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XRF analysis of tooth enamel under conditions of experimental erosion in vitro

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Abstract
INTRODUCTION. Dental erosion is a result of chemical processes – specifically, "acid attacks" on the tooth surface that occur without bacterial involvement – leading to alterations in the mineral structure of dental tissues. In recent years, the erosive potential of fruit juices, carbonated and non-carbonated soft drinks, as well as alcoholic beverages, has been actively studied in an effort to better understand the mechanisms of demineralization and to assess the impact of drink acidity. However, there is a lack of data on experiments that provide a comprehensive profile of macro- and microelement content in erosion zones of varying severity compared to intact enamel and dentin.
AIM. To examine, using X-ray fluorescence analysis, the dynamics of changes in the calcium-to-phosphorus (Ca/P) ratio–an important indicator of tooth mineral content–under conditions of artificial erosion caused by various acidic food and beverage solutions.
MATERIALS AND METHODS. The exogenous acidic agents used included solutions of lactic, acetic, and hydrochloric acids; lemon juice; dry red wine; Dobry Cola; and a solution of Acidin-Pepsin tablets (a drug prescribed for hypoacid and anacid gastritis). Recently extracted intact teeth were immersed in the test liquids for three days. Demineralization was assessed based on observed changes in elemental composition. The chemical analysis of the solid dental tissues was performed using an M4 TORNADO X-ray fluorescence spectrometer (Bruker).
RESULTS. In all test conditions, demineralization occurred as evidenced by the active release of calcium and phosphorus – the main macroelements – from the crystal lattices of hydroxyapatite, carbonate apatite, chlorapatite, fluorapatite, and other mixed apatite forms found in enamel. Notably, the kinetics of calcium and phosphorus loss differed significantly. In all cases, the Ca/P ratio increased substantially after three days of exposure to the erosive medium, compared to baseline values in intact enamel. This finding indicates that phosphate groups are the first to be lost during erosion, dissolving into the oral environment, followed by calcium loss as a less intense secondary process.
CONCLUSIONS. Based on analysis of Ca/P ratios, enamel erosion appears to begin with dephosphorylation of the crystalline lattice, followed by decalcification.

Keywords: erosive wear, tooth erosion, artificial dental erosion, X-ray fluorescence analysis, tooth mineral structure

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Рентгенофлуоресцентный анализ (РФА) эмали зуба в условиях экспериментальной эрозии in vitro

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Резюме
ВВЕДЕНИЕ. Эрозия есть следствие химических процессов, а именно «кислотной атаки» на зуб без участия бактерий, в результате чего искажается минеральная структура тканей зуба. Эрозивный потенциал фруктовых соков, газированных и негазированных безалкогольных и алкогольных напитков

в последние годы изучается особенно интенсивно с целью понять тонкий механизм деминерализации, оценить влияние степени кислотности напитка. Однако сведений об экспериментах, в которых была бы получена полная картина по содержанию макро- и микроэлементов в зонах эрозии разной степени в сравнении с неповрежденными участками эмали и дентина, в литературе не обнаружено.
ЦЕЛЬ. Изучить с помощью рентгенофлуоресцентного анализа динамики изменения наиболее показательного индекса Ca/P тканей зуба в условиях искусственной эрозии, вызванной различными пищевыми жидкостями с пониженным значением pH.
МАТЕРИАЛЫ И МЕТОДЫ. В качестве экзогенных источников кислотного фактора использовали растворы кислот (молочная, уксусная, соляная), сок лимона, красное сухое вино, Добрый Cola, а также раствор таблеток препарата Ацидин-пепсин, назначаемого при анацидном и гипоацидном гастритах. Интактные свежеудаленные зубы погружали в исследуемые жидкости на трое суток, после чего оценивали степень происшедшей деминерализации по наблюдаемым изменениям содержания элементов. Химический состав образцов твердых тканей зуба выполняли на рентгенофлуоресцентном спектрометре M4 TORNADO (Bruker).
РЕЗУЛЬТАТЫ. Во всех случаях наблюдался процесс деминерализации, связанный с активным выходом из кристаллических решеток гидроксиапатита, карбонатапатита, хлорапатита, фторапатита и других, смешанных форм апатитов, из которых состоит эмаль, основных макроэлементов – кальция и фосфора, причем кинетика этих двух процессов имела существенные различия. Во всех случаях индекс Ca/P через трое суток пребывания в эрозионной среде значительно отклонился в сторону увеличения по сравнению с исходными значениями, характерными для неповрежденной эмали. Это обстоятельство прямо указывает на то, что кристаллы апатитов, составляющие эмалевые призмы, при эрозии в первую очередь лишаются фосфатных групп, уходящих в ротовую жидкость, с последующей декальцинацией в статусе менее интенсивного вторичного процесса.
ВЫВОДЫ. На основе анализа значений индекса Ca/P установлено, что при эрозии дефосфорилирование кристаллической решетки эмали является первичным процессом, за которым следует декальцификация.

