Archives of Materials Science and Engineering

Volume 62
Issue 1
July 2013
Pages 15-21

International Scientific Journal published monthly by the World Academy of Materials and Manufacturing Engineering

Effect of water dilution and alcohol mixing on erosive potential of orange juice on bovine enamel

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Received 21.04.2013; published in revised form 01.07.2013

ABSTRACT

Purpose: This in vitro study aimed to evaluate the changes of erosive potential of an orange juice before and after mixing with water and alcohol.

Design/methodology/approach: Fifty enamel specimens obtained from bovine teeth were prepared and randomly assigned into the following groups (n=10) with different erosive solution: I. commercially available pure orange juice (Cappy, Coca Cola Co.), II. orange juice diluted with distilled water at a ratio 3:1 (v/v), III. orange juice mixed with vodka (Smirnoff, 40% ethanol, Polmos) at a ratio 3:1 (v/v); IV. Orange juice diluted with distilled water (1:1, v/v), V. Orange juice mixed with vodka (1:1 v/v). The enamel specimens were submitted to a short-term erosion-remineralization cycling model (five 1-min erosion challenges in-between six 10-min remineralization periods in artificial saliva). Erosive potential of the drinks was assessed on the basis of chemical analysis and percent surface microhardness change (%SMHC) calculated from Vickers surface microhardness measurements before and after cycling. In chemical analysis of the experimental drinks, the pH value, titratable acidity, and buffer capacity (β) were determined.

Findings: The pH of tested drink remained low even after dilution with water or mixing with alcohol, however, titratable acidity decreased after addition of water and alcohol. Short-term erosion-remineralization cycling resulted in significantly decreased surface microhardness of enamel specimens in all experimental groups. In the proportion of 3:1, juice mixed with alcohol resulted in significantly smaller %SMHC than juice diluted with water. This difference was not observed at a ratio 1:1.

Practical implications: Consumers should be aware of tooth damage by drinks with low pH and high titratable acidity, even when mixing them with water and alcohol.

Originality/value: To the best of our knowledge, this is the first study comparing the erosive effect of water-diluted orange juice with that of juice mixed with alcohol.

Keywords: Erosion; Tooth; Enamel; Orange juice; Alcohol; Microhardness

Reference to this paper should be given in the following way:

P. Malara, A. Kloc-Ptaszna, L.A. Dobrzański, M. Górniak, T. Stefański, Effect of water dilution and alcohol mixing on erosive potential of orange juice on bovine enamel, Archives of Materials Science and Engineering 62/1 (2013) 15-21.

PROPERTIES

1. Introduction

There is a large body of evidence, based on the results of growing number of *in vitro* and *in situ* studies, that the excessive consumption of acidic drinks and foodstuffs poses a risk to the dentition [1]. The acid-induced chemical dissolution of mineral from tooth surface is defined as dental erosion, although it could be more appropriately termed 'dental corrosion' or 'biocorrosion' [2], in analogy to metallic corrosion [3]. Unlike in the caries process, where the destruction of hard dental tissues is caused by acids produced by bacteria in dental plaque, dental erosion is due to acids of non-microbiological origin. Severe erosive lesions might lead to the exposure of the dentin and cause a dentine hypersensitivity [4].

A wide range of acids are involved in the process of dental erosion. They may be extrinsic or intrinsic. The intrinsic causes include recurrent vomiting as part of the eating disorders (anorexia or bulimia nervosa) or due to the regurgitation of the gastric contents (gastro-oesophageal reflux) [5]. Extrinsic erosion is caused by low-pH beverages (like fruit juices, carbonated soft drinks), foods (any citrus food, tomato ketchup, salad dressings, pickles), medications (aspirin tablets, effervescent vitamin C) and environmental or occupational exposure to acidic agents (e.g. battery factory workers) [6].

Dietary acids are thought to be the main aetiologic agent, since the consumption of soft drinks has increased considerably over the last few decades, and in the United Kingdom has been reported to have reached 235.3 litres per person per year in 2011 [7].

Alcoholic beverages, such as wine (white and red), beer, cider, are also known to cause dental erosion [7]. Drinks based on citrus fruits were found to have a high erosive potential [8]. A ready-made, pre-mixed alcoholic soft drinks (the so-called 'alcopops') have been implicated as an etiological factor in dental erosion in young adults [8-11].

