In vitro effect of beer, red and white wine on the morphology and surface roughness of human enamel

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Abstract

Background. Beer, red and white wine are acidic drinks whose frequent consumption can increase the risk of dental erosion.

Objectives. To establish the effect of beer, red and white wine on the morphology and surface roughness (SR) of human enamel using different exposure times in a cyclic de- and remineralization model in vitro.

Materials and methods. The experiment included 33 surgically extracted impacted human third molars from patients aged 18–25 years. Enamel samples obtained by cutting crowns (n = 132) were submitted to alternate cycles of demineralization in (1) beer, (2) red wine, (3) white wine, (PC) positive control (orange juice), and remineralization in artificial saliva, which also represented a medium for negative control (NC). The experiment included cycles with different exposure times in alcoholic beverages and orange juice of 15, 30 and 60 min. Thus, 12 groups were formed (for each drink and each exposure time) containing 10 samples each, while the NC group consisted of 12 samples. Experiments were repeated 3x/day for 10 days. Enamel surface alterations were determined by stylus profilometry (average surface roughness (R_a)) and scanning electron microscopy (SEM). The Shapiro–Wilk test, independent samples Kruskal–Wallis test and multiple comparisons (all pairwise) were performed.

Results. With increasing exposure time, there was a positive correlation with R_a for white wine- and orange juice-immersed samples (60 min compared to 15 min), which was also observed using SEM. There was no significant difference in the R_a between the other experimental samples for the same exposure time.

Conclusions. This study confirms a certain erosive potential of beer, red and white wine, and a significant relationship with pH, titratable acidity (TA) and SR, but not with the exposure time for all tested alcoholic beverages. Moreover, differences among the ultrastructural patterns caused by alcoholic beverages over the enamel surface were observed.

Key words: alcoholic beverages, dental erosion, SEM, stylus profilometry

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Background

The most cited definition of tooth erosion is Imfeld's, which implies the loss of tooth substance by chemical processes without bacterial involvement.[1] The etiology of dental erosion is multifactorial and can arise from extrinsic acidic substances (acidic beverages/food or medications)[2,3] or intrinsic factors that involve the migration of gastric juice into the oral cavity (reflux disease, laryngopharyngeal reflux, eating disorders, chronic alcoholism, pregnancy, etc.).[4]

While the erosive potential of soft drinks is well documented, there is insufficient data on the impact of alcoholic beverages on dental tissues. High and frequent use of alcoholic beverages can be seen as both an internal and external factor of dental erosion. People who often and excessively consume alcohol with, for example, the habit of keeping drinks in their mouths, prolong the contact of alcoholic beverages with the tooth surface and increase the risk of erosion.[5] Alcoholics also have poor dietary control and tend to consume more acidic foods and drinks. Additionally, the chemical properties of alcohol can cause vomiting, resulting in frequent contact of gastric acid with the tooth surface.[4]

The most studied alcoholic beverage in terms of erosion is wine, mainly in special occupational groups. Wine tasters who consume over 20 types of this drink a day have a higher risk of dental tissue erosion than people who enjoy alcohol occasionally.[6,7] Previous data on the prolonged action of wines show their high erosive potential. Moreover, continuous exposure of enamel samples to white wines for 24 h may lead to severe dental erosion, a conclusion established based on surface roughness (SR) and the amount of released calcium.[6] On the other hand, some types of red wine have been reported to significantly reduce enamel microhardness when in contact for at least 120 s.[8]

There is little data on the analysis of the erosive potential of beer, and most evidence is based on the detection of released calcium and phosphate.[9] Other researchers have examined the effect of beer on the enamel surface hardness and concluded that some brands of beer have a potential dental effect that is much less pronounced compared to soft drinks.[2,10]

Interestingly, the previously mentioned studies used single, shorter or longer exposures of enamel samples to alcoholic beverages. In contrast, cyclic erosion experiments better reflect the challenges faced by dentition by alternately exposing samples to de- and re-mineralizing solutions.[11–13] To the best of our knowledge, only 1 study used such a model to verify the erosion kinetics of an alcoholic beverage (red wine) on enamel. The cyclic procedure caused the polyphenols from red wine to modify the acquired enamel pellicle, reducing the erosive potential of the beverage.[14]

In the present research, the null hypothesis was that in the multiple exposure model, beer, red and white wine do not affect the increase in SR and ultrastructure changes of the enamel surface in relation to the exposure time.

