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Evaluation of pH and Acidity in Xylitol Lollipops, Propolis Mouthwash, and Probiotic Lozenges: An in Vitro Study

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Abstract: ***Recently, attention has focused on studying the physicochemical parameters of certain prophylactic products and their erosive and cariogenic potential. These characteristics include sugar content, endogenous pH, and titratable acidity. This study aims to assess the endogenous pH and titratable acidity of xylitol lollipops, propolis mouthwash, and probiotic lozenges in vitro. The results showed that the lollipop had the lowest endogenous pH, followed by propolis mouth rinse. The probiotic tablet had the highest pH value but was still below the neutral level. The average values for titratable acidity ranked the products in the same order: xylitol lollipops had the highest average titratable acidity 27.3 geq, followed by the propolis oral solution 25.1 geq, and the probiotic tablets were last 5.0 geq. The prophylactic products in this study are acidic, and their frequent use negatively impacts dental structures. Their regular intake can lead to erosion and favor the development of a carious process in the future.***

Keywords: pH, titratable acidity, xylitol, propolis, probiotic

1. Introduction

Dental caries is a widespread oral disease affecting most of the world's population (1). Modern biointervention trends aim to restore the disturbed microbial balance by introducing beneficial microorganisms and inhibiting cariogenic ones (1). People commonly use probiotics to maintain their health, particularly for the gastrointestinal tract. However, they can effectively prevent and treat various oral diseases, such as dental caries, oral mucositis, and halitosis (2). A greater frequency of colds characterizes childhood due to the undeveloped immune system and the lack of built-up prevention habits. Pediatricians often prescribe antibiotics in combination with probiotics as a treatment for several diseases in childhood.

One of the main factors in the development of caries is the frequent consumption of sugar (3). Therefore, as an alternative, its substitutes are often used - polyols (such as xylitol, erythritol, and others), which do not allow the formation of lactic acid in the dental plaque (3). Several countries have approved the use of xylitol in foods, pharmaceuticals, and oral health products e.g., gums, toothpaste, syrups, lollipops, and its use is steadily increasing (4).

A modern trend is the use of natural products in the creation of medicines for the treatment of several diseases and products for oral care, especially in childhood (5). Propolis has antimicrobial, anticancer, antifungal, antiviral, and anti-inflammatory properties and is used in dentistry for various purposes (5). In the pharmacy network, there are propolis oral solutions that natural parents prefer as an alternative to solutions containing chemical plaque control agents.

A number of products (propolis, probiotic and xylitol) are available as caries preventives and their use is increasing. These products have been extensively studied, with various research overwhelmingly confirming their positive effects on oral health, prevention of dental caries, control of dental biofilm and other risk factors for the development of caries (2, 6, 7).

Caries is a multifactorial disease, and over the years, attention has been directed to studying the physico-chemical parameters of some pharmaceutical and non-medicinal products concerning their cariogenic potential. Such characteristics are sugar content, endogenous pH, titratable acidity, etc. (8-10).

The hydrogen indicator, the so-called pH, is a parameter for the alkalinity or acidity of a given solution or product, determining its chemical character (11). pH is measured using two methods: colorimetric (using colored indicators or lacmus papers) and potentiometric (using a pH meter) (11). *Endogenous pH* is a crucial variable providing information on the cariogenic and erosive potential of the studied products (11, 12).
Titratable acidity represents the total content of acids - free protons and undissociated acids - in a given solution that can

react with strong bases and be neutralized (11, 13). The higher the percentage content (titratable acidity), the more pronounced the erosive effect of the product on enamel due to its dissolution (14-18).

However, products' erosive potential is related to both their endogenous pH and titratable acidity (13). We are not aware that the literature has investigated these characteristics of xylitol lollipops, propolis mouthwash, and probiotic lozenges, which directed our attention to conducting the present study.

Aim: The study **aim**ed to assess the endogenous pH and titratable acidity of xylitol lollipops, propolis mouthwash, and probiotic lozenges in vitro. The null hypothesis is that there is no statistically significant difference between the indicated parameters of the studied products.

2. Material and methods

The objects of this experimental study are three products from the pharmacy network - children's probiotic tablets, xylitol lollipop, and propolis mouthwash, selected due to their frequent use, widespread among society, and advertised as having a positive effect on oral health. The units of study are the endogenous pH of the respective products and their titratable acidity.
The composition of the studied products is presented in Table 1.