In a cluster analyses of five consecutive surveys conducted among Warsaw adolescents from 1988 to 2004, it has been shown that drinking of various kinds of alcoholic beverages became more frequent, and there is a markedly increase of vodka abuse by teenagers [12]. The use of alcohol mixed with acidic beverages is a popular pattern of alcohol intake.

To our knowledge, no previously published study has investigated the changes in erosive potential of orange juice after mixing with alcohol. In addition, there are currently only few studies about diluting drinks with respect to dental erosion. Therefore, the objective of this *in vitro* study is to evaluate the changes of erosive potential of an orange juice before and after mixing with water and alcohol, in form of vodka.

2. Investigation methodology

2.1. Material

The dental enamel was prepared from freshly extracted, non-damaged bovine permanent mandibular incisors that were obtained from 3-4-year-old cattle, bred locally for human consumption, after negative bovine spongiform encephalopathy (BSE) test. The teeth were stored in 0.5% aqueous thymol solution at 4°C when not in use.

2.2. Specimen preparation

The pulp tissue was removed from the coronal part of the tooth with endodontic files. Rectangular enamel slabs (5 x 5 mm and x 2.5 mm thick) were prepared from labial surfaces of the teeth using low speed water-cooled diamond saw (Minitom, Struers, Copenhagen, Denmark). The specimens were embedded in acrylic resin (DuroFast, Struers) using hot mounting press machine (CitoPress-20, Struers). Then, the enamel surfaces were subjected to wet-grinding with abrasive paper (500-4000 grit, Water Proof Silicon Carbide Grinding Paper, Struers, Erkrath, Germany) and polishing with felt paper wet by diamond suspension (3 μm, 1 μm Diamont Paste, Struers). This procedure was performed with semi-automatic grinding/polishing device (Tegramin-30, Struers), and resulted in removal of approximately 250 μm of the outermost enamel layer as it was measured with micrometer. Finally, the specimens were ultrasonically cleaned for 5 min with distilled water to remove the smear layer. Specimen with visible surface defects, such as cracks, scratches, white spots, were discarded. Schematic illustration of the preparation sequence is presented in Fig. 1.

One hundred acceptable specimens having a mean Vickers Hardness Number (VHN) above 300 were selected. Prepared slabs were stored in a saturated mineral solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 150 mM KCl, 1 mM NaN_3, 20 mM TRIS, pH 7.0).

2.3. Experimental design

The erosive potential of the experimental solutions was investigated in short-term erosion-remineralization model. Fifty specimens were randomly allocated (*http://www.random.org*) into 5 experimental groups (n=10), as shown in Table 1.

Table 1.
Experimental groups

Group	Solution	Mixing ratio (v/v)
I	pure orange juice[1]	-
II	orange juice[1] + distilled water	3:1
III	orange juice[1] + 40% ethanol[2]	3:1
IV	orange juice[1] + distilled water	1:1
V	orange juice[1] + 40% ethanol[2]	1:1

[1]Cappy, The Coca Cola Co., HBC Poland
[2]Smirnoff Vladimir vodka, 40% alcohol, Polmos

The specimens were alternately immersed in acidic test solution (1 min; 10 cm^3/specimen, at 21°C with slow stirring) and in artificial saliva (10 min: 0.213 g/dm^3 of $CaCl_2 \cdot 2H_2O$; 0.738 g/dm^3 of KH_2PO_4; 1.114 g/dm^3 of KCl; 0.381 g/dm^3 of

NaCl; HEPES, and 2.2 g/dm^3 of porcine gastric mucin, pH adjusted to 7.0 with 0.1 M NaOH) for six times (Fig. 2) [13]. All solutions were freshly prepared in the morning of each experimental day. During cycling they were not renewed. Before changing solutions, the specimens were washed in deionized water and gently dried with paper towel.

