The Effect of Dried Fruits on Children's Salivary Bacteria

BY

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THESIS

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TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
A. Background	1
B. Purpose of the Study	4
C. Hypothesis	4
II. CONCENTUAL FRAMEWORK AND RELATED LITERATURE	5
A. Saliva	5
1. Components and Functions	5
2. Secretion and Oral Clearance	6
3. Bacterial Clearance	7
4. Saliva Collection	8
B. Dental Caries and Sugars	9
C. Dietary Plant-derived Polyphenols	10
D. Dried Fruits and Oral Bacteria	11
1. Raisins	11
2. Banana Chips	11
3. Cranberries	12
III. MATERIALS AND METHODS	14
A. Overview	14
B. Inclusion Criteria	14
C. Exclusion Criteria	15
D. Test Foods	15

TABLE OF CONTENTS (Cont')

CHAPTER			PAGE
	E.	Study Design	16
		1. Pre-Procedural Instructions	16
		2. Initial Whole Saliva Sample Collections	16
		3. Preparation and Test Food Consumption	17
		4. Post Whole Saliva Sample Collections	17
		5. Determination of Viable Bacterial Count in Saliva Samples.	18
		6. Statistical Analysis	19
IV.	RESULTS		20
V.	DISCUSSIONS		27
VI.	CONCLUSIONS		32
VII.	CITED LITERATURE		33
VIII.	APPENDICES		36
	1)	Appendix A	36
	2)	Appendix B	39
	3)	Appendix C	42
	4)	Appendix D	47
IX.	VITA		48

TABLE OF CONTENTS (Cont')

TABLES PAGE

I. Salivary bacterial counts (colony forming unit CFU/ml) before and after consuming Craisins………………………………………………………………………….. 20

II. Salivary bacterial counts (colony forming unit CFU/ml) before and after consuming Raisins…………………………………………………………………………. 21

III. Salivary bacterial counts (colony forming unit CFU/ml) before and after consuming Banana Chips ………………………………………………………………….. 21

IV. Salivary bacterial counts (colony forming unit CFU/ml) before and after consuming Gum Base……………………………………………………………………….. 22

V. Effect of Consuming Dried Fruits on Children's Total Salivary Bacteria….. 22

FIGURES PAGE

I. Study Design…………………………………………………………………………………………. 18

II. Test Effect of Consuming Dried Fruits on Children's Total Salivary Bacteria (% = CFUpost/CFUpre)……………………………………………………………………………. 23

III. Study Demographics (N=15)………………………………………………………… 23

IV. Pearson Correlation number of visits (N=15)…………………………………….. 24

V. Effect of Consuming Dried fruits on Children's Total Salivary Bacteria: Chi Squared Anaylsis (N=15)……………………………………………………………………….. 25

VI. Effect of Consuming Dried Fruits on Children's Total Salivary Bacteria (N=15)………………………………………………………………………………………….. 25

SUMMARY

Although sweet and sticky, selected dried fruits contain plant-derived antimicrobial polyphenols that inhibit growth of oral pathogens and have shown to benefit oral health, with anti-gingivitis and anti-caries properties. **Objective:** To investigate the effect of chewing and consumption of selected dried fruits on viability of bacteria in children's saliva. **Methods:** Fifteen 7 to 12 years old children of all genders and race enrolled in this randomized controlled, crossover study. Children with systemic diseases or orthodontic appliance were excluded. The participants refrained from oral hygiene the night before and the morning of the testing. A baseline non-stimulated whole saliva sample was first collected over a five-minute period. The participants then chewed 20 g of one of the test foods (raisins, craisins or banana chips) or the control (a non-flavored gum base) for five minutes and saliva sample was collected 30 minutes after test food consumption. The total viable salivary bacterial counts before and after foods consumption was determined in the laboratory. The reduction in bacterial counts was determined. There was a three-day washout period between visits. **Results:** Based on data obtained thus far, chewing and consumption of raisins or craisins (dried cranberries) were found to reduce the total salivary bacterial count in study participants. However, chewing and consuming dried banana chips or chewing a gum base did not reduce viability of salivary bacteria. **Conclusions:** Unlike Banana chips, dried fruits such as craisins or raisins reduced salivary bacteria in children between 7-12 years of age. These dried fruits may be a healthier alternative over the popular sugary snacks and provide protective benefits against oral pathogens and contribute to oral health.

I. INTRODUCTION

A. Background

Dental caries and its associated consequences are one of the most prevalent health problems in children in the United States and currently on the rise (Peterson, 2003). Dental caries is a multifactorial disease that involves the presence and interaction of four factors: host defense, dental plaque bacteria, availability of carbohydrate as bacterial energy source, and duration of bacterial challenge that results in dental caries (Usha, 2009). As stated in the AAPD Policy on Dietary Recommendation for Infants, Children, and Adolescents; dietary habits that include prolonged contact of fruit juices and sticky snacks are significant risk factors for dental caries.

Dental caries result from demineralization of tooth enamel from an acid challenge source (Tahmassebi *et al.*, 2006; Skinner *et al*., 2015). Dental plaque biofilm plays an important role in the caries process. It is a complex multispecies microbial community that adheres to tooth surfaces and is thought to be one of the prime etiologic agents of dental caries. Oral bacteria, especially *Streptococcus mutans,* ferment sucrose and other carbohydrates and produce lactic acid from metabolism. The lactic acid in the dental plaque subsequently lowers the pH level in the oral cavity to 5.5, which leads to demineralization of the enamel and dentin. *S. mutans* also produces glycosyltranferases (GTFs) which hydrolyze sucrose to produce the adherent polysaccharide matrix facilitating accumulation and stickiness of the plaque biofilm (Tahmassebi *et al*, 2006). If the plaque biofilm consists of mainly acidogenic bacteria, it facilitates caries formation.

In the oral cavity saliva is the mixed glandular secretion, which constantly bathes the teeth and the oral

Mucosa. The functions of saliva are largely protective which aid in the following: fluid lubricant, ion reservoir, buffer, cleansing, antimicrobial actions, agglutination, pellicle formation, digestion, taste excretion and water balance (Dodds, 2005). Difference in the rates of salivary clearance of specific foods and substances help to explain differences in disease susceptibility among individuals and at various sites within the mouth. Thus, a rapid salivary clearance of harmful substances is beneficial for oral health, while the reverse is true for protective substances (Dawes, 1995).

Saliva also contains many different species of oral bacteria such as *Streptococcus mutans* (S. mutans), *Porphyromonas gingivalis*, *Staphylococcus epidermidis*, *Streptococcus salivarius*, and *Lactobacillus sp.* Not only due these bacteria have a negative impact on oral structures, they have been shown to affect overall health and wellness. In 2005 researchers from the Forsyth Institute in Boston discovered high levels of mouth bacteria *C. gingivalis, P. melaninogenica* and *S. mitis*, present in oral cancer patients, strongly suggesting a link. In 2009 University of Buffalo researchers reported a link between the amount of mouth bacteria and an increased risk of a heart attack. They suggest that the link is not due to the types of bacteria but their numbers. If mouth bacteria are linked to diseases elsewhere in the body they will provide the basis for simple diagnostic tests that can be used as early predictors of disease. Knowledge of friendly mouth bacteria is spearheading the development of more mouth friendly foods.

In order to maintain oral bacterial numbers at a level that doesn't initiate oral disease, the control of bacteria in the plaque and saliva is of importance. This is often achieved by using antimicrobial oral hygiene products. In recent years, natural antimicrobial agents have gained acceptance by the general public. Polyphenols are natural-occurring phytochemicals that can be found in many edible plants, and the major classes of them are flavonoids, phenolic acids or alcohols, stilbenes, and lignans, with flavonoids being the most abundant one found in dietary food products (Wu, 2009; Ferrazzano 2009; Jeon *et al*., 2011). Dietary polyphenols in both edible and non-edible plants have been shown to benefit health, including their antioxidant, anticancer, and anti-inflammatory properties within the last decade (Pandey and Rizvi, 2009; Gupta *et al*., 2011). Many widely consumed polyphenol-rich foods or beverages such as fruits, tea, and coffee have been shown to benefit oral health, with antigingivitis and anticaries properties (Yoo *et al*., 2011; Gupta *et al*., 2011). Polyphenols from fruits and berries such as cranberries (*Vaccinium macrocarpon*) or lingonberries have also been shown to have bacteriostatic effect on *Streptococcus mutans* and evident anti-adhesion properties (Kontiokari *et al*., 2005; Koo *et al*., 2006; Duarte *et al*., 2006; Yoo *et al*., 2011; Riihinen *et al*., 2014). Many commercially available snacks contain dietary plant-derived polyphenols, which include various dried fruits such as cranberries, raisins and plums. However it is unknown whether these sticky dried fruit snacks affect cariogenic bacteria or provide oral health benefits for its consumers.

Children nowadays have access to a wide variety of snacks including healthy energy dense dried fruit snacks. With the sugar content, and their sticky qualities, there is a concern that some of these dried fruits snacks may contribute to dental caries. The influence of dietary habits towards oral health is indisputable, and understanding how commercially available snacks may affect dental health can serve as a new approach to promote these mouth friendly foods. There are limited studies connecting polyphenols-containing dried fruit snacks and their affect on oral bacterial counts especially in children, making these concepts less well understood. The oral health benefit of these dried fruits is worth investigating. In this study we examined if dried fruits containing antimicrobial polyphenols affect the viability of bacteria in children's saliva.

B. Purpose of the Study

The purpose of the study is to investigate the effect of chewing and consumption of selected dried fruits on viability of bacteria in children's saliva. The dried fruits tested were dried cranberries (craisins), raisins, dried bananas. Gumbase was used as a control.

C. Hypotheses

We hypothesize that commercially available dried fruits, especially those containing plant-derived antimicrobial polyphenols may benefit children's oral health by reducing the salivary bacteria in the oral cavity.

