



## Effects of chlorophenol / hydrogen peroxide versus xylitol or chlorhexidine as chewing gum on salivary flow rate, pH, buffer capacity and salivary *Streptococcus mutans* scores

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### Abstract

**Objectives:** A medicated chewing gum is a solid, single-dose preparation intended to be chewed for a certain period of time and deliver the drug. It may contain one or more than one active pharmaceutical ingredient. Within this context, we formulated a medicated gum with three active pharmaceutical ingredients: Camphor, p-chlorophenol and hydrogen peroxide, to be evaluated as therapeutic agents in dental caries. The aim of this study was to compare the effects on salivary streptococcus mutans, pH, buffer capacity and secretion rate of three medicated gums containing chlorophenol/hydrogen peroxide, xylitol or chlorhexidine.

**Methods:** Double-blind, randomized crossover treatment in 24 subjects. The patients were randomized and subjected to six different treatment sequences. The subjects used, 1 gum tablet three times a day for 20 minutes, morning, noon and night. At the beginning and end of the three experimental phases saliva samples were obtained for determining the pH, buffer capacity, salivary flow rate and quantitative enumeration of *S. mutans*.

**Results:** The use of medicated gum with camphor/p-chlorophenol and hydrogen peroxide did not modify the salivary chemical parameters measured, and not significantly reduced the number of *S. mutans*, after 7 days. Chewing gums with chlorhexidine significantly reduced the quantitative counting of *S. mutans* salivary and flow rate in a period of 7 days.

**Conclusions:** The use of medicated chewing gums based on camphor/p-chlorophenol and hydrogen peroxide does not alter the chemical salivary parameters and does not significantly reduce the number of *S. mutans*, after a use over a period of 7 days.

**Key words:** Medicated Chewing Gum, Camphor, p-Chlorophenol, Hydrogen Peroxide, Saliva, chlorhexidine, xylitol.

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### Efeito de gomas de mascar contendo clorofenol / peróxido de hidrogênio, xilitol ou clorexidina no fluxo salivar, pH, capacidade tampão e escores salivares de *Streptococcus mutans*

#### Resumo

**Objetivos:** Gomas de mascar medicadas são preparações sólidas, de dose única, que devem ser mastigadas por um determinado período de tempo a fim de que um ou mais agentes farmacológicos sejam administrados. Neste contexto, gomas de mascar medicadas com três ingredientes farmacêuticos ativos – cânfora, p-clorofenol e peróxido de hidrogênio – foram avaliadas como agentes terapêuticos para cárie dentária. O objetivo deste estudo foi comparar o efeito de gomas de mascar contendo chlorophenol / peróxido de hidrogênio, xilitol ou clorexidina sobre *Streptococcus mutans* salivares, pH, capacidade tampão, a taxa de secreção salivar.

**Métodos:** Foi realizado estudo duplo-cego, com delimitação de randomização cruzada de tratamento em 24 pacientes. Esses foram submetidos a seis seqüências diferentes de tratamento. Gomas de mascar foram administradas três vezes ao dia durante 20 minutos, pela manhã, tarde e noite. No início e no final das três fases de amostras de saliva experimentais foram obtidas para a determinação de pH, capacidade tampão, fluxo salivar e enumeração quantitativa de *S. mutans*.

**Resultados:** O uso de goma medicado de cânfora com p-clorofenol e peróxido de hidrogênio não modificaram os parâmetros químicos salivares medidos, e reduziram o número de *S. mutans*, após 7 dias. Gomas de mascar contendo clorexidina reduziram significativamente a contagem quantitativa de *S. mutans* e a taxa de fluxo salivar em um período de 7 dias.

**Conclusões:** O uso de gomas de mascar medicadas com base de cânfora/p-clorofenol ou com peróxido de hidrogênio não altera significativamente os parâmetros químicos salivares e não reduz significativamente o número de *S. mutans* após a utilização por período de 7 dias.

**Palavras-chave:** Goma de mascar medicada, cânfora, p-clorofenol, peróxido de hidrogênio, saliva, clorexidina, xilitol.