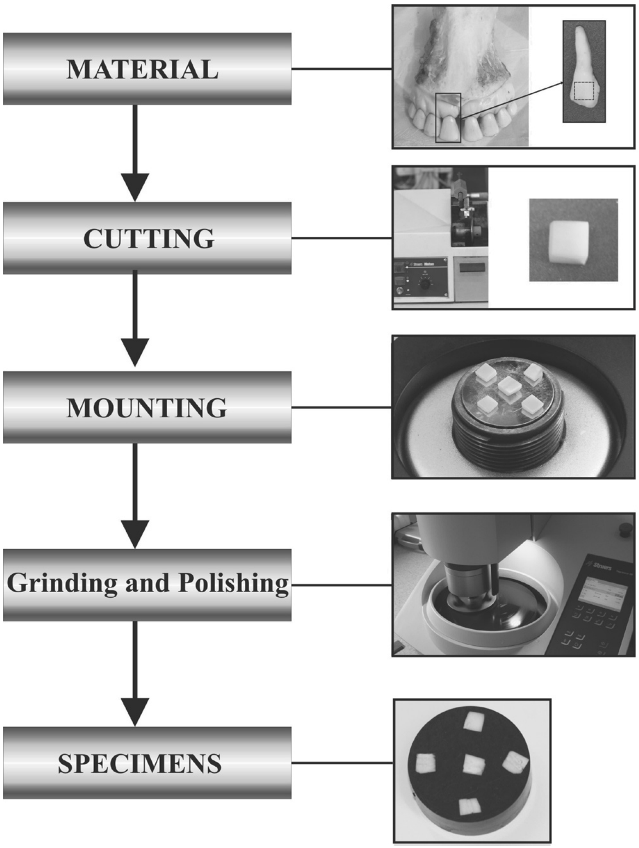

Fig. 1. Enamel specimen preparation

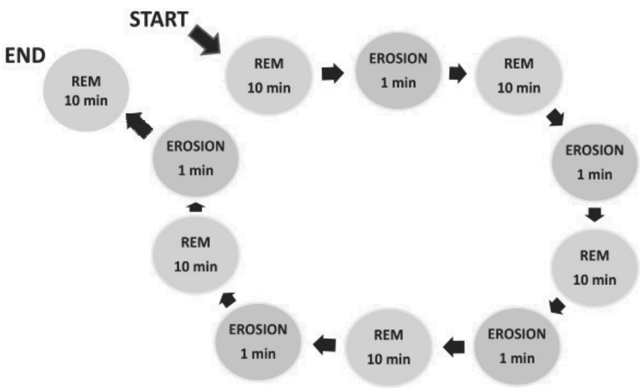

Fig. 2. Short-term erosion-remineralization protocol (REM - remineralizing period in artificial saliva)

2.4. Surface microhardness measurement

Specimen surface microhardness (SMH) was determined at baseline (SMH_0) and after exposure (SMH_1) to the solutions by operator blinded to the experimental conditions to which the specimens had been exposed. The indentations were made using a computer-aided FM-700 microhardness tester, coupled to FM ARS software (Future Tech Corp., Tokyo, Japan). A Vickers diamond was used with a 50-g load and dwell time of 15 s [14]. Five indentations at an interval of 100 µm were made for each specimen and the mean SMH was calculated (Fig 3). The percentage SMH change (%SMHC) was determined, as follows:

$$\%SMHC = \frac{SMH1 - SMH0}{SMH0} \times 100 \quad (1)$$

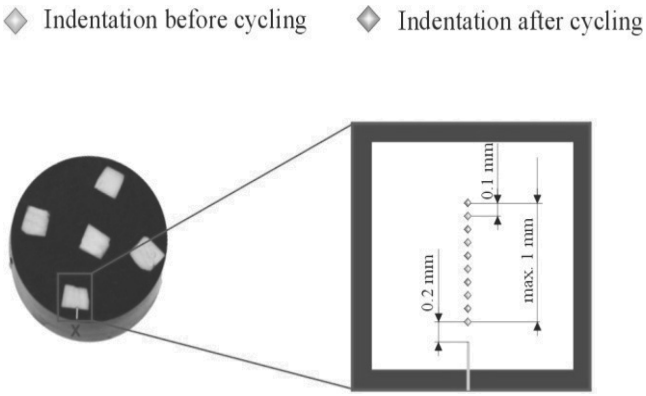

Fig. 3. Diagram of the microhardness indents scheme on the 5 x 5 mm bovine enamel specimens

2.5. Chemical analysis of the drinks

The initial pH of the test drinks was measured three times at 21°C using a glass electrode connected to a standard pH meter (Elmetron CP 401), calibrated with reference buffers of pH 4.00 and pH 7.00. Before measurement, the solutions were mixed thoroughly with a magnetic stirrer for 3 min.