II. CONCEPTUAL FRAMEWORK AND RELATED LITERATURE

A. Saliva

1. Components and functions

Human saliva not only lubricates oral tissues, making oral functions such as swallowing and speaking possible, but it also protects teeth and mucosal surfaces in many ways. Salivary fluid is an exocrine secretion consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides of importance to oral health (*Berkovitz 2002*). There are also glucose and nitrogenous products, such as urea and ammonia. The components interact and are responsible for the various functions attributed to saliva (*Humphrey 2001*).

Total or whole saliva refers to the complex mixture of fluids from the salivary glands, the gingival fold, oral mucosa transudate, in addition to mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial and blood cells, as well as traces of medications or chemical products (*Edgar 2004*). At rest, without exogenous or pharmacological stimulation, there is a small, continuous salivary flow present in the form of a film that covers, moisturizes, and lubricates the oral tissues. Whereas, stimulated saliva is produced in the face of some mechanical, gustatory, olfactory, or pharmacological stimulus, contributing to around 80% to 90% of daily salivary production (*Jenkins 1978*).

2. Secretion and Oral Clearance

Differences in the rates of salivary clearance of carbohydrates from food, acids from plaque, and therapeutic substances for example fluoride help to explain differences in disease susceptibility among individuals. The effect of incoming freshly secreted saliva, together with the swallowing process, is to reduce the concentration of exogenous substances also known as salivary clearance. Thus, a rapid salivary clearance of harmful substances is beneficial for oral health, while the reverse is true for protective substances (Dawes *et al*, 1983). Saliva, when secreted by the salivary glands is sterile but whole saliva in the mouth may contain bacteria at levels up to 10^9/ml. For bacteria to survive in the mouth they must be able to attach to and proliferate on oral surfaces since the unstimulated salivary flow rate is too high for saliva to act as a continuous culture system (Dawes *et al*, 2003).

The unstimulated flow rate is normally about 0.3-0.4 ml/min but may vary among individuals. According to the Dawes model, the lower the unstimulated salivary flow rate, the more prolonged clearance of a substance (Dawes, 2005). Since individuals with severe hyposalivation may have, unstimulated flow rates even lower than the minimum value of 0.05ml/min the delayed clearance of carbohydrates may help to account for their high susceptibility to dental caries (Lagerlof 1984). The stimulated salivary flow rate may remain above the unstimulated rate for only about a minute after food consumption, however, it is proven to play an important role. The longer the salivary sucrose concentration remains high, the more sucrose will diffuse into the dental plaque. Lagerlof *et al* has shown that the stimulated flow rate can also have a great effect on the clearance pattern of fluoride. As with sucrose, the faster clearance rate caused by stimulation of salivary flow reduces the amount of fluoride diffusing into dental plaque. Factors affecting the unstimulated salivary flow rate includes: degree of hydration, body position, exposure to light, previous stimulation, and circadian rhythms (*Dawes et al, 2004*).

3. Bacterial Clearance

Human saliva has several critical functions, including lubrication, digestion, formation of a bioactive semipermeable barrier (pellicle) that coats oral surfaces and regulation of the composition of the oral flora. Saliva fulfills the latter function by its antimicrobial activity and by promoting selective microbial clearance or adherence (Gillece-Castro, 1991). Salivary agglutinin is a high molecular mass component of human saliva that binds *Streptococcus mutans,* an oral bacterium implicated in dental caries. Salivary agglutinins were identified as a protein fraction that mediates specific adhesion and aggregation of *Streptococcus mutans*. They consist of monoclonal antibodies to agglutinin and block adherence of *S. mutans* to the pellicle and aggregation of the bacterial cells by saliva (Carlen, 1995). Saliva which contains salivary agglutinins adheres bad oral bacteria allowing it to be cleared to the stomach before attaching to the pellicle on dentition. Bacterial clearance is a topic of great interest and more studies are continually being done to find out more about the mechanism of these salivary agglutinins.

4. Saliva Collection

Human saliva is a unique biological fluid with numerous functions within the oral cavity, predominantly facilitating the maintenance of oral health and creating an appropriate ecological balance in the mouth (Humphrey, 2001). To date, most of the saliva collection devices that are commercially available allow a person to collect resting/unstimulated saliva and/or stimulated saliva either via mechanical stimulation or acid stimulation (Pfaffe, 2011). When a person is in a resting state, saliva production is largely produced by the submandibular gland, while only 20% and 8% are produced by parotid and sublingual glands, respectively. In contrast, when saliva production is stimulated either via chewing gum, food or through acid stimulation, most of the saliva produced is primarily derived from the parotid gland (Dodds, 2005). The results of the drooling versus spitting are of considerable interest with saliva collection. Research shows that with 5-minute saliva collections, spitting introduced more than twice as many bacteria into saliva as did drooling without oral movements. When collection times were reduced to 1 min, this difference was amplified to 14 times (Togelius et al., 1984; Bentley et al., 1988; Karjalainen et al., 1992).

B. Dental Caries and Sugars

The U.S. Food Guide Pyramid and Dietary Guidelines for Americans along with the European National Guidelines promote a diet rich in carbohydrates, whole grains, fruit, and vegetables. However, foods within these categories are also sources of fermentable carbohydrates. Intake of sugars in the United States has increased significantly in the latter part of the 20th century; per capita consumption of added sugars has increased by 23% from 1990 to 1998 (*Kantor 1998*). Fermentable carbohydrates including sugars or starch are carbohydrates (sugars and starch) that are digested in the oral cavity via salivary amylase. Sugars enter the diet in two forms: those found naturally in foods (ex. fruit, honey, and dairy products) and those that are added to foods during processing to alter the flavor, taste, or texture of the food (*Johnson, 2001).* Caries risk also depends on individual host factors.

The presence of any individual characteristics such as low or high salivary pH, genetic predisposition, prior caries history, use of medications, incidence of systemic or local diseases that affect the immune system, and personal hygiene habits which also play a role in the associated caries risks of particular foods. The cariogenic risk associated with individual foods is challenging to determine in human studies because of the variability in salivary flow and salivary and plaque pH, the eating experience (frequency and food combinations), bioavailability of starch-derived sugars, retention time of food in the oral cavity, and potential interactions between starches and sugars (*Mobley 1998*). It is best to eat a balanced diet rich in whole grains, fruit, and vegetables and practice good oral hygiene.

C. Dietary Plant-derived Polyphenols

Polyphenol is a generic term for the several thousand plant-based molecules that have antioxidant properties. Polyphenols are natural phytochemicals that can be found in many fruits, plants, and vegetables (Pandey and Rizvi, 2009). Polyphenols are divided into four primary groups: phenolic acids, lignans, stilbenes, and flavonoids and are well known and polyphenols are also helpful for regulating enzyme function and stimulating cell receptors (Elliott, 2000). Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular diseases, cancers, and osteoporosis and suggests a role in the prevention of neurodegenerative diseases and diabetes mellitus (Scalbert, 2000). Polyphenol-rich plants such as grapes (raisins), tea plant, cranberries, apples, and cherries can contain 200-300 mg polyphenols per 100 grams of weight, and even the food products or beverages manufactured from them contain a significant amount of polyphenols (Pandey and Rizvi, 2009). Polyphenols may contribute to the astringent or bitter taste of the foods, and tend to have high oxidative stability (Pandey and Rizvi, 2009). Epidemiological studies and meta-analyses have strongly suggest that consuming polyphenol-rich foods offer long term health benefits and protect from cardiovascular diseases, diabetes, and osteoporosis (Graf *et al*., 2005; Pandey and Rizvi, 2009).

Traditionally, the notion of dental caries prevention has placed much focus on mechanical removal of dental plaque and food debris, but in recent years there has been an increased interest in using natural food product to aid in dental caries prevention in the research field (Gupta *et al*, 2011). Dietary plant-derived polyphenols, such as flavonoids and tannins, which can be found in grapes (*Vitis vinifera*), raisins, American cranberry fruit

(*Vaccinium macrocarpon*), and Cinnamon (*Cinnamomun cassia*) have been found to possess antimicrobial activities against selected oral bacteria. Their anti-caries and anti-gingivitis potential have been suggested. (Rivero-Cruz, 2008, Yoo *et al*., 2011; Gupta *et al*., 2011).

D. Dried Fruits and Oral Bacteria

1. Raisins

Besides being a traditional and popular snack food, raisins contain polyphenols, antioxidants, flavonoids and iron that may benefit overall human health. The sweetness of raisins is contributed by mainly glucose and fructose, but not sucrose. It is well documented that sucrose, the main dietary sugar, serves as a substrate for the synthesis of adherent glucans in human dental plaque associated with tooth decay and gum disease (Cury et al., 2000). Although various in vitro studies have been performed to investigate the mode of actions of these phytochemicals and their effects on bodily functions, much less attention has been paid to their effects on oral health and disease prevention. The various phytochemicals reported in raisins include triterpenes, flavonoids hydroxycinnamic acids and 5-hydroxymethyl-2-furaldehyde. Rivero-Cruz et al. have reported that antimicrobial compounds present in raisins are capable of suppressing growth and/or virulence properties of oral pathogens (Rivero-Cruz, 2008).