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## Introduction

Currently, oral health problems constitute pathologies of high prevalence in almost all age groups, and they affect importantly people's life quality. Dental caries are the most frequently presented in our population. This is one of the most common bacterial etiology diseases among human beings considered as a public health problem in many places in the world. Several studies have been devoted to investigate about dental caries prevention, with special emphasis on the measures which control dental bacterial plaque formation, thus reducing the presence of the pathogenic agent. That is how antimicrobial and natural sweeteners, including chlorhexidine and xylitol, have been used against *Streptococcus mutans* to reduce plaque-mediated diseases [1], because it has been implicated as the main etiological agent of dental caries [2].

Like chlorhexidine (CHX), camphorated parachlorophenol (CMPC) and hydrogen peroxide (HP) are chemical inhibitors of bacterial plaque [3]. Parachlorophenol is one of the most used phenolic compounds whose antibacterial and antiseptic power is evident, reason why it has been used during many years in dentistry, specifically in Endodontics [4]. Hydrogen peroxide (HP) is a weak acid, used as general antiseptic and disinfectant at 3%. It possesses a bactericidal effect, and an *in vitro* study has demonstrated that it prevents the bacterial growing of *Streptococcus mutans* [5].

The use of chewing gums is, nowadays, wide-spread among population. Their benefits for oral health are well-known, because it is an effective physical and mechanical method for food rests removal and teeth cleaning, but only secondary to toothbrushing. Chew gums also increments the salivary flow through a combination of mechanical and gustatory stimulation; it increments the plaque, and saliva pH; and, what is more important, it can serve as a means of drug delivery and release, such as chlorhexidine and antiseptics [6]. Chewing gum as a delivery system for various topical dental prophylactic and therapeutic agents has been repeatedly studied, and a few dental chewing gum products are registered and marketed in various countries. Thus, there are gums containing fluoride, enzymes, mineral salts, metal salts, xylitol, carbamide and CHX diacetate [16].

In the literature, no studies have described chewing containing camphorated p-chlorophenol and hydrogen peroxide but four mouthwashes research in the same composition as the gum was published in the IADR-Chile in 2006 [7] 2007 [7] and 2008 [9] and another publication of 2010 [3]. The first and fourth study indicated that the mouthwash reduces plaque formation and found no statistically significant differences with chlorhexidine. In the second and third study the effect of mouthwash on salivary parameters and antibacterial activity was measured.

Medicated chewing gums are used in various therapeutic purposes as treatment of dental caries. Within this context, this study has established the following objectives: to compare the effects of three medicated gum chlorophenol/

hydrogen peroxide, xylitol and chlorhexidine on salivary *mutans streptococci* counts, pH, buffer capacity and secretion rate of saliva.

## Methods

### Ethical Aspects

This study was approved by the Ethics Committee of the Dental School of University of Chile, and all participants signed an informed consent before initiating their participation in it.

### Study Population

The sample consisted of a total of 24 subjects (7 males and 17 females) whose age fluctuated from 22 to 40 years old. They were randomly distributed to be treated in a cross way with three experimental medicated chewing gums. Inclusion criteria were healthy volunteers from both sexes, older than 18 years old and younger than 60 years old. Exclusion criteria were evident signs of caries or periodontitis, smokers, patients under removable prosthesis and/or orthodontics treatment, and patients under antibiotic, antiseptic or other medication treatment of those known as capable of interfering with salivation.

### Study Design and Clinical Procedure

Crossover, double-blinded study, with random distribution. All participants received prophylaxis, carried out with a smooth brush, fine pumice stone, and water. The subjects were carefully instructed in the sense that it was forbidden, all along the research, to use any other oral hygienic product different than those handed by the study (toothbrush and toothpaste). Likewise, it was remembered that the existence of concomitant medication was banned. During the experimental phases, the subjects used one kind of chewing gum 3 times a day, during 20 minutes: in the morning (after breakfast), at midday (after lunch), and by night (after dinner). Between each experimental phase, the subjects entered into a "resting" stage of two weeks, which included another initial prophylaxis, and maintenance of the oral personal hygiene.

### Chewing Gum Composition

Each tablet of all chewing gum weighted 0.8 g.

