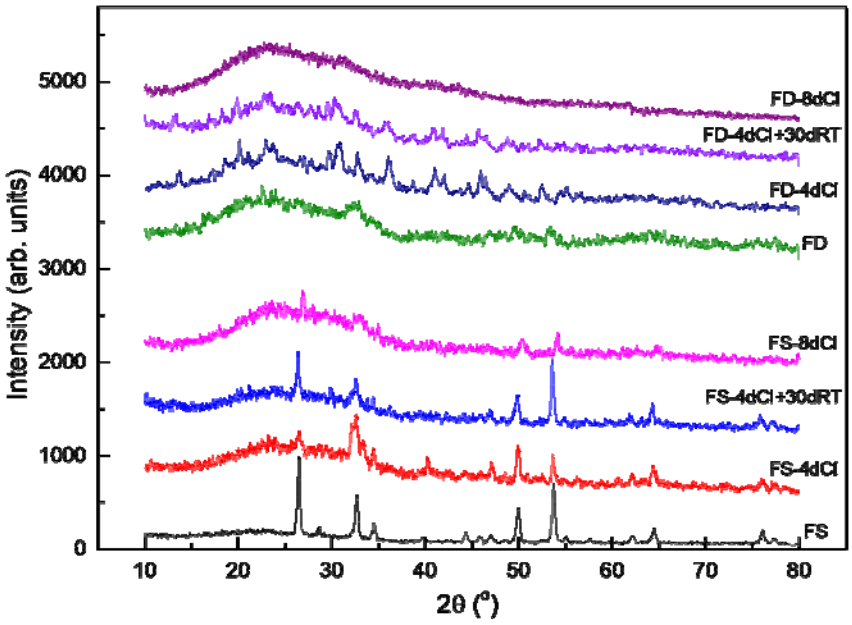

Fig. 3 – Comparison of the XRD spectra of the enamel and dentine after 4 days of immersion in citric acid, (FS-4dCI and FD-4dCI), 30 days of conservation, (FS-4dCI+30dRT and FD-4dCI+30dRT) and 8 days of immersion in citric acid, (FS-8dCI and FD-8dCI).

Information on molecular scale, concerning possible chemical reaction between the citric acid and the tooth, are provided by FTIR investigation. Every new component arising from chemical reaction is followed by the modification of

the initial vibrational spectrum of the samples. Before immersion into the acid, the spectrum of the enamel contains vibration bands located at 1632, 1461, 1418, 1035, 873, 604, 564 cm^{-1} and small band shoulders at 1095, 957, 873 cm^{-1}, (Fig. 4a). The bands 1095, 1035 and 957 cm^{-1} are assigned to PO_4^{-3} groups. The bands (1551, 1461, 1418 and 873 cm^{-1}) are assigned to the carbonate groups of the hydroxyapatite, [17]. The carbonate bands at 1551 and 1461 cm^{-1} indicate the inclusion of CO_3^{2-} in the OH^- position [9].

The bands in the domain 1600–1700 cm^{-1} and the vibration at 561 and 604 cm^{-1} are associated to the absorbed water [18].

Some modifications can be seen after 4 days immersion of the enamel in the citric acid, (FS-4dCI). A shoulder appears at 567 cm^{-1} and new peaks at 660, 840, 1150 and 1551 cm^{-1}. These peaks cannot be seen before. That means the apparition of new vibration modes determined by the interaction of the enamel with the citric acid. The peak located at 1632 cm^{-1}, which can be seen into the spectrum before immersion, appears here with high intensity at 1625 cm^{-1}. This shift indicates the increasing of the amount of water after immersion into the citric acid. The spectrum contains also some peaks at 1084 and 1393 cm^{-1} determined by the interaction with the citric acid. The peak 1084 cm^{-1} corresponds to the peak 1082 cm^{-1} of the citric acid, slowly shifted. The small shoulders which appear in the spectrum of FD-4dCI at 1702 and 1735 cm^{-1} are due to the absorption of citric acid. They correspond to the peaks 1704 and 1749 cm^{-1} of the citric acid. These peaks indicate the penetration of the acid into the structure of the enamel. It is in concordance with the XRD investigation.