Table 1: Composition of the studied products

Trademark	Product	Composition
Lactoflor	Lactoflor BioPlus probiotic and prebiotic tablets	Filler (isomalt), probiotic blend (1x109 CFU/tab - Natural Bulgarian Lactobacillus bulgaricus LKZ-200, isolated from blood geranium leaves; Bulgarian Lactobacillus rhamnosus LLR-L1 with high bioavailability and increased activity, against candida/ e.coli/ st. aureus; Streptococcus thermophiles, prebiotic (fiber-inulin), stabilizer (gum arabic), anti-caking agents (magnesium salts of fatty acids, silicon dioxide)
Miradent	XyliPOP xylitol lollipops strawberry without sugar	3.2 g xylitol (sweetener), isomalt (stabilizer), gum arabic (thickener), flavors, calcium lactate (acidity regulator), magnesium stearate (anti-caking agent), citric acid (acidity regulator), betanin, strawberry powder
Bioaptheka	Bio Apteka mouthwash with honey and propolis	Aqua, Propolis Extract, Glycerin, PEG-40 Hydrogenated Castor Oil, Organic Honey Extract, Aroma, Zinc Gluconate, Sodium Saccharin, Pentylene Glycol, Methyl Diisopropyl Propionamide, Citric Acid, Potassium Sorbate, Sodium Benzoate.

Assessment of endogenous pH of the studied products
All reagents in the indicated concentrations are prepared according to the European Pharmacopoeia with an experimental setup according to an already established methodology (19, 20). An electronic scale with an accuracy of 0.0001 (Mettler Toledo, model AT200, Switzerland), and a pH-meter with an accuracy of 0.01 with a built-in thermometer (model pHTestr 50S Spear-Tip Waterproof Pocket tester, Premium 50 Series, Oakton, USA) were used for the needs of the experiment. Six samples of each product were measured on the electronic scale (18 samples in total). The mass of the probiotic tablet and xylitol lollipop is approximately 1.0 g with an accuracy of +/- 0.0001 g. The mass of the propolis mouth rinse is approximately 10.0 g with an accuracy of +/- 0.0001 g. To each of the test samples, deionized water was added in a ratio of 1:5. Measuring flasks of suitable volume were used for this purpose.

Each test sample was placed in a glass beaker to measure the pH and temperature of the solution. The pH meter electrode was immersed in the solution for one minute while the solution was stirred continuously using the tip. The mean pH value, the mean value of the measured temperature, and the standard deviation were calculated from the results obtained from each sample.

Assessment of titratable acidity of the studied products
Preparation of test solutions: 1.0 g of the product - accurately measured mass on an analytical balance, is transferred to a 100 ml Erlenmeyer flask with 50.0 ml of deionized water. Add 0.2 ml of 0.1% alcoholic solution of phenolphthalein.

The obtained solutions were titrated with a 0.1 M solution of sodium base under strong shaking until a pink color appeared, which did not disappear for 20-30 s. A control solution is also titrated in parallel. The spent milliliters of 0.1 M sodium hydroxide solution multiplied by 10 represents the free acidity expressed in g/eq.

Statistical analysis
Statistical analysis was conducted with a statistics computer software SPSS v.19.0 (SPSS Inc., Chicago, IL, USA). The significance level was set at p=0.05.

3. Results

The comparison between the studied products based on the average value of endogenous pH is presented in Table 2.

Table 2: Comparative analysis of the endogenous acidity of the studied products

pH / Product	Mean ± SD	Min	Max	Ind Samples Test
Mouthwash[1]	4.73 ± 0.011	4.72	4.75	$T_{1,2}$ = 154.923, p = 0,000
Lollipop[2]	3.74 ± 0.010	3.73	3.76	$T_{2,3}$ = -129.472, p = 0,000
Probiotic lozenges[3]	5.991 ± 0.041	5.95	6.06	$T_{1,3}$ = -71.700, p = 0,000

We analyzed the mean pH values of the three products and found statistically significant differences. The lollipop had the lowest endogenous pH, and the probiotic tablet had the highest but still below the neutral value (Table 2).

Table 3 presents the obtained results from the assessment of titratable acidity for each of the investigated prophylactics - lollipop, propolis solution, and probiotic tablet.

Table 3: Comparative analysis of free titrated acidity of the studied products

Titrated acidity / Product	Mean ± SD	Min	Max	Ind Samples Test
Mouth rinse[1]	2.516 ± 0.204	2.400	2.900	$T_{1,2}$ = -2.320, p = 0,043
Lollipop[2]	2.733 ± 0.103	2.600	2.800	$T_{2,3}$ = 52.968, p = 0,000
Probiotic lozenges[3]	0.500 ± 0.000	0.500	0.500	$T_{1,3}$ = 24.200, p = 0,000

The obtained average values for this indicator ranked the studied products in the following order: the xylitol lollipops had the highest average titratable acidity (27.3 g/eq), the propolis oral solution was second (25.1 g/eq), and the probiotic tablets were last (5.0 g/eq). The same arrangement of the products is shown in the study of their endogenous pH (Table 2).