Chewing gum with camphor, p-chlorophenol, and hydrogen peroxide (HP) was comprised of Camphor (1.6 mg), p-chlorophenol (0.8 mg), and hydrogen peroxide (3.2 mg), and excipients q.s.p. (0.8 g).

Chewing gum with xylitol was formulated with Xylitol (286 mg), sorbitol (141 mg), and excipient q.s.p (0.8 g).

Chewing gum with chlorhexidine contained Chlorhexidine acetate (5.33 mg per 0.8 g of chewing gum).

### Clinical Assessment

Volunteers were handed solid paraffin and two sterilized tubes. They were asked to chew the solid paraffin during one minute and thirty seconds, and, then, to pour the

collected saliva into the sterilized tube. Quantitative count of *Streptococcus mutans* was made in this tube.

### Sialometrical Analysis

Patients were indicated to chew the paraffin for 5 minutes, and to pour it into another tube. This sample was used to determine the salivary flow rate, pH, and the buffer capacity.

### Salivary Flow Rate

To calculate the salivary flow rate (SFR), we applied the following formula [10]: the tube weight with saliva less the empty tube, divided by the collection time, divided by 1,005 (saliva specific weight) (g/ml). Results were expressed in ml/minute.

### Sialochemical Analysis

Saliva samples were centrifuged (5000 rpm per 10 minutes) to remove bacteria and other extraneous material. The resulting clarified fluid was used for the biochemical assays to measure salivary pH and Buffer Capacity.

**pH Determination.** We calibrated the pHmeter between pH 4 and 7. Then, we measured the pH directly in the test tube. Finally, we measured again and expressed the results in pH units [11].

### Buffer Capacity

We took 1 ml saliva aliquot, and added it to 3 ml of 0.005 M HCl. We added 2-octanol 1 drop. We shook it for 20 minutes, and we measured the final pH in a previously calibrated pHmeter. After, we repeated the procedure. The averaged results were then expressed in pH units [11].

### Microbiological Processing

Saliva samples were homogenized in a vortex mixer (maximir type) for 45 seconds. Subsequently, they were diluted in series of 10, 100, and 1000 times ( $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ ), respectively, in phosphate-buffered saline (PBS) under a laminar flow hood. From each dilution, 100  $\mu$ l was deposited in Petri plates with TYCSB selective agar (trypticase, L-casitone, yeast extract, sodium sulphite, sodium chloride, disodium phosphate, sodium acetate, sucrose, agar, and bacitracin). Those plates were placed in jars with microaerophilic atmosphere, which were incubated for 48 hours at 36° in the heat cabinet. After it, *Streptococcus mutans* count was done using the stereoscopic magnifying glass, expressing the result in number of colony forming units by millimeter of saliva (CFU/ml) [12,13]. The whole procedure was carried out under a laminar air flow hood in order to avoid the sample contamination. *Streptococcus mutans* colonies developed during 48 hours in Petri plates were counted using a Spencer magnifying glass with 10 $\times$  magnification, only considering those adhesive, grayish-white, with a rough surface, resembling a frosted glass, and hard consistence, which cannot be divided when manipulated with a platinum handle. The total quantity of colonies presented in the Petri plate was obtained using

the dilution coefficient. They were called Colony Forming Units per ml of saliva (CFU/ml). The colonies were counted one by one, and the final number was multiplied by 10,000 (dilution prior to planting) [12,13].

### Place of Study

The procedures to collect saliva samples for the microbiological and chemical part were done at the Microbiology and Chemistry Laboratory of the Dental School of University of Chile. Oral cavity exploration was carried out at the diagnosis clinic on the first floor of the Dental School of the same University.

### Statistical Analysis

The Statistical Package for Social Science (SPSS INC Chicago link), version 17 was used for analysis. Difference in proportions was tested using Kruskal-Wallis ANOVA followed by Mann-Whitney U test for intra group comparison, and difference in means was tested using ANOVA followed by Tukey's post hoc and Independent sample *t*-test if necessary. Level of statistical significance was assumed at  $p < 0.05$ .

**Apparition of Adverse Reactions to Medication (ARM):** The patients of this study filled a reception card of possible adverse events at the end of each experimental phase.

