

Effectiveness of an Oral Moisturizer with Malic Acid/Xylitol as Anti-caries Therapy in Children

Efectividad de un hidratante bucal con ácido málico/xilitol como terapia anticaries en niños

Received: October 20, 2021 | Accepted: January 27, 2022

ANTONIO ARMANDO AGUIRRE-AGUILAR^a

Adjunct Professor, School of Stomatology, Universidad Nacional de Trujillo, Perú
ORCID: <https://orcid.org/0000-0002-4785-2660>

EDITH ESTHER DELGADO-ASMAT

Second specialty graduate, School of Stomatology, Universidad Nacional de Trujillo, Perú

ORCID: <https://orcid.org/0000-0002-0554-3368>

TERESA ETELVINA RÍOS-CARO

Adjunct Professor, School of Stomatology, Universidad Nacional de Trujillo, Perú

ORCID: <https://orcid.org/0000-0002-2069-8675>

AUGUSTO ALBERTO AGUIRRE-AGUILAR

Adjunct Professor, School of Stomatology, Universidad Nacional de Trujillo, Perú

ORCID: <https://orcid.org/0000-0003-4638-433X>

FRANZ TITO CORONEL-ZUBIATE

Second specialty graduate, School of Stomatology, Universidad Nacional de Trujillo, Perú

ORCID: <https://orcid.org/0000-0003-4747-947X>

^a Corresponding Author: aaguirrea@unitru.edu.pe

How to cite: Aguirre-Aguilar AA, Delgado-Asmat EE, Ríos-Caro TE, Aguirre-Aguilar AA, Coronel-Zubiata FT. Effectiveness of an oral moisturizer with malic acid/xylitol as anti-caries therapy in children. Univ. Med. 2022;63(2). <https://doi.org/10.11144/Javeriana.umed63-2.bucal>

ABSTRACT

Objective: To determine the effectiveness of an oral moisturizer containing malic acid/xylitol as anticaries therapy in 5-year-old children free of caries and at different levels of the Simplified Oral Hygiene Index (OHI-s). **Materials and methods:** Randomized uncontrolled clinical trial in 96 salivary samples from children divided into two groups of 48 children with acceptable (n = 16), deficient (n = 16) and adequate (n = 16) s-OHI, with one and two applications of the moisturizer per group per day, for 21 days. The effectiveness was evaluated according to the salivary profile (salivary volume, salivary flow, *Streptococcus mutans* count, salivary pH, buffer capacity and fluoride level). **Results:** With an application at the appropriate level of OHI-s there was a positive change in salivary volume (7.5 ml) and pH (7.47), at the acceptable level its effectiveness was manifested in the pH (7.51), and buffer capacity (7.22), and at the deficient level in buffer capacity (6.23). With two applications, it is efficient in all the components of the salivary profile, except for the level of fluoride and the count of *Streptococcus mutans* for the 3 levels of OHI-s. **Conclusions:** Two daily applications of Xeros Dentaïd® Spray have a positive effect on salivary profile values and can be used as anti-caries therapy.

Keywords

saliva; oral hygiene index; effectiveness; dental caries; wetting agents.

RESUMEN

Objetivo: Conocer la efectividad de un hidratante oral que contiene ácido málico/xilitol como terapia anticaries en niños de 5 años libres de caries y diferentes niveles de Índice de Higiene Oral simplificado (IHO-s). **Materiales y métodos:** Ensayo clínico no controlado aleatorizado en 96 muestras salivales de niños, distribuidos en dos grupos de 48 niños con IHO-s aceptable ($n = 16$), deficiente ($n = 16$) y adecuado ($n = 16$), con una y dos aplicaciones del hidratante por grupo al día, durante 21 días. La efectividad fue evaluada de acuerdo con el perfil salival (volumen salival, flujo salival, recuento de *Streptococcus mutans*, pH salival, capacidad amortiguadora y cantidad de flúor). **Resultados:** Con una aplicación en el nivel adecuado de IHO-s hubo un cambio positivo en el volumen (7,5 ml) y pH (7,47) salivales; en el nivel aceptable, su efectividad se manifestó en el pH (7,51) y capacidad amortiguadora (7,22), y en el nivel deficiente, en la capacidad amortiguadora (6,23). Con dos aplicaciones es eficiente en todos los componentes del perfil salival, excepto en nivel de flúor y recuento de *Streptococcus mutans* para los 3 niveles de IHO-s. **Conclusiones:** Dos aplicaciones diarias del spray Xeros Dentaïd® tienen un efecto positivo sobre los valores del perfil salival y puede utilizarse como terapia anticaries.