2. Banana Chips

Banana chips, made by deep-frying and drying slices of ripe bananas, make for a convenient snack. Like whole bananas, banana chips contain many essential nutrients that nourish the body and benefit health. Bananas naturally contain sugar, but refined sugar used at the time of processing banana chips can be quite unhealthy. It is used to enhance the flavor of the chips. In one-half cup of banana chips, there is 12g of added sugar in your body. These sugars are usually referred to as dietary carbohydrates (Moynihan, 2004). Dietary carbohydrates can be divided into simple sugars and complex carbohydrates, e.g., starches. Among the sugars, sucrose is a major component of the modern diet of Western countries and, increasingly, of developing countries (Walker, 2004). Its consumption far exceeds that of other common dietary sugars (glucose, fructose, lactose), and it appears also to be the most frequently consumed sugar. The increasing use of high-fructose corn syrups (e.g., 42% and 55%o High Fructose Corn Syrup) instead of sucrose in beverages, for example, is of more recent date (Lingstrom, 2000). Sucrose, upon entering dental plaque, can be readily fermented by a wide variety of plaque bacteria to organic acids, which are responsible for caries formation. Sucrose is also unique among the common dietary sugars by serving as a specific substrate for the bacterial synthesis of extracellular polysaccharides (glucans) that have been implicated in caries causation (Wu, 2009).

3. Cranberries

Dried cranberries from American Cranberry fruit (*Vaccinium macrocarpon)* and are distinctively abundant in polyphenols, such as flavonoids (Bonifait and Grenier, 2010). Its consumption is widely accepted by the public, and its therapeutic effect in treating urinary tract infection via antibacterial activity against *Eschericia coli* is well established (Kontiokari *et al*., 2005). The potential benefits of cranberry juice or dried cranberry extrat toward inhibition of *Streptococcus mutans* have been investigated extensively in the past decade. Koo *et al*. (2006) had investigated the effect of

Cranberries on biofilm development and discovered that cranberries inhibited bacterial adhesion through blocking the biding sites in salivary pellicle and in glucans. Yamanaka *et al*. (2004) had further delineated that the anti-adhesion property of cranberries was due to a reduction of hydrophobicity in the cell surface of *Streptococcus mutans,* which led to an inability to attach to hydroxyapatite surfaces. It has been reported that cranberry contains molecules with anti-inflammatory properties. For instance, cranberry polyphenols reduce TNF-α-induced up-regulation of various inflammatory mediator production by human microvascular endothelial cells (Youdim et al., 2002). Cranberry components may affect oral supra-gingival biofilm formation and influence the tooth decay process by affecting glucan/fructan formation. In addition, it may lower the hydrophobicity of the bacteria, further reducing their capacity to adhere. The ability of cranberries to decrease acid production and possessing anti-adhesion properties is yet another means by which it may act as a mouth friendly food (Steinberg et al., 2005).

III. MATERIALS AND METHODS

A. Overview

This randomized controlled crossover study consists of 15 healthy children subjects between the ages of 7 and 12 year old recruited regardless of race or gender. Subjects were recruited using word of mouth and flyers posted on the University of Illinois-Chicago (UIC) campus. Respondants to the flyers who were legal guardians of potential subjects were contacted by telephone and study procedures, criteria and purpose were described via phone script. Once verbally screened using the phone script, a set of inclusion and exclusions criterias were reviewed. Subjects and their legal guardians were then invited to the UIC College of Dentistry building for a screening visit. Subjects were instructed to refrain from oral hygiene practices for at least 24 hours prior to the screening procedure to allow adequate accumulation of dental plaque and to refrain from eating anything except water for 2 hours prior to the testing procedures. At the screening visit, a clinical oral examination was conducted to determine the eligibility of the subject using inclusion and exclusion criteria. The following inclusion and exclusion criteria were utilized.

B. Inclusion Criteria

1. Give written informed consent and assent to participate

2. Be between the ages of 7-12 year old

3. Be in good general health without any class II mobile teeth

4. Ability to refrain from oral hygiene maintenance for 24 hours and eating/drinking (with the exception of water) for 2 hours prior to testing

5. Willing to participate in four 45 minute appointments at UIC college of Dentistry

6. No allergies or oppositions to any of the food products to be tested

C. <u>Exclusion Criteria</u>

1.Systemic diseases such as diabetes that affect oral health

2. Use of oral products containing antimicrobial agents or taking antibotics 21 days prior to study

3. Having fixed orthodontic appliances

4. History of irritation or sensitivity to the test foods

D. <u>Test Foods</u>

The subjects and their legal guardian will attend 4 visits (including screening visit) to complete the full study. If a subject drops out of the study, their data was not used. The test foods included commercially marketed products.

The test food groups were:

- 20 g Craisins (C)- Ocean Spray (Ocean Spray Cranberries, Inc. Lakeville-Middleboro, MA 02349 USA)

- 20 g Raisins (R)- Sun-Maid (Sun-Maid Growers of California, Kingsburg, CA 93631 USA)

- 20 g Banana Chips (B)- Jewel Osco Departmental Store (Jewel Osco, Chicago IL 60612 USA).

- 1 g of Gum Base (G)- Wrigley (Blackhawk St, Chicago, IL 60642 USA).

E. <u>Study Design</u>

1. Pre-Procedural Instructions

Participants were instructed to refrain from oral hygiene practices like brushing and flossing for 24 hour period while also refraining from eating and drinking for minimum of 2 hours prior to the study visit. Once the participants arrived, they were asked to lightly rinse their mouth out with water. A randomized selection of the food group was done. Four coins were labeled with C, R, B, G representing the test food groups. The participants took all four coins and threw them on the table. The coins were recorded in a left to right manner depicting which test food was first and which was fourth. The sequence of test groups for a particular participant was recorded to ensure that all participants go through each test food group by the end of the study. The total time for each visit is approximately 45-60 minutes with a washout period of two days minimum between study visits. Figure 1 summarizes the procedures for the study

2. Inital Whole Saliva Samples Collection

Initial whole saliva samples were collected from 15 qualified children. No demographic or protected health information were obtained or recorded from the subjects. Saliva samples were collected using the passive drool method for five minutes. The participant was seated in a chair, directed to tilt head down and passively drool without swallowing for the directed amount of time. During the procedure, the saliva was collected via a disposable funnel 65mm x 69mm (*Simport*®, 462021, Bernard-Pillon, Canada) into a removable capped 15ml Conical tube (*Falcon*® *,* 352097, Corning Science, Reynosa, Tamaulipas, Mexico) for storage, and labeled with an identification number (Pre#1 -#15). All tubes containing saliva samples were stored on ice until laboratory processing.

3. **Preparation and Test Food Consumption**

Following the collection of initial saliva (labeled as “pre-“), participates were given a specific test food from which was randomly chosen from a coin toss. The test foods were pre-weighed on a laboratory scale accurately. 20 g samples were used for Craisins, Raisins and Banana Chips while the Gum Base was 1 g. Each test food was chewed and consumed over a period of five minutes at a chewing rate of 50 chews per minute. This was regulated by a metronome, which was played at 50 beats per minute. Each test food was distributed evenly throughout the oral cavity and chewed thoroughly on both the left and right side of their dentition. Immediately following the test food consumption, participants were brought to a waiting room and waited for 30 minutes before saliva was collected again.

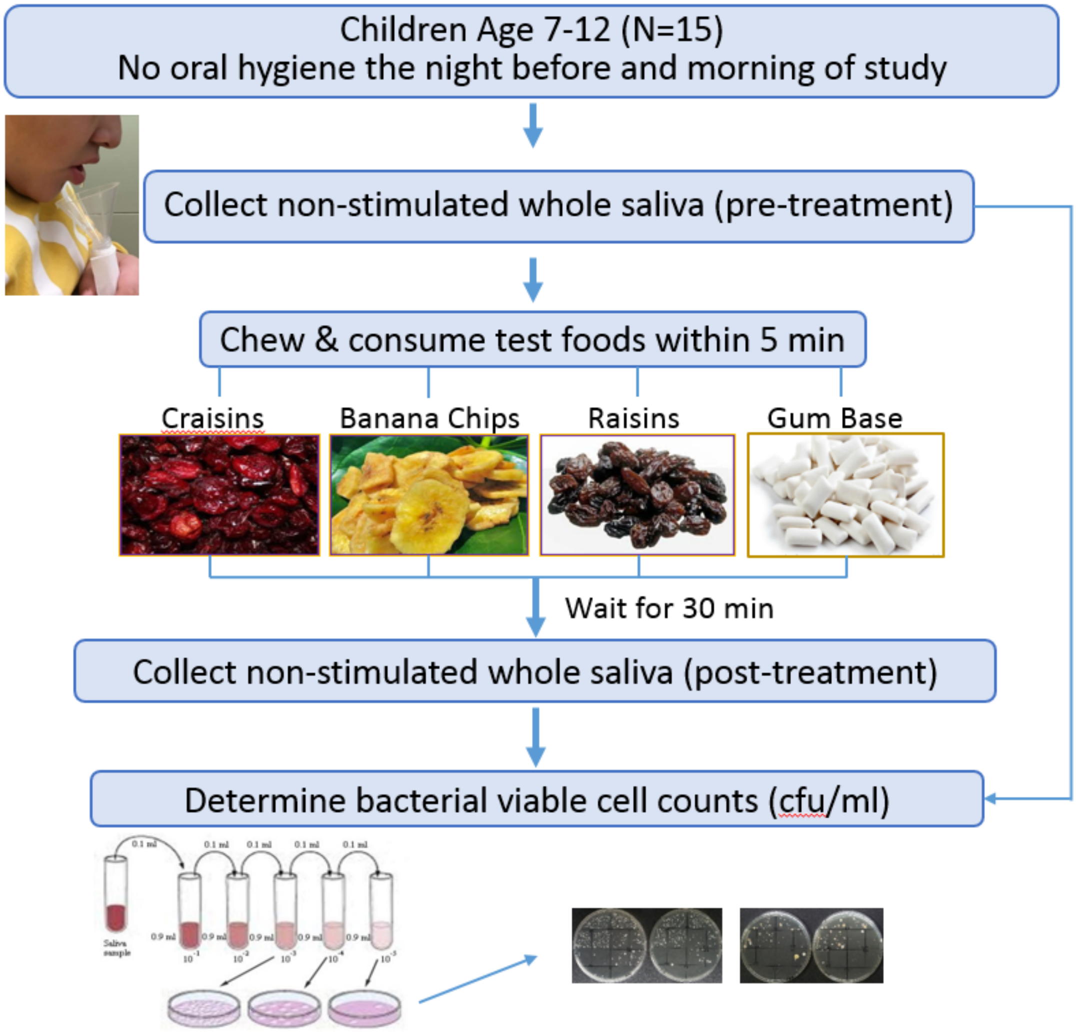
4. **Post Whole Saliva Sample Collections**

Just as before, the samples were collected using the passive drool method for five minutes. The participant was seat in a chair, directed to tilt head down and passively drool without swallowing for the directed amount of time. During the procedure, the saliva was collected via a disposable funnel 65mm x 69mm (*Simport*®, 462021, Bernard-Pillon, Canada) into a removable capped 15ml Conical tube (*Falcon®*, 352097, Corning Science, Reynosa, Tamaulipas, Mexico) for storage, and labeled with an identification number (Post#1 -#15). All tubes containing saliva samples ("post-") were stored on ice until laboratory processing.