Ключевые слова: эрозивный износ, эрозия зубов, искусственная эрозия зубов, рентгенофлуоресцентный анализ, минеральная структура зуба

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INTRODUCTION

Erosive tooth wear (erosion, erosive attrition) is a contemporary medical issue closely related to oral health. Data from various sources indicate a growing prevalence of erosion, ranging between 30–50% of the population [1–3], while in certain geochemical provinces, substantially higher figures have been reported [4; 5]. It is generally accepted that erosion results from chemical processes triggered by an “acid attack” on the tooth surface without bacterial involvement. This process leads to the alteration of the mineral structure of dental tissues, initially affecting superficial layers and subsequently extending to deeper ones.

Erosion develops under the influence of dietary, and consequently chemical, biological, and behavioral factors, which interact and produce diverse clinical manifestations. Early detection of erosion remains a critical challenge for dental practitioners, as functional and aesthetic complications typically arise at more advanced stages. Understanding the etiological factors of erosive tooth wear is essential for preventing disease progression and for designing effective preventive strategies [6; 7].

In recent years, the erosive potential of fruit juices, carbonated and non-carbonated soft drinks, as well as alcoholic beverages, has become increasingly evident and has been the subject of intensive study [8]. These investigations aim to elucidate the mechanisms of demineralization and to assess how beverage acidity and the combined effect of dissolved substances influence the intensity of the process. To date, numerous highly cited publications have reported the results of in vitro experiments on extracted teeth immersed in various food-derived liquids or their analogues [9–11]. Researchers have employed advanced methods such as three-dimensional confocal laser microscopy, atomic force microscopy, laser speckle imaging, microradiography, and electron probe microanalysis.

However, we have not encountered studies that provide a comprehensive characterization of macro- and microelement composition within erosion zones of varying severity compared with intact areas of enamel and dentin. Such information would be of significant practical value, as it could contribute to a more detailed understanding of the chemical dimension of the erosive process, which comprises a complex interplay of reversible and irreversible chemical reactions. The crystalline lattice components of hard dental tissues are induced into these reactions by the surrounding liquid medium.

X-ray fluorescence analysis (XRF), which we have repeatedly applied in dental research, has proven to be an effective method for obtaining novel insights that allow for a re-evaluation of the etiology and development of oral pathologies. This, in turn, provides clinicians with the opportunity to implement more evidence-based and thus more effective therapeutic strategies. In particular, the use of XRF has demonstrated fundamental differences in the chemical mechanisms underlying caries and erosion [12; 13].

AIM

To investigate, using X-ray fluorescence analysis (XRF), the temporal dynamics of the calcium-to-phosphorus (Ca/P) ratio in dental hard tissues under conditions of artificial erosion induced by various low-pH food and beverage solutions with differing erosive potentials.

MATERIALS AND METHODS

The study was conducted at the Departments of Therapeutic Dentistry and Endodontics, and General and Bioorganic Chemistry of the Russian University of Medicine, as well as in the laboratories of the N.S. Kurnakov Institute of General and Inorganic Chemistry of the Russian Academy of Sciences. Intact human teeth extracted for periodontal and orthodontic reasons, without signs of prior dental treatment, were used. Samples were cleaned of soft tissues, disinfected, and stored in distilled water at 4°C. The hydrogen ion concentration (pH) was measured using a portable electronic pH-meter (Hanna Instruments) at 25°C.

As exogenous sources of the acidic factor, we used solutions of lactic, acetic, and hydrochloric acids; fresh lemon juice; commercial beverages (dry red wine, Dobry Cola); and a solution of Acidin-Pepsin tablets, a preparation prescribed for anacidic and hypoacidic gastritis. Healthy teeth were immersed in the test solutions for three days, after which the degree of demineralization was evaluated based on changes in elemental composition.

