Form for the Submission of Protocols for Observational and Interventional Research in Humans.
(Except Clinical Trials)

Ethics Committee for Human Research at the Catholic University of Cuenca (CEISH-UCACUE)

GENERAL INFORMATION OF THE RESEARCH

Title: The effect of xylitol-based chewing gum, both sugar-free and sugared, on salivary flow and pH in young adults: a randomized controlled trial in students from the Dentistry Career at the Catholic University of Cuenca, 2023

The study will be conducted on students from the Faculty of Dentistry at the Catholic University of Cuenca, main campus in Cuenca, enrolled during the period September 2023 to February 2024, aged 18 to 25, who wish to participate voluntarily. It will be executed in the Molecular Biology laboratory, which is equipped with a pH meter (to measure the amount of hydrogen ion in saliva) belonging to the Department of Research of the Dentistry program. This study will commence in December 2023 pending approval from the Ethics Committee.

In healthy and balanced diets with reduced sugar concentration, physiological mechanisms in the mouth (such as saliva) can prevent the onset of tissue lesions in the teeth at the level of hydroxyapatite crystals before cavitation (dental caries) occurs, through the restoration of pH and remineralization of tooth tissue. Saliva plays protective roles as an adjunct to dilution and cleaning of the oral cavity, as well as buffering and enabling ion exchange. The activity depends on the consistency and rate of flow, with the level of salivary pH defining how ionic exchange occurs during enamel remineralization and demineralization processes.[2]

Global increases in sugar consumption have prompted the development of sugar substitutes, including polyols or non-fermentable sugars, of which the most commonly used are nutritive sweeteners such as sorbitol and xylitol. Chewing gum stimulates salivation, neutralizes and raises pH levels, and serves as a means to administer therapeutic agents. Therefore, there are chewing gums that contain sugar substitutes like sorbitol and xylitol[4]. Vantipalli et al.[5] indicate that chewing sugar-free gum significantly reduces caries compared to the use of rinses, tablets, and candies. These authors report an increase in salivary pH within 10 minutes of starting consumption. German[6] researchers claim that the consumption of this product reduces health insurance company costs by 29.13% and improves oral health status by 5.38%.

The study possesses a degree of originality, as there are studies conducted in Ecuador that use reactive strips as instruments to evaluate salivary pH. Therefore, appropriate and high-quality equipment is needed to determine salivary pH. The use of chewing gum containing polyols could play a role in preventing dental caries compared to not using gum or using sugared gum, most likely due to the increased salivary flow and pH, improving enamel lesion remineralization. However, xylitol has anticariogenic properties influencing the reduction of dental plaque. Therefore, the objective of the study is to evaluate the effect of xylitol-based chewing gum, both sugar-free and sugared, on salivary flow and pH in young adults through a randomized controlled trial.

TYPE OF RESEARCH

Observational Studies		Intervention Studies	
• **Cross-sectional Studies**	☐	• Quasi-Experimental Studies	☐
• Ecological Studies	☐	• Field Trial	☐
• Case Reports	☐	Randomized Controlled Trials without the use of drugs and/or medical devices	☒
• Case Series	☐		
• Case-Control Studies	☐		
• Cohort Studies	☐		
Others			
• Specify			☐

PROJECT EXECUTION TIME

The study will last for 6 months, from December 12, 2023, to July 31, 2024.

RESEARCH FUNDING	
Total amount of research funding.	$4.054,00
Sources of funding.	Catholic University of Cuenca

GENERAL RESEARCH INFORMATION

SPONSOR INFORMATION				
Name of the individual/institution conducting the research				
Sponsor Vice-Rector of Research	[redacted]	National ID/RUC /RUC		PAB256196
Institutional telephone number	0964038407	Extensión	Email address	[redacted]
Address	Av. Américas and Humboldt, Cuenca			
Institutional website	https://www.ucacue.edu.ec/			
Executing body	Catholic University of Cuenca			

