

## ESTIMATION OF pH, TOTAL ACID AND ETHANOL CONTENT OF COMMERCIALY AVAILABLE ALCOHOL-CONTAINING MOUTHWASHES AND ITS EFFECT ON SALIVARY pH

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### ABSTRACT

#### BACKGROUND

Use of mouthwash has become widespread as a part of routine oral hygiene. Mouthwashes may have deleterious effects on oral tissues because of its low pH, high acid content and use of ethanol as an antiseptic agent.

#### MATERIALS AND METHODS

A cross-sectional experimental study was conducted among 48 undergraduate dental students. Eight commercially available alcohol-containing mouthwashes, which are available in Indian market were selected for which the pH, total acid and ethanol content was estimated. 48 subjects were randomly divided into 8 groups. Unstimulated salivary samples were collected before rinsing, then subjects were asked to rinse with 10 mL mouthwash for 60 seconds and salivary samples were collected immediately after rinsing. Salivary pH before and after rinsing was estimated using digital pH meter.

#### RESULTS

Listerine original mouthwash was found to have lowest pH (3.84) and highest acid content (26.8%). High alcohol content was seen in Listerine fresh burst mouthwash (26%). Mean salivary pH before and after rinsing showed a significant difference among all the groups with highest mean salivary pH difference for Listerine fresh burst mouthwash (1.21). Post-hoc Turkey's analysis to compare the change in mean salivary pH between study groups showed a significant difference between Listerine original, Listerine fresh burst and Listerine cool mint mouthwash with other mouthwashes.

#### CONCLUSION

There was a significant reduction in salivary pH after rinsing with low pH, high acid and high alcohol-containing mouthwashes.

#### KEYWORDS

Mouthwashes, pH, Total Acid, Ethanol.

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#### BACKGROUND

A mouthwash is defined as a non-sterile aqueous solution used mostly for its deodorant, refreshing or antiseptic effect. Mouthwashes are usually recommended for consumers to reduce halitosis, prevent and control dental caries and periodontal diseases.<sup>1</sup>

Mouthwashes or rinses are designed to reduce oral bacteria, remove food particles, temporarily reduce bad breathe and provide a pleasant taste. The amount of the

different components in mouthwashes varies from product to product. Some practically have the same composition as toothpastes, although they do not contain abrasives. Distinct from toothpastes, most mouth rinses contain alcohol as a preservative and a semi-active ingredient. The amount of alcohol is usually ranging from 18-26%.<sup>1</sup>

Although, mouthwashes are effective in reducing plaque-induced gingivitis and providing fluoride to prevent dental caries. Some studies have addressed the risks associated with the daily use of mouthwashes.<sup>2</sup> These risks include dry mouth, an increase in the incidence of head and neck cancer and extrinsic pigmentation. Paradoxically, oral health mouthwashes may also cause dental caries largely due to the inclusion of ethanol as an antiseptic agent, which can be oxidised to acetic acid. These changes increase acidity and thus demonstrate an increased risk of dental caries.<sup>3</sup> So, the present study was aimed to estimate the pH, total acid and total ethanol of the commercially available mouthwashes and the salivary pH change before and after rinsing.

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**MATERIALS AND METHODS**

An experimental study was carried out over a period of two months from September 2014 to October 2014 among eight commercially available alcohol-containing mouthwashes, which were available in Indian market and dental under graduate students who were residing in Narayana Dental College Hostel, Nellore, to estimate the pH, total acid and total ethanol of the commercially available alcohol-containing mouthwashes and to estimate the salivary pH before and after rinsing.

**Source of Data-** The source of data was primary in nature and the data was obtained from the eight commercially available alcohol-containing mouthwashes, which were available in Indian market and dental undergraduate students who were residing in Narayana Dental College Hostel, Nellore.

**Study Setting-** The study was carried out in Narayana Pharmacy College, Nellore, and Narayana Dental College and Hospital, Nellore.