4. Discussion

Using some prophylactic products can create a local cariogenic situation and cause erosion of dental structures. The reasons for this can be the composition and consistency of the product, the frequency and duration of intake, the method of administration, etc. (21). The present study compared the endogenous pH and titratable acidity of popular over-the-counter products widely used by children and adults. The obtained results reject the null hypothesis.

In specialized literature, a solution with pH<7 is considered acidic (22). Using a descriptive analysis of the data obtained when examining the endogenous pH of the three prophylactic products, we established their acidic nature (Table 2). Xylitol lollipops had the lowest average endogenous pH value. It is a product created as an alternative to sugary lollipops, which children prefer and are readily available everywhere. Products containing xylitol, including chewing gum, candies, lollipops, and beverages, are also widely available on the market. Much of the dental community even recommends their use to prevent dental caries. Sugar alcohols (xylitol, erythritol, etc.) have been shown in various tests to be non-acidogenic or hypoacidogenic and, therefore, to have no or low cariogenicity (23). Xylitol mouthwashes have a pH above six and relatively low titratable acidity (24). In the composition of xylitol-containing lollipops, other products with an acidic composition are also included, and they would have an erosive potential on tooth enamel, especially with prolonged and frequent use. The results of the present study show that xylitol lollipops have a highly acidic nature and low endogenous pH values (Table 2).

Erosion is defined as the dissolution of the tooth crystal caused by acids other than those formed in the dental biofilm (25). Sources of such acids can be medications, foods, and drinks (26). Due to their acidic nature, the products of the present study can initiate demineralization even in the absence of biofilm on the tooth surface, putting the tooth surfaces in contact with them at risk.

Mouthwash solutions stay in the mouth for a short period (20-30 seconds). The propolis mouthwash we studied has acidic characteristics that make it risky for daily usage by children. A recent study evaluating the pH values and titratable acidity of different mouthwashes in vitro found that 43% of the products tested showed a pH below the critical value of 5.5 (17). Below this pH value in the dental plaque, the irreversible release of mineral ions from the apatite structure of the enamel begins (27). Oral hygiene products such as toothpaste and mouthwash often have a low pH, increasing their ingredients' chemical stability (17). Although our and other studies prove that the pH value of mouthwashes is below the critical level for enamel, which could lead to tooth erosion, these values fall within the norm of the European Standard for Oral Care Products - NEN- EN-ISO16408:2015 (17, 28).

The highest endogenous pH values were reported for the probiotic tablets (Table 2). When taking them, manufacturers recommend chewing them first, which means they can also affect the balance in the oral cavity and lead to enamel erosion. The frequent use of acidic medicines and products that come into direct contact with the teeth is an etiological factor for dental erosion in children and adults (29). Our research proved the acidic nature of all three prophylactic products studied. In comparison, other studies of conventional medications also found an acidic pH (30).

Besides the pH value, titratable acidity plays a vital role in erosion (18). By determining it, the salivary buffer capacity needed to neutralize the pH of the products can be indirectly measured (10). The higher the titratable acidity of a drug, the more pronounced its erosive potential on enamel will be (16). Our study determined the highest titratable acidity and the highest erosive effect in xylitol lollipops, followed by propolis mouthwash and probiotic tablets (Table 3). The propolis mouthwash results of the present study were confirmed by another publication, which found that the titratable acidity of mouthwash solutions ranged from 0 to 48 g/eq (17). The value we determined for the propolis solution was 25.1 g/eq (Table 3). The pH value is a more important characteristic for the residence time of various products in the oral cavity. However, titratable acidity is a factor that delays the recovery of salivary pH and prolongs the time during which there is a risk of mineral loss (13). The results of the present in vitro study suggest an increased erosive potential of the products. However, in vivo conditions of the oral cavity influence many biological factors, such as dental pellicle, buffer capacity, and remineralizing action of saliva, which would reduce this negative effect (31).

5. Conclusion

The study confirms that the prophylactic products analyzed exhibit acidic properties, which may contribute to dental erosion and caries with frequent use. Xylitol lollipops have

the highest erosive potential, followed by propolis mouthwash, with probiotic lozenges being the least erosive. This information is crucial for making informed decisions regarding the use of these products in daily dental care routines.

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Conflict of interest: Nil

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