5. Determination of Viable Bacterial count in Saliva Samples

All saliva samples ("Pre-"and "Post-") were stored in ice immediately after collection and transported to the microbiology laboratory for further analysis. Saliva samples serially diluted 10-fold and 20 µL of each dilution were plated on to BHI argot plate containing 0.001% hemin and 0.0001% vitamin K. On plates were incubated at 37°C anaerobically (anaerobic chamber, 5% CO_2; 10% H_2; 85% N_2) for 48 hrs. Viable colony counts were enumerated and expressed as CFU/ml. The (% Pre-) = $CFU_{post}/CFU_{Pre;}$ % bacterial reduction = 100% - % Pre.

Figure I. Study Design



6. Statistical Analysis

To compare the % change of bacterial counts after chewing, between different foods, GEE (Generalized Estimating Equation) model was used to take account of within-subject correlation. Outcome was transformed using $\log_{10}$ base. Some descriptive statistics of mean (std) and proportions were checked and their correlation with age and gender were tested using Pearson correlation and t-test. No statistical association between outcome and gender or age was detected, so final GEE model was conducted without them.

IV. Results

This randomized controlled crossover study included fifteen healthy children subjects between the ages of 7 and 12. Thirty children (eligible subjects) were screened. Of the thirty, only twenty qualified for the study (qualified subjects). Of these twenty subjects, only fifteen subjects completed the study. Reasons for incompletion included inability to follow protocol, unpleasant taste of test foods and absent for appointment. Figure II represents the range of age and sex of each subject, the mean age was 9 years old and more females than males were enrolled and completed the study.

The salivary bacterial counts before and after chewing test foods were determined. As shown in Table I, chewing craisins did not increase bacterial counts in saliva. The viable bacteria counts reduced by 3.39%. Table II shows salivary bacterial counts (CFU/ml) before and after consuming raisins. Unlike craisins, chewing raisins did not reduce saliva bacterial counts but an increased of approximately 39% was observed. Like raisins, consuming banana chips showed an increase in salivary bacterial counts (~18%, Table III). After chewing a flavorless gum base, the saliva bacterial counts also showed an increase of approximately 25 % (Table IV). In summary, the only test food group that did not demonstrate an increase in bacterial counts after consumption was craisins (Table V). Figure II is a bar graph to easily visualize the % increase and reduction of salivary bacteria for each test food. As noted, craisins were the only test food to show a reduction of saliva bacteria counts after consuming. Our study consisted of four males and eleven females ranging from ages 7-11 (Figure III). Figure IV represents a Pearson correlation showing that there is a significant reduction in bacterial counts supporting our hypothesis with craisins ($P<.05$). Figure V shows a Chi square analysis of each test food compared with each other. There is significant reduction in bacteria counts with comparing craisins vs. banana chips and craisins with raisins. These results help support our hypothesis that craisins are showing a significant reduction comparatively to raisins and banana chips. Figure VI provides the mean net reduction of salivary bacteria after test food consumption. It displays the change from post/pre while showing the average percent difference in reduction with a '+'.

TABLE I
Salivary bacterial counts (colony forming unit CFU/ml) before and after consuming Craisins (N=15)

Subjects #	pre	post	% post/pre*	% Reduction**
#1	5.31E+07	2.35E+07	44.20	55.80
#2	4.43E+07	4.20E+07	94.80	5.20
#3	6.10E+07	4.50E+07	73.77	26.23
#4	8.15E+06	8.50E+06	104.29	-4.29
#5	3.60E+07	3.00E+07	83.32	16.68
#6	2.15E+07	2.52E+07	117.48	-17.48
#7	1.41E+08	1.72E+08	121.63	-21.63
#8	2.35E+07	3.36E+07	142.77	-42.77
#10	3.09E+07	1.86E+07	60.13	39.87
#13	5.84E+07	3.76E+07	64.38	35.62
#14	2.07E+07	2.29E+07	110.77	-10.77
#15	3.80E+05	6.00E+05	157.89	-57.89
#17	3.96E+06	2.75E+06	69.53	30.47
#19	5.24E+07	5.30E+07	101.15	-1.15
#20	4.22E+07	4.34E+07	102.97	-2.97
avg			96.61	3.39

TABLE II
Salivary bacterial counts (colony forming unit CFU/ml) before and after consuming Raisins (N=15)

Subjects #	pre	post	% post/pre*	% Reduction**
#1	1.19E+07	9.40E+06	78.99	21.01
#2	3.10E+06	8.80E+06	283.87	-183.87
#3	3.40E+06	7.50E+06	220.59	-120.59
#4	2.90E+06	5.55E+06	191.38	-91.38
#5	3.13E+07	2.05E+07	65.44	34.56
#6	3.82E+07	1.40E+08	365.18	-265.18
#7	3.22E+07	3.73E+07	115.68	-15.68
#8	4.15E+07	4.07E+07	98.19	1.81
#10	1.73E+07	1.24E+07	71.90	28.10
#13	6.69E+07	7.50E+07	112.12	-12.12
#14	1.56E+07	1.81E+07	116.40	-16.40
#15	1.10E+06	6.95E+05	63.18	36.82
#17	1.79E+06	1.84E+06	102.51	-2.51
#19	5.00E+07	5.41E+07	108.20	-8.20
#20	5.64E+07	4.95E+07	87.77	12.23
avg			138.76	-38.76

TABLE III
Salivary bacterial counts (colony forming unit CFU/ml) before and after consuming Banana Chips (N=15)

Subjects #	pre	post	% post/pre*	% Reduction**
#1	8.34E+07	6.72E+07	80.56	19.44
#2	1.83E+07	1.85E+07	101.09	-1.09
#3	6.95E+06	6.70E+06	96.40	3.60
#4	6.75E+06	1.07E+07	158.52	-58.52
#5	1.82E+07	3.59E+07	197.25	-97.25
#6	2.01E+07	1.69E+07	84.08	15.92
#7	2.98E+07	4.30E+07	144.13	-44.13
#8	1.98E+07	2.53E+07	127.53	-27.53
#10	1.12E+07	9.85E+06	87.75	12.25
#13	5.99E+07	6.50E+07	108.51	-8.51
#14	1.91E+07	2.18E+07	114.44	-14.44
#15	1.86E+06	2.80E+06	150.27	-50.27
#17	3.90E+06	3.15E+06	80.74	19.26
#19	4.63E+07	5.76E+07	124.41	-24.41
#20	4.12E+07	5.01E+07	121.63	-21.63
avg			118.48	-18.48

TABLE VI
Salivary bacterial counts (colony forming unit CFU/ml) before and after chewing Gum Base (N=15)

Subjects #	pre	post	% post/pre*	% Reduction**
#1	1.73E+07	1.20E+07	69.57	30.43
#2	1.50E+07	1.77E+07	118.06	-18.06
#3	6.85E+08	5.00E+08	72.99	27.01
#4	2.83E+06	2.67E+06	94.51	5.49
#5	2.61E+07	3.00E+07	114.94	-14.94
#6	2.20E+07	1.44E+07	65.38	34.62
#7	3.70E+07	1.30E+08	350.00	-250.00
#8	5.48E+07	6.69E+07	122.02	-22.02
#10	2.01E+06	2.37E+06	117.96	-17.96
#13	5.88E+07	7.00E+07	118.96	-18.96
#14	1.22E+07	1.15E+07	93.85	6.15
#15	1.04E+06	2.03E+06	194.71	-94.71
#17	2.04E+06	2.06E+06	100.98	-0.98
#19	5.73E+07	5.05E+07	88.21	11.79
#20	3.42E+07	4.98E+07	145.83	-45.83
avg			124.53	-24.53

*Post/Pre= % of Control
** % Reduction=1 – Post/Pre (%)

TABLE V
Effect of Consuming Dried Fruits on Children's Total Salivary Bacteria

	% (post/pre)	% reduction
Craisin	96.61	3.39
Raisin	138.7603	-38.76
Banana Chips	118.4874	-18.49
Gum base	124.5317	-24.53

Figure II. Effect of Consuming Dried Fruits on Children's Total Salivary Bacteria (% = CFUpost/CFUpre)