**Study Duration-** Study was conducted out over a period of two months from September 2014 to October 2014.

**Inclusion Criteria-** Subjects who were caries free, willing to participate and who gave informed consent were included in the study.

**Exclusion Criteria-** Subjects who were using tobacco or alcohol in any of the forms, those who were suffering from any systemic illness or using any medication at the time of study or in period of last 15 days prior to the study were excluded from the study.

**Sample Size Estimation-** The sample size was estimated based on standard deviation and difference in salivary pH before and after reported in the previous study by Chand S et al (2013).<sup>4</sup> The estimated sample size was 48, which were proportionately selected into eight groups. The formula used for the estimation of the sample size was-

$$n = \frac{(Z_{\alpha} + Z_{\beta})^2 \times 2 \times (S)^2}{(d)^2}$$

$Z_{\alpha}$  = Z value for alpha error (1.96).

$Z_{\beta}$  = Z value for beta error (0.84).

S = standard deviation = 0.15.

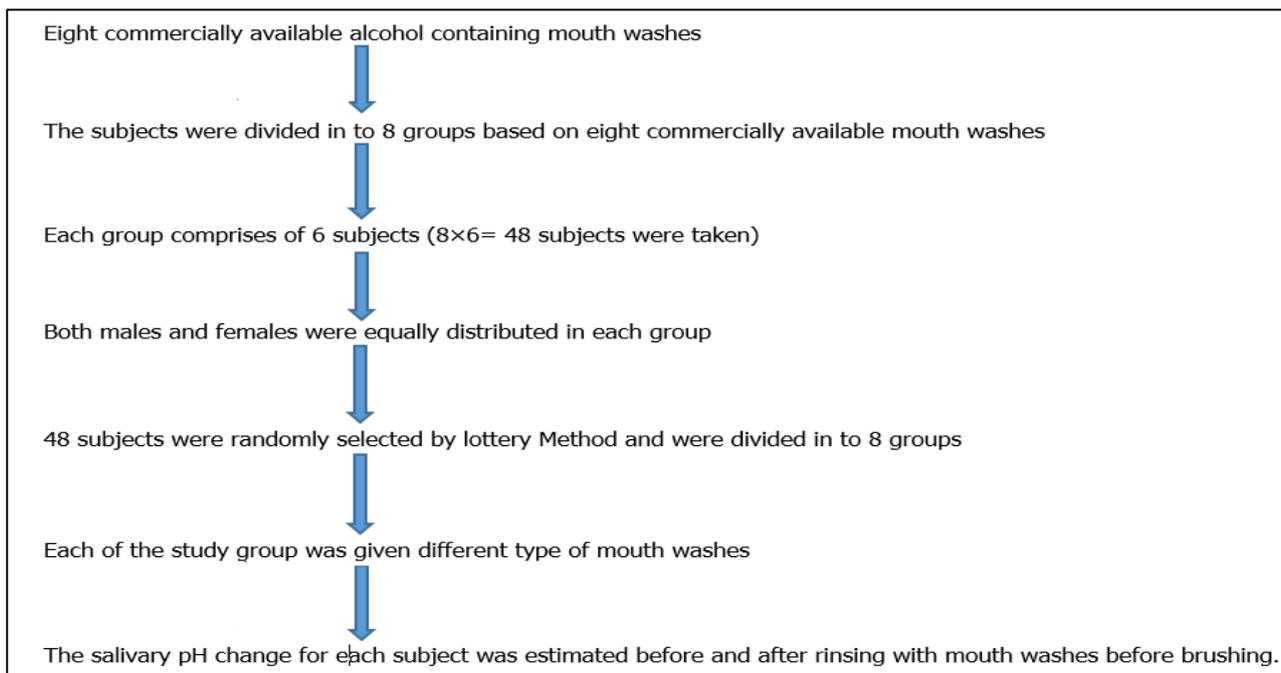
d = difference.

d = before = 7.06.

d = after = 6.81.

d = before - after = 7.06 - 6.81 = 0.25.