Palabras clave

saliva; índice de higiene oral; efectividad; caries dental; agentes mojanantes.

Introduction

Xeros Dentaïd® spray is a product that was created as an oral moisturizer for the treatment of xerostomia. It stimulates salivary production since it contains malic acid (1.00%), xylitol (10.00%) and sodium fluoride (0.05%). Its malic acid/xylitol content would provide the anticaries properties, having demonstrated in previous research its effectiveness in controlling the colony forming units (CFU) of *Streptococcus mutans*, the main microorganism responsible for carious processes (1-4), which leads us to evaluate its possible use as an alternative anticariogenic therapy.

Saliva is a mixed secretion resulting from the mixture of fluids from the major and minor salivary glands and crevicular fluid. It can have a very liquid or viscous consistency, depending on the gland that produces it (5-9). Ninety-nine percent is water and 1% is made up of dissolved solids, including electrolytes and proteins (5,10,11). Its main function is to lubricate the mucosa and

hard tissues, in addition to participating in biofilm formation and preventing desiccation, among other characteristics (12-15). It also facilitates the formation of the food bolus during mastication (14,16,17), since it mechanically drags microorganisms and transports them to the stomach, where they are destroyed by the action of gastric juice (17-19).

Saliva maintains oral pH, preserves tooth stability by cleaning carbohydrates, and regulates ion exchange, which favors remineralization (13). Given the importance of fluoride, we will mention that the optimal value of fluoride in saliva is 0.006 to 0.016 parts per million (ppm), which depends on whether it is in areas with or without fluoridation of drinking water (13). Salivary pH is usually neutral, with an average value of 6.7 when food is not present, but it decreases when food is ingested (19). After 2 to 5 minutes of rinsing with glucose or sucrose medium, the oral pH decreases and returns to its basal state about 40 minutes later, which is known as Stephan's curve (19,20). There is no specific value considered as critical pH, but values between 5.3 and 5.7 for enamel and 6.6 and 6.7 for dentin are acceptable (20). Another important aspect is the buffering capacity of saliva, since there is a close relationship between the acid-base regulation mechanism of saliva and the incidence of caries, due to its capacity to moderate the decrease in pH that results from bacterial action in the fermentation of carbohydrates (20-22).

Caries is one of the main diseases in children, to the point that it is five times more common than asthma (21). A number of factors have been proposed to play a role in the development of caries, so this disease is considered to be multifactorial. The main factors recognized are the agent (microorganism), the environment (substrate) and the susceptible host (tooth) (1-3).

There is great interest in the prevention of dental caries, and the National Institute of Health includes the following preventive actions to avoid its formation: oral hygiene advice, nutritional advice and counseling, use of fluoride products (such as fluoride varnish, fluoride

pastes and fluoridated water), application of chlorhexidine as an antimicrobial, pit and fissure sealants to prevent the development of dental caries. Several scientific studies have revealed that Xeros Dentaïd® spray activates the salivary glands and increases the flow of saliva in the mouth thanks to its content of malic acid and xylitol; while fluoride confers remineralizing properties to the enamel. Thus, it can be used as a conservative and preventive anticaries alternative. It is important to note that malic acid is a popular ingredient added to toothpastes and mouthwashes because of its antiseptic effect in reducing bacteria in the mouth, as does xylitol (4).

Several studies have been carried out to evaluate the salivary profile. Namoc (22) found that there is no relationship between the amount of bacterial plaque, salivary flow, salivary pH and salivary buffering capacity according to sex. Aguirre Aguilar and Vargas Armas (23) concluded that after chocolate consumption, salivary pH decreases proportionally to oral cleanliness. Cevallos Zumarán and Aguirre Aguilar (24) found that with chocolate consumption salivary pH decreases proportionally to the level of oral hygiene. Rodríguez-Alayo and Aguirre-Aguilar (25) determined that the effect of 1% xylitol toothpaste used in oral hygiene (without modification of brushing technique or brushing times) in 5-year-old children, after 30 days of use, reduced the population density of salivary *Streptococcus mutans* from 338,800 to 113,695 CFU and increased salivary fluoride concentration from 0.033 to 0.262 ppm, without producing significant variation in average salivary volume, flow, pH and buffering capacity values. Aguirre Aguilar and Rebaza Honores (26) established that the salivary profile does not differ between the different levels of dentobacterial plaque index in 5-year-old children free of caries.