## Results

### Sialochemical Analysis Results

#### a) Salivary pH Determination

There was a slight increase in the salivary pH after 7 days of use in treatments for chewing gums 1 (camphor/p-chlorophenol/hydrogen peroxide) and 2 (xylitol), as we can observe in Table 1. A very slight pH decrease was observed in chewing gums with chlorhexidine (chewing gum 3).

#### b) Buffer Capacity Determination

No statistically significant differences in the buffering capacity before and after use for 7 days for any of medicated chewing gum used were found ( $p > 0.05$ ) (Table 1). For the three used gum (chewing gum 1, 2, 3) it shows a decrease in the buffering capacity.

#### c) Salivary Flow Rate Determination

The average of the salivary flow rate expressed in ml/min for each treatment, obtained before and after 7 days of use, can be appreciated in Table 1. A slight increase in the salivary flow rate is observed in chewing gums with camphor, p-chlorophenol, and hydrogen peroxide (chewing gum 1), and in chewing gums with xylitol (chewing gum 2). On the contrary, a decrease is observed in the average flow in patients that goes from 2.38 to 2.11 ml/min in chewing gums with chlorhexidine (chewing gum 3). Statistically significant differences in the salivary flow rates before and after use for 7 days for medicated chewing gum 3 used were found ( $p < 0.05$ )

**Table 1.** Effect of chewing gum (chlorophenol/hydrogen peroxide, xylitol, chlorhexidine) on salivary flow rate, pH and buffer capacity salivary.

Gums	Parameters	Before	After 7 days	p-value			
Chewing Gum 1	pH	Media	7.81	7.88	0.44		
		SD	0.30	0.31			
		RD	1.32	1.15			
	BC	Media	5.99	5.60		0.16	
		SD	1.25	1.51			
		RD	4.0	4.9			
	SFR	Media	2.16	2.19			0.79
		SD	0.80	0.89			
		RD	3.15	2.90			
Chewing Gum 2	pH	Media	7.95	7.99	0.74		
		SD	0.43	0.36			
		RD	1.83	1.57			
	BC	Media	6.18	6.01		0.51	
		SD	1.68	1.68			
		RD	5.43	4.82			
	SFR	Media	2.36	2.38			0.90
		SD	0.93	1.07			
		RD	4.08	5.14			
Chewing Gum 3	pH	Media	7.92	7.88	0.38		
		SD	0.37	0.38			
		RD	1.44	1.54			
	BC	Media	6.10	5.60		0.10	
		SD	1.52	1.67			
		RD	4.86	4.65			
	SFR	Media	2.32	2.11			0.02*
		SD	0.94	0.87			
		RD	3.83	3.89			

Chewing Gum 1: Camphor, p-chlorophenol, hydrogen; Chewing Gum 2: Xylitol, sorbitol; Chewing Gum 3: Chlorhexidine acetate; BC: buffer capacity; SFR: salivary flow rate; SD: Standard Deviation; RD: Range of Data; \* Significant difference.

### Microbiological Analysis Results

Table 2 shows the average of the quantitative count for *Streptococcus mutans* expressed as logarithm (log) CFU/ml for each treatment before and after 7 days of use. Products with camphor, p-chlorophenol, and hydrogen peroxide (chewing gum 1), and with xylitol (chewing gum 2) presented a non significant decrease in *Streptococcus mutans* log CFU after using chewing gum. On the contrary, a significant decrease was observed from 4.48 a 3.75 log CFU/ml of saliva for the product with chlorhexidine (chewing gum 3).

### Statistical Analysis before and after Use of Chewing Gum for 7 Days

It is observed that the use of a chewing gum containing camphor/p-chlorophenol, and hydrogen peroxide (chewing gum 1) during 7 days did not produce significant statistical changes in any of the three chemical parameters analyzed, as

**Table 2.** Effect of chewing gum (chlorophenol/hydrogen peroxide, xylitol, chlorhexidine) on *Streptococcus mutans* salivary scores (log CFU/ml saliva).