$= \frac{(1.96 + 0.84)^2 \times 2 \times 0.15 \times 0.15}{0.25 \times 0.25}$ $= \frac{0.3528}{0.0625}$ $= 5.6448 \sim 6$ <p>6 per group.</p>
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**Study Procedure-** Eight commercially available alcohol-containing mouthwashes were taken and the pH, total acid and the total ethanol content were estimated. The pH for each mouthwash was estimated using the digital pH meter.<sup>3</sup> The pH values were estimated for each of these 8 different types of mouthwashes. Take 25 mL of mouthwash in a sterile test tube. To measure the pH of mouthwash, pH sensitive electrode was dipped into the sterile test tube. The digital reading was allowed to stabilise for a few seconds and the pH reading was recorded. In between the readings, the electrode was cleaned with a stream of distilled water and placed in a standard solution of pH 7. This ensured stable readings and provided a constant check on any drift. Same procedure was repeated for all 8 types of mouthwashes to find out their pH.

The total acid in each mouthwash was determined via acid-base titration;<sup>3</sup> 10 mL of each sample is taken and to this, 50 mL of equal volumes of alcohol and solvent ether are added and has been neutralised. Then, 1 mL of phenolphthalein is added and heated gently on a water bath. Finally, titrate with 0.1 N KOH and shake consistently until a pink colour is obtained and the reading is noted at this point. The acid value is calculated by the following formula as follows-

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{W \text{ (ml)}}$$

a = 1N NaOH absorbed  
0.00561 = equivalent factors  
w = Weight in ml of the sample.

The total ethanol in each mouthwash was determined by preparing reagents like potassium bromate, potassium iodide and starch-indicated solutions.<sup>3</sup> A sample of 0.0025 mL of each mouthwash was taken and to this 5 mL distilled water is added and mixed with 25 mL of distilled water mixed with 5 mL concentration of HCl. To this, 20 mL of both potassium bromide and potassium iodide and starch indicator was added (1 to 2 drops). Finally, titrate with 0.1N sodium thiosulphate and add few drops of indicators and shake gently until blue colour is obtained and then readings are noted at this point. The ethanol content is calculated by the formula as follows-

$$\% \text{ of alcohol hydroxy group} = \frac{(x-y) \times N \times 17.01 \times 100}{w \times 100}$$

x = Volume of Na thiosulphate used for blank sample  
y = Volume of Na thiosulphate used for sample  
N = Normality of Na thiosulphate  
W = Weight of sample

The salivary pH for each subject was estimated before and after rinsing with mouthwashes before brushing, because there is significant effect of pH of the dentifrices on

the salivary pH. The unstimulated salivary samples were collected from the subjects in morning before rinsing and then the subjects were asked to rinse with 10 mL mouthwash for 60 seconds and salivary samples were collected immediately after rinsing in sterile test tube. The salivary pH was directly estimated using the digital pH meter calibrated using buffers of pH 4 and 7 for standardisation. To measure the salivary pH, pH sensitive electrode was dipped into the sterile test tube. The digital reading was allowed to stabilise for a few seconds and the pH reading was recorded. In between the readings, the electrode was cleaned with a stream of distilled water and placed in a standard solution of pH 7. This ensured stable readings and provided a constant check on any drift. Same procedure was carried out for each subject to find out their salivary pH before and after rinsing with mouthwashes. The accuracy of pH meter was checked at regular intervals to ensure that readings were correct.

### Statistical Analysis

The collected data was entered in the Microsoft Excel Sheet and analysed using the SPSS Version 18 statistical package. The mean and the standard deviation calculated for the mouthwash pH, total acid and total ethanol using descriptive statistics. Paired t-test was used to compare the changes in the mean salivary pH after rinsing in each group. One way ANOVA and post-hoc Turkey's test was used to compare the changes in mean salivary pH after rinsing between different groups.