Objectives

This study aimed to determine the erosive effect of beer, red and white wine of well-known Serbian brands on human enamel in a cyclic de- and remineralization model in vitro. Effects were assessed based on the analysis of average SR and scanning electron microscopy (SEM) observations using different exposure times.

Materials and methods

Tested alcoholic beverages

Three alcoholic beverages were tested: beer, red wine and white wine, which could be found in the free sale. Orange juice was used as the positive control. Table 1 shows the compositions of the experimental beverages as listed on their packaging.

Sample preparation and group divisions

This study was approved by the Research Ethics Committee of Faculty of Medicine, University of Niš, Serbia (approval No. 12- 14250-2/5-2018). The experiment included 33 impacted human third molars, which had been surgically extracted for medical reasons from patients aged 18–25 years.

After extraction and the usual cleaning procedure (storage in 1% sodium hypochlorite for 24 h and organic debris

Table 1. Compositions of the tested drinks as listed on their packaging

Drink	Manufacturer	Composition
Life Premium 100% orange juice	NECTAR Group, Bačka Palanka, Serbia	water, concentrated orange fruit juice, citric acid
Zaječar beer	Heineken, Zaječar, Serbia	water, barley malt, corn grits, hop extract
Rubin Vranac red wine	Rubin A.D., Kruševac, Serbia	water, alcohol 12%, glycerol, organic acids, tannins, phenols, anthocyanins, flavan-3-ols
Royal Riesling white wine	Levač Winery, Rekovac, Serbia	water, alcohol 10.5%, lactic acid, malic acid, tartaric acid, citric acid, succinic acid, acetic acid, sulphates

removal), the roots were removed, and the crowns were cut into quarters (distal, mesial, buccal, and lingual), using a diamond saw under water irrigation.[3] In this way, 104 samples were obtained for enamel SR analysis and 28 for SEM observations. If any sample was damaged during cutting, it was replaced with a new one, which was prepared from a newly extracted impacted molar.

Circular molds were made and filled with self-cured resin for samples that were being tested for SR. Each sample was immersed in resin so that the enamel surface was accessible for average surface roughness (R_a) measurement. Before the erosive challenge, the samples were cleaned with non-fluoridated pumice, rinsed with water and air-dried. After preparation, the samples planned for SEM observation were immediately placed in an ultrasonic water bath to remove cutting debris, washed with water and air-dried.

The samples were randomly assigned to 3 experimental groups: 1) beer, 2) red wine and 3) white wine; and 2 control groups: (positive control (PC)) orange juice and (negative control (NC)) artificial saliva, taking into account the planned number of samples with/without circular molds. Experimental groups, including the PC, consisted of 30 samples (24 for SR analysis and 6 for SEM observation), 10 (8+2) for each planned beverage exposure time: 15 min, 30 min and 60 min, while the NC group consisted of 12 (8+4) samples.

Artificial saliva (1.5 mM $Ca(NO_3)_2$, 0.90 mM KH_2PO_4, 130 mM KCl, and 60 mM Tris buffer, pH = 7.4)[15] was used as a medium for the NC, as well as a medium for experimental and PC samples between demineralization cycles.

pH and titratable acidity measurement

The pH of beverages was measured immediately after opening at 25°C using a previously calibrated multifunctional electronic device CONSORT C830 (Consort BVBA, Turnhout, Belgium). A total of 50 mL of the beverage was placed in a beaker and stirred using a non-heating magnetic stirrer until a stable reading was reached. Titratable acidity (TA) was calculated as the volume of 0.9613 M NaOH solution required to increase the pH of each beverage to 5.5 and 7.0. The solution was added in aliquots of 0.3 mL until a stable pH reading was achieved. The pH and TA of the beverages were measured in triplicate, and an average value was calculated (Table 2).