In the third decade of the 21st century, it is essential for dentistry that people incorporate into their oral hygiene biocompatible products with active ingredients or substances that provide a change in the salivary profile and help prevent enamel demineralization, and that are also

effective in controlling bacterial colonization in the oral cavity. According to the last official report of the Ministry of Health in 2005, in Peru there is an average of 90% prevalence of dental caries in the school population aged 5.84 years.

According to updated reports from the Epidemiological Surveillance System of Oral Pathologies in Mexico, the ceo-d index (decayed, extracted or obturated) was found to be 2.4, 3.8, 4.3 and 4.7, respectively, in children 2, 3, 4 and 5 years of age. The Chilean Ministry of Health provides data on the prevalence of dental caries for 2007: the ceo-d index in 2-year-olds is 0.54; in 4-year-olds it is 2.32, and in 6-year-olds it is 3.71. In Paraguay, according to a National Oral Health Survey conducted in 2008, it was concluded that 98% of the population suffers from oral health problems, and that the prevalence and incidence in schoolchildren are very high, with a ceo-d index of 5.6 in 6-year-old children. In Brazil, there is a national oral health research program called SBBrazil that maintains an updated database for monitoring national oral health policies. According to SBBrazil, in 2010, 5-year-old children had a ceo-d index of 2.3 (27). With these arguments, there is a need to incorporate alternative therapies that are easy to apply, that are clinically useful for the treatment of dental caries, that do not have side effects, and that can be included in preventive programs.

Therefore, this study sought to determine the effectiveness of an oral cavity moisturizer containing malic acid/xylitol as an anticaries therapy in caries-free 5-year-old children with different levels of oral hygiene.

Material and methods

A non-randomized, prospective, longitudinal and comparative non-controlled clinical trial was carried out; the probabilistic sample consisted of 96 5-year-old children selected from the Rafael Narváez Cadenillas Experimental Educational School of Universidad Nacional de Trujillo (Peru).

The project was approved by the Standing Committee for Scientific Research of the School

of Stomatology of Universidad Nacional de Trujillo, and was authorized to work at the educational institution.

In a discussion with parents, they were informed about the likelihood of their children participating in the research. Once informed consent was obtained, all 5-year-old children were screened and caries-free children were selected by evaluating their Simplified Oral Hygiene Index (OHI-S) and divided into 2 groups: one with once-daily application and the other with twice-daily application.

In each group, children were selected according to oral hygiene levels: adequate, acceptable, deficient; the group was closed when 16 participants completed each level. After the selection, saliva samples were taken and collected in airtight beakers for laboratory analysis of the salivary profile prior to application of Xeros Dentaïd® spray.

These same children were regrouped into two groups: one group with one application of Xeros Dentaïd® spray per day (one hour before eating their lunch box and applied by the teacher or mother) and a second group with two applications of Xeros Dentaïd® spray per day (one hour before lunch box, applied by the teacher or mother, and one hour before dinner, applied by the mother). Xeros Dentaïd® was applied for 21 days and then a new saliva sample was taken and placed in a hermetically sealed collection container for subsequent transfer and analysis of the salivary profile of each group.

The non-stimulated saliva collection technique of Tomas Seif (28), which uses a saliva collection container and a spit funnel, was used to collect the sample. The child was kept seated and was instructed not to swallow or move while the test was being performed. He was also asked to hold the saliva in his mouth for 2 minutes without swallowing, and then spit it into the container. The procedure was repeated 4 more times for the same 2 minutes each, for a total of 10 minutes. The volume was measured in a calibrated container in milliliters.

To determine the salivary flow, the amount of saliva obtained in milliliters in the calibrated container was divided by 10. Thus the non-

stimulated salivary density per minute was obtained. The “isolation and quantification of *Streptococcus mutans* present in saliva” test was used by the surface method in CFU per milliliter (28), which consists of diluting the saliva at 1:10, 1:100 and 1:1000 with sterile isotonic saline. 100 μ L of each dilution was then seeded on TYS20B agar and incubated at 37 °C for 48 hours under anaerobic conditions.