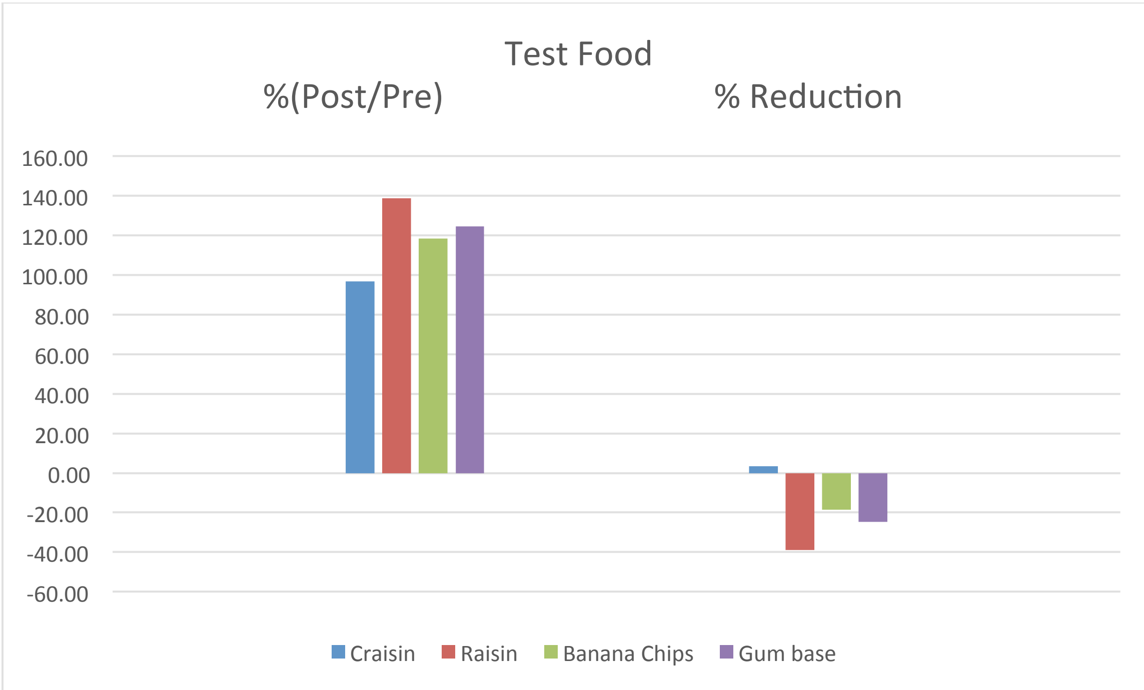


Figure III. Study Demographics (N=15)

Age	Gender	N=15
7	F	5
7	M	2
8	F	2
8	M	1
9	F	2
9	M	1
10	M	1
11	F	2

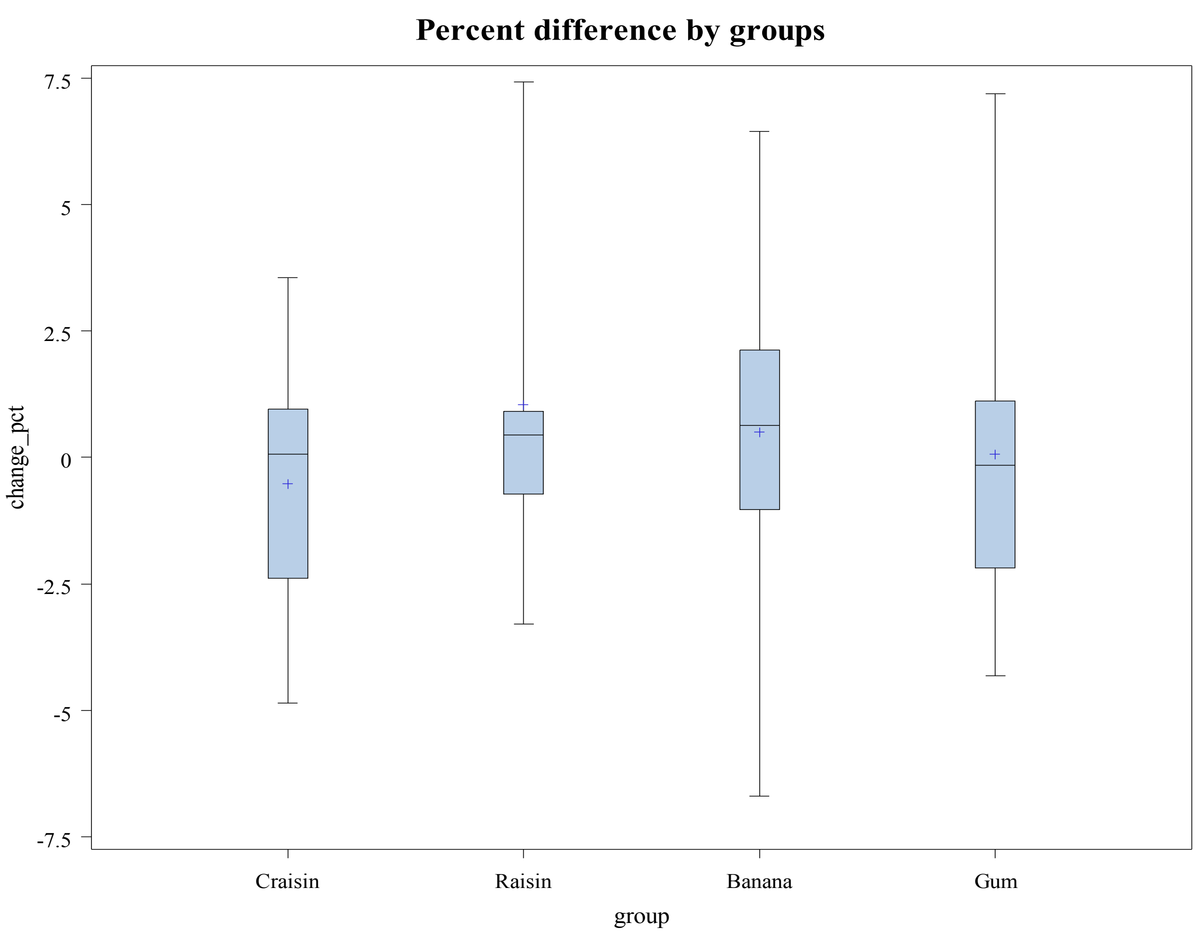
Figure IV. Pearson Correlation number of visits (N=15)

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations	
	age
c_pctchange	-0.07766 0.7832 15
r_pctchange	-0.37192 0.1722 15
b_pctchange	-0.15212 0.5884 15
g_pctchange	-0.11726 0.6773 15
c_pre	-0.55502 0.0317 15
c_post	-0.61847 0.0140 15
r_pre	-0.36576 0.1800 15
r_post	-0.49128 0.0629 15
b_pre	-0.19714 0.4813 15
b_post	-0.25250 0.3639 15
g_pre	-0.44056 0.1003 15
g_post	-0.48251 0.0685 15

Figure V. Effect of consuming dried fruits on children's total salivary bacteria: Chi Square Analysis

Contrast Estimate Results										
		Mean					L'Beta			
Label	Mean Estimate	Confidence Limits		L'Beta Estimate	Standard Error	Alpha	Confidence Limits		Chi-Square	Pr > ChiSq
banana vs gum	0.6024	-0.7924	1.9971	0.6024	0.7116	0.05	-0.7924	1.9971	0.72	0.3973
craisin vs gum	-0.5377	-1.7328	0.6575	-0.5377	0.6098	0.05	-1.7328	0.6575	0.78	0.3779
raisin vs gum	1.6316	-0.3806	3.6437	1.6316	1.0266	0.05	-0.3806	3.6437	2.53	0.1120
craisin vs banana	-1.1400	-1.9912	-0.2888	-1.1400	0.4343	0.05	-1.9912	-0.2888	6.89	0.0087
raisin vs banana	1.0292	-1.0945	3.1530	1.0292	1.0836	0.05	-1.0945	3.1530	0.90	0.3422
craisin vs raisin	-2.1692	-3.9854	-0.3531	-2.1692	0.9266	0.05	-3.9854	-0.3531	5.48	0.0192

Figure VI. Effect of consuming dried fruits on children's total salivary bacteria



V. DISCUSSION

Dental caries is one of the most prevalent chronic diseases, and it affects U.S. children significantly. Since the introduction of water fluoridation in 1940s, there has been a decline in the caries prevalence; however dental caries still remains a national issue (Guideline on Fluoride Therapy; Brunelle and Carlos, 2015). Since dental plaque and dental caries are closely associated, mechanical removal of plaque and food debris has been emphasized for caries prevention. In recent years, effort has been made to investigate foods and diet that are anti-cariogenic and beneficial for oral health (Ferrazzano *et al*., 2009). Dietary plant-derived polyphenols, which can be found in many plants such as grapes (raisins), tea plant, cranberries (craisins), apples, and cherries (Pandey and Rizvi, 2009). Their potential for anti-caries and anti-gingivitis properties has been suggested and attracted attention in preventive dentistry research (Gupta *et al*., 2011; Yoo *et al.*, 2015). These components have been widely used in the food industry and are commonly consumed in the forms of dried fruit snacks and fruit beverages. In this study, we examined the effects of various commercially available dried fruits viability of oral bacteria in the saliva of children. Our study has found that select dried fruits may inhibit and possibly even have a germ kill effect on children's oral bacteria.

The human oral cavity is colonized by numerous and diverse microorganisms. These bacteria constitute complex microbial communities on intraoral surfaces, and dental plaque microbiota that form on the teeth are the cause of two major oral diseases, dental caries and periodontitis. *Streptococci mutans* are the major etiologic agent of dental caries and *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola* are prime suspects in periodontitis (Loesche, 1986). Saliva is a biological fluid secreted from the salivary glands into the oral cavity and contains bacteria shed from adhering microbial communities on various intraoral surfaces, including tooth surfaces, gingival crevices, tongue dorsum, and buccal mucosa. Oral bacteria in a planktonic state (as in saliva) are not generally regarded as direct causal agents of the oral diseases. However, intraoral transmission of pathogenic bacteria is likely to be mediated by bacteria dispersed by saliva (Greenstein, 1997).