### RESULTS

Listerine original mouthwash was found to have lowest pH (3.84) followed by Listerine fresh burst (4.88) and Listerine cool mint mouthwash (4.92). Rexitin plus mouth showed highest pH (6.50) followed by Senquel AD mouthwash (6.43) and Clohex mouthwash (6.25). Listerine original mouthwash was found to have highest acid content (26.8%) followed by Listerine fresh burst (22.4) and Listerine cool mint mouthwash (22.4). Colgate plax peppermint mouthwash and Colgate plax fresh mint mouthwash was found to have lowest acid content (8%). High alcohol content was seen for Listerine fresh burst mouthwash (26%) followed by Listerine original mouthwash (21.6%) and Listerine cool mint mouthwash (21.6%). Colgate plax peppermint mouthwash and Colgate plax fresh mint mouthwash was found to have lowest alcohol content (7.2%) (Table 1).

Highest pH drop of 5.84 after rinsing was seen for Listerine fresh burst mouthwash with mean pH difference of 1.21 followed by Listerine original mouthwash with pH drop 6.23 and mean pH difference of 1.17 and Listerine cool mint mouthwash with pH drop 6.05 and mean pH difference of 1.03. Lowest pH drop of 7.68 after rinsing was seen for Senquel AD mouthwash with mean pH difference of 0.26 followed by Rexitin plus mouth with pH drop 7.29 and mean pH difference of 0.28. Mean salivary pH before and after rinsing showed a significant difference among all the groups (Table 2).

The change in mean salivary pH between study groups on post-hoc Turkey's analysis showed a significant difference between Listerine original, Listerine fresh burst and Listerine cool mint mouthwash with other mouthwashes

indicating that Listerine group of mouthwashes showed low pH, high acid and alcohol content (Table 3).

Mouthwashes	pH	Acid Content	Alcohol Content
Listerine original mouthwash	3.84	26.8%	21.6%
Listerine fresh burst mouthwash	4.88	22.4%	26%
Listerine cool mint mouthwash	4.92	22.4%	21.6%
Colgate plax peppermint mouthwash	6.05	8%	7.2%
Senquel AD mouthwash	6.43	8.3%	7.5%
Colgate plax fresh mint mouthwash	5.84	8%	7.2%
Clohex mouthwash	6.25	13.8%	13%
Rexidin mouthwash	6.50	12.4%	11.6%

**Table 1. pH, Total Acid and Ethanol Content of Different Commercially Available Alcohol-Containing Mouthwashes**

Mouthwash	n	Mean pH of Saliva Before Rinsing	Mean pH of Saliva After Rinsing	Mean Difference in pH	t-value	p-value
Listerine original mouthwash	6	7.02	5.84	1.17	40.9	0.0277*
Listerine fresh burst mouthwash	6	7.43	6.23	1.21	41.9	0.002*
Listerine cool mint mouthwash	6	7.08	6.05	1.03	37.4	0.0277*
Colgate plax peppermint mouthwash	6	7.32	7.78	0.46	13.3	0.008*
Senquel AD mouthwash	6	7.44	7.68	0.26	4.87	0.0277*
Colgate plax fresh mint mouthwash	6	7.98	7.58	0.54	10.12	0.0277*
Clohex mouthwash	6	6.85	7.38	0.52	10.62	0.0277*
Rexidin mouthwash	6	7.29	7.59	0.28	4.34	0.0464*

**Table 2. Comparison of Mean Salivary pH Within the Study Groups Before and After Rinsing (Paired t-Test)**

Mouthwashes	Listerine Original Mouthwash	Listerine Fresh Burst Mouthwash	Listerine Cool Mint Mouthwash	Colgate Plax Peppermint Mouthwash	Senquel AD Mouthwash	Colgate Plax Fresh Mint Mouthwash	Clohex Mouthwash	Rexidin Plus Mouthwash
Listerine original mouthwash	-	0.7488	0.4233	0.0040*	0.0040*	0.0040*	0.0040*	0.0040*
Listerine fresh burst mouthwash	-	-	0.5228	0.0040*	0.0040*	0.0040*	0.0040*	0.0040*
Listerine cool mint mouthwash	-	-	-	0.0040*	0.0040*	0.0040*	0.0040*	0.0040*
Colgate plax peppermint mouthwash	-	-	-	-	0.3367	0.5752	0.6320	0.3367
Senquel AD mouthwash	-	-	-	-	-	0.2496	0.6320	0.6320
Colgate plax fresh mint mouthwash	-	-	-	-	-	-	1.0000	0.2496
Clohex mouthwash	-	-	-	-	-	-	-	0.6320
Rexidin plus mouthwash	-	-	-	-	-	-	-	-

