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Abstract: Acid-base balance of saliva is an important factor for oral health because lowering the pH is associated with the development of oral diseases. The physiological value of saliva pH is in the range of 6.2-7.6. A saliva sample was taken before consuming the liquid and four samples at a certain time interval after that, in 10 volunteers. PH was determined using a digital pH meter (Lutron Electronics Enterprise pH meter). The tested liquids lead to an initial decrease in pH which after 2 hours approaches the values before the experiment. Sweetened carbonated drinks have the most significant effect on the pH of saliva.

Key words: saliva, pH, acidic beverages, mouthwashes

1. INTRODUCTION

The normal pH value of the saliva of healthy individuals ranges from 6.2 to 7.6. The concentration of the buffer system is directly related to both the pH level and the degree of saliva secretion. Thanks to it, the pH of all physiological liquids generally remains constant. In this regard, even after consuming acidic foods or applying acidic solutions, with the help of a buffer system, they are neutralized, so the pH of saliva remains relatively stable [1].

Many dental patients report drinking acidic beverages on a regular basis, and their increasing prevalence has made them a source of concern for both dental professionals and the general public [2]. Importantly, recent research has discovered that drinking acidic beverages can lower oral pH below critical level of 5.5. Significant research on the effects of acidic beverages on oral pH suggests that the pH drop is greatest immediately after consumption, and that pH returns to pre-exposure levels within a half-hour [3-5].

There are studies in the literature that examined the use of medications before and after exposure to enhance salivary pH neutralization and enamel protection. Turssi et al. found that a calcium lactate pre-rinse followed by a sodium fluoride rinse protected enamel surfaces from citric acid erosion better than sodium fluoride alone [6]. Other study results by Lindquist et al. looked into the effectiveness of post-drink mouthrinses and other products in accelerating the return of salivary pH to acceptable levels [7,8]. The pH-buffering abilities of neutralizing agents such as antacid tablets, chewing gum, and mineral water have been noted by researchers.

Through a series of researches and comparative studies, it has been determined that Listerine,

which is used as an additional means of maintaining oral hygiene, has the strongest effect of all antiseptics against the formation of dental biofilm, as well as inflammatory processes. It is effective in preventing supragingival plaque and gingivitis. It is considered that products with essential oils are more suitable for use than chlorhexidine, because, unlike it, they do not cause stains on teeth and that during their long-term daily use, resistant microorganisms and opportunistic microorganisms do not appear [9].

In addition to Listerine, mouthwashes with fluorides have a great advantage, because their local application dissolves the surface layers of enamel and releases various ions [10]. Fluoride ions bind the released calcium ions to form calcium fluoride, which is deposited on the demineralized enamel. When the pH value falls below 5.5, calcium fluoride dissolves and fluoride ions are released, the increased presence of which plays a significant role in forcing the remineralization process and inhibiting the enamel surface demineralization process [11]. It is considered that these solutions should be used in certain concentrations depending on the frequency of application. Thus, if they are applied once a day, it is necessary to use a concentration of 200-300 ppm of fluorine; if applied once a week, a concentration of 1000 ppm fluorine is used; and if applied once every two weeks, a concentration of 2000 ppm fluorine is used [12].

Because of its antimicrobial properties, chlorhexidine (CHX) is a widely used antiseptic mouthwash among dental professionals and the general public. It's also generally biocompatible, popular with dental professionals and the patients using it orally as an antiseptic mouthwash to prevent bacterial biofilm and plaque accumulation [13].

The majority of the mouthrinses are acidic, some with a pH as low as 3 [14].

The aim of this research study is to evaluate accessible options for returning salivary pH to a physiological level after acidic exposure.

2. MATERIALS AND METHODOLOGY

In the research, unstimulated saliva of 10 volunteers of different ages was analyzed. 6 male and 4 female volunteers were included, without oral and systemic diseases present. The ethical consent of the Ethics Committee of the Dental Clinic of Vojvodina (01-18 / 22-2020) was obtained for the conducted research.

