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Physicochemical properties, labeling and antimicrobial activity of mouthwashes for children

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Abstract

Objective: To analyze the physicochemical properties, labeling and antimicrobial activity of mouthwashes for children against oral biofilm microorganisms.

Material and Methods: We selected a total of eight brands of mouthwashes and used distilled water and chlorhexidine as negative and positive controls, respectively. The packages were analyzed by direct observation. The assessment of pH, °Brix and kinematic viscosity was carried out using a digital pHmeter, specific field refractometer and capillary viscometer, respectively. For this study, we used strains of *Streptococcus oralis, Streptococcus mutans, Streptococcus salivarius* and *Lactobacillus acidophilus*, which were reactivated in BHI and plated on blood agar. Susceptibility tests were made by the agar diffusion method, followed by incubation at 37 °C for 48 hours.

Results: The pH, °Brix and viscosity values ranged from 4.89 to 7.23, 2.8 to 20.0, and 1.47 mm^2/s to 2.73 mm^2/s, respectively. Chlorhexidine was found to show the largest zones of inhibition against *S. salivarius* and *L. acidophilus*. This effect was observed for the mouthwashes against *S. oralis* and *S. mutans*.

Conclusion: The mouthwashes for children tested herein were found to show a neutral or close to neutral pH, presence of soluble solids in their composition and little variation of viscosity. These products proved to show antimicrobial activity on the microorganisms tested, excepting one of the mouthwashes, which showed no effect on *S. mutans*.

Key words: Mouthwashes; Hydrogen-Ion Concentration; Microbiology

Propriedades físico-químicas, apresentação de rotulagem e atividade antimicrobiana de colutórios infantis

Resumo

Objetivo: Analisar propriedades físico-químicas, modo de apresentação e ação antimicrobiana de colutórios infantis sobre micrmisms do biofilme dentário.

Metodologia: Selecionaram-se oito marcas de colutórios, adotando-se água destilada e clorexidina como controle negativo e positivo, respectivamente. As embalagens foram analisadas por meio de observação direta. A avaliação do pH, °Brix e viscosidade cinemática foi realizada mediante phmetro digital, refratômetro específico de campo e viscosímetro capilar, respectivamente. Utilizou-se cepas de *Streptococcus oralis, Streptococcus. mutans, Streptococcus salivarius* e *Lactobacillus acidophilus*, sendo elas reativadas em BHI e semeadas em placas contendo ágar sangue. Pelo método da difusão em ágar, realizaram-se testes de susceptibilidade, com incubação a 37°C/48 horas.

Resultados: Os valores de pH, °Brix e viscosidade variaram de 4,89 a 7,23; 2,8 a 20,0; 1,47 mm^2/s a 2,73 mm^2/s, respectivamente. A clorexidina demonstrou os maiores halos de inibição sobre *S. salivarius* e *L. acidophilus*, sendo este efeito observado para os colutórios frente ao *S. oralis* e *S. mutans*.

Conclusão: O pH dos colutórios infantis foi neutro ou próximo a ele, havendo presença de sólidos solúveis em sua composição, enquanto a viscosidade mostrou pouca variação. Estes produtos apresentaram ação antimicrobiana sobre os microrganismos estudados, exceto um dos colutórios que não demonstrou efeito sobre *S. mutans*.

Palavras-chave: Antissépticos Bucais; Concentração de Íons de Hidrogênio; Microbiologia

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Introduction

Over the past years, preventive dentistry has developed several mechanisms aimed at the control of dental biofilm. Mechanical and chemical methods or even the combination of both have shown to be effective alternatives against oral biofilms. The mechanical methods, e.g. brushing and flossing, are known to be the most effective ones. However, chemical methods such as the use of mouthwashes also stand out as adjuncts to oral hygiene for significantly reducing the amount of oral biofilm [1-3].

In Brazil, the majority of mouthwashes are readily available in retail establishments. As it is not usually necessary to have in hands a prescription given by the dentist to obtain them, these products have become very accessible to children and adults. Hence, the unreasonable use of mouthwashes by the general population has provoked concern due to some of the components included in their formulations, which can cause side effects [4]. The potential risks and/or side effects of mouthwashes may be associated with three main factors: physicochemical properties such as pH, which plays an important role in tooth enamel erosion [5,6]; active substances, namely essential oils, chlorhexidine, triclosan and cetylpyridinium chloride, which have antimicrobial activity [6]; and other ingredients, such as alcohol, which can cause tooth staining, and desquamation and hyper-keratinized lesions in the oral mucosa [6].