RESEARCH EXECUTION COVERAGE		
National ☐		
Planning zones ☐	Zona 1 (Esmeraldas, Carchi, Imbabura y Sucumbíos.) ☐ Zona 2 (Napo, Orellana y Pichincha) ☐ Zona 3 (Cotopaxi, Chimborazo, Pastaza y Tungurahua) ☐ Zona 4 (Manabí y Santo Domingo de los Tsáchilas) ☐ Zona 5 (Los Ríos, Guayas, Santa Elena, Bolívar) ☐ Zona 6 (Azuay, Cañar y Morona Santiago) ☐ Zona 7 (El Oro, Loja y Zamora Chinchipe) ☐ Zona 8 (Guayaquil, Durán y Samborondón) ☐ Zona 9 (Distrito Metropolitano de Quito) ☐	
Provincial ☐	Please specify the provinces where your research will be conducted.	☐
Local ☒	Please specify the province and cantons where your research will be conducted: Azuay-Cuenca	☒

RESEARCH PERSONNEL

ROLE	Full Name	Identification Card	Academic Background	Institution Affiliation	Personal and Institutional Email Address	Cellular Phone Number
Sponsor	Pedro Martínez Suárez	PAB256196	Doctor of Philosophy	Catholic University of Cuenca	pmartinezs@ucacue.edu.ec	0964038407
Principal Investigator	Miriam Verónica Lima Illescas	0103711925	Doctora en Ciencias Estomalógicas	Catholic University of Cuenca	mlimai@ucacue.edu.ec	0999975073
Investigator 1	Andrea Karina Maurat Argudo	0104981444	Master of Bioethics	Catholic University of Cuenca	andrea.maurat@ucacue.edu.ec	0995800436

Universidad Católica de Cuenca

RESEARCH DETAILS

STRUCTURED SUMMARY

Chewing gum stimulates salivation, neutralizes, and raises pH levels, serving as a medium for administering therapeutic agents. However, global increases in sugar consumption have led to the development of chewing gums containing sugar substitutes such as polyols or non-fermentable sugars, with the most common being nutritive sweeteners like sorbitol and xylitol. The use of polyol-containing chewing gum could play a role in preventing dental caries compared to not using gum or using sugared gum, most likely due to increased salivary flow and pH, thereby improving enamel lesion remineralization. However, xylitol has anticariogenic properties influencing the reduction of dental plaque. Objective: To evaluate the effects of xylitol-based chewing gum, both sugar-free and sugared, on salivary flow and pH in young adults. Methodology: This is a quantitative, analytical, experimental study through a double-blind parallel randomized controlled trial. The population will consist of all students enrolled (1275 students in the period October 2022-February 2023) in the Dentistry program at the Catholic University, aged 18 to 25, who voluntarily meet the inclusion criteria. The sample size will be 21 participants in each group, adjusted for a 15% loss of information or study dropout, thus requiring 25 participants in each group. Participants meeting the selection criteria will be assigned probabilistically using simple random sampling through a lottery system to four groups: the first group (G1) will consume gum with 100% xylitol, the second group (G2) will consume sugar-free gum with sorbitol, maltitol, mannitol, and xylitol, the third group (G3) will consume sugared gum containing sugar, dextrose, glucose, sweeteners such as aspartame and acesulfame-K, and the fourth group (G4, control) will chew paraffin. Data will be entered into an Excel matrix and analyzed using SPSS v.25. Descriptive analysis of the data will be conducted. Chi-square will be used for demographic data, Levene's test will determine data homogeneity, and ANOVA will determine mean differences, followed by Tukey's post-hoc comparison to find significant differences between group means, with a significance level of $p < 0.05$.Expected Results: It is expected that salivary pH and flow means will differ among the different chewing gums.

RESEARCH PROBLEM

Global increases in sugar consumption have prompted the development of sugar substitutes, including polyols or non-fermentable sugars, of which the most commonly used are nutritive sweeteners like sorbitol and xylitol. Chewing gum is a means to administer therapeutic agents and contribute to improving oral conditions. In the national market, there are sugared chewing gums (sucrose) and sugar-free gums that include xylitol plus sorbitol. These gums are consumed in high quantities, especially by teenagers and young adults, and the few studies conducted in Ecuador indicate a significant elevation in salivary pH from 6.4 to 7.14 [7,8] after consuming sugar-free chewing gums, compared to sugared gums. However, these investigations evaluate salivary pH levels using reactive strips. Additionally, there are no locally available chewing gums solely based on xylitol, and there are also sugar-free chewing gums that use a blend of compounds as sweeteners without indicating the percentages. Therefore, high-quality randomized controlled trials are needed to determine the effectiveness on salivary pH.