The salivary pH of the samples was determined using the HANNA HI98128 potentiometer, according to the manufacturer’s specifications. The buffering capacity was determined using Erickson’s method (28), where once the non-stimulated saliva was collected, 1.0 ml was transferred to 3.0 ml of hydrochloric acid (0.0033 mol/L), adding a drop of 2-octanol to avoid foaming. It was then mixed for 20 minutes to remove carbon dioxide and evaluated with the pH meter. A potentiometric measurement of fluoride levels was also performed, from which information about the concentration of fluoride ion in saliva samples was obtained (28,29).

The data collected in the data sheets were processed with SPSS statistical software, version 24.0. The variation in each characteristic of the salivary profile before and after the application of Xeros Dentaïd® spray was evaluated using Student’s t-test to compare means in paired data. Significance was considered if $p < 0.05$.

Results

Ninety-six saliva samples from caries-free 5-year-old children were analyzed, divided into two groups: 48 with one daily application and 48 with two daily applications. Each sample group was analyzed in six dimensions: saliva volume, saliva flow, *Streptococcus mutans* CFU count, salivary pH, buffering capacity and salivary fluoride concentrations.

The results show the effectiveness of the oral moisturizer with an application at the adequate level of OHI-s only on salivary volume and pH; while at the acceptable level, its effectiveness is manifested on pH and buffering capacity, and at the deficient level, only for buffering capacity.

It was evident that two applications of the oral moisturizer are efficient in all components of the salivary profile, except fluoride concentration and *Streptococcus mutans* count, for almost all OHI-s levels (Table 1).

Table 1
Salivary profile according to number of applications, level of Oral Hygiene Index OHI-s and time of evaluation, in application of Xeros Dentaid® Spray in caries-free five-year-old children

Component of the salivary profile	One application			Two applications		
	Adequate	Acceptable	Deficient	Adequate	Acceptable	Deficient
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Salivary volume						
Before	6.90 ± 0.17	7.00 ± 0.00	7.00 ± 0.00	6.71 ± 0.49	6.91 ± 0.15	6.70 ± 0.45
After	7.50 ± 0.00	7.50 ± 0.00	7.67 ± 0.29	7.57 ± 0.19	7.64 ± 0.24	7.80 ± 0.28
Test Each Level	<i>t</i> = 6.00 <i>p</i> = 0.03	<i>t</i> = +	<i>t</i> = 4.00 <i>p</i> = 0.06	<i>t</i> = 4.77 <i>p</i> = 0.00	<i>t</i> = 8.42 <i>p</i> = 0.00	<i>t</i> = 4.49 <i>p</i> = 0.01
Salivary flow						
Before	0.68 ± 0.03	0.70 ± 0.00	0.70 ± 0.00	0.69 ± 0.02	0.69 ± 0.02	0.67 ± 0.04
After	0.75 ± 0.00	0.75 ± 0.00	0.77 ± 0.03	0.76 ± 0.02	0.76 ± 0.02	0.78 ± 0.03
Test Each Level	<i>t</i> = 4.00 <i>p</i> = 0.06	<i>t</i> = +	<i>t</i> = 4.00 <i>p</i> = 0.06	<i>t</i> = 7.07 <i>p</i> = 0.00	<i>t</i> = 7.78 <i>p</i> = 0.00	<i>t</i> = 4.49 <i>p</i> = 0.01
Streptococcus mutans CFUs						
Before	295 800 ± 54 170	180 692 ± 28 516	357 907 ± 156 298	329 973 ± 110 914	209 066 ± 56 935	268 688 ± 12 088
After	183 667 ± 83 919	154 025 ± 28 875	261 333 ± 199 084	320 943 ± 346 047	182 171 ± 61 709	185 200 ± 121 143
Test Each Level	<i>t</i> = 3.45 <i>p</i> = 0.075	<i>t</i> = 2.34 <i>p</i> = 0.052	<i>t</i> = 3.77 <i>p</i> = 0.064	<i>t</i> = 0.074 <i>p</i> = 0.942	<i>t</i> = 2.098 <i>p</i> = 0.081	<i>t</i> = 1.535 <i>p</i> = 0.199
Salivary pH						
Before	7.05 ± 0.24	7.01 ± 0.36	6.12 ± 0.10	7.46 ± 0.38	6.92 ± 0.48	6.19 ± 0.12
After	7.47 ± 0.31	7.51 ± 0.34	6.65 ± 0.38	7.94 ± 0.37	7.54 ± 0.50	6.83 ± 0.11
Test Each Level	<i>t</i> = 5.38 <i>p</i> = 0.03	<i>t</i> = 8.17 <i>p</i> = 0.00	<i>t</i> = 1.94 <i>p</i> = 0.19	<i>t</i> = 24.18 <i>p</i> = 0.00	<i>t</i> = 1.94 <i>p</i> = 0.19	<i>t</i> = 15.09 <i>p</i> = 0.00
Buffering capacity						
Before	6.69 ± 0.22	6.43 ± 0.36	5.55 ± 0.13	7.04 ± 0.40	6.42 ± 0.45	5.74 ± 0.16
After	7.22 ± 0.39	7.22 ± 0.42	6.23 ± 0.10	7.62 ± 0.34	7.06 ± 0.47	6.35 ± 0.24
Test Each Level	<i>t</i> = 3.65 <i>p</i> = 0.07	<i>t</i> = 10.15 <i>p</i> = 0.00	<i>t</i> = 5.08 <i>p</i> = 0.04	<i>t</i> = 13.69 <i>p</i> = 0.00	<i>t</i> = 15.06 <i>p</i> = 0.00	<i>t</i> = 10.39 <i>p</i> = 0.00
Fluoride concentration						
Before	0.97 ± 0.75	0.70 ± 0.58	0.73 ± 0.12	1.01 ± 1.00	0.40 ± 0.47	1.52 ± 1.09
After	0.30 ± 0.26	0.26 ± 0.16	0.37 ± 0.38	0.43 ± 0.23	0.21 ± 0.23	0.50 ± 0.26
Test Each Level	<i>t</i> = 1.14 <i>p</i> = 0.37	<i>t</i> = 2.38 <i>p</i> = 0.05	<i>t</i> = 1.98 <i>p</i> = 0.19	<i>t</i> = 1.63 <i>p</i> = 0.15	<i>t</i> = 0.84 <i>p</i> = 0.84	<i>t</i> = 2.11 <i>p</i> = 0.10