Among the potential damages resulting from mouthwash use, erosion is one of the most reported in the literature. It is considered as a progressive and irreversible loss of tooth enamel, resulting from chemical processes not involved with bacterial action. Yet, it can be changed by the interplay of biological and behavioral factors [5]. In this perspective, there is a need to analyze the physicochemical characteristics of these products.

With regard to pH, when the enamel surface is subjected to the action of aqueous inorganic solutions with acidic pH (between 4 and 5) – if unsaturated compared to fluorapatite and hydroxyapatite –, it is observed the presence of surface alterations that confer erosion-like features to enamel similar to those observed under clinical conditions [8]. This happens in the cases when the salivary pH is lower than 4.5 or when one makes use of acidic products [8].

Nevertheless, the erosive potential of a substance is not totally dependent on its pH, but also influenced by its viscosity. Kinematic viscosity is a flow resistance controlled by internal attrition forces between the atoms of a liquid agent. This property plays a role in the erosive process, taking into account that the possible erosive phenomenon triggered by a mouthwash is more intense depending on the ability of the liquid to adhere onto the tooth surface [6].

Another feature of interest in the study of dental erosion is the amount of total soluble solids (sugar, salt, proteins, and acids, among others) present in a substance, as a greater concentration of these components may be considered a coadjuvant factor for the establishment of erosive lesions.

The limitations of oral hygiene mechanical methods on the part of children, in addition to the broad advertising made by companies, have made the use of mouthwashes for children grow significantly in recent years. Nevertheless, the advertising made by the manufacturers of mouthwashes for children does not specify correctly their indications and does not emphasize proper care with regard to their use [6].

It is worth noting that the Board Resolution (RDC) # 79 (2000) of the Brazilian National Health Surveillance Agency (ANVISA) classifies the mouthwashes for children into the grade-2 risk category, i.e., mouthwashes are considered as products with potential risk [8].

In this perspective, two important issues must be considered. The first one relates to the significant number of mouthwashes for children available in the market of distinct trademarks and presenting in their compositions different agents with varied modes of action [2,5). The second one concerns the information labeled on their packages, as these are considered products for oral and dental hygiene with an assumed potential risk. According to ANVISA, fluoridated mouthwashes should minimally contain in their labels the name of the fluoride compound used, its concentration in ppm (part *per* million), mode of use, and warnings of not using these products in children under 6 years of age and of potential risks to consumers' health [9].

Thus, the present study analyzed the physicochemical properties and labeling of mouthwashes for children and investigated the *in vitro* antimicrobial action of these products on microorganisms present in the oral biofilm.

Material and Methods

Samples

We obtained mouthwashes for children of all commercial brands available in drugstores and supermarkets in the city of João Pessoa, PB, Brazil. This study sample was composed by one of each of the following mouthwashes: Colgate Plax Kids®, Listerine Agent Coolblue®, Cepacol Teen®, Sanifill®, Condor Junior®, Johnson & Johnson®, Equate®, and Bitufo® (Table 1). Examiners checked for manufacturing and expiry date, and kept the products at room temperature as recommended by their manufacturers.

Table 1. Mouthwashes analyzed and their respective manufacturers.

Product	Manufacturer
Colgate Plax Kids®	Colgate-Palmolive Ind. and Com. Ltd
Listerine Agent Coolblue®	Johnson & Johnson
Cepacol Teen®	Aventis Pharma Ltd
Sanifill Sanikids®	Facilit Odontológica e Perfum. Ltd
Condor Junior®	Condor Ltd
Johnson & Johnson®	Johnson & Johnson
Equate®	Ind. Com. and Repr. Poli Products Ltd
Bitufo®	Bitufo Ltd

Analysis of physicochemical properties

The pH measurements were made at the Laboratory of Oral Microbiology (UFPB) using a bench digital pHmeter (Orion 4 Star, Analyzer®). A total of 30 ml of each sample were inserted into a graduated beaker checking the volume and proceeding with the analysis. Each sample was measured three times. The final pH was set as the arithmetic mean of the values recorded.

Refractometry was used for the measurement of total soluble solids content. To this end, we used three drops of each sample to obtain the °Brix value by using a specific field refractometer, model N1, Atago® with ºBrix reading range between 0~32% and accuracy of 0.2. The results were given by the mean of three measurements. These analyses were performed at the Laboratory for Flavor Analysis (UFPB).

The kinematic viscosity was measured with the aid of a Cannon-Fenske capillary viscometer at room temperature (25 °C). Initially, we determined the time, in seconds, for a volume of a liquid (20 ml) to flow by gravity through the capillary of a calibrated viscometer. Then, the kinematic viscosity was calculated by means of a standard equation:

$$V = K \cdot t,$$

where:

K is a constant: 0.2326 mm²/s²;

t is the time, in seconds, for the volume of a liquid to flow by gravity through the capillary of a viscometer;

V is the viscosity value, whose measure unit is mm²/s.