Table 2. Average of initial pH values and TA for pHs 5.5 and 7.0 of analyzed drinks

Analyzed drinks	Initial pH	TA	
		pH 5.5	pH 7.0
Orange juice	3.82 ±0.04	4.28 ±0.03	5.83 ±0.05
Beer	3.96 ±0.05	0.64 ±0.05	1.59 ±0.07
Red wine	3.49 ±0.05	1.82 ±0.04	2.34 ±0.03
White wine	3.02 ±0.06	2.69 ±0.03	3.18 ±0.05

TA – titratable acidity: amount of base (mL of 0.9613 M NaOH) needed to raise the pH to 5.5 and 7.0. Data are presented as mean ± standard deviation (M ±SD).

Erosive challenge

The experimental samples and the PC group had the following treatment: 1) immersion in 50 mL of alcoholic beverage at room temperature for 15 min, 30 min and 60 min, with occasional shaking; 2) rinsing with 5 mL of distilled water; 3) storage in artificial saliva until the next immersion.[11] This daily cycle was performed with 3 immersions for 10 consecutive days. Experimental solutions, including the PC, were changed every 24 h. At the end of the experiment, the samples were washed with distilled water, dried and prepared for the SR analysis/SEM observation.

Determination of surface roughness

The R_a was assessed using a stylus profilometer (Surftest SJ-301; Mitutoyo, Kawasaki, Japan).[3] The points of roughness measurement were randomly selected on the sample surface. Measurements were carried out at right angles to the samples. Three measurements were performed for each sample, and the mean value was calculated. For each reading, the device needle ran 0.25 mm/s, the length of the measuring line was 0.5 mm and the cutoff was 2.5 mm. To exclude possible errors, the measurement of SR was performed by only 1 investigator.

SEM observation

Scanning electron microscopy was used as an additional method to observe the enamel surface at each step. After preparation (mounting on stubs, fixing and sputter coating with gold/palladium), the samples were examined using a scanning electron microscope (JEOL-JSM-5300; JEOL, Akishima, Japan). Photomicrographs of representative areas were taken at ×2000 magnification.

Statistical analyses

The data obtained by this research were statistically analyzed using SPSS v. 15.0 (SPSS Inc., Chicago, USA). Continuous variables are presented as mean ± standard deviation (M ±SD) (for normal distribution) or by median (Me, i.e., 2nd quartile (Q2), 1st quartile (Q1)–3rd quartile (Q3)) and 95% confidence interval (95% CI), if the data distribution deviated from normal. Data normality was tested using a Shapiro–Wilk test. Because some variables presented distribution that deviated from normal, an independent samples Kruskal–Wallis test with multiple comparisons (all pairwise) was performed. An estimation error level of less than 5% ($p < 0.05$) was used as the threshold of statistical significance.

Results

pH results and TA measurement

The initial pH values were below critical (5.5) for the evaluated acidic beverages. White wine had the lowest average pH value (3.02 ±0.06), while beer had the highest average pH value (3.96 ±0.05), greater than orange juice selected for PC.

Furthermore, white wine gave the highest TA, requiring 2.69 mL of NaOH to reach a pH value of 5.5 (and 3.18 mL to reach a pH of 7.0). Beer showed a rapid response when NaOH was added, requiring only 0.64 mL of NaOH to reach a pH value of 5.5 (and 1.59 mL to reach a pH of 7.0). Orange juice had the greatest TA (4.28 or 5.83 mL) of NaOH to reach the equivalent pH values.