**Table 3. Comparison of Change in Mean Salivary pH (Before and After Rinsing) Between Study Groups (Post-Hoc Turkey's Test)**

**DISCUSSION**

Mouthwashes have been used for centuries for medicinal and cosmetic purposes, but it is only in recent years that the rationale for use of the active ingredients of these products has been subject to scientific research and clinical trials.<sup>5</sup> Based on studies published in the Brazilian<sup>6,7</sup> and International<sup>8,9,10,11</sup> dental literature, the present study was conducted to estimate the pH, total acid and ethanol content

of different commercially available alcohol-containing mouthwashes and to evaluate the effect of these mouthwashes on salivary pH after rinsing. The salivary pH is an important biomarker for dental caries.<sup>4</sup> The mouthwashes varying in their chemical composition, pH range might influence the salivary pH.

The present study found that the pH of different commercially available alcohol-containing mouthwashes

used in the study were in the range of 3.84-6.50, highest pH being reported for Rexidin plus and lowest being reported for Listerine original, respectively. Although, a pH value equal to or less than 5.5 is considered critical for enamel dissolution, mineral loss may begin even at higher pH 6. Therefore, the prolonged use of oral rinses with pH below this value maybe potentially harmful to dental tissues. In the present study, three mouthwashes were classified as potentially erosive (pH <5.5), corroborating the findings of previous investigations.<sup>8,12,6,7,9,11</sup> Previous studies have demonstrated convincingly that such acidic conditions in solution can lead to significant enamel loss within the first few minutes of contact.<sup>13</sup> Such a timeframe is within the duration of most mouthwash regimens.

The low pH of oral care products increases the chemical stability of some fluoride compounds and favours the incorporation of fluoride ions into the lattice of hydroxyapatite and the precipitation of calcium fluoride onto the tooth surface.<sup>14</sup> Based on this statement, product labels were examined to identify mouthwashes containing fluoride. Low pH may make these products harmful to dental tissues if not used carefully. Although, mouth rinses have been formulated as pre- and post-brushing products for routine use, findings of a previous in situ study evaluating the erosive effects of some mouth rinses on enamel have suggested that low pH mouth rinses should not be considered for long-term or continuous use and never as pre-brushing rinses.<sup>6</sup> However, it must be emphasised that erosive potential of a substance cannot be attributed exclusively to pH.<sup>7</sup>

Substances with low titratable acidity are readily neutralised by oral fluids, while those with high titratable acidity cause a prolonged drop in pH and greater demineralisation of dental tissues.<sup>15</sup> In the present study, three of the mouthwashes exhibited acid content of greater than 20%. Among the tested mouthwashes, Listerine original mouthwash presented the highest acidity of 26.8%. Lack of similar studies evaluating of oral rinse products hinders comparison of the present results to data published in the literature. Acidity contributes to the development of dental caries. The source of this acidity is often attributed to sugar fermentation by endogenous bacteria in the mouth or to acidic foods and drinks, which lower salivary pH.<sup>3</sup> Studies have demonstrated that significant loss of enamel can occur within the first few minutes of contact with such acidic solutions.<sup>13,14,17</sup>