The final kinematic viscosity was given by the mean of three measurements.

Analysis of labeling

The packages of the eight mouthwashes for children selected to compose the sample were analyzed by direct observation. The parameters considered for evaluation were: a) amount of fluoride (ppm); b) recommended time of rinsing; c) chemical composition, including: presence of alcohol, type of emulsifiers and flavorings; d) presence of safety lock on the lid; and e) reference to age restrictions for product use.

Antimicrobial activity

All products were evaluated for their antimicrobial activity without being diluted, as indicated for use. For the analyses, we used the agar diffusion method with the wells technique [10]. Caution was taken to use sterile culture media and instruments throughout the whole experiment.

The antiseptics were tested on cultures of standard strains of *Streptococcus oralis* (ATCC 10557), *Streptococcus mutans* (ATCC 25175), *Streptococcus salivarius* (ATCC 7073) and *Lactobacillus acidophilus* (ATCC 4356) originated from the National Institute of Quality Control in Health (INCQS), Oswaldo Cruz Foundation. The choice for these bacteria was based on the recognized role that they play in the early biofilm formation and/or in the subsequent colonization [11].

The cultures were activated in tubes containing 10 mL of BHI broth. After one day, they were seeded in Petri plates containing 20 ml of BHI blood agar.

Then, five 6-mm diameter wells were bored in each plate to posteriorly store the mouthwash samples. Chlorhexidine (0.12%) and distilled water were used as positive and negative controls, respectively. The experiments were performed in duplicate.

These susceptibility tests were carried out with incubation at 37 °C for 48 hours. The reading was done by observing the formation of zones of growth inhibition, indicating bacteriostatic or bactericidal properties of the mouthwashes. These zones were measured with the aid of a manual caliper. The results were expressed as the mean diameter (mm) of the zones of growth inhibition formed around the wells. Data were analyzed descriptively using the statistical package SPSS, version 17.0.

Results

The pH and °Brix values of the mouthwashes ranged from 4.89 to 7.23 and 2.8 to 20.0, respectively. Viscosity values were found to be between 1.47 and 2.73 mm²/s (Table 2).

Table 2. Results of the analyses of pH, °Brix and Kinematic Viscosity (KV) of mouthwashes for children.

	pH	°Brix (%)	KV (mV)
Colgate Plax Kids®	4.9	20.0	2.7
Listerine Agent Coolblue®	5.6	13.5	1.7
Cepacol Teen®	7.3	13.7	1.9
Sanifill®	7.1	6.7	1.7
Condor Junior®	7.2	4.7	1.5
Johnson & Johnson®	7.1	2.8	1.7
Equate®	6.6	14.5	2.2
Bitufo®	5.8	9.0	1.7

Listerine Agent Coolblue® was the only product without fluoride in its composition, differing from the others that had fluoride concentration ranging from 225 to 226.2 ppm. Only Condor Junior® made no reference to the rinsing time in its package, and all mouthwashes had no alcohol in their composition. Tutti-frutti was the most frequent flavor (62.5%) and most of the products (75%) had sorbitol as a sweetener. Half of the samples had no safety lock in the lids, and a large part of which (37.5%) failed to indicate age restriction in their labels (Table 3).

With regard to the results of antimicrobial activity, zones of microbial growth inhibition were produced by all mouthwashes against *S. mutans*, excepting Bitufo®, which showed no ability to inhibit bacterial growth (Table 4).

Table 3. Labeling analysis of the mouthwashes packages.

	Colgate®	Listerine®	Cepacol®	Sanifill®	Condor®	Johnson®	Equate®	Bitufo®
Fluoride concentration (ppm)	225	–	226.2	226	225	226	225	225
Rinsing time	1 min	30 s	30 s	30 s	–	1 min	30 s	1 min
Aromatizers	tutti-frutti	mint	strawberry	tutti-frutti	strawberry	tutti-frutti	tutti-frutti	tutti-frutti
Sweeteners	sodium saccharin and sorbtiol	sorbitol and sucralose	sodium saccharin	sorbitol	sorbitol	sucralose	sorbitol	sorbitol and xylitol
Age restriction	> 6	–	–	> 6	> 6	> 6	–	> 6
Safety lock	absent	present	present	absent	present	present	Absent	absent
Presence of alcohol	absent	absent	absent	absent	absent	absent	Absent	absent

Table 4. Means of the zones of microbial growth inhibition, in millimeters.