Similar investigation was done on the dentine (Fig. 4b). Before the immersion, the spectrum contains some distinctive peaks at 561, 604, 872, 1031, and 1663 cm^{-1}. These peaks can be seen also in the spectrum of the enamel before immersion (FS) (Fig. 4a) at 564, 604, 873, 1035 and 1632 cm^{-1} but they are slightly shifted. The shift of the bands 561, 604 and 1031 cm^{-1} of the PO_3^{-4} groups, is determined by the difference in the crystallinity of the enamel and dentine, [19, 20]. The peak 1551 cm^{-1} of the carbonate groups of the hydroxyapatite appear here shifted at 1546 cm^{-1}.

The spectrum after 4 days of immersion in citric acid is shown in Figure 4b, (FD-4dCI). The peaks 601 and 1550 cm^{-1} represent the shifted peaks 604 and 1546 cm^{-1} of the dentine before immersion. The peak 1085 cm^{-1} appear here distinctively, it appears as small shoulders in the spectrum of the dentine before immersion, and distinctively in the spectrum of the citric acid at 1082 cm^{-1}. The peaks of the citric acid in the domain 1150–1392 cm^{-1} are missing in the spectrum of the dentine before immersion, but they appear distinctively after 4 days of immersion. Same situation for the peaks 1704 and 1749 cm^{-1} of the acid, which are missing in the spectrum of the initial dentine, but they appear distinctively at 1702 and 1735 cm^{-1} after immersion. That indicates progressively penetration of the acid into the structure of the dentine in function of the time of immersion. The presence of the water inside the dentine after immersion is confirmed by the presence of the broad

peak at 1632 cm^{-1}. New peaks appear at 528, 660, 840, 1150 cm^{-1} assigned to different phosphate and carbonate structures.