To determine the titratable (neutralizable) acidity, 20 ml of each drink were titrated with 0.1 M NaOH (standard solution, POCH, Gliwice, Poland) in steps of 0.5 mL in 30-s intervals. The amount of base needed to raise the initial pH to 5.5 and 7.00 was measured in triplicate for each drink, which was stirred constantly at 21°C.

The differential buffer capacity (β) was determined from the slope of a titration curve and calculated at the original pH of the tested solutions according to the equation:

$$\beta = - \Delta C_B / \Delta pH \quad (2)$$

where ΔC_b denotes the amount of base (0.1 M NaOH) added to the drink and ΔpH is the change in the pH of the drink caused by the addition of the base.

2.6. Statistical analysis

The data were tested for normality distribution using the Shapiro-Wilk test. Since the assumption were satisfied, the comparisons were performed with Student's t test in order to test for statistically significant difference in the severity of dental erosion between the experimental groups. The level of significance was set at $p \leq 0.05$. Statistical analyses were carried out using Excel 2007 (Microsoft) and Statistica software package (Statsoft, ver. 8.0).

3. Results

3.1. Chemical analysis

Table 2 presents the initial pH values, titratable acidity, and buffer capacity data for all five tested solutions.

Table 2.
Chemical characteristics of the experimental solutions: mean pH, titratable acidity (TA) to pH 5.5 and 7.0 in mmol OH^-/dm^3, buffer capacity (β)

Group	pH	TA pH 5.5	TA pH 7.0	β
I	3.81	40.50	49.03	30.98
II	3.81	36.79	44.87	27.77
III	3.84	36.85	45.27	27.90
IV	3.84	25.53	32.83	18.27
V	4.03	24.70	36.12	21.69

The water dilution (1:3, 1:1) and alcohol addition (1:3) had almost no influence on the initial pH of the juice (measurement error of the pH-meter: ±0.05). Only juice mixed with alcohol in the proportion of 1:1 increased the pH. The amount of 0.1 M NaOH needed to increase the pH to 5.5 and 7.0 was considerably higher to pure orange juice. In addition, undiluted juice had the highest buffer capacity. Dilution of the juice with water and alcohol at a ratio of 1:3 resulted in a slight decrease of titratable acidity, while dilution in the proportion of 1:1 produced a markedly reduction of the titratable acidity of the orange juice (Fig. 4).

3.2. Surface microhardness measurement

Fig. 5 shows the mean percent surface microhardness changes (%SMHC). Mean values, standard deviations, and 95% confidence levels are expressed in Table 3. A short-term erosion-remineralization cycling resulted in a significant decrease ($p<0.001$) in the enamel surface microhardness in all experimental groups. At baseline, the mean microhardness (±SD) of the samples was 350.50 ± 34.5 VHN, while at the end of the cycling was 329.1 ± 32.1 VHN. Diluting the orange juice with water at ratio 1:3 showed no significant difference in %SMHC when compared with undiluted juice ($p=0.94$), whereas juice mixed with alcohol in the same proportion did ($p=0.02$). No statistically significant differences in %SMHC were found between juices diluted with water and alcohol in the ratio of 1:1 ($p=0.27$).

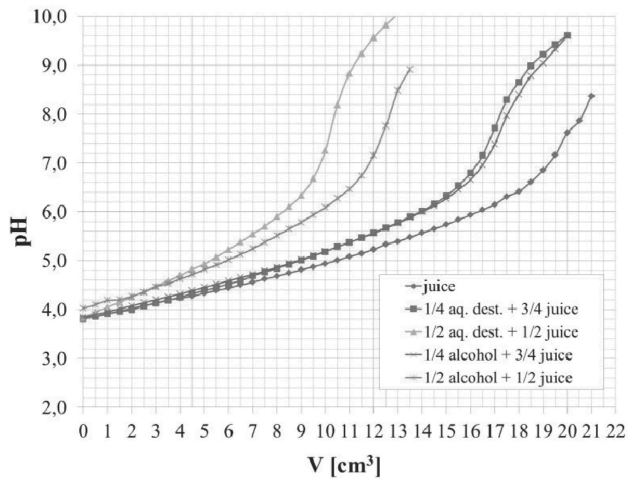

Fig. 4. Profile of the titration curves of 20-ml samples of orange juice drinks titrated with 0.1 M NaOH