The sampling method belongs to the passive saliva collection (SalivaBio Passive Drool Method), in this way a sample of mixed saliva is obtained, which is poured directly from the mouth into a plastic tube. This method can collect a small amount of sample. In this way, the collection of high quality samples is ensured, and thus precise analytical results. It is a very simple method of collecting saliva, which makes it suitable for both adults and children. Before collecting the sample, it was advised that the subjects do not consume food and colored drinks, except water, smoking tobacco, as well as brushing their teeth, since all these factors can affect the level of saliva secretion.

During this research, 4 mouthwashes were used: Oral B, Listerine, Denta dent and Perioplus and 3 commercial carbonated drinks: Coca Cola, Coca Cola zero and Fanta.

In all volunteers, saliva sampling was performed as follows. Saliva samples were collected before drinking or rinsing the mouth, and then liquids were used. Subsequently, subjects had given a saliva sample after 15 minutes, half an hour, an hour, and 2 hours after the start of the experiment. The pH value was determined with a pH meter. (Lutron Electronics Enterprise pH meter) (Figure 1).

When it comes to statistical analyses, the data were presented in the form of mean values and standard deviation. To evaluate how far data were from normality, the Shapiro–Wilk test was used. In cases when data were with disturbed distribution from normality, the significance of the difference between the examined research groups, was tested by the Wilcoxon signed-rank test, while for the data that exhibited normal distribution one way ANOVA and paired samples T-tests were used. The level of statistical significance was set at 5% ($p < 0.05$). For all statistical calculations Jamovi software (version 0.9.2.8) was used.

3. RESULTS

Figure 2 and table 1 depict changes in pH compared to baseline values following liquid exposure and post-exposure effect of four post treatment measurements. It can be observed that for the entire sample, initial drop 15 minutes after all liquids consumption has been observed, while pH values uniformly increased 30 minutes after the initial measurement and remained stable until the end of the experiment. Figure 3 and table 2 depict these results in relation to specific mouthwash and carbonated beverage. It is important to note that Coca Cola demonstrated the highest initial pH decrease following acidic consumption.



Fig. 1. pH measurement

Table 1. Descriptive statistics for the entire sample

	Baseline	15 min	30 min	60 min	120 min
Mean	7.62	7.55	7.80	7.74	7.75
Median	7.62	7.74	7.92	7.76	7.74
Standard deviation	0.273	0.700	0.508	0.414	0.363
Minimum	6.99	4.84	5.42	6.17	6.66
Maximum	8.26	8.40	8.35	8.38	8.36
Shapiro-Wilk W	0.985	0.761	0.716	0.899	0.963
Shapiro-Wilk p	0.368	< .001	< .001	< .001	0.010

Since data distribution significantly deviated from normality non parametric tests had been conducted. Table 3 shows that statistically significant differences were observed between baseline measurements and the measurement after 15 minutes ($p < 0.05$).

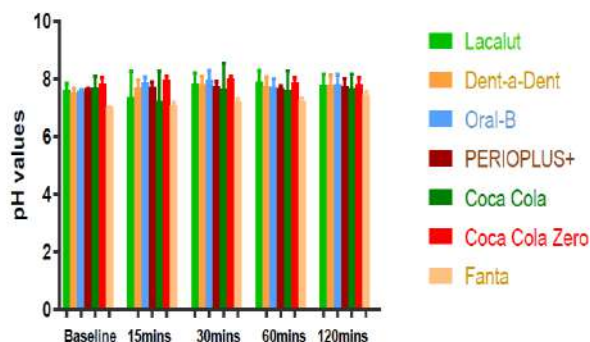


Fig. 2. PH values at the baseline and throughout the experiment

Table 2 pH values in relation to liquids throughout the experiment

Liquid	Baseline	15 min	30 min	60 min	120 min
Coca Cola Zero	7.83	7.97	8.01	7.87	7.8
Coca Cola	7.69	7.24	7.64	7.62	7.66
Dent-a-Dent	7.53	7.69	7.82	7.76	7.78
Fanta	7.02	7.08	7.19	7.23	7.45
Listerine	7.61	7.37	7.84	7.88	7.78
Oral-B	7.58	7.87	7.96	7.72	7.82
PERIOPUS+	7.67	7.72	7.76	7.67	7.77