A study conducted in India[5] compared the efficacy of two sugar-free chewing gums. The first group showed a significant increase in salivary pH levels up to 2 minutes after consuming sugar-free chewing gums (pH 7.98 ± 0.19), which gradually decreased to 7.60 ± 0.12 at 30 minutes, compared to another group of sugar-free chewing gums where salivary pH levels at two minutes were higher (pH 7.49 ± 0.16) than at 30 minutes (pH 6.82 ± 0.28), showing statistically significant differences within each group. Regarding salivary flow, there was a higher average at two minutes (1.89 ± 0.42), which gradually decreased to 1.09 ± 0.31 at 30 minutes after consuming sugar-free chewing gums. Similar results were observed in the second group of sugar-free chewing gums at two minutes (1.89 ± 0.45), which decreased to 1.04 ± 0.31 at 30 minutes, showing statistically significant differences.

Therefore, chewing gums stimulate an increase in salivary flow, which contributes to diluting and cleaning the oral cavity, as well as buffering and allowing ionic exchange. Additionally, they promote a neutral or alkaline salivary pH level, which may contribute to enamel remineralization and, therefore, prevent dental caries.[2] Furthermore, they could serve as a complement to oral hygiene in situations where tooth brushing is not possible.

Based on the above, the following question is raised: Does the consumption of xylitol-based chewing gums positively influence salivary flow and pH compared to sugared or sugar-free gums?

JUSTIFICATION

This research is focused on determining the effects of xylitol-based chewing gums (100% xylitol, Xylichew brand), sugar-free gums (sorbitol, maltitol, mannitol, and xylitol, Trident brand), and sugared gums (sugar, dextrose, glucose, sweeteners such as aspartame and acesulfame-K, Agogó brand) on salivary flow and pH in young adults: a randomized controlled trial in students of the Dentistry Career at the Catholic University of Cuenca, 2023. In our context, the distribution of sugar-free gums includes xylitol and sorbitol among its ingredients, without indicating the exact proportions of each. Additionally, there are no chewing gums in the Ecuadorian market that contain xylitol as the sole ingredient, hence the need to compare with a foreign chewing gum, which indicates the presence of only xylitol.

Vantipalli et al.[5] showed statistically significant differences between two sugar-free chewing gums in terms of unstimulated salivary pH and salivary pH stimulated at 2 minutes, showing an increase at this time compared to 30 minutes after gum consumption. Similar results were reported for salivary flow, showing a higher flow at two minutes compared to 30 minutes after gum consumption. This study demonstrates significant changes within each group and between subgroups for salivary pH levels, while salivary flow showed significant changes only within each gum group evaluated.

This study has a degree of originality, as there are studies conducted in Ecuador that found significant changes with an increase in salivary pH levels in chewing gums containing sorbitol and maltitol. However, they did not evaluate chewing gum containing xylitol as the sole ingredient, and they used reactive strips as equipment to evaluate salivary pH. Therefore, high-quality and appropriate equipment is needed to determine salivary pH, and the Molecular Biology Laboratory has a pH meter, providing a precise tool for assessment.

The research topic is of interest to researchers and institutions as it is part of the Medical and Health Sciences research line, within the field of health education and promotion. The results will be published through an article in an indexed journal, benefiting clinicians such as dentists, physicians, undergraduate and postgraduate students in health-related fields, and the general population who will learn about the benefits of consuming xylitol-based and sugar-free chewing gums compared to sugared gums. In situations where differences are found, it can represent an oral hygiene tool in conditions where oral hygiene with brushing technique is not feasible, and the use of chewing gum would improve salivary pH, preventing enamel demineralization and thus dental caries.

The pH meter from BOECO brand (model BT-675) is a precision tool that measures pH, mV, and temperature. It has a built-in microprocessor that stores, calculates, and compensates all parameters related to pH determinations, including electrode temperature characteristics, electrode slope deviations, compensated solutions, and buffers.