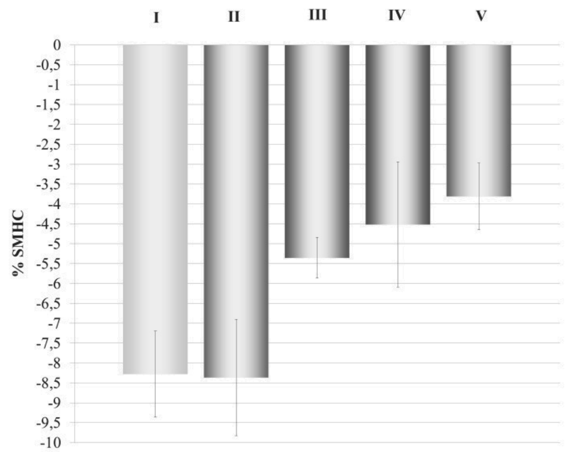

Fig. 5. Means and standard deviations of %SMHC of the experimental groups

Table 3.
Mean percent surface microhardness change (%SMHC) with 95% confidence levels after short-term erosion-remineralization cycling

Group	%SMHC	95% CI
I	-8.28 (2.16)[a]	-6.94 to -9.62
II	-8.36 (2.92)[a]	-6.55 to -10.17
III	-5.35 (3.15)[b]	-3.40 to -7.30
IV	-4.51 (1.02)[b,c]	-3.88 to -5.14
V	-3.81 (1.68)[c]	-2.77 to -4.85

Figures in parentheses are SD (standard deviation)
Means within columns sharing the same superscript letter do not differ significantly ($p > 0.05$)

4. Discussion

The erosive potential of a drink is influenced by a number of chemical parameters, including: type of acid (pK_A), pH, titratable/neutralizable acidity, buffering capacity, calcium chelating properties, viscosity (adhesiveness) and concentration of calcium, phosphate and fluoride ions [15,16].

In this investigation, the erosive potential of the drinks was assessed on the basis of their pH, titratable acidity and buffer capacity. In addition, we observed a changes in surface microhardness of the dental enamel, since this method is appropriate for measurement dental erosion in short-term erosion model [17]. This is because the initial stage of acid erosion involves demineralization and softening of the tooth surface without loss of tooth structure [18]. In long-term erosion-remineralization protocol (lasting for a several days), irreversible loss of dental hard tissue occurs, hence other methods for assessment should be applied, such as surface profilometry or confocal laser scanning microscopy - CLSM [19]. On extensively demineralized enamel surface it is not possible to accurately measure microhardness from the indentations. In the future, we are going to visualise and quantify the loss of enamel using a CLSM and profilometry. Fig. 6 shows an exemplary 3D scan of an eroded enamel specimen after 150-min erosive challenge with pure orange juice, while Fig. 7 presents enamel loss measurement.

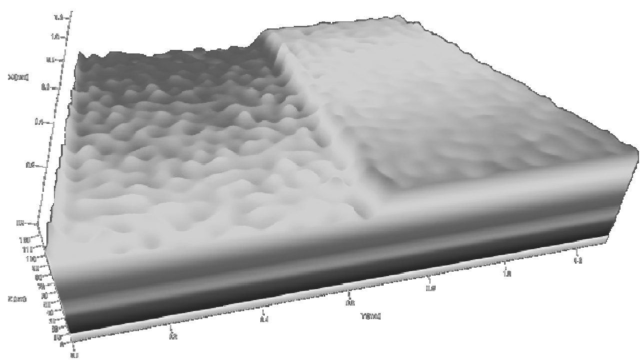

Fig. 6. Three-dimensional image of an eroded enamel surface from confocal laser scanning microscope (LSM Exciter 5, Zeiss). The left hand side area of the surface was exposed to pure orange juice (pH 3.8) for 150 min. Right part of the specimen was protected during erosive challenges with adhesive tape. Enamel loss measurement (in µm)

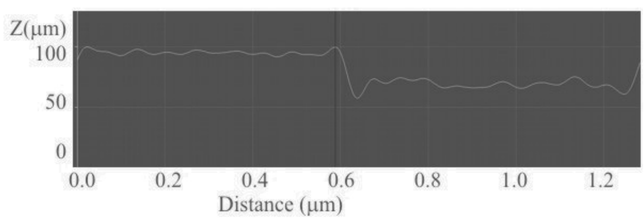

Fig. 7. Measurement of enamel loss (in µm)

In this study, erosive potential was investigated with short-term erosion-remineralization model in an attempt to replicate the situation during consumption of acidic drink [20]. Specimens were immersed in artificial saliva, because saliva seems to play an important role in reducing the effects of erosive challenges due to its remineralizing and buffering properties as well as the ability to form a protective pellicle layer on dental hard tissues [21].