	S. mutans	*S. oralis*	*S. salivarius*	*L. acidophilus*
Colgate Plax Kids®	12.0	9.5	10.5	19.5
Listerine Agent Coolblue®	9.5	8.5	9.5	14.5
Cepacol Teen®	8.5	10.5	10.0	20.0
Sanifill®	18.0	8.5	9.5	17.0
Condor Junior®	17.5	9.5	13.0	18.0
Johnson & Johnson®	12.0	8.0	11.0	15.5
Equate®	10.0	8.5	10.0	20.0
Bitufo®	-	20.5	11.5	8.5
0.12% chlorhexidine	15.0	15.5	16.0	28.0
Distilled water	–	–	–	–

Discussion

Even though some limitations of *in vitro* studies can be found when one considers the natural reproduction of oral conditions, namely: eating habits, buffer capacity of saliva, and individual characteristics [12], the present study allowed us to evaluate the physicochemical properties of mouthwashes for children, in addition to elucidate their antimicrobial potential. Even so, *in vitro* studies have the benefit of providing isolated data of variables of interest, with no influence of other factors. Hence, this type of study has been widely reported in the literature as it allows evaluating the physicochemical properties of beverages, providing important information about the characteristics of the analyzed products [13].

Although the value of critical pH for enamel dissolution is equal to or less than 5.5 [1], mineral loss can occur even with a higher pH and, thus, prolonged use of mouthwashes with pH around these values may be potentially harmful to dental tissues, that is, may cause loss of structure of tooth surfaces exposed to the acidic environment [1,4-6,13,14].

In the present study, 50% of the samples (Colgate Plax Kids®, Listerine Agent Coolblue®, Bitufo® and Equate®) showed acidic pH (pH<7), but only Colgate Plax Kids® showed pH values that could cause tooth enamel dissolution (pH<5.5). These findings are in disagreement with those obtained by Oliveira [14], who found that 66.7% of mouthwashes commercially available in the city of João Pessoa, PB, Brazil, had pH values below 5.5. In a study by Corso et al. [4], 66.3% of the 11 mouthwashes analyzed were proven to show pH value below that considered critical for enamel dissolution, unlike what was found in the present study. Results more similar to those obtained herein were observed in the study by Delbem et al. [15], who assessed 14 brands of mouthwash, of which 28.5% showed pH values below 5.5.

Some fluoride compounds have their chemical stability increased when exposed to low pH, thus favoring the incorporation of fluoride ions into the hydroxyapatite structure and precipitation of calcium fluoride on the tooth surface [16]. This fact could be a reason for manufacturers to develop products with lowered pH. Nevertheless, it is worth noting that in recent years there has been an increased frequency of mouthwashes for children manufactured with pH close to neutral values. In 2005, Oliveira [14] found that 66.7% of the products had pH below 5.5, whereas in the present study we found that only 12.5% had critical pH for enamel dissolution.

Refractometry is a physical method for measuring the amount of total soluble solids (sugar, salt, proteins, acids, among others) present in an aqueous solution. The ºBrix scale is calibrated by the number of grams of sugar *per* 100 g of solution [17].

Of the products analyzed, Johnson & Johnson® and Colgate Plax Kids® mouthwashes were found to have the lowest (2.8%) and the highest (20%) average content of total soluble solids (TSS) on the °Brix scale, respectively. In the study by Cavalcanti et al. [5], Oral B® and Clinerize® mouthwashes had the lowest (4.7%) and the highest (23.70%) content of TSS, respectively. In addition, four out of the ten brands investigated by the authors showed TSS above 20 %, whereas in the present study and in another conducted by Souza [6], only one brand (Colgate Plax Kids®) exceeded this percentage.

Viscosity values ranged between 1.5 mm²/s (Condor Junior®) and 2.7 mm²/s (Colgate Plax Kids®). These findings are in agreement with those obtained by Souza [6], who found Colgate Plax Kids® to be the most viscous mouthwash.

In the study by Lima et al [18], the product with the lowest viscosity value (4.0 mPa.s) was also found to be the one with the lowest pH value (5.36), whereas in our study and in another carried out by Souza [6] the mouthwash that showed the lowest pH (4.89 and 4.75, respectively) showed the highest value for viscosity (2.7 and 2.5 mm^2/s, respectively).

According to the analysis of labeling, the samples had a similar concentration of fluoride in their composition, ranging between 225 ppm and 226.2 ppm, with the exception of Listerine Agent Coolblue®, which did not have fluoride among its compounds. These findings confirm the study by Souza [6], in which all mouthwashes showed fluoride concentration between 225 ppm and 226.2 ppm.