THEORETICAL FRAMEWORK

One of the most prevalent oral health conditions is dental caries, affecting 35% of the global population. [1] Since 1960, it has been considered an infectious and transmissible disease, related to the presence of microorganisms, susceptible teeth, and fermentable substrates. The ecological plaque hypothesis is one of the most accepted, stating that oral environments with a low pH level disrupt the resident microflora, leading to an enrichment of pathogenic bacteria. [9]

En dietas saludables y equilibradas con reducida concentración de azúcar, los mecanismos fisiológicos de la boca (como la saliva) detiene la aparición de lesiones en los tejidos del diente a nivel de los cristales de la hidroxiapatita antes de la cavitación (caries dental) al restaurar el pH y remineralizar.[1] One of the most prevalent conditions in oral health is dental caries, affecting 35% of the world's population.[1] Since the 1960s, it has been considered an infectious and transmissible disease, related to the presence of microorganisms, susceptible teeth, and fermentable substrates. The ecological plaque hypothesis is one of the most accepted, stating that oral environments with a low pH level create an imbalance in the resident microflora, leading to bacterial pathogenic enrichment. [9] In healthy and balanced diets with reduced sugar concentration, the physiological mechanisms of the mouth (such as saliva) prevent the onset of lesions in tooth tissues at the hydroxyapatite crystal level before cavitation (dental caries) by restoring pH and remineralizing.[1]

Excessive consumption of sugary foods creates an imbalance in oral microbiota (dysbiosis), which favors bacterial competitiveness in dental biofilm (accumulation of bacteria and food deposits). Fructans and glucans are polymers produced from sucrose that stimulate microbial cohesion and adhesion. [10,11]

Studies link the consumption of fermentable carbohydrates containing sucrose to the risk of dental caries because microorganisms depend on them for their survival and on the diet provided by the metabolic activity of carbohydrates in oral microbiota. Additionally, bacteria require fermentable carbohydrates for the synthesis of insoluble glucans and fructans, which allow adhesion to the tooth surface, thereby promoting the formation of bacterial plaque. [12]

High sugar intake and low levels of salivary pH appear to be pivotal components that disrupt oral cavity homeostasis and lead to the development of dental caries. A few minutes after sugar intake, the pH of dental plaque decreases, causing enamel demineralization when the acidity level is below the critical pH, which varies between individuals within an approximate range of 5.2 to 5.57. At this point, acids affect the enamel by dissolving apatite crystals and releasing calcium and phosphate ions into saliva. Although this process occurs naturally, over time it leads to the destruction of enamel infrastructure. [12]

Therefore, proposed strategies to prevent the disease should include inhibition of acid production, reduction in sugar consumption primarily between meals, and the use of non-fermentable sugars. [13]
Chewing generates salivary stimulation, neutralizes and raises pH levels, and serves as a means to administer therapeutic agents. [3] As a result, there is an improvement in the removal of fermentable carbohydrates in the oral cavity and it promotes mineral oversaturation, which promotes enamel remineralization. Vantipalli et al.[5] indicate that chewing sugar-free gum significantly reduces caries compared to the use of rinses, tablets, and candies. These authors report an increase in salivary pH within 10 minutes of starting consumption.

Chewing gum is primarily made from gum resin and certain amounts of preservatives, flavorings, and sweeteners. Nowadays, there is a wide variety of sweeteners that replace sugar in chewing gum. These non-cariogenic products hinder bacterial metabolism and prevent acid production because they are non-fermentable. They also increase their anticariogenic potential due to the provocation of a palatability reflex by the sweet taste, which, along with the chewing process, increases salivary flow stimulation. [12]
The labeling of "sugar-free" is allowed for gums that do not decrease the pH below 5.7 during or 30 minutes after consumption.[12] The use of sugar-free gum can be particularly effective in conditions where daily oral hygiene and maintenance are difficult to carry out conventionally.