Discussion

Current dentistry has been conducting research on salivary function and its importance as part of oral health maintenance and as a mechanism for caries prevention therapy. Thus, caries control agents such as xylitol (25) have been incorporated in toothpastes, oral rinses, chewing gums, sprays, and tablets, among others.

Saliva has a protective function such as lubrication and an antimicrobial function, as well as assisting in the remineralization of teeth. Secretion is a process controlled by the nervous system, and the amounts of saliva secreted vary daily according to age, number of teeth, food intake, etc. The average salivary flow in a 5-year-old child with caries is 0.48 ml/min (30) and without caries is 0.62 ml/min (26). The present research showed that after treatment with two applications per day, the salivary flow was 0.77 ml/min (1.1 L per day), which is a value very close to those reported for caries-free children (8.9 L per day) (26).

For the study, the population density of *Streptococcus mutans* was evaluated, since this bacterium has been found to be the main cause of the onset of caries. In this study, for the group of two applications per day, the post-treatment average ended at 18×10^4 CFU/ml, a value that is close to that indicated by Boj (15), who determined that to consider that a person has a low cariogenic risk, their values should be less than 100,000 CFU/ml. Previous studies have determined that xylitol is effective in reducing *Streptococcus mutans* colonies from day 14, and that only after 3 months of treatment could they be completely eliminated from the oral cavity (25). So, although Table 1 shows that the decrease in *Streptococcus mutans* counts is not significant, it could be justified by the time of application and because all the children in the study were caries-free.

Salivary pH has an average neutral value of 6.7, varying in the range of 6.2 and 7.6 (11). The salivary pH reported in the study after treatment with two applications at the acceptable OHI-s level rose from 6.92 to 7.5. This shows a significant difference ($p = 0.00$) that would favor remineralization and resistance to acid attack in caries disease. When comparing salivary pH values at all OHI-s levels and in both application groups, a favorable increase is noted, which confirms its anticaries protective capacity, considered as a remineralization promoter (11,23).

The buffering capacity was evaluated to establish the effectiveness of the product in

salivary acid-base regulation, which is essential during food intake and chewing. This raised pH from 6.47 to 7.08, on average, in the group of two applications per day and at all 3 OHI-s levels, showing a positive significant difference in response to acid attack.