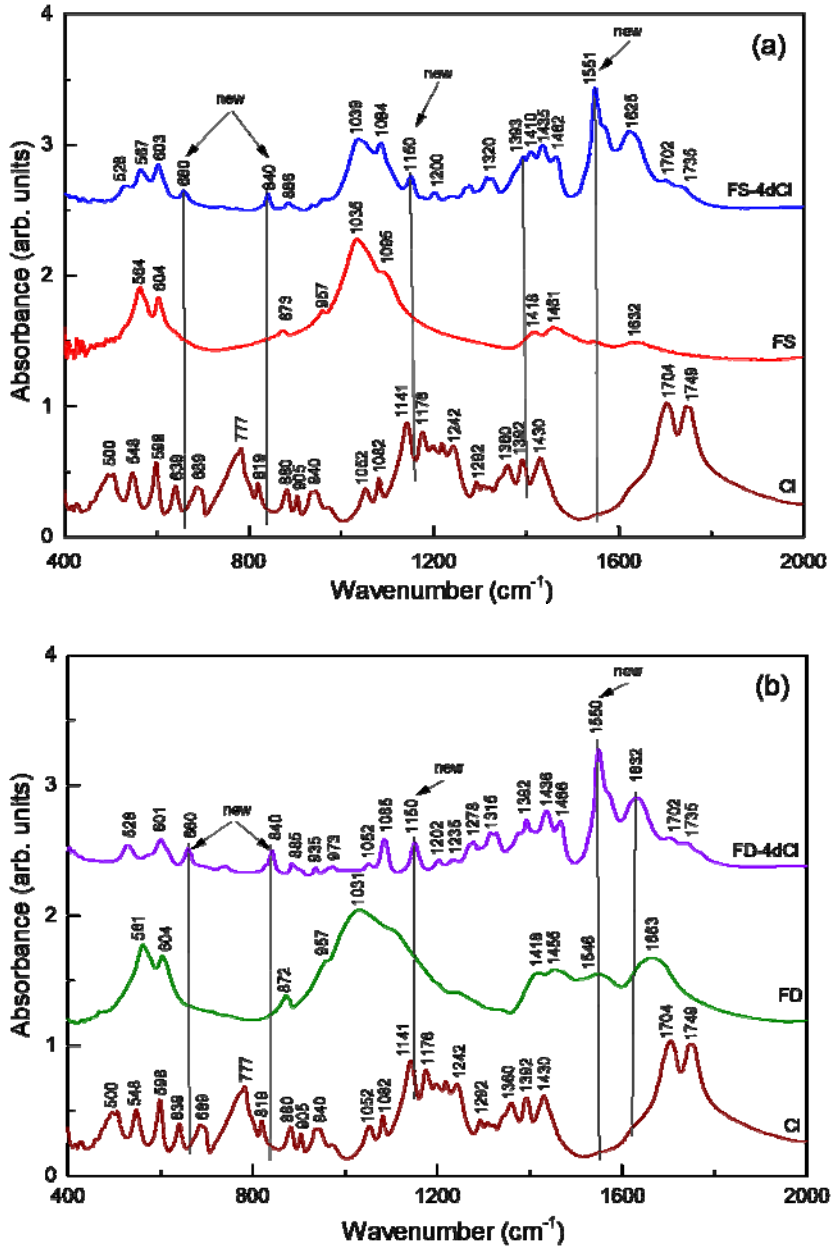

Fig. 4 – The FTIR spectra of the: a) CI and FS; b) CI and FD before and after 4 days immersion in citric acid in the range 400–2000 cm^{-1}.

The IR spectra indicate only weak interaction of the acid with the enamel. The enamel plays an efficient protective role of the inner part of the tooth. On the other hand, the dentine, without the protection of the enamel, is strongly affected by the acid. The evolution of the FTIR spectra indicates a progressively penetration of the acid into the structure of the dentine.