The chemical composition of dental hard tissues was analyzed using an M4 TORNADO (Bruker) X-ray diffractometer, a micro-XRF spectrometer designed for nondestructive qualitative and quantitative material characterization. The method is based on the interaction of high-energy X-rays with elements in the sample, resulting in the emission of secondary X-rays (X-ray fluorescence) characteristic for each atom. The instrument was equipped with a rhodium fine-focus X-ray tube (maximum excitation 50 kV, 30 W, 600 μA). The MultiPoint mode was applied (analysis at several points across the selected enamel surface area).

During measurements, the X-ray beam was directed to a defined point of the enamel surface, and the elemental composition was determined for a spot diameter of 20 μm. For each point, the system averaged 100 pulses and displayed the detected elements in the form of a spectrum. The resulting spectrum was converted into a table showing the percentage mass ratios of the detected atoms relative to the total elemental content, with the spectrum normalized to 100%. It is well established that XRF cannot determine with sufficient accuracy the elements of the first two periods of the Periodic Table and sodium; however, this limitation did not affect the objectives of our study.

The spectrometer was calibrated against a standard sample of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) for calcium and phosphorus content. The measurement accuracy was 0.01%. Statistical analysis of the obtained data was performed using a personal computer and the software package *Statistica 9.0*.

For interpretation of deviations in elemental content, the calcium-to-phosphorus (Ca/P) ratio was used as a reference index. According to the literature, enamel resistant to acidic challenge demonstrates a Ca/P ratio in the range of 1.6–2.1 (by mass). In our study, the Ca/P ratio for intact enamel samples from healthy teeth ranged between 1.9–2.1. The trace elements identified by XRF spectra corresponded to the typical elemental composition of enamel both in nomenclature and quantitative distribution.

RESULTS

The three-day immersion period of intact teeth in liquids with a predetermined erosive potential was chosen in order to avoid random fluctuations characteristic of shorter exposure intervals, while simultaneously establishing a high acid load from the outset. In our view, the obtained results largely reflect the clinical picture that develops in vivo under conditions of regular and systematic consumption of such beverages.

In all cases, demineralization was observed, associated with the active release of calcium and phosphorus – the principal macroelements – from the crystal lattices of hydroxyapatite, carbonate-apatite, chloroapatite, fluoroapatite, and other mixed apatite forms constituting enamel. Notably, the kinetics of calcium and phosphorus release demonstrated substantial differences.

Under normal conditions, the heterogeneous equilibrium of "demineralization–remineralization" between the solid phase (enamel) and the saturated solution (oral fluid) can conventionally [14] be represented as:

$$Ca_{10}(PO_4)_6(OH)_2 + 8H_3O^+ \leftrightarrows 10Ca^{2+} + 6HPO_4^{2-} + 10H_2O,$$

whereas at a critical pH value of 5.5 or below, a cascade of ion-exchange reactions is initiated, beginning with the hydration of apatites as calcium ions are replaced by an equivalent charge of hydronium ions:

$$Ca_{10}(PO_4)_6(OH)_2 + 2H_3O^+ \leftrightarrows Ca_9(H_3O^+)_2(PO_4)_6(OH)_2 + Ca^{2+}.$$

If the buffering capacity of saliva is insufficient to neutralize the exogenous acid challenge, enamel destruction ensues. In vivo, however, the kinetics of equilibrium processes is considerably more complex, being additionally influenced by factors such as the electrolyte composition and ionic strength of oral fluids and beverages, as well as gender, age, circadian rhythms, and other systemic variables.

As an illustration, Fig. 1 presents a fragment of a tooth sample immersed in "Dobry Cola", accompanied by the corresponding XRF spectrum (Fig. 2) and the numerical values of relative elemental content at analytical points (Fig. 3).

DISCUSSION

The obtained results for the studied media, arranged in order of increasing acidity, are presented in the table. It was found that in all cases the Ca/P ratio, after three days of immersion in erosive media, showed a significant upward deviation compared to the baseline values characteristic of intact enamel. This finding directly indicates that, during erosion, the apatite crystals forming enamel prisms initially lose phosphate groups, which migrate into the oral fluid, with subsequent decalcification occurring as a less intensive secondary process. This phenomenon has previously been noted in studies of enamel affected by erosion both *in vivo* [15] and *in vitro* [16], although without detailed consideration of the underlying chemical processes.