Fluoride is present in saliva in very low concentrations, but it plays an important role in remineralization, because when it combines with enamel crystals it forms fluorapatite, which is a mineral that is more resistant to attack by bacterial acids (29). When salivary fluoride was evaluated in each group, the difference between the initial and post-treatment values was notable. Mean salivary fluoride rose from 0.052 ppm and 0.049 ppm with one and two applications to 0.057 ppm and 0.058 ppm, respectively. There were no significant differences in the different OHI-s levels; these values were similar to those reported by Aguirre Aguilar and Rebaza Honores (26) in the saliva of children free of caries, and higher than the common values of 0.006 ppm for areas without fluoridation of drinking water (30). This would confirm the influence of dental products on the amounts of fluoride in saliva.

In view of the above, the use of Xeros Dentaïd® oral moisturizer spray improves the values of the components of the salivary profile and acts as an ally in the prevention of caries.

Conclusions

Applying Xeros Dentaïd® oral moisturizer twice a day, which contains malic acid and xylitol, has a positive effect on salivary profile values (salivary volume, salivary flow, *Streptococcus mutans* count, salivary pH, buffering capacity and fluoride concentration) in caries-free 5-year-old children with different OHI-s levels. This shows a better oral condition after application, so its use as an oral moisturizer can be extended as an adjuvant to anticaries therapy.

It is recommended to extend the work to other ages and to include variation by other factors, such as caries risk levels and gender, in order to increase the sample.

References

1. Delfín Soto OA, González Sabín C, Sardiña Valdés M, Pérez Ruiz A. Determinación del flujo, el pH y la actividad peroxidásica salival en niños con diferentes grados de caries dental. Rev Habanera Cienc Med [Internet]. 2005;4(3). Available from: <https://www.redalyc.org/articulo.oa?id=180417676005>
2. Téllez M. pH salival y su capacidad amortiguadora como factor de riesgo de caries en niños en la Escuela Primaria Federal “Ignacio Ramírez” [thesis degree]. Universidad Veracruzana, México; 2011.
3. Fontana M, Young D, Wolff M, Pitts N, Longbotton C. Definiendo la caries dental para 2010 y en adelante. Gac Dent [Internet]. 2010 [cited 2013 Jun 17];226:104-29. Available from: http://www.gacetadental.com/pdf/226_ciencia_definiendo_caries_dental.pdf
4. Aguilar-Salvatierra A, Guardia J, Calvo-Giraldo JL, Herrera D, Gómez-Moreno G. Eficacia del spray de ácido málico 1% Xeros DENTAID spray, en el tratamiento de la xerostomía inducida por fármacos. Dentaïd expertise [Internet]. 2010 nov [cited 2011 Nov]. Available from: <http://www.dentaïdexpertise.com/es/eficacia-del-spray-de-acido-malico-1-xeros-dentaïd-spray-en-el-tratamiento-de-la-xerostomia-inducida-por-farmacos/141>
5. Sacsquispe S. La saliva y su rol en el diagnóstico. Act Odonto. 2009;6(1).
6. Loyo Molina K, Balda Zavarce R, González Blanco O, Solórzano Peláez AL, González M. Actividad cariogénica y su relación con el flujo salival y capacidad amortiguadora de la saliva. Acta Odontol [Internet]. 1999;37(3).

Available from: <https://www.actaodontologica.com/ediciones/1999/3/art-12/>

7. Llana C. La saliva en el mantenimiento de la salud oral y como ayuda en el diagnóstico de algunas patologías. *Med Oral Patol Oral Cir Bucal* [Internet]. 2006 [cited 2019 May 27];11:E449-55. Available from: <http://www.medicinaoral.com/medoralfree01/v11i5/medoralv11i5p449e.pdf>

8. Baños Román FF, Aranda Jacobo R. Placa dentobacteriana. *Rev AMD* [Internet]. 2003;60(1):34-6. Available from: <https://www.medigraphic.com/pdfs/adm/od-2003/od031g.pdf>

9. Hörsted-Binslev P, Mjør IA. *Modern concepts in operative dentistry*. Copenhagen: Munksgaard; 1988.

10. Thylstrup A, Ferjerskov O. *Textbook of clinical cariology*. 2.^a ed. Copenhagen: Munksgaard; 1994.

11. Ayala Luis JV. Determinación del pH salival después del consumo de una dieta cariogénica con y sin cepillado dental previo en niños [Internet thesis degree]. Universidad Nacional Mayor de San Marcos, Perú; 2008. Available from: <https://hdl.handle.net/20.500.12672/2179>

12. Barrancos J, Barrancos P. *Operatoria dental: integración clínica*. 4th ed. Buenos Aires: Editorial Médica Panamericana; 2006.