Gums	Parameters	Before	After 7 day	p-value			
Chewing Gum 1	SMS	Media	5.12	4.91	0.11		
		SD	5.66	5.51			
		RD	6.34	6.19			
Chewing Gum 2	SMS	Media	4.96	4.63		0.17	
		SD	5.31	4.89			
		RD	5.90	5.46			
Chewing Gum 3	SMS	Media	4.48	3.75			0.024*
		SD	4.75	4.13			
		RD	5.38	4.73			

Chewin Gum 1: Camphor, p-chlorophenol, hydrogen; Chewing Gum 2: Xylitol, sorbitol; Chewing Gum 3: Chlorhexidine acetate; SMS: *Streptococcus mutans* scores (in logarithmic form); SD: Standard Deviation; RD: Range of Data; \* Significant difference.

it neither did in the *Streptococcus mutans* count. The use of chewing gums with xylitol (chewing gum 2) did not show significant statistical differences in any of the parameters studied.

Instead, in the case of the chewing gum with chlorhexidine (chewing gum 3), with respect to the *Streptococcus mutans* count, there was a significant statistical decrease in the number of CFU/ml of saliva after using the product during 7 days, where  $p < 0.02$ . The salivary pH did not show significant differences, but the buffer capacity suffered a significant statistical decrease after 7 days using the product, where  $p < 0.02$ .

### Statistical Analysis inter Chewing Gums

To compare statistically between the gums and their effect on the pH, buffering capacity and flow rate parameters, the difference between the initial and final value was calculated for each parameter and for each gum. pH delta, buffer capacity delta and flow rate delta were obtained for each gum. Then deltas were compared by Kruskal-Wallis test. No differences were found in pH ( $p = 0.174$ ), buffer capacity ( $p = 0.343$ ) and salivary flow ( $p = 0.089$ ) changes for the three chewing gum.

Similarly for counting bacteria *Streptococcus mutans*, we proceeded to calculate the difference between the initial and final count after application of chewing gum. The differences in counts were analyzed by the Kruskal-Wallis test and there were no statistical differences between the chewing gum.

### Adverse Events

The most common adverse events found after using these three chewing gums corresponded to dental stains, which were easily removed with oral prophylaxis; neck and head muscles pain or problems; and, TMJ sounds and/or pain. Only one person (4.3%) experimented dental stains with chewing gums with camphor, p-chlorophenol, and hydrogen peroxide (chewing gum 1); two persons (8.7%) told about problems and/or pain mainly when palpating the area of temporal and

masseter muscles; and, two persons (8.7%) presented TMJ sounds or pains. In the case of chewing gums with xylitol (chewing gum 2), there were no dental stains. A higher number of neck and head muscles pain or problems were shown in five persons, which corresponds to a 21.7%. And, four persons (17.4%) presented TMJ sounds or pain. The highest number of people with dental stains (3 persons corresponding to a 12.5%) corresponded to the chewing gums with chlorhexidine (chewing gum 3). Four persons (16.6%) presented neck and shoulder muscles pain or problems. And, two persons (8.3%) presented TMJ sounds and/or pain.

## Discussion

As it is known thanks to many studies, as that of Dawes [14] in 2005, gum chewing increases the salivary flow rate. Flow rate is considerably increased while chewing, reaching its peak in 2 minutes and then decreases progressively in time, but even after 90 minutes of chewing SFR stimulated by the chewing gum, it is significant higher than the initial or unstimulated saliva [14]. In this study we wanted to verify such increase in the salivary flow rate during gum chewing, but in a longer term. It was intended to know if after a week of daily use the flow rate kept increasing. Chewing gums 1 and 2 (camphor, p-chlorophenol, and hydrogen peroxide; and, xylitol, respectively) did not, since flow rate was kept as the initial one. This coincides with the studies carried out by Dawes [14,15], where it was proved that the SFR of unstimulated saliva 15 minutes after gum chewing for a period of 90 minutes was kept as its initial one without a chewing gum. This lead to the conclusion that an extended period of gum chewing does not tire out the salivary glands, and that the flow of unstimulated saliva does not decrease after a long time chewing a gum [14,15]. On the contrary, a significant decrease of the salivary flow rate was produced in the case of chewing gum 3 with chlorhexidine, which does not coincide with Dawes' report [14]. Perhaps, this is due to a "fatigue" of the salivary glands when being stimulated 20 minutes, 3 times a day, during 7 days. These observations have not been informed in literature, reason why more research is required. Still, all results obtained are kept within the normal SFR values, corresponding to 1-2 ml/min. This study makes clear that the increase of the SFR when consuming chewing gums is only produced at the moment of chewing, and this is not maintained over time in any of the three medications. With respect to salivary pH, we can affirm that 7 days after using chewing gums, it remained similar to its initial level in the three treatments, which also tells us that pH only increases during gum chewing, as it is stated in many reports [14, 15, 16, 17, 18], and that this effect is not maintained over time. We cannot talk about a salivary pH decrease either. Buffer capacity did not suffer significant statistical variations in any of the three treatments. Nevertheless, we can highlight that in the case of chewing gums with chlorhexidine (chewing gum 3) a bigger decrease of it was produced. This can be explained by the diminished salivary flow also presented,