The initial pH values of the analyzed drinks and TA were expressed as mean values of triple measurement ±SD (Table 2).

Results of enamel roughness measurement

The R_a values obtained after immersing the samples in different beverages for different exposure times are shown in Table 3.

By comparing independent samples defined in relation to the beverage exposure time, the Kruskal–Wallis test allowed for establishing a statistically significant difference in the R_a of samples immersed in orange juice ($p = 0.008$) and white wine ($p = 0.041$) (Table 4). The subsequent multiple comparisons revealed a statistically significant difference in the R_a of samples exposed for 15 min compared to 60 min to orange juice ($p = 0.006$) and 15 min compared to 60 min to white wine ($p = 0.044$) (Table 5).

By comparing independent samples defined in relation to the beverages used for the same exposure time, the Kruskal–Wallis test allowed for establishing a statistically significant difference at all exposure times ($p < 0.001$) (Table 6). The subsequent multiple

Table 6. Independent samples Kruskal–Wallis test average surface roughness (R_a) in relation to beverage type

Statistical parameter	Exposure time 15 min	Exposure time 30 min	Exposure time 60 min
Test statistics	24.681[a]	32.075[a]	27.748[a]
df	4	4	4
p-value	0.000	0.000	0.000

df – degrees of freedom; [a] the test statistics is adjusted for ties.

Table 3. Average surface roughness (R_a) by groups (control and experimental with different exposure times)

Exposure time [min]	Negative control (artificial saliva)	Beer	Red wine	White wine	Positive control (orange juice)
15	1.67 (1.60–1.82)	1.96 (1.68–3.03)	2.40 (1.81–3.68)	2.54 (2.29–3.13)	3.23 (3.05–3.62)
30		2.29 (1.80–3.32)	2.48 (1.86–3.67)	3.03 (2.31–3.78)	5.22 (4.55–6.12)
60		2.63 (1.61–3.68)	2.82 (1.78–4.10)	3.56 (3.29–3.73)	6.58 (5.43–6.99)

Data are given as medians: 2nd quartile (Q2) (1st quartile (Q1)–3rd quartile (Q3)).

Table 4. Independent samples Kruskal–Wallis test average surface roughness (R_a) in relation to exposure time

Statistical parameter	Negative control	Beer	Red wine	White wine	Positive control
Test statistics	0[ab]	0.665[ab]	0.180[ab]	6.405[a]	9.765[a]
df	2	2	2	2	2
p-value	1.000	0.717	0.914	**0.041**	**0.008**

df – degrees of freedom; [a] test statistics is adjusted for ties; [b] multiple comparisons were not performed because the overall test did not show significant differences across samples. Values in bold indicate statistically significant results.

Table 5. Pairwise comparisons of average surface roughness (R_a) in relation to exposure time [min] to orange juice (positive control) and white wine

Group	Exposure time [min]	Test statistic	SE	Standardized test statistic	Sig.	Adj. Sig.[a]
Positive control (orange juice)	15–30	–7.125	3.536	–2.015	0.044	0.132
	15–60	–10.875	3.536	–3.076	0.002	**0.006**
	30–60	–3.750	3.536	–1.061	0.289	0.867
White wine	15–30	–2.250	3.536	–0.638	0.525	1.000
	15–60	–8.625	3.536	–2.440	0.015	**0.044**
	30–60	–6.375	3.536	–1.803	0.071	0.214

SE – standard error; Sig. – significance; Adj. – adjusted; [a] significance values have been adjusted by the Bonferroni correction for multiple tests. Values in bold indicate statistically significant results.

comparisons revealed that at all beverage exposure times, R_a was significantly different between the NC and PC samples ($p < 0.001$), and NC and white wine ($p = 0.006$ – exposure time 15 min, $p = 0.010$ – exposure time 30 min, and $p = 0.005$ – exposure time 60 min). Furthermore, the 60-minute exposure displayed differences between samples immersed in orange juice and beer ($p = 0.039$; Table 7). There was no significant difference in the R_a between the experimental samples for the same exposure time.