In this study we recruited subjects from the ages of 7 to 12 and 15 of them were eligibly qualified. Of these subjects, 11 of them were females and 4 males with a mean age of 9. The subjects were of all race and ethnicities. The passive drool method was utilized to properly collect un-stimulated saliva samples before and 30 minutes after consumption of a test food. The drool method we used was the most simple and universally accepted method for saliva collection. The saliva flow of children seemed to vary based on age, showing a direct correlation between saliva flow and increase in age. We noticed that young children find it harder to passively drool saliva into the collection tube without swallowing throughout the 5 minutes. Older children seemed to follow directions and logically think through the task of drooling allowing ample amount of saliva to be collected. Also younger children were shown to spit more into the collection tube instead of allowing saliva to drool and be collected. They often pooled saliva in their mouth and forcefully spit resulting in the collection of particles to enter the collection tube. Children who discontinued with the study were younger. They were unable to follow protocol or did not like the taste of certain test foods such as raisins or craisins. Those who did not return for the scheduled appointments were excluded from the study. Almost all studies performed to date have been carried out on adults however when working with children, it was challenging to regulate and stay consistent with collection. Once collected these samples were placed on ice and brought to the microbiology laboratory, serially diluted and plated on agar plates. We found the method for bacterial colonies counting to yield consistent results.

In the past decades, mouth rinses with antimicrobial or antiplaque compounds have been popular for plaque control. These rinses have been proven to aid in oral health by reducing plaque bacteria. For example, Listerine, an antimicrobial mouth rinse, has been shown to kill microorganisms in 10 to 30 seconds; the microbes includes methicillin-resistant *Staphylococcus aureus, Streptococcus pyogenes, Helicobacter pylori, Candida albicans, Streptococcus mutans, Actinomyces viscosus, Porphyromonas gingivalis, Prevotella intermedia,* and *Actinobacillus actinomycetemcomitans* (Okuda, 1998). There are also mouth rinses containing natural and artificial compounds that promote plaque control. Antimicrobial polyphenolic compounds found in raisins or dried cranberries have been shown to inhibit bacteria growth but whether or not they affect oral bacteria in plaque or saliva is not well understood. Our study showed that chewing and consumption of craisins reduced bacterial level in children's saliva. Chewing and consuming banana chips or raisins did not affect bacterial counts in the saliva. Although craisins contain added sugar and are sticky in natural, our results suggest that repeated consumption over a short snacking time may reduce saliva bacterial count, therefore, may benefit oral health. Further studies are warranted.

Dried fruits, such as raisins, cranberries and dried plums have been associated with dental caries due to their sweet and stickiness (Sadler, 2016). However, studies have shown that sticky foods are not necessarily retentive and may be cleared relatively fast from the oral cavity and may not be as harmful as other commonly consumed sugary snacks or candies. The red pigments in dried cranberries (Craisins) and have been shown to inhibit biofilm formation (Bonifait, 2010). These proanthocyanidins [PACs] are the most abundant phytochemicals extracted from cranberry fruit, and have been reported to possess antimicrobial, anti-adhesion, antioxidant, and anti-inflammatory properties. These unique flavonoids may play an important preventive role in oral infections Like Teflon, they prevent the attachment of pathogens to host tissues, and can inhibit the formation of biofilms in the mouth. Cranberry PACs inhibited adherence and biofilm formation by *Porphyromonas gingivalis*, a bacterium associated with gum disease, and markedly reduced its invasiveness (Feghali, 2002).

Wu's lab has reported that raisins contain plant-derived antimicrobial polyphenols that inhibited oral pathogens associated with dental caries. Consumption of raisins did not reduce plaque pH below 5.5, the "threshold" pH that is harmful to the enamel (Wu, 2009). In this study consuming raisins did not reduce saliva bacteria as much as craisins. But again, studies with more consumption of these dried fruits need to be performed.

Children's frequent consumption of sugar-sweetened snacks is one of the risk factors for dental caries. Investigating the anti-cariogenic potential of natural foods or commercially available beverages containing dietary plant-derived polyphenols can help the consumers, especially children, make better decisions in their diet to promote oral health. The awareness of healthy diet should not only emphasize on general health but also promote oral health, which is intricately associated with overall well being. The concept of oral disease prevention or oral health promotion using natural foods or beverages from the diet is a novel, practical, and acceptable approach that also promotes overall health and wellness. If sequenced properly between meals and oral hygiene, selected dried fruits may have added oral health benefits by controlling, “through prevention”, the most prevalent infectious diseases of mankind.

VI. CONCLUSIONS

The following conclusions can be drawn from this study:

1. Consumption of dried fruits, such as craisins, reduced bacterial level in children's saliva. They may represent a healthier alternative over the popular sugary snacks.
2. If sequenced properly between meals and oral hygiene, selected dried fruits may have added oral health benefits by controlling, "through prevention", the most prevalent infectious diseases of mankind.
3. The concept of oral diseases prevention using natural foods or beverages in the diet may be a novel, practical, and acceptable approach to promote oral health in children.

VII. CITED LITERATURES

Abd Allah, A. A., Ibrahium, M. I., Al-atrouny, A. M.: Effect of dried fruit on some cariogenic bacteria. World Appl. Sci. J. 12 (4): 552-558, 2011.

Aimutis, W. R.: Bioactive properties of milk proteins with particular focus on anticariogenesis. J. Nutr. 134(4): 989S-95S, 2004

American Academy of Pediatric Dentistry. Guideline on Fluoride Therapy. Reference Manual. 36(6):171-174, 2015.

American Academy of Pediatric Dentistry. Policy on Dietary Recommendations for Infants, Children, and Adolescents. Reference Manual. 36(6):56-58, 2015.

Anderson, R. A.: Chromium and polyphenols from cinnamon improve insulin sensitivity. Proc Nutr Soc. 67(1): 48-53, 2008.

Armfield, J. M., Spencer, A. J., Roberts-Thompson, K. F., Plastow, K.: Water fluoridation and the association of sugar-sweetened beverage consumption and dental caries in Australian children. Am J Public Health. 103: 494-500, 2013.

Bonifait, L., Grenier, D.: Cranberry Polyphenols: Potential Benefits for Dental Caries and Periodontal Disease. J. Can. Dent. Assoc. 76:a130, 2010.

Bratthall, D. and Hänsel Petersson, G. (2005), Cariogram – a multifactorial risk assessment model for a multifactorial disease. Community Dentistry and Oral Epidemiology, 33: 256–264.

Brunelle J, Carlos J.: Recent trends in dental caries in U.S. children and the effect of water fluoridation. Journal of Dental Research. 69:723-727, 2015.

Dawes C. (2005): Effects of Diet on Salivary Secretion and Composition, J Dent Res 49: 1263–1272.

Dawes C, Dong C. The flow rate and electrolyte composition of whole saliva elicited by the use of sucrose-containing and sugar-free chewing-gums. Arch Oral Biol. 1995 Aug; 40(8): 699–705.

Duarte S, Gregoire S, Singh A et al.: Inhibitory effects of cranberry polyphenols on formation and acidogenicity of Streptococcus mutans biofilms. FEMS Microbiology Letters. 257(1):50-56, 2006.

Feghali K, Feldman M, Dang La V, Santos J, Grenier D. Cranberry proanthocyanidins: natural weapons against periodontal diseases. J Agric Food Chem. 2012 Jun 13; 60(23): 5728–5735. Published online 2011 Nov 29

Ferrazzano G, Amato I, Ingenito A, De Natale A, Pollio A.: Anti-cariogenic effects of polyphenols from plant stimulant beverages. Fitoterapia. 80(5):255-262, 2009.

Graf, B.A., Milbury, P. E., Blumberg, J. B.: Flavonols, flavonones, flavanones and human health: Epidemiological evidence. J. Med. Food. 8:281-290, 2005.

Greenstein G, Lamster I. Bacterial transmission in periodontal diseases: a critical review. J Periodontol. 1997 May;68(5):421-31.

Gupta C, Kumari A, Garg A, Catanzaro R, Marotta F.: Comparative study of cinnamon oil and clove oil on some oral microbiota. ACTA BIOMED. 82:197-199, 2011.

Jeon J, Rosalen P, Falsetta M, Koo H.: Natural Products in Caries Research: Current (Limited) Knowledge, Challenges and Future Perspective. Caries Res. 45(3):243263, 2011.

Kontiokari T, Salo J, Eerola E, Uhari M.: Cranberry juice and bacterial colonization in children—A placebo-controlled randomized trial. Clinical Nutrition. 24(6):10651072, 2005.

Koo H, Nino de Guzman P, Schobel B, Vacca Smith A, Bowen W.: Influence of Cranberry Juice on Glucan-Mediated Processes Involved in Streptococcus mutans Biofilm Development. Caries Research. 40:20-27, 2006.

Okuda K, Adachi M, Iijima K. The efficacy of antimicrobial mouth rinses in oral health care.Bull Tokyo Dent Coll. 1998 Feb; 39(1): 7–14.

Pandey K, Rizvi S.: Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. Oxidative Medicine and Cellular Longevity. 2(5):270-278, 2009.

Pelczar M, Chan E, Krieg N.: Control of microorganisms, the control of microorganisms by physical agents. In: Microbiology. McGraw-Hill International. 469-509, 1988.

Petersen P.: The World Oral Health Report 2003: continuous improvement of oral health in the 21st century -- the approach of the WHO Global Oral Health Programme. Community Dentistry and Oral Epidemiology. 31:3-24, 2003.

Qin, B., Dawson, H.D., Schoene, N.W., Polansky, M. M., Anderson, R.A.: Cinnamon polyphenols regulate multiple metabolic pathways involved in insulin signaling and intestinal lipoprotein metabolism of small intestinal enterocytes. Journal of Nutrition. 28: 1172-1179, 2012.

Ramos-Nono, M. E., Cliford, M. N., Adams, M. R.: Quantitative structure activity relationship for the effect of benzoic acid, cinnamic acids and benzaldehyde on *Listeria monocytogenes*. J. Appl. Microbiol. 80: 303-310, 1996.