The sweeteners most commonly used in gums are xylitol and sorbitol. [12,13] They are referred to as "sugar alcohols" because their chemical structure is similar to both sugar and alcohol. [12]
Xylitol is a pentahydroxy alcohol derived from D-xylose that was approved by the Food and Drug Administration (FDA) in 1960 and has since been used as a substitute for refined white sugar. [2,13]

It is estimated that to prevent dental caries with the use of xylitol, it is not necessary to completely replace sucrose in the diet, and relatively small daily doses of xylitol ranging from 4 to 10 grams per day have been shown to provide anticaries protection. It has been suggested that chewing gum sweetened with xylitol three to five times a day, for a minimum of five minutes each time, ideally after meals, is effective in achieving the desired effect on cariogenic bacteria. [4,12,14]

Xylitol is considered cariostatic due to its ability to decrease counts of Streptococcus mutans (SM) [12,14] and Lactobacillus (LB) [4], as well as its role in controlling dental plaque formation and, consequently, the incidence of dental caries. Therefore, it is particularly effective in conditions where proper oral hygiene is not possible [12], or as a complement to toothbrushing for oral hygiene. [14,15]
Additionally, it stimulates salivary secretion, an important factor in maintaining alkaline pH, and promotes mechanical cleansing of tooth surfaces. [13]
Its mechanism of action is to create an unfavorable environment for bacterial growth, especially for Streptococcus mutans (SM), as it reduces its ability to adhere to oral tissues by affecting the insoluble glucans involved in this process. This property reduces the possibility of dental plaque formation and, therefore, the local effect of lactic acid. Additionally, xylitol penetrates the bacterial

cytoplasm, interfering with glycolysis and inhibiting the growth of microorganisms. [12]

Furthermore, its action affects other species such as Streptococcus sobrinus (SS) and Actinomyces viscosus (AV). As a result, a less cariogenic environment is created. [12] Tapiainen et al. [16] stated that the effect of xylitol disappears from saliva within 15 minutes.

Sorbitol is a hexitol derived from glucose with low cariogenic potential. Like xylitol, it causes a decrease in SM, SS, AV, LB, and insoluble glucans, along with an increase in salivary pH, giving it anticariogenic potential. Current evidence establishes that pentitols like xylitol, as well as hexitols like sorbitol, have good results in reducing dental caries. However, pentitols are more effective and therefore more commonly used. [4,12]

RESEARCH OBJECTIVES

General Objective

Compare the effects of xylitol-based chewing gum (Xylichew), sugar-free (Trident), and sugared (Agogó) on salivary flow rate and pH in young adults

Specific Objectives

Determine the initial salivary flow, at 10, 20, and 30 minutes after the consumption of xylitol-based, sugar-free, and sugared chewing gums in young adults.

Determine the initial salivary pH, at 10, 20, and 30 minutes after the consumption of xylitol-based, sugar-free, and sugared chewing gums in young adults.

RESEARCH QUESTION OR STUDY HYPOTHESIS

H0: The consumption of different chewing gums does not produce changes in the level and flow of initial salivary pH at 10, 20, and 30 minutes.

H1: The consumption of different chewing gums results in changes in the level and flow of initial salivary pH at 10, 20, and 30 minutes.

METHODOLOGY

- **Research Design**

It is a quantitative, analytical, experimental study conducted through a double-blind, parallel randomized controlled trial.

- **Definition of the study population and, if applicable, it is necessary to detail how the sample size calculation was performed (formulas and development).**

The population will consist of all enrolled students (1275 students in the period September 2023-February 2024) in the Dentistry program at the Catholic University, aged between 18 and 25 years old, who voluntarily meet the inclusion criteria.

For the sample size calculation, an online calculator was used, employing the formula for comparing two means. A confidence level of 95% and a statistical power of 90% were set. Based on a previous study that reported a desired difference of 1 unit, with a precision and variance of 1 in the reference or control group, it was determined that 21 participants would be needed in each group. However, to account for potential information loss or dropout from the study, the sample size was adjusted by 15%, resulting in the requirement of 25 participants in each group. (Figure 1)

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

Figure 1. Hypothesis testing formula for determining means.[14]

Replacing the formula

n= participants needed in each of the groups

Zα= Z value corresponding to the desired risk 0.05 =1.96

Zβ= Z value corresponding to the desired risk 90%= 1.282

S^2= Variance of the quantitative variable in the control group =1

d= Minimum detectable difference desired = 1

$$n = \frac{2(1.96+1.282)^2 * 1^2}{1^2} = 20.99$$

- **List of establishments where observational or intervention research will be conducted on human subjects. It is necessary to specify whether the institutions are public or private and include their postal address and letter of interest (Appendix B)**

The intervention will take place among students of the Dentistry program at the University of Cuenca, main campus, in the Molecular Biology laboratory. Sampling and determination of salivary flow and pH level will be carried out there. **(Appendix B)**

- **Inclusion Criteria**

Within the selection criteria, the inclusion criteria will be participants with at least 20 teeth, providing written informed consent, and being willing to comply with the study procedures.