Results of SEM observations

Photomicrographs of the enamel surface after immersion in artificial saliva show an unchanged surface with perikymata, weak roughness and developing pores. After the erosive challenge with orange juice, a generalized irregularity with atypical etching, as well as the presence of wrinkles and cracks that deepen with increased exposure time were manifested (Fig. 1). Differences in the quality of erosive changes were observed between samples immersed in beer and red wine, but the degree of erosive damage did not increase with exposure time. In contrast, differences in the erosive ultrastructural pattern after 30 min and 60 min of cycled exposure to white wine were observed (Fig. 2).

Discussion

Although in vitro models provide limited information on intraoral erosion, significant conclusions based on this type of research have been drawn. A large number of experiments used single exposures of samples to acidic substances, mainly to predict the erosive potential. This includes different exposure times from 10 s to 60 min,[10,16,17]

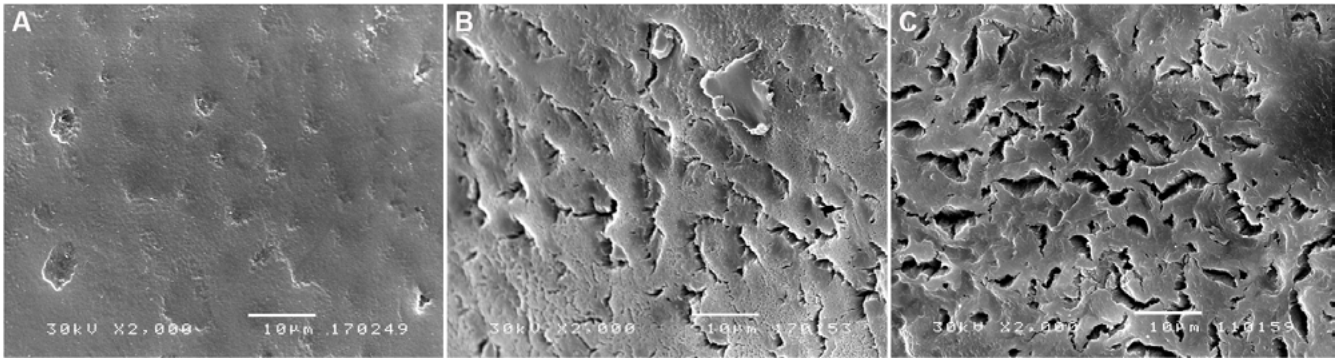

Fig. 1. Scanning electron microscopy (SEM) image of the control samples (×2000 magnification). A. Artificial saliva: unaltered surface, slight rugosity and development pores; B. Orange juice: 30 min of cyclic exposure, atypical etching of the enamel surface with deep creases and furrows, partially covered with granular crystals; C. Orange juice – 60 min of cyclic exposure, densely wrinkled areas with loss of enamel morphology