Riihinen, K. R., Ou, Z. M., Gödecke, T., Lankin, D. C., Pauli, G. F., Wu, C. D.: The antibiofilm activity of lingonberry flavonoids against oral pathogens is a case connected to residual complexity. Fitoterapia. 97:78-86, 2014.

Rivero-Cruz, J. F., Zhu, M., Kinghorn, A. D., & Wu, C. D. (2008). Antimicrobial constituents of Thompson seedless raisins (Vitis vinifera) against selected oral pathogens. Phytochemistry Letters, 1(3), 151-154.

Sadler, MJ. Dried Fruit and Dental Health. Int J Food Sci Nutr. 2016 Dec;67(8):944-59 Epub 2016 Jul 14.

Schilling, K. M., Bowen, W. H.: Glucans synthesized in situ in experimental salivary pellicle function as specific binding sites for *Streptococcus mutans*. Infect. Immun. 60: 284295, 1992.

Tahmassebi, J., Duggal M., Malik-Kotru G., Curzon M.: Fruits and dental health: A review of the current literature. Journal of Dentistry. 34(1):2-11, 2006.

Usha C, R S. Dental caries - A complete changeover (Part I). Journal of Conservative Dentistry : JCD. 2009;12(2):46-54. doi:10.4103/0972-0707.55617. Dodds MWJ, Johnson DA, Yeh C-K. Health Benefits of saliva: a review. J Dent 2005; 33: 223-233.

Wu, C., Wei, G.,: Food Constituents And Oral Health: current status and future prospect. Woodhead Publishing, Cambridge, United Kingdom. 396-417, 2009.

Xu, X., Zhou X., Wu C.: Tea catechin epigallocatechin gallate inhibits Streptococcus mutans biofilm formation by suppressing gtf genes. Archives of Oral Biology. 57(6):678-683, 2012.

Yamanaka, A., Kimizuka, R., Kato, T., Okuda, K.: Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbial. Immunol. 19:150-154, 2004.

Yoo, S., Murata R., Duarte S.: Antimicrobial traits of tea- and cranberry-derived polyphenols against Streptococcus mutans. Caries Research. 45:327-335, 2015.

Zero, D.: Etiology of dental erosion extrinsic factors. European Journal of Oral Sciences. 104(2):162-177, 1996.

Zhu, M., Carvalho, R., Scher, A., Wu, C.D.: Short-term germ-killing effect of sugar-sweetened cinnamon chewing gum on salivary anaerobes associated with halitosis. J. Clin dent. 22(1):23-2, 2011.

VIII.
APPENDIX A
Initial IRB Review

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Initial Review (Response To Modifications)

July 25, 2016

Lucas Carubia, DDS
Pediatric Dentistry
801 S. Paulina Street
M/C 850
Chicago, IL 60612
Phone: (312) 545-5592 / Fax: (312) 996-1994

RE: Protocol # 2016-0634
"The Effect of Dried Fruits on Children's Salivary Bacteria"

Dear Dr. Carubia:

Your Initial Review (Response To Modifications) was reviewed and approved by the Expedited review process on July 22, 2016. You may now begin your research

Please note the following information about your approved research protocol:

Protocol Approval Period: July 22, 2016 - July 22, 2017
Approved Subject Enrollment #: 30
Additional Determinations for Research Involving Minors: The Board determined that this research satisfies 45CFR46.404, research not involving greater than minimal risk. Therefore, in accordance with 45CFR46.408, the IRB determined that only one parent's/legal guardian's permission/signature is needed. Wards of the State may not be enrolled unless the IRB grants specific approval and assures inclusion of additional protections in the research required under 45CFR46.409. If you wish to enroll Wards of the State contact OPRS and refer to the tip sheet.

Performance Sites: UIC
Sponsor: None
Research Protocol(s):

a) The Effect of Dried Fruits on Children's Salivary Bacteria; Version 1.1; 07/13/2016

Recruitment Material(s):

a) Volunteers Needed Flyer; Version 1.1; 07/13/2016
b) Telephone Script; Version 1.2; 07/22/2016

Assent(s):

a) The Effect of Dried Fruits on Children's Salivary Bacteria Assent; Version 1.2; 07/22/2016

Parental Permission(s):

a) The Effect of Dried Fruits on Children's Salivary Bacteria; Version 1.1; 07/13/2016

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific category:

(3) Prospective collection of biological specimens for research purposes by noninvasive means. Examples: (a) hair and nail clippings in a nondisfiguring manner; (b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction; (c) permanent teeth if routine patient care indicates a need for extraction; (d) excreta and external secretions (including sweat); (e) uncannulated saliva collected either in an unstimulated fashion or stimulated by chewing gumbase or wax by applying a dilute citric solution to the tongue; (f) placenta removed at delivery; (g) amniotic fluid obtained at the time of rupture of the membrane prior to or during labor; (h) supra- and subgingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylactic scaling of the teeth and the process is accomplished in accordance with accepted prophylactic techniques; (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; (j) sputum collected after saline mist nebulization.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
06/13/2016	Initial Review	Expedited	06/24/2016	Modifications Required
07/14/2016	Response To Modifications	Expedited	07/22/2016	Approved

Please remember to:

→ Use your **research protocol number** (2016-0634) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-9680. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jovana Ljuboje
IRB Coordinator, IRB #3
Office for the Protection of Research Subjects

Enclosures to be sent via separate email:

1. **Assent Document(s):**
   a) The Effect of Dried Fruits on Children's Salivary Bacteria Assent; Version 1.2; 07/22/2016
2. **Parental Permission(s):**
   a) The Effect of Dried Fruits on Children's Salivary Bacteria; Version 1.1; 07/13/2016
3. **Recruiting Material(s):**
   a) Volunteers Needed Flyer; Version 1.1; 07/13/2016
   b) Telephone Script; Version 1.2; 07/22/2016

cc: Marcio Da. Fonseca, Pediatric Dentistry, M/C 850
Christine D. Wu, Faculty Sponsor, M/C 850

APPENDIX B
Approval Notice Continuing Review

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Initial Review (Response To Modifications)

July 25, 2016

Lucas Carubia, DDS
Pediatric Dentistry
801 S. Paulina Street
M/C 850
Chicago, IL 60612
Phone: (312) 545-5592 / Fax: (312) 996-1994

RE: Protocol # 2016-0634
"The Effect of Dried Fruits on Children's Salivary Bacteria"

Dear Dr. Carubia:

Your Initial Review (Response To Modifications) was reviewed and approved by the Expedited review process on July 22, 2016. You may now begin your research

Please note the following information about your approved research protocol:

Protocol Approval Period: July 22, 2016 - July 22, 2017
Approved Subject Enrollment #: 30
Additional Determinations for Research Involving Minors: The Board determined that this research satisfies 45CFR46.404, research not involving greater than minimal risk. Therefore, in accordance with 45CFR46.408, the IRB determined that only one parent's/legal guardian's permission/signature is needed. Wards of the State may not be enrolled unless the IRB grants specific approval and assures inclusion of additional protections in the research required under 45CFR46.409. If you wish to enroll Wards of the State contact OPRS and refer to the tip sheet.
Performance Sites: UIC

Sponsor: None

Research Protocol(s):

b) The Effect of Dried Fruits on Children's Salivary Bacteria; Version 1.1; 07/13/2016

Recruitment Material(s):

c) Volunteers Needed Flyer; Version 1.1; 07/13/2016
d) Telephone Script; Version 1.2; 07/22/2016

Assent(s):

b) The Effect of Dried Fruits on Children's Salivary Bacteria Assent; Version 1.2; 07/22/2016

Parental Permission(s):

b) The Effect of Dried Fruits on Children's Salivary Bacteria; Version 1.1; 07/13/2016

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific category:

(3) Prospective collection of biological specimens for research purposes by noninvasive means. Examples: (a) hair and nail clippings in a nondisfiguring manner; (b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction; (c) permanent teeth if routine patient care indicates a need for extraction; (d) excreta and external secretions (including sweat); (e) uncannulated saliva collected either in an unstimulated fashion or stimulated by chewing gumbase or wax by applying a dilute citric solution to the tongue; (f) placenta removed at delivery; (g) amniotic fluid obtained at the time of rupture of the membrane prior to or during labor; (h) supra- and subgingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylactic scaling of the teeth and the process is accomplished in accordance with accepted prophylactic techniques; (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; (j) sputum collected after saline mist nebulization.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
06/13/2016	Initial Review	Expedited	06/24/2016	Modifications Required
07/14/2016	Response To Modifications	Expedited	07/22/2016	Approved

Please remember to:

→ Use your **research protocol number** (2016-0634) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-9680. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jovana Ljuboje
IRB Coordinator, IRB #3
Office for the Protection of Research Subjects

Enclosures to be sent via separate email:

4. **Assent Document(s):**
   b) The Effect of Dried Fruits on Children's Salivary Bacteria Assent; Version 1.2; 07/22/2016
5. **Parental Permission(s):**
   b) The Effect of Dried Fruits on Children's Salivary Bacteria; Version 1.1; 07/13/2016
6. **Recruiting Material(s):**
   c) Volunteers Needed Flyer; Version 1.1; 07/13/2016
   d) Telephone Script; Version 1.2; 07/22/2016

cc: Marcio Da. Fonseca, Pediatric Dentistry, M/C 850
Christine D. Wu, Faculty Sponsor, M/C

APPENDIX C
Consent

APPROVAL
STARTS — EXPIRES
07/22/2016 — 07/22/2017
UIC UNIVERSITY OF ILLINOIS AT CHICAGO
INSTITUTIONAL REVIEW BOARD

University of Illinois at Chicago
Research Information and Parental Permission for Participation in Biomedical Research
The Effect of Dried Fruits on Children's Salivary Bacteria

Your child is being asked to participate in a research study. Researchers are required to provide a consent form such as this one to tell you about the research, to explain that taking part is voluntary, to describe the risks and benefits of participation, and to help you to make an informed decision. You should feel free to ask the researchers any questions you may have.