A bovine enamel was used as an alternative to human enamel. Bovine enamel has the advantage that it is easy to obtain in large quantities with good quality (the dental caries in bovine teeth is quite rare). Bovine enamel is thicker and has more uniform chemical composition than that of human teeth, and thus provides a less variable response to erosive agent. Moreover, bovine teeth are easier to prepare due to a large and relatively flat surfaces. On the other hand, bovine enamel is more porous than human enamel and less resistant to acid diffusion, which results in more rapid erosion progression [22]. Therefore, the actual change of surface microhardness might be overestimated and should be interpreted with caution when comparing with human enamel [23]. Nevertheless, in our study bovine enamel specimens were in all experimental groups, thus the above-mentioned phenomenon would affect all the groups. An advantage, however, is that bovine enamel is more susceptible to demineralization than human enamel, and small erosive changes could be observed between the groups.

The results of this study indicate that orange juice is very acidic and harmful to the dental enamel. Even after being diluted with water and alcohol (vodka), juice retains a significant erosive potential due to high concentration of citric acid. Our study confirms the fact that pH of the drink strongly affects the erosion of enamel since hydrogen ion concentration provides the main driving force for dissolution of hydroxyapatite [24]:

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \leftrightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O \quad (3)$$

Although citric acid is so-called weak acid, it is very damaging to the tooth. From one molecule of citric acid three hydrogen ions could be produced. Additionally, the citric anion has the possibility of complexing of calcium cations dissolved from hydroxyapatite [25].

It was observed that the higher proportion of mixed alcohol, the lower surface microhardness change of the enamel. In contrary, erosive exposition to water-diluted juice in the ratio 1:3 resulted in the similar %SMHC as after exposition to pure orange juice. Significantly less %SMHC was observed only after increasing water dilution to 1:1.

Only two previous studies have investigated the effect of dilution on erosive potential of popular dilutable fruit drinks. Cairns et al. [26] were concentrated only on chemical assessment of the diluted drinks (pH, titratable acidity), while Hunter et al. [27] employed also surface loss measurements with profilometry. Both Cairns et al. and Hunter et al. found that despite dilution has little effect on initial pH, increasing diluting factor produces decrease in titratable acidity of the drinks [26,27]. These finding are in accordance with the present results. Cairns et al. observed that despite diluting fruit drinks to 1:100 (at this ratio, solution appears indistinguishable from water), the measured pH is below 5.0. To attain a neutral pH some drinks require to be diluted between 1:500 and 1:10,000 [26].

Although titratable acidity of a drink has been stated to be a better indicator of its erosive potential [28], also pH is a relevant predictor of this capability, as it was observed in present study. The pH is a good predictor for the first minutes of an acidic exposure, whereas the titratable acidity better characterizes the erosive potential during longer erosive periods [29].

At smaller diluting ratio (3:1), alcohol significantly decreased erosive potential of orange juice when compared with water-diluted juice, although the pH, titratable acidity and buffer capacity of these solutions was similar. Therefore, other factors than hydrogen ion concentration might be the reason for different erosiveness of the drinks. The interaction between ethanol and orange juice should be further evaluated. Our results should be also validated with more clinically relevant models (e.g. *in situ* studies) [14]. In the near future, preventive measures for dental erosion will be investigated [30].

5. Conclusions

Within the limitations of this in vitro study it might be concluded that:

- orange juice is highly acidic and erosive to the enamel, even when used half-diluted with water and alcohol for 5 min,
- erosive potential of orange juice mixed with 40% alcohol at a ratio of 3:1 is lower as compared to that of water-diluted juice in the same proportions,
- in 1:1 dilution, orange juice mixed with alcohol and water demonstrate comparable erosive potential, lower than that of pure orange juice.

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