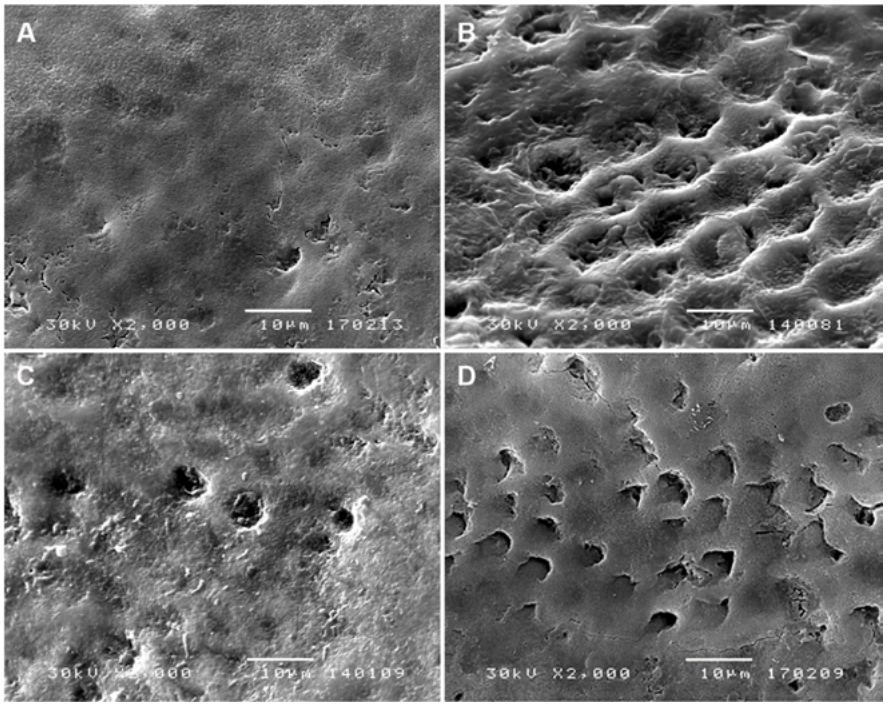

Fig. 2. Scanning electron microscopy (SEM) image of the experimental samples after cyclic exposure (×2000 magnification). A. 30 min to beer: shallow depressions, slight porosity and 2 smaller fields of atypical etching (lower part of the picture, left and right corner); B. 60 min to red wine: shallow indentations with pronounced "honeycomb" structure; C. 30 min to white wine: a greater number of increased diameter pores, rare wrinkled fields, demineralization of some enamel rods; D. 60 min to white wine: irregular areas with accentuated rod contours, rod demineralization, the visible "hoof-like" form of the rods

Table 7. Pairwise comparisons of average surface roughness (R_a) in relation to beverage type for the same exposure time

Exposure time	Groups compared	Test statistic	SE	Standardized test statistic	Sig.	Adj. Sig.[a]
15 min	NC, white wine	−20.304	5.948	−3.413	0.001	**0.006**
	NC, PC	−27.179	5.948	−4.569	0.000	**0.000**
	beer, red wine	−3.250	6.711	−0.484	0.628	1.000
	beer, white wine	−8.500	6.711	−1.267	0.205	1.000
	red wine, white wine	−5.250	6.711	−0.782	0.434	1.000
30 min	NC, white wine	−19.589	5.949	−3.293	0.001	**0.010**
	NC, PC	−32.589	5.949	−5.479	0.000	**0.000**
	beer, red wine	−1.750	6.711	−0.261	0.794	1.000
	beer, white wine	−5.687	6.711	−0.848	0.397	1.000
	red wine, white wine	−3.937	6.711	−0.587	0.557	1.000
60 min	NC, white wine	−20.643	5.948	−3.470	0.001	**0.005**
	NC, PC	−29.143	5.948	−4.899	0.000	**0.000**
	beer, PC	19.375	6.710	2.887	0.004	**0.039**
	beer, red wine	−1.937	6.710	−0.289	0.773	1.000
	beer, white wine	−10.875	6.710	−1.621	0.105	1.000
	red wine, white wine	−8.937	6.710	−1.332	0.183	1.000

PC – positive control; NC – negative control; SE – standard error; Sig. – significance; Adj. – adjusted; [a] significance values have been adjusted by the Bonferroni correction for multiple tests. Only pairs with statistical significance, as well as pairs that include experimental drinks (beer, red and white wine) are included. Values in bold indicate statistically significant results.

ending with 24 h.[6] The present research used the cyclic de- and remineralization model, i.e., samples immersion in acidic (alcoholic) beverages, including occasional agitation, followed by exposure of samples to artificial (or natural) saliva, then repeating the challenge several times.