13. García W. *Análisis de la cadena de golosinas de chocolate*. 1998.

14. Liébana J. *Microbiología oral*. Madrid: Latinoamericana; 1995.

15. Boj JR. *Odontopediatría: la evolución del niño al adulto joven*. Madrid: Médica Rífano; 2010.

16. Gutiérrez J. *Fundamentos de ciencias aplicadas a la odontología*. Bogotá: Pontificia Universidad Javeriana; 2006.

17. Lindhe J, Lang NP, Karring T. *Clinical periology and implant dentistry*. 5th ed. Blackwell Munksgaard; 2008.

18. Jenkins GN. *Fisiología y bioquímica bucal*. Ciudad de México: Limusa; 1993.

19. Yabar E, Aguirre A. Variación de pH salival en jóvenes por consumo de chocolate de leche. *Vist dent*. 2011;14(1):729-33.

20. Genco R, Golman HM, Cohem DW. *Periodoncia*. México: McGraw-Hill Interamericana; 1990.

21. Rahman Zamani A. *Caries en niños pequeños* [Internet]. California Childcare Health Program; 2006 [cited 2013 Jun 20]. Available from: <https://studylib.es/doc/5115556/caries-en-ni%C3%B1os-peque%C3%B1os---california-childcare-health-pr...>

22. Namoc J. Relación entre el nivel de biofilm dental con flujo, pH y capacidad buffersalivales en 58 estudiantes de 15 y 16 años [thesis degree]. Trujillo: Universidad Nacional de Trujillo, Perú; 2011.

23. Aguirre Aguilar AA, Vargas Armas SS. Variación del nivel del pH salival por consumo de chocolate y su relación con el IHO en adolescentes. *Oral* [Internet]. 2012;13(41):857-61. Available from: <http://www.cmd.buap.mx/oral/43%20Ano%2013%20::%20Numero%2041/05%20Variacion%20del%20pH%20salival%20por%20consumo%20de%20chocolate%20y%20su%20relacion%20con%20el%20IHO%20en%20adolescentes.pdf>

24. Cevallos Zumarán JF, Aguirre Aguilar AA. Método pronóstico de valoración de riesgo para caries dental por consumo de chocolate. *Rev Odontol Mex* [Internet]. 2015;19(1):27-32. Available from: <https://www.medigraphic.com/revista-odontologica>

hic.com/pdfs/odon/uo-2015/uo151d.pdf

25. Rodríguez-Alayo G, Aguirre-Aguilar AA. Efecto de una pasta dental con xilitol sobre el perfil salival en niños de cinco años. *Oral* [Internet]. 2020;21(66):1851-9. Available from: <https://www.imbiomed.com.mx/articulo.php?id=114865>

26. Aguirre Aguilar AA, Rebaza Honores ML. Perfil salival de niños de cinco años libres de caries y su relación con el nivel de placa dentobacteriana. *Oral* [Internet]. 2014;15(49):1173-8. Available from: <https://www.imbiomed.com.mx/articulo.php?id=104068>

27. Martins Paiva S, Álvarez Vidigal E, Abanto J, Cabrera Matta A, López Robles RA, Masoli C, et al. Epidemiología de la caries dental en américa latina. *Rev Odontopediatr Latinoam*. 2012;4(2). <https://doi.org/10.47990/alop.v4i2.21>

28. Seif T. *Cariología*. Caracas: Actualidades Odontológicas Latinoamericana; 1997.

29. Moritsuka M, Kotasako Y, Burrow MF, Ikeda M, Tagami J. The pH change after titration into resting and stimulated saliva for a buffering capacity test. *Australian Dental Journal*. 2006;5(2):170-4.

30. Aguirre Aguilar AA, Narro Sebastián FG. Perfil salival y su relación con el índice CEOD en niños de 5 años. *Rev Odontol Mex* [Internet]. 2016;20(3):159-65. Available from: <https://www.medigraphic.com/pdfs/odon/uo-2016/uo163b.pdf>