- **Exclusion Criteria**

The exclusion criteria will be individuals with systemic, infectious, or inflammatory diseases or those taking medications, antibiotics, or fluoride in the last month; regular consumers of products and mouthwashes containing xylitol or sorbitol, with abnormal salivary flow (<1 ml/min), pregnant women or those on contraceptive pill treatment, or with eating disorders (anorexia and bulimia); subjects with periodontal disease or presence of dental caries.

- **The variables described in this section should be consistent with those mentioned in (APPENDIX C) the table of operationalization of the variables**

The variables of interest will be chewing gums, salivary flow level, and salivary pH during 4 time periods: initial (without chewing gums), at 10, 20, and 30 minutes after consuming the chewing gums.

Detailed and sequential description of all procedures that will be carried out to achieve each of the stated objectives. At least the following should be included.

7.1 Procedures: Students will be invited to participate through the study's presentation in each and every classroom of the students enrolled in the period September 2023-February 2024. At the end of the presentation, they will be invited to sign the informed consent, a document in which participants will provide their cell phone numbers so that they can be contacted via WhatsApp messages for the preliminary examination of their oral cavity to verify compliance with the inclusion criteria, as well as for sample

collection.

7.2 Sample Selection: Participants who meet the selection criteria will be probabilistically assigned using simple random sampling through a lottery system and will be assigned to each group. There will be 4 groups: the first group, G1, will consume chewing gums with 100% xylitol; the second group, G2, will consist of individuals who consume sugar-free chewing gums; the third group, G3, will consist of participants who chew sugared gums; and the fourth group, G4, will serve as the control group and will chew paraffin (Figure 2). This procedure will be carried out by investigator 1 (**Appendix D - Chewing Gum Information**)

The principal investigator, investigator 1 and 2, and technician 1, as well as the participants who will form the different groups consuming the chewing gums, will be unaware of the type of gum (double-blind). Both the chewing gums and the paraffin will have the same weight (1.3 g), and the blinding method will involve the presence of identical, pre-numbered containers containing the different chewing gums. This information will only be accessible to technician 2. Saliva samples will be collected by the principal investigator, and the data will be recorded on a form specifically designed for this purpose by technician 1, which will be executed in the Molecular Biology laboratory (**Appendix E - Data Collection Form**).

7.3 Saliva Sample Collection: Saliva samples will be taken between 8 to 10 am, ensuring that subjects have not ingested food, smoked, or brushed their teeth at least 1 hour prior to sample collection. Participants will be asked to rinse their mouths with pure water to remove any food residue. Subsequently, they will be seated in a chair in an upright position with their head slightly tilted forward and eyes open, and the basal saliva sample will be taken as indicated by the established protocol for collecting unstimulated saliva by Tomas Seif. Before starting the test, participants will be asked to swallow saliva. They should keep their mouth slightly open and allow saliva to drain into the tube. The test will last five minutes, during which salivary pH will be evaluated and weighed, denoted as T1. For stimulated saliva, the protocol dictated by the Faculty of Dentistry at the University of Southern California will be used, utilizing the drainage method. Stimulated saliva will be collected in another pre-weighed test tube at three additional time intervals: T2 from 5 to 10 minutes, T3 from 15 to 20 minutes, and T4 from 25 to 30 minutes after the start of chewing gum consumption. Salivary flow and pH level measurements will be evaluated immediately using a pH meter (BT-675 meter, Boeco-Germany), performed by the principal investigator. Sample collection will be conducted over a period of two weeks.