To achieve better comparability between the tested substances, enamel samples from impacted third molars were used. Their surfaces were completely intact (they were not exposed to chewing forces), without any scratches or notches that are otherwise characteristic of teeth in function. Furthermore, they came from individuals of approximately the same age and with a similar degree of tooth mineralization.

As a medium for remineralization, we used artificial saliva with electrolytes of the same or similar formulation in previous, related studies.[13,18] Also, gentle agitation of the solutions was applied to "imitate" the usual way of drinking (no shaking or retention).

To assess the erosive damage, a stylus profilometer was used, which can read all surface irregularities along the length of the object. Although this method is flawed in that it does not register the amount of enamel loss,[19] it is applied in a large number of studies to assess the impact of erosive substances on hard dental tissues.[3,12,19,20] Of the 4 parameters registered using the stylus profilometer, R_a was singled out, which shows the average roughness value. While this parameter does not provide information about the characteristics of surface irregularities, it is a common analytical tool in the investigation of the surface of dental tissues and materials after erosive challenges (acidic beverages, bleaches, etc.).[6,21,22]

The absolute R_a values were higher compared to those observed in previously published studies.[3] A possible reason is our use of a 0.75 mN low-pressure detector with a 2-µm stylus radius. This allowed for a more precise measurement to be taken due to the recording of narrower and deeper irregularities without fear of damaging the sample surface.[20]

The enamel samples analyzed for SR were not flattened and polished before immersion in the experimental and control solutions. This methodology is justified by the fact that polishing removes significant amounts of enamel, probably a complete aprismatic layer, which leads to faster lesion progression[23]; since natural enamel surfaces require longer periods of erosion, we found that cyclic exposure to erosive solution of 15 min, 30 min or 60 min during 10 days is long enough for measurable change (such as SR) to be quantified[16]; measurement of 1 central cluster roughness of unpolished enamel represents the total SR of enamel, before and after erosion, the same as in the polished sample.[24]

In studies of the erosive potential of acidic substances, the determination of the initial pH and TA (and/or buffering capacity) is mandatory. Erosion occurs at low pH, but there is no fixed "critical" pH for tooth erosion. This value is calculated from the calcium and phosphate concentrations in the erosive solution itself.[5] From the critical values (pHc) published by Lussi and Carvalho, we singled out those that are important for this study, namely orange juice (3.6), beer (5.0) and red and white wine (5.1).[5]

Wine derives its acidity mostly from weak mono- and di-basic acids, since white wine contains malic acid and a certain amount of lactic acid, while the share of citric acid

is almost negligible. Lactic, and to a lesser extent tartaric, acid dominate in red wine.[25]

Chemical analysis indicates that beer contains phenolic acids whose presence affects its pH (around 4.0).[26] In contrast, orange juice contains citric acid, whose strong erosive effect comes from hydrogen ions, and acid anions (citrates) that build complexes with calcium, as well as undissociated acid molecules.[25]

Of the possible buffer properties, this study focused on the determination of TA, which has a "closer" relationship with the concentration of undissociated acid than the buffering capacity. Unsaturated substances with low pH and high TA have a higher erosive potential.[5,25] In the present study, we demonstrated that white wine has higher TA values than red wine and beer, which is generally consistent with other research.[2] Moreover, the high values of TA for orange juice ($4.28_{5.5}$ and $5.83_{7.0}$) are in line with the findings of other studies.[2,14,18] Cyclic exposure of 30 min and 60 min was long enough to show significantly stronger erosive potential of orange juice compared to the shorter exposure (15 min).