In our view, the cascade of processes described above should be regarded as the key mechanism in caries development, where the tooth is exposed to a complex mixture of acids produced by bacterial metabolism in the presence of numerous enzymes with affinity to phosphate groups [14]. These enzymes facilitate conjugation and thereby contribute, to some extent, to the

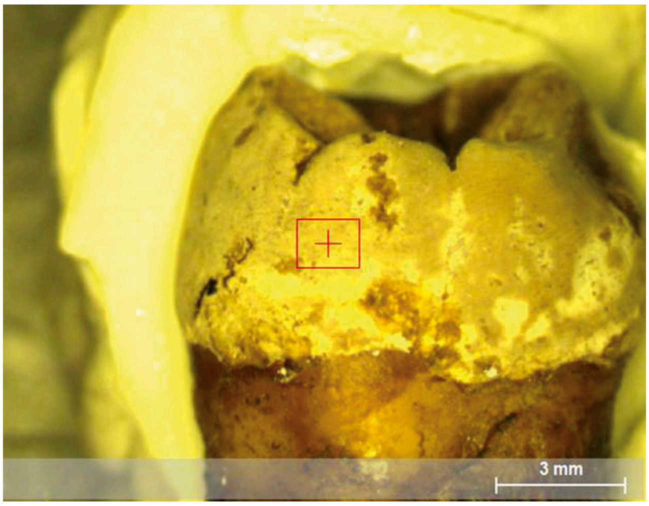

Fig. 1. Enamel area after tooth exposure to “Dobry Cola” (RFA, MultiPoint mode)

Рис. 1. Участок эмали после выдержки зуба в «Добрый Cola» (РФА, режим MultiPoint)

Mass percent (%)

P	Cl	Ca	Zn
8,40	0,39	90,95	0,26
8,20	0,45	91,10	0,24
9,04	0,32	90,37	0,27
8,39	0,49	90,81	0,31
7,80	0,41	91,50	0,29
8,47	0,43	90,81	0,29

	P	Cl	Ca	Zn
Mean value:	8,38	0,42	90,92	0,28
Sigma:	0,40	0,06	0,37	0,02
Sigma mean:	0,16	0,02	0,15	0,01

Fig. 2. X-ray diffraction spectra corresponding to the tooth after exposure to “Dobry Cola”

Рис. 2. Спектры РФА, соответствующие зубу после выдержки в «Добрый Cola»

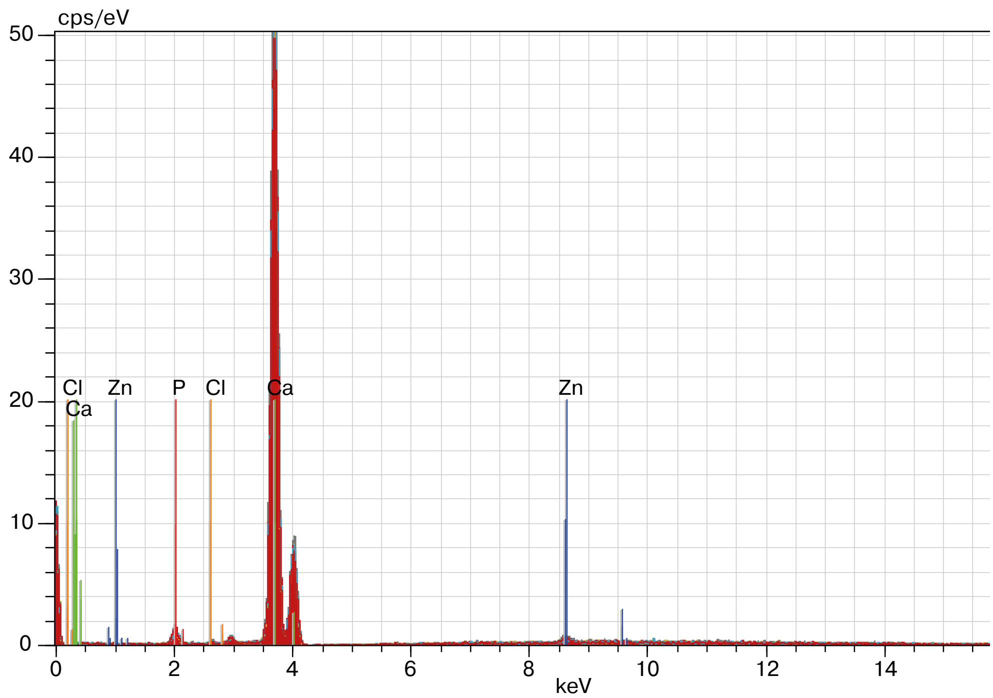

Fig. 3. Data on the analysis of the tooth surface after exposure to «Dobry Cola» (RFA, MultiPoint mode)