The present study also estimated total ethanol content of different commercially available alcohol-containing mouthwashes. Ethanol content was 7.2-26%, highest ethanol being reported for Listerine fresh burst and lowest being reported for Colgate plax peppermint and Colgate plax fresh mint respectively corroborating the findings of previous investigation.<sup>18</sup> Alcohol is an irritant to epithelium and in animal studies; the topically applied alcohol was linked with increased occurrence of tumours.<sup>19</sup> Inflammatory changes may also develop that may induce hyperkeratosis and atrophy of mucosa. In a study that involved 40 hamsters, two hamsters showed areas of hyperparakeratinisation, loss

of cellular cohesion in basal cell layers with mononuclear inflammatory cells when treated with 23% alcohol-based mouthwash.<sup>20</sup>

Although, not therapeutically active, ethanol in the mouthwash has been proven to produce multiple other effects many of which are not beneficial and unnecessary for the user. These range from a characteristic burning sensation upon contact with oral mucosa by activation of vanilloid receptor-1; a heat gated ion channel to a dehydrating effect on the oral mucosa.<sup>21,22</sup> Due to the astringent action of ethanol, the use of high alcohol mouthwashes in patients with radiation mucositis is not recommended.<sup>23</sup> Patients with Sjogren's syndrome should avoid alcohol-based mouthwash as it may aggravate xerostomia and with decreased salivary flow locally, atrophic changes of oral epithelium may occur. Similarly, people on alcohol withdrawal therapy or patients on disulfiram therapy should also avoid using such formulations. American Dental Association advises patients of burning mouth syndrome to avoid irritating substances such as mouthwashes.<sup>24</sup>

Studies and case reports link such a high percentage of alcohol in certain mouthwashes with development of leukoplakia. The lesion was irreversible when the mouthwash was stopped.<sup>25</sup> There has also been concern about the correlation of high level of alcohol in the mouthwash with increased susceptibility towards oral cancer.<sup>26</sup> Although to date, no scientific data establishes it as an aetiological factor, but few cases have been documented in the literature in which alcohol-containing mouthwashes are suspected, especially of a patient who suffered from multiple recurrent oral cancers. The patient gave a history of using 14% alcohol-based mouthwash. However, it must be stated that other local and systemic factors can aggravate the susceptibility towards oral cancer. Mouthwashes may also cause dental caries largely due to the inclusion of ethanol as an antiseptic agent, which can be oxidised to acetic acid.<sup>1</sup>

The present study also attempted to find the effect of these mouth rinses on the salivary pH of the study subjects after rinsing. Salivary pH is the important salivary parameter affecting the carious process. Demineralisation and remineralisation processes of the teeth occurring in the oral cavity are dependent on the pH of the saliva.<sup>4</sup> Our study found that there was a significant reduction in salivary pH between Listerine original, Listerine fresh burst and Listerine cool mint mouthwash compared to other alcohol-containing mouthwashes indicating that Listerine group of mouthwashes showed low pH, high acid and alcohol content after rinsing with low pH, high acid and high alcohol-containing mouthwashes.

## CONCLUSION

Listerine group of mouthwashes showed low pH, high acid and alcohol content. There was a significant reduction in salivary pH following rinsing with Listerine original, Listerine fresh burst and Listerine cool mint mouthwash when compared to other alcohol-containing mouthwashes.

**Recommendations**

- Addition of chemicals that keep mouthwashes above pH 6. This is necessary because it has been demonstrated that pH less than 5.5 at the surface of the tooth leads to a net loss of enamel and mineral structure on the tooth's surface. This can increase the incidence of dental caries. Such dental caries have previously been shown to be mainly caused by the frequency of acid exposure. Some chemicals that increase pH of the mouthwashes are the strong base-producing salts- Ca<sup>2+</sup>/Na<sup>+</sup>/K<sup>+</sup>-containing salts, etc.
- The proper concentration of antioxidants for the ethanol-containing mouthwashes should be used to prevent and reduce the oxidation of the alcohol to acetic acid.
- Chronic use of alcohol should be discouraged because it can increase the incidence of cancer of the mouth.
- The establishment of proper dental care, education to protect consumers from dental caries and promotes dental health.
- We recommend that mouthwash manufacturers routinely check the change of pH of the mouthwashes and use preservatives that maintain desirable pH homeostasis in the products.

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