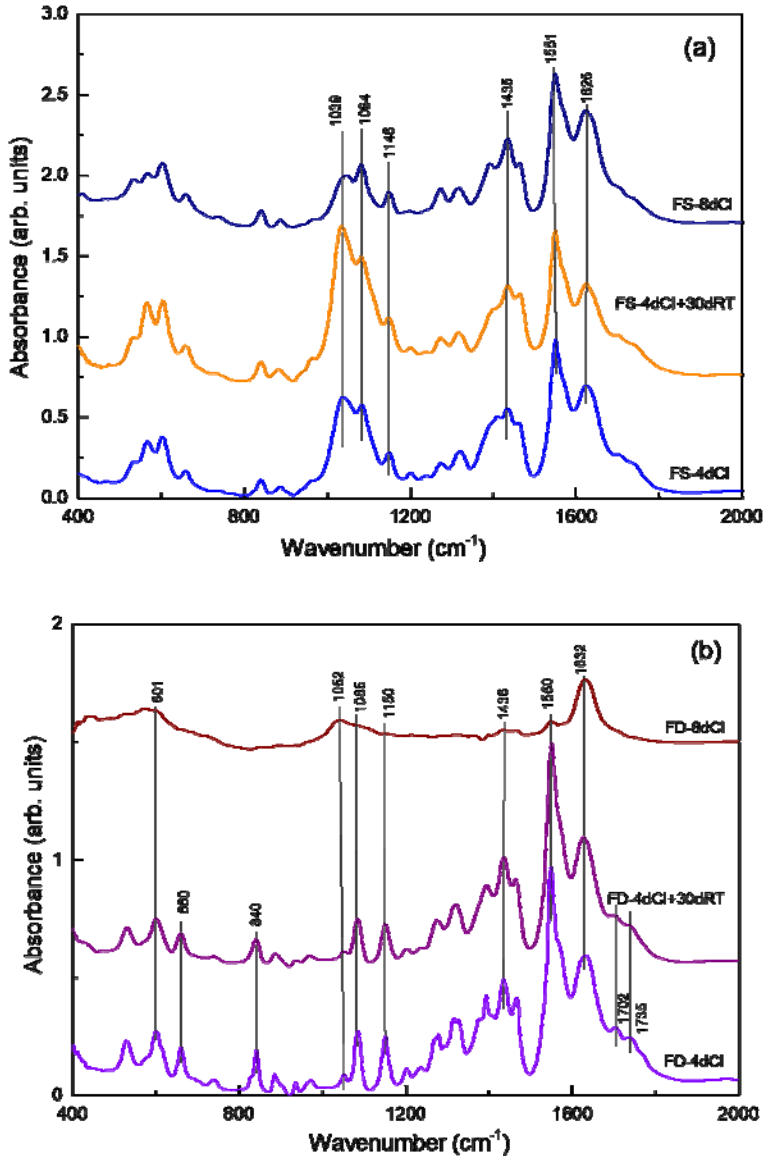

Fig. 5 – FTIR spectra in the range 400–2000 cm^{-1} of the FS (a) and FD (b) after 30 days of conservation, and after 8 days immersion in citric acid.

As in the case of the XRD investigation, after immersion in citric acid the samples were stored 30 days in closed containers in dark at room temperature and then were investigated again by FTIR spectroscopy. The FTIR spectrum of the enamel is almost identical with its spectrum before conservation (Fig. 5a, FS-4dCI and FS-4dCI+30dRT). That means good stability of the enamel after this period of conservation. After a new immersion in citric acid for 4 days, the spectrum is almost similar with the spectrum before the conservation, (Fig. 5a, FS-8dCI). Only the amplitude of the bands 1039 and 1084 cm^{-1} decreases. Other bands, 1146, 1435, 1551 1625 cm^{-1} remain unchanged, that confirms the good chemical stability of the enamel against the citric acid.

The spectrum of the dentine after 30 days of conservation is similar with the spectrum before the conservation. The vibration bands appear without shift or important modification of the intensities, (Fig. 5b). That means no deterioration of the structure of the dentine during this period. Important modifications appear after 8 days of total immersion in citric acid. Distinctive bands can be seen at 1632, 1550, 1052 and 601 cm^{-1}. Some vibration bands are missing or appear with reduced intensity, 660, 840, 1085, 1150, 1200–1500, 1702, 1735 cm^{-1}. That indicates strongly effect of the acid on the structure of the dentine after 8 days of immersion in the acid.

4. CONCLUSIONS

The structural stability of the enamel and dentine of human tooth exposed to the action of the citric acid during different time intervals was analyzed by XRD and FTIR methods. Before any degradation of the enamel the XRD investigation shows an ordered structure determined mainly by the hydroxyapatite, as well as an amorphous state determined by the organic components of the enamel. This structure remains almost unaffected after 4 days of immersion in citric acid. The conservation of the samples in closed containers in dark at room temperature during 30 days, has no effect on theirs structure. However, after a total period of 8 days of exposure to the acid, the ordered structure of the enamel is affected. This fact is determined by the interaction of the enamel with the acid. A reduction of the crystalline phase is observed. This behavior is confirmed by the FTIR investigation. The molecular bonds of the enamel become to be affected by the action of the acid after 4 days of exposure, process that is amplified after a total exposure of 8 days. We can identify some vibration bands of the acid after long period of immersion. That demonstrates the penetration of the citric acid into the structure of the enamel after 8 days of immersion.

The dentine is more affected by the action of the acid. This is demonstrated by the evolution of the XRD spectra. After 8 days of exposure the ordered structure vanishes completely. This fact is confirmed also by the FTIR spectra. After the first 4 days of the action of the acid we can identify some vibration bands of the citric

acid into the spectrum of the sample, and after a total period of 8 days of exposure to the acid, the FTIR spectrum is completely different in comparison with the initial one. That indicates a penetration of the acid into the structure of the dentine and a chemical reaction with the acid.

As general conclusion, the enamel confers an acceptable protection of the inner part of the tooth for short time of exposure to the citric acid. The dentine, without the protection of the enamel, is strongly affected by acid.

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