Only Condor Junior® mouthwash failed to indicate its rinsing time in the package. The others ranged from 30 seconds to 60 seconds, confirming the study by Souza [6], who verified that most of the studied products recommended those times using 10 to 20 ml of solution. This is of clinical importance when it is taken into account the risks associated with the use of fluoride-containing mouthwashes, because the inadvertent ingestion of the product during rinsing is related to the risk of having an acute intoxication by this ion [19]. Therefore, the lack of information in the label about the rinsing time of these products raises concern.

In this study, no sample had alcohol included in the composition, whereas in the study by Souza [6], only Cepacol® Flavor Tutti-Frutti had alcohol in its formulation. Bussadori and Masuda [20] pointed out that dentists should not prescribe alcohol-containing mouthwashes for individuals in this age group as these can cause physical and chemical dependence due to their composition.

Aromatizers are substances used to add or enhance the aroma of the food. They are usually combined with flavoring agents able to confer or enhance the flavor [21]. Most of the products mentioned tutti-frutti as aromatizer (62.5%), followed by strawberry (25 %) and mint (12.5%). This is due to the attraction that these aromas and flavors have on children, especially tutti-frutti. With regard to sweeteners, sorbitol was mentioned in 75% of the products. Sweeteners are substances with a high sweetening power, when compared to sucrose, often used in replacement of this sugar in foods and dietary beverages in order to reduce their caloric value [22] and cariogenic potential [23].

Half of the mouthwashes had no safety lock on their lids. As these products are aimed at children, this parameter has an important purpose and may prevent unwanted consumption of antiseptics by the young users. Cepacol Teen® Equate® and Bitufo® did not stamp on their labels any information about age restriction. As for the other samples, the use of mouthwash was reported for more than six years only, according to the RDC nº 79/2000 of ANVISA [9]. This warning is of utmost importance to consumers, because fluoride-containing mouthwashes offer potential risks to systemic toxicity and dental fluorosis, since children under 6 years of age do not have complete control over their swallowing reflexes [1,8].

With regard to the results of antimicrobial activity, all mouthwashes produced inhibition zones on the microorganisms studied, except Bitufo® against *Streptococcus mutans*, which did not inhibit bacterial growth.

The samples that showed the largest inhibition zones against *Streptococcus mutans* were Sanifill® and Condor Junior®. On *Streptococcus oralis*, Bitufo® demonstrated the best result; on *Streptococcus salivarius*, Junior Condor® had the largest zones, and on *Lactobacillus acidophilus,* Equate® and Cepacol Teen® showed the best results.

It is important to point out that few studies have investigated the antimicrobial action of mouthwashes on biofilm-forming bacteria, most of which restricted to *S. mutans*.

Pinto Filho et al. [24] conducted an in *vitro* study investigating the antimicrobial effectiveness of different types of oral rinses on *S. mutans*. The authors found that triclosan-containing mouthwash (Colgate Plax®) was the most effective agent against this bacterial species, corroborating the data of this study, in which Colgate Plax® was proven to inhibit *S. mutans* growth producing a 12-mm zone. However, the authors [24] found that the essential oils-containing mouthwash (Listerine®) was unable to produce inhibition zones, which is in disagreement with the data presented herein, where zones of 9.5 mm were observed for Listerine Agent Coolblue®.

When evaluating the *in vitro* antimicrobial activity of mouthwashes, Moreira et al. [25] found that Colgate Plax® and Cepacol® showed inhibition zones of 20 mm and 11 mm against *S. mutans*, respectively, similarly to what was found in this study. However, as observed by Pinto Filho et al. [24], Listerine® showed no antimicrobial activity against *S. mutans*.

The antimicrobial effect of these products can be explained by the agents included in their formulations. Agents such as sodium fluoride, cetylpyridinium chloride, triclosan, menthol, eucalyptol, xylitol, thymol, among others, have proven antimicrobial action [3].

Conclusion

The hydrogen potential of the mouthwashes analyzed ranged from 4.89 to 7.23, of which 83.3% had acidic pH, but only one reached the critical value for enamel dissolution.

As to the viscosity of the mouthwashes, we found values ranging from 1.47 to 2.73 mm²/s, while the values of total soluble solids content ranged from 2.8% to 20%. Some mouthwashes failed to label important information on their packages, namely: rinsing time and age restriction, and also to present safety lock. All mouthwashes proved to have antimicrobial activity against the microorganisms tested in this study, excepting one of the products against *Streptococcus mutans*.

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