Рис. 3. Данные по анализу поверхности зуба после его выдержки в «Добрый Cola» (РФА, режим MultiPoint) stabilization of phosphate groups within the crystal lattice. By contrast, in erosion, enamel is subjected to the direct action of protons with varying degrees of hydration derived from exogenous acids. In this scenario, the dominant reaction becomes:

$$PO_4^{3-} + H_3O^+ \rightarrow HPO_4^{2-} + H_2O,$$

driven by the high stability of the resulting hydrogen phosphate ion, as indicated by its dissociation constant (1.26×10^{-12}).

As shown in the table, erosion occurred in all tested solutions within the physiological pH values typical of food products. Moreover, across the broad acidity range ($\Delta pH = 3.68 - 1.86 = 1.82$, corresponding to a two-order difference in proton concentration), no correlation was observed between the degree of acidity and the severity of erosive damage. At the same time, the ratio of dephosphorylation to decalcification intensity was found to remain statistically constant, with some variability of recorded values within the examined enamel zone being consistent with its natural heterogeneity.

It should be emphasized that the present observations refer to pH values characteristic of food products. Exceeding these physiological boundaries exposes the enamel crystal lattice to extreme conditions that overwhelm compensatory mechanisms. This was confirmed in our analysis of tooth surfaces exposed to 14% hydrochloric acid, where the measured calcium and phosphorus contents and the corresponding Ca/P ratio indicated profound, irreversible fragmentation of both enamel and dentin (Table 1).

CONCLUSIONS

1. Using X-ray fluorescence analysis (*in vitro*), we demonstrated that various food and beverage solutions exhibit high erosive activity, which is virtually independent of their pH values within the range of 3.58–1.86.

2. Based on the analysis of the Ca/P ratio, it was established that during erosion, dephosphorylation of the enamel crystal lattice represents the primary process, followed by decalcification. A chemical rationale for this observation has been proposed.

Table 1. Dynamics of the content of Ca, P, and the value of the Ca/P index in the enamel of healthy teeth after three days of exposure to an erosive environment

Таблица 1. Динамика содержания Ca, P и значения индекса Ca/P в эмали здоровых зубов после трех суток пребывания в эрозионной среде

Liquid	pH	Tooth №	Calcium content, % mean (range)	Phosphorus content, % mean (range)	Ca/P ratio mean (range)
80% food-grade lactic acid solution	3.68	1	85.51 (84.66–86.18)	14.11 (13.44–14.98)	6.06 (5.65–6.41)
		2	86.16 (84.74–87.33)	13.50 (12.34–14.89)	6.38 (5.69–7.08)
Wine	2.83	1	86.54 (85.67–88.21)	12.36 (10.78–13.24)	7.01 (6.47–8.18)
		2	85.13 (83.70–85.92)	13.86 (13.06–15.19)	6.14 (5.51–6.58)
Dobry Cola	2.70	1	85.84 (84.96–86.73)	13.46 (12.57–14.45)	6.38 (5.88–6.90)
		2	85.58 (85.10–86.14)	13.96 (13.35–14.47)	6.13 (5.88–6.45)
Lemon juice	2.38	1	85.55 (85.08–86.19)	13.69 (13.10–14.16)	6.25 (6.01–6.58)
		2	85.89 (85.74–86.21)	13.28 (12.93–13.42)	6.47 (6.39–6.67)
		3	85.70 (85.03–86.73)	13.48 (12.55–14.10)	6.36 (6.03–6.91)
9% acetic acid solution	2.26	1	86.77 (85.99–87.59)	12.59 (11.74–13.41)	6.89 (6.41–7.46)
		2	85.35 (84.37–85.90)	13.63 (13.00–14.57)	6.26 (5.79–6.61)
Solution of Acidin-Pepsin tablets (15 tablets in 100 mL of water)	1.86	1	84.25 (83.40–85.53)	13.31 (12.13–14.18)	6.33 (5.88–7.05)
		2	86.08 (84.62–88.37)	12.18 (9.36–13.80)	7.07 (6.13–9.44)
14% hydrochloric acid solution	<0	1	73.85 (69.69–82.43)	21.22 (11.96–26.60)	3.98 (2.62–6.89)
		2	67.17 (57.14–75.60)	19.81 (12.86–25.62)	3.39 (2.23–5.58)

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