producing also a decrease in bicarbonate concentration, the main buffer agent of saliva, and so explaining this slight decrease in buffer capacity. Another possible explanation is the acidity of chlorhexidine, which may react with the bicarbonate in acid-base reaction that would decrease concentration and affect their pH and also the buffering capacity of the saliva. This observation has not been informed in literature yet, reason why more research is required. As for the two prior parameters, we can state that the buffer or buffer capacity is increased only at the moment of gum chewing, as many studies show, effect which is not maintained over time. Regarding the microbiological analysis, the results obtained in this study show that chewing gums with chlorhexidine (chewing gum 3) are still the more efficient ones in diminishing the bacterial count of *Streptococcus mutans*, coinciding with what was reported by Imfeld [16] in 2006. The results for the other two chewing gums show a decrease in the quantitative count of *Streptococcus mutans*, but it is not statistically significant. Some explanations for these results could be: the release of medication in the saliva quickly disappears from the oral cavity due to an involuntary deglutition [19]. The medication concentration in the oral cavity always tend to decrease as a result of dilution with saliva, or releasing the medication gum is strongly influenced by the formulation of the gum and the way in which the patient chews the gum [19].

Another explanation for this can be the reduced number of days during which the patients were subjected to a daily use of chewing gums (only a week), since in most of the studies carried out with chewing gums with xylitol the time of use was longer (between 90 days and 40 months) [20, 21,22].

In analyzing the results salivary comparative parameters and the *mutans streptococci* count the activity of the three chewing gum, no significant differences were seen, although the difference between the initial count and *mutans streptococci* after 7 days was significant for gum chlorhexidine.

The statistical study inter gum, through assessed differences between before and after the use of the gum shows no difference for physicochemical and microbiological analysis.

The adverse events occurred after chewing these three chewing gums per 20 minutes, 3 times a day, during 7 days, can be explained due to the fact that both neck and head muscles and TMJ problems and/or pains were produced in a higher percentage in chewing gum 2 (with xylitol), reaching a 21.7%, and a 17.4%, respectively, which presents the harder consistency of the three chewing gums. In addition, patients told that these problems for chewing gums 1, 2, and 3 presented when chewing the gums for a time longer than the 20 minutes agreed for the study. There is no documentation regarding the maximum chewing time in which the muscles and articulations problems and alterations start to be produced, which also deserves more research. Dental stains, that occurred in three patients, are highly associated to the use of chewing gums with chlorhexidine (chewing gum 3), corresponding to a 12.5%. This occurrence



of pigmentations corresponds to one of the main disadvantages of chlorhexidine [16]. It is worth noting that these stains were easily removed with prophylaxis with a soft brush, and a fine pumice stone. This finding does not coincide with what was reported by Imfeld [16] in 2006, where it was stated that chewing gums with chlorhexidine would produce less stains than using a mouthwash. It is outstanding that in this study there was no report about adverse events, such as skin and mucus irritation, or any kind of injuries or taste sensitivity loss in any of the three chewing gums studied.

## Conclusions

Medicated gums based on camphor, p-chlorophenol and hydrogen peroxide for 7 days did not significantly modify the pH, buffer capacity and the flow rate. The number of *Streptococcus mutans* does not decrease significantly. By comparing the chewing gums activity between the three salivary chemical parameters on salivary bacterial counts, there were no significant differences.

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