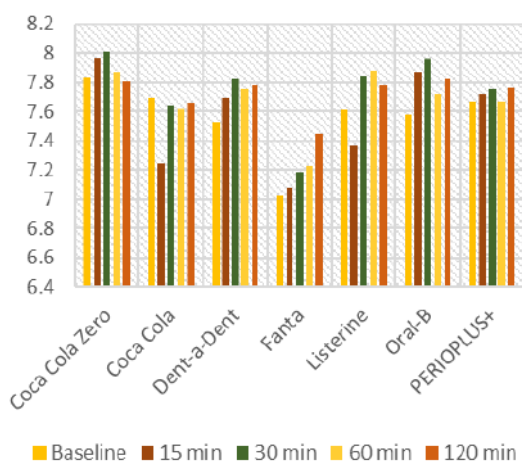


Fig. 3. PH values in relation to solution

The increase in pH values 30 minutes after the baseline measurement was also statistically significant ($p < 0.05$, Wilcoxon signed-rank test). After 60 and 120 minutes from the beginning of the experiment there was no statistically significant differences ($p > 0.05$)

Table 3. Wilcoxon signed-rank test intergroup analysis

	χ^2	Df	P
Baseline	20.55	6	0.002
15 min	16.65	6	0.011
30 min	13.42	6	0.037
60 min	11.28	6	0.080
120 min	4.53	6	0.606

4. DISCUSSION AND CONCLUSION

In the present investigation during this experimental research, data were obtained in order to show how the use of antibacterial mouthwashes on one side, and the use of carbonated beverages, on the other side, affects the level of pH and thus influences the protective role of saliva. The protective role of saliva includes cleaning, lubrication, maintaining the integrity of the oral mucosa, buffering properties, antimicrobial properties, remineralization and assisting in the digestion of food, but the ability to resist the acidic challenge presents one of the most important protective mechanisms [4,7,8].

Based on the obtained results, we determined that the use of various antiseptic agents for rinsing the mouth, as well as the use of carbonated beverages significantly affects the acido-basic salivary balance, which thus can have significant effect on oral health.

While the use of antiseptic solutions has its other mechanisms in maintaining oral health, the initial adverse effect on the acidity of the oral cavity is much lower compared to the frequent use of carbonated beverages. Also, mouthwashes are used according to a strictly defined protocol of oral hygiene and diet upon the advice of a dentist, immediately after brushing teeth, when the pH in the oral cavity is highest and the duration of use of mouthwash is limited to a few minutes. On the other hand, consuming carbonated drinks is a habit that patients can pursue for a longer period of time during the day, in conditions when plaque is already present on tooth surfaces, so the pH drop in this case repeats, lasts longer and certainly has greater clinical significance. In the present investigation, the most frequently used types of active ingredients in mouthwashes had been evaluated (fluoride mouthwash, chlorhexidine and essential oil based mouth rinse), together with most widely used carbonated beverages (Coca Cola and Fanta). In this study, it was shown that saliva has a strong buffering capacity and that the pH values return to the initial values within half an hour after the use of the analyzed solutions. These findings should be taken with caution because completely healthy volunteers, young adults, with extremely low caries activity and no caries lesions participated in this study. These results cannot therefore be directly extrapolated to the general population,

For that reason, it is very important to take into account the extent to which and how often these solutions are used. Since there is a possibility of lowering the pH of the oral cavity below 5.5 when maintaining oral hygiene by brushing teeth and using chemoprophylactic agents, patients should be advised to avoid consuming acidic foods and beverages or use other chemoprophylactic agents to

avoid further lowering the pH. Addy and his coworkers were the first to suggest that mouthrinses could cause dentine smear layer erosion, especially when used in conjunction with mechanical toothbrushing [15].

Many dental patients report drinking a lot of acidic beverages, which is a known and significant health risk for exposed enamel and dentin tooth surfaces. In general, giving dental professionals and patients more information about acidic beverages could help them better educate their patients on how to avoid further erosion and create an oral cavity that is less favorable to unfavorable acidic attack.

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