It seems that beer is not a strong erosive substance. Although Zaječar beer has a relatively low pH (3.9) and $TK_{5.5}$ = 0.64, the R_a values were significantly lower than the R_a for orange juice samples. Zanatta et al. examined the microhardness of bovine enamel after immersing samples in 3 different beer brands for 5 min, 30 min and 60 min.[10] Only Heineken beer showed a decrease in microhardness after exposure for 30 min, although its pH was slightly higher (pH = 4.35) than the other 2 beers tested. They assumed the reason was the larger amount of citrate, which was not completely consumed during the brewing process.[10] Similarly, Lussi et al. found that Carlsberg beer and Montagne red wine did not produce any significant changes in enamel surface hardness.[2] The present results are comparable to those of the mentioned authors, although they used a different method (microhardness) for erosion assessment in examining the erosive potential of several types and brands of beer[2,10] and wine.[2]

Willershausen et al. examined the impact of white wine and red wine on human enamel for a continuous period of 24 h. In addition to the R_a parameter analysis, the amount of released calcium was calculated. Riesling white wine was observed to have the lowest pH and highest TA, as well as significantly higher Ca release from the eroded samples. In the current study, white wine of the same type had a lower pH (3.02 compared to 3.49) and a higher TA (2.69 compared to 1.82) than red wine (Vranac). Although the absolute R_a values for white wine were higher, no statistical significance was found. Most of the previous studies have found that white wine is more erosive than red white,[2,6] and explained this by the higher amount of polyphenols in red wine.[2,14] Polyphenol molecules can react with salivary proteins to form protein–polyphenol complexes that bind to proteins of the acquired enamel pellicle. Exposing the acquired pellicle to liquids rich in polyphenols facilitates further adhesion of these complexes to the pellicle and increases its thickness and resistance to removal.[14] The present research used artificial saliva that does not contain proteins, but some studies have shown that spontaneous formation of thin polyphenolic coatings is possible on polymeric, metallic and native-oxide surfaces that are exposed to liquids rich in polyphenols.[27]

Although quantitative analyses of hard dental tissues altered by erosion provide far more objective results, SEM with grading (scoring) of the alterations can be applied for qualitative assessment of tissue surface morphology.[28,29] Acid attacks lead to a surface etching pattern with more or less exposure of enamel rods (prisms), which depends on the severity of the erosive challenge. Beyer et al. studied the ultrastructure of the enamel surface after immersing the samples in different acids for 60 s. The SEM micrographs of lactic, phosphoric and ascorbic acid-treated samples showed "cobblestone" type enamel etching with a rough surface and tiny crystals, unlike samples exposed to tartaric, malic and citric acid, which had smooth and less eroded areas.[30] Apart from the analogy regarding the acids that are an integral part of alcoholic beverages, there is no other data on the enamel surface SEM examination after exposure to beer, red and white wine. Only in the case of Bordeaux red wine, slight signs of erosion were found after a single immersion for 90 s.[8] Considering our experimental setup (cyclic model), comparisons with the results of other authors were not possible.

In contrast, our results showing the atypical erosion of the enamel surface treated with orange juice are in accordance with the results of Braga et al., who compared enamel morphology after an erosive challenge with gastric and orange juice in a cyclic procedure.[11]

In the present study, cyclic exposure to alcoholic beverages led to an increase in SR along with exposure time, but only in samples immersed in white wine (60 min compared to 15 min). However, no such result was observed with samples immersed in beer and red wine. The SEM observation showed the same result, so the null hypothesis was partially accepted.

Limitations

Erosion is a complex condition that depends on numerous factors and their interaction. Due to the limited effect of in vitro studies, several types of analyses should be conducted to allow both qualitative and quantitative assessment of tooth tissue loss. In the present study, SEM observation contributed to the qualitative analysis of enamel surfaces, but due to the small number of samples, it could not be supported by scores that would indicate the degree of erosive damage.

Conclusions

This study confirms the limited erosive potential of beer, red and white wine, and a significant relationship with pH, TA and SR, but not with the exposure time for all tested

alcoholic beverages. It also provides information on morphological differences in the intensity of erosive changes with time of exposure to white wine, as well as qualitative differences among the ultrastructural patterns caused by beer, red and white wine on the enamel surface.

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