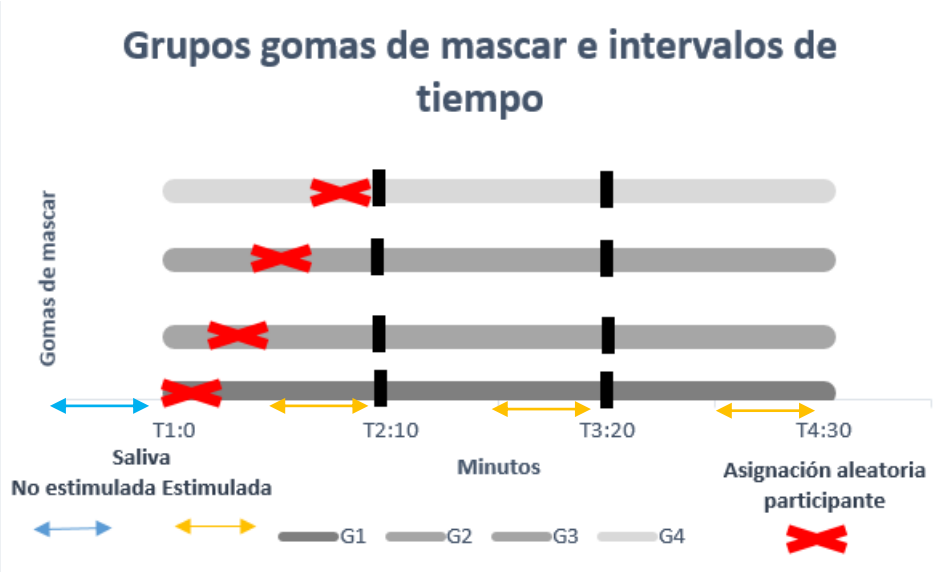

Figure 2. Random allocation of participants for the determination of salivary flow and pH level.

7.4 Operation mechanism of the desktop pH meter BT-675 BOECO-Germany

The to take pH measurements, the BT-675 must be calibrated before first use and will be calibrated by the company Record Dental. Immediately after sample collection, the pH level and salivary flow will be measured. (**Appendix F - Certification of the pH meter BT-675 BOECO-Germany**)

Procedure for using the desktop meter:

1. Connect the pH electrode to the BNC connector and the ATC/Temp probe to the ATC/Temp connector on the unit. The "ATC" icon will light up.
2. Press the "Mode" button until the "pH" icon and the "AUTO" icon light up.
3. Rinse the pH electrode and the ATC/temp probe with distilled water and immerse them in the sample to be measured. Remove any trapped air bubbles around the probe by shaking or agitating the probe.
4. Press the "Mea. / Eff." button. The "WAIT" icon will start flashing. The unit is waiting for a stable reading. The display will track the pH value as detected by the pH electrode and the ATC/Temp probe.
5. When the "WAIT" icon disappears, the reading is "LOCKED" and will not respond to further changes in the sample. The pH value displayed is the pH value of the sample at the sample temperature shown.

7.5 Disposal of biological samples: Immediately after determining the salivary flow and pH level in the Molecular Biology Laboratory, disposal will be carried out using the system for managing biological waste. This will involve the disposal of biohazardous waste, for which the Falcon sample tubes will be capped, and the samples will be placed in red bags identified as biohazardous waste. Custody of the waste will be the responsibility of the principal investigator.

7.6 Handling of data sheets: The physical documents will be handed over to technician 1, who will then receive them and immediately, the laboratory coordinator will assign technician 1 a physical space for handling the information. The information will not be provided to third parties, as only investigator 1 will have knowledge and access to that physical space and will develop the data matrix in Excel. It is worth noting that the laboratory coordinator will assign the necessary physical space, and thereafter, only technician 1 will have access to it.

7.7 Information Management: Technician 1 will handle the physical documents until the laboratory process is completed. Subsequently, Technician 1 will provide the data matrix in Excel for further analysis by Investigator 1. Participants will not receive individual research results; instead, the final research results will be disseminated.

8. Human biological samples do not require exportation as they will be disposed of immediately after the determination of salivary flow and pH level.

9. Data Analysis Plan

The data analysis will be conducted using SPSS v.25. Descriptive analysis of the data will be performed. Chi-square test will be used for demographic data, Levene's test will determine the homogeneity of the data, and ANOVA test will be used to determine differences in means. Post-hoc Tukey's test will be employed to find significant differences between the means of the groups, with a significance level set at $p<0.05$. All analyses will be conducted by Investigator 1.