Principal Investigator Name and Title: Dr. Lucas Carubia, Pediatric Dentistry Resident
Department: Pediatric Dentistry
Address and Contact Information: 801 S. Paulina Street, Room 469, College of Dentistry, University of IL at Chicago, Chicago, Il 60612
Emergency Contact Name and Information: Dr. Christine D. Wu, 312-355-1990

Why am I being asked?

Your child is being asked to be a subject in a research study about the effect dried fruits have on oral health because you have responded to our flyer, and your child is between 7-12 years of age and may be eligible to participate.

Dental caries also known as tooth decay is a very serious problem among children. Diet, especially sugars, play an important role in causing dental caries. It is a common chronic disease that results from an increased production of acid by bacteria in the mouth. Dried fruits, such as raisins and cranberries have been thought to cause dental caries because they are sticky, sweet, and cling to the teeth. The plaque bacteria on tooth surfaces use these sugars and produce acids. The acids dissolve the enamel of the tooth, which leads to tooth decay. However, research has shown that some dried fruits contain plant-derived antimicrobial compounds that may inhibit oral bacteria associated with dental caries. These antimicrobials may also kill oral bacteria in saliva.

Please read this form and ask any questions you may have before agreeing to allow your child to participate in the research study.

Your child's participation in this research is voluntary. Your decision whether or not to allow your child to participate will not affect your current or future dealings with the University of Illinois at Chicago. If you decide to participate, you are free to withdraw from the study at any time without affecting the relationship with the University of Illinois at Chicago

What is the purpose of this research?

The purpose of this consent is to better understand the effect of eating dried fruits on oral health in children.

What procedures are involved?

This research will be performed at the College of Dentistry at 801 S. Paulina Street, Chicago, IL, 60612. Your child will need to come to the study site at least four times including this visit. Each of the visits will take about 30-45 minutes.

Study procedures:

- Before each visit, we request that your child does not brush, floss or use a mouthrinse the night before and the morning of the test. Also your child should not eat or drink for 2 hours prior to the test.
- The test will involve collecting saliva using a passive drool techique.
- Your child will be asked to rinse his/her mouth. Shortly after, saliva will be collected using the passive drool method. Your child will be seated and will be asked to swallow and then with head tilted down, allow the saliva to drain off the lower lip into a Salimetric passive drool kit for 5 minutes. The tube with saliva is placed immediately into ice after collection. After this your child will be asked to chew 20 g (4 tsp) of a test food within five minutes. She/he will then wait for another 30 minutes. After this, your child will be asked to rinse his/her mouth very lightly just to remove any food particles remaining and then saliva will be collected again using the same methods previously described.
- At each visit, we will give your child one test food to eat. The test food groups will be randomly assigned (that is, random just like flipping a coin) for each visit. The four different food groups are 1) gum base, 2) raisins, 3) craisins (dried cranberries) and 4) dried banana.
- The estimated time for each visit is 30-45 minutes. There is a minimum of 1 day in between each visit.

What are the potential risks and discomforts?

There are no known risks from collection of salvia using the passive drool technique. To protect confidentiality, your child's information will only be identified by a numeric code.

Will I be told about new information that may affect my decision to participate?

During the course of the study, you will be informed of any new findings (either good or bad), such as changes in the risks or benefits resulting from participation in the research or new alternatives to participation that might cause you to change your mind about continuing in the study. If new information is provided to you, your consent to continue your child's participation in this study may be re-obtained.

Are there benefits to taking part in the research?

You or your child will not directly benefit from participation in the research. It is hoped that knowledge gained from this research may benefit all to understand the effect of dried fruits on oral health in children in the future.

What about privacy and confidentiality?

A possible risk of research is that your child's participation in the research or information about your child and your child's health might become known to individuals outside the research. To protect confidentiality, your child will only be identified by a numeric code. No information about your child, or provided by you, during the research, will be disclosed to others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care or when the UIC Office for the Protection of Research Subjects monitors the research or consent process) or if required by law.

Study information which identifies you and the consent form signed by you will be looked at and/or copied for examining the research by:

- UIC Office for the Protection of Research Subjects, State of Illinois Auditors, or
- Principal Investigator.

Your child's information will only be identified by a numeric code and your child's name will not be included with the data. Two lists will be assembled for this study: one list links your child's name, date of birth and contact information with his or her numeric code, and the second list will be of your child's study data identified only by the assigned numeric code. The numeric identifiers will be removed once the study is completed. The de-identified data will be stored in a locked file cabinet within a locked room. When the results of the research are published or discussed in conferences, no information will be included that could reveal your child's identity.

What are the costs for participating in this research?

There are no costs to you or your child for participating in this research.

Will I be reimbursed for any of my expenses or paid for my participation in this research?

You will receive $15 for each completed study visit. If you wish to withdraw at any time from the study and do not complete all the visits in the study, you will be compensated for the visits you have completed. If you complete the study, you will receive a total of $60 for completing the four study visits at the end of the study.

Can I withdraw or be removed from the study?

If you allow your child to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at UIC.

The researchers also have the right to stop your child's participation in this study without your consent if, they believe it is in your child's best interests.

In the event you withdraw or are asked to leave the study, you will still be compensated for the visits that you participated.

<u>Who should I contact if I have questions?</u>

Contact the researchers Dr. Christine D. Wu at 312-355-1990 or email address: Chriswu@uic.edu

- if you have any questions about this study or your part in it,
- if you feel you have had a research-related injury (or a bad reaction to the study treatment), and/or
- if you have questions, concerns or complaints about the research.

<u>What are my rights as a research subject?</u>

If you have questions about your rights as a research subject or concerns, complaints, or to offer input you may call the Office for the Protection of Research Subjects (OPRS) at 312-996-1711 or 1-866-789-6215 (toll-free) or e-mail OPRS at uicirb@uic.edu.

<u>Remember</u>:

Your child's participation in this research is voluntary. Your decision whether or not to let him/her participate will not affect your current or future relations with the University. If you allow your child to participate, you are free to withdraw him/her at any time without affecting that relationship.

<u>Signature of Parent or Legal Guardian</u>

I have read (or someone has read to me) the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to allow my child to participate in this research. I will be given a copy of this signed and dated form.

____________________________ ___________
Signature of parent or guardian Date

____
Printed Name of parent or guardian

____
Printed Name of Child

____________________________ ___________
Signature of Person Obtaining Consent Date

____
Printed Name of Person Obtaining Consent

APPENDIX D
ASSENT

APPROVAL
STARTS 07/22/2016 — EXPIRES 07/22/2017
UNIVERSITY OF ILLINOIS AT CHICAGO INSTITUTIONAL REVIEW BOARD

University of Illinois at Chicago
ASSENT TO PARTICIPATE IN RESEARCH
Form Template – 7/22/16
The Effect of Dried Fruits on Children's Salivary Bacteria

My name is Dr. Lucas Carubia and my research partners are named Dr. Christine Wu, Dr. Larry Salzmann and Dr. Evelina Kratunova. We are asking you to take part in a research study because we are trying to learn more about the effect of dried fruits on oral health. If you agree to be in this study you will visit College of Dentistry 4 times including this visit. At the first visit you will be asked to rinse your mouth with tap water and an initial saliva sample will be collected. You will be asked to swallow and then with your head tilted to let your saliva flow off your lower lip into a collection tube, which is placed immediately into ice after collection. after this, you will be asked to chew 20 g (4tsp) of one of the test foods for five minutes. Your saliva will then be collected again after 30 minutes. The test foods you will be chewing and swallow are raisins, craisins (dried cranberries) and dried banana chips. You will only chew gum base. You will come to the College of Dentistry for three more times to complete the study using the same procedure explained above. We will do laboratory tests to see changes in bacteria numbers in the saliva before and after eating the test foods.

- There is no risk or harm involved in letting us collect your saliva sample. It does not cause any pain.
- The study has no direct benefit to you but will allow us to learn more about the effects of certain foods on oral health.
- Please talk this over with your parents before you decide whether or not to participate. We will also ask your parents to give their permission for you to take part in this study. But even if your parents say "yes" you can still decide not to do this.
- If you don't want to be in this study, you don't have to participate. Remember, being in this study is up to you and no one will be upset if you don't want to participate or even if you change your mind later and want to stop.
- You can ask any questions that you have about the study. If you have a question later that you didn't think of now, you can call me at 312-355-1990 or ask me next time.
- Signing your name at the bottom means that you agree to be in this study. You and your parents will be given a copy of this form after you have signed it.

______________________________ _______________
Name of Subject Date

____________________________ _____ __________
Signature Age Grade in School

Lucas R. Carubia, DDS

lucas.carubia@gmail.com
719-640-1962

EDUCATION

Pediatric Dentistry Residency and Masters in Oral Sciences
June 2017
University of Illinois (Chicago)
Chicago, IL

Doctor of Dental Surgery
May 2015
University of Colorado School of Dental Medicine
Denver, CO

Bachelor of Science in Nutrition Health and Exercise Science
May 2011
University of Nebraska-Lincoln
Lincoln, NE

AWARDS AND HONORS

- Clinic and Research Day-First Place Literature Review
- Delta Sigma Delta Fraternity Leadership Award
- Alpha Lambda Delta Honor Society
- Phi Eta Sigma Honor Society
- Phi Upsilon Omicron Honor Society
- George Beadle Scholarship
- Henry & Julianne Bauermeister Scholarship
- Lincoln Community Scholarship
- FarmHouse Founders Award
- FarmHouse Freshman 4.0 GPA Award
- The National Society of Collegiate Scholars
- International Scholar Laureate Program
- College of Education and Health Science Dean's List (2007-2011)
- Lewis-Palmer High School Valedictorian (2007)