HUMAN AND MATERIAL RESOURCES

1. Enumerate the human resources that will participate in the research, detailing the functions each one will fulfill; it should coincide with the section.

1. Principal Investigator	[redacted]	Collecting the sample and measuring salivary flow and pH
2. Investigator 1	[redacted]	Inviting participation via email and conducting statistical analysis
3. Investigator 2	[redacted]	Sending study participation information via email.
4. Technician 1	[redacted]	Collecting quantitative data on the data sheets.
5. Technician 2	[redacted]	Collecting the sample.

2. Provide a detailed description of all the material resources used for the execution of the project, including necessary information

Among the materials are nitrile gloves, masks, Falcon tubes, funnel, hand towels, xylitol chewing gums, Trident, Agogo, and paraffin. As for the equipment, a pH meter and a precision scale (Boeco) are needed.

ETHICAL AND GENDER CONSIDERATIONS

1. **Provide a detailed description of the measures to be taken to ensure the rights of individuals to minimal risk, autonomy, and confidentiality**

For the conduct of this research, reference will be made to the Universal Declaration on Bioethics and Human Rights, the Helsinki Declaration, International Ethical Guidelines for Biomedical Research Involving Human Subjects, Organic Health Law, and considering that research in health, particularly involving human beings or the use of biological samples of human origin, in this case, as it involves an intervention trial with human subjects, permission will be sought from a duly recognized entity by the Ecuadorian state, in this case, the Ethics Committee in Human Research at the Catholic University of Cuenca (Approval of the study, Declaration of Confidentiality, and Informed Consent). Subsequently, approval will be sought from the National Agency for Regulation, Control, and Sanitary Surveillance (ARCSA) for the registration, review, and approval of the trial.[19]

2. Describe the process of anonymizing human biological samples, specifying if applicable, how they will be coded (example: First letter of the name, first letter of the surname, first digits of ID card LH1715).

The process of anonymizing data involves:

Purpose

Minimizing the number of variables necessary for identifying individuals, restricting access to confidential information to the research team involved in the present investigation

Coding

Participants will be coded in an alphanumeric manner.

Anonymization Process

The process will be determined by a sequential number, with the appended single letter corresponding to the chewing gum: Xylitol, Non-sugared, Sugared, Paraffin (X, N, A, P), followed by the participant's age in years and a letter, which in this case is the letter A for all participants as shown in Table 1. The code consists of a length of 6 characters. Table 1. Anonymization Process

Current Code	Anonymization
1. Miranda Ojeda Adriana Estefanía, Xilitol, 18 Años	**1.X18A**

1. Miranda Ojeda Adriana Estefanía **X**ilitol **F**emenino **18 A**ños

3. Define the person or institution responsible for safeguarding the human biological samples.

On the same day that the sample is taken, the salivary flow and pH level will be measured, and the samples will be disposed of in red biohazard bags. The custody will be under the responsibility of the principal investigator. The Molecular Biology Laboratory has a system for managing biological waste

INFORMED CONSENT

The informed consent is presented in **Appendix** 4, and the participants are individuals aged 18 to 25 years old, therefore, there is no need for assent.

EXPECTED RESULTS

The possible results according to the study objectives:

Identify the benefits of chewing gum consumption on salivary flow.
Determine significant differences in salivary pH among different types of chewing gum. In addition to finding positive results, it could be implemented in oral health promotion programs as an alternative, especially where oral hygiene is not practiced three times a day.
Among the possible risks, allergy to chewing gum components could occur. (Annex D - Information on chewing gum)

CITED REFERENCES

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- That the content, authorship, and responsibility for the study results correspond to the Sponsor and the Principal Investigator, and that the Ministry of Public Health is exempted from any legal action arising from this cause.
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- That they will ensure compliance with this research in the terms approved by both the Committee of Ethics of Research in Human Beings and by the Ministry of Public Health.
- That a final report of the research with the obtained results will be sent to the Ministry of Public Health.

Location: Cuenca, Ecuador

Date: December 12, 2023

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[redacted]