**Proposal Cover Sheet**

**Term:   Fall**  X       **Spring \_\_\_\_\_ Year**2011

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Present Year in Education (e.g., freshman, sophomore, etc.):  Junior

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Have you identified a research mentor for a senior thesis (if applicable)?

\_\_\_\_\_ Yes  \_\_\_\_X\_ No.

If yes, please identify.

Name:  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Title of Proposal:**

Amount of Raisins (*Vitis vinifera*) consumption that is needed to effect the growth and

acid production by *Streptococcus mutans* on dental plaque.

**Checklist:**

All required portions of the first submission are included  \_\_\_\_X \_ Yes  \_\_\_\_\_ No

I had an external reviewer read the proposal  \_\_\_\_\_ Yes  \_\_\_X\_\_ No

If Yes, who \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ When  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

I authorize the use of this proposal as an example in future courses \_\_X\_\_\_ Yes  \_\_\_\_\_ No

**Project Summary**

**Abstract**

Many researches have shown that Raisins contains phytochemicals produce an effect on growth and Acid Production by Streptococcus mutans on dental plaque. The smallest amount of raisins that is needed to be consumed to produce that effect will be examined in this proposal. Eighty adolescents plaque pH will be measured, after consuming raisins from TSG, rinsing with ten percent of sucrose or sorbitol, with a touch microelectrode prior to baseline using time intervals. Although the plaque pH of individuals who consume greater amount of raisins are expecting to be higher, the end result, overall, of all the groups should be the same.

**Keywords**: Thompless seedless Grapes, Raisins, *Streptoccocus mutans*, Dental plaque, Plaque pH

**Table of Contents**

Coversheet……………………………………………………………………………………….1

Project summary)………………………………………………………………………………...2

* Abstract

Table of Contents……………………………………………………………………………….3

Project Description……………………………………………………………………………4-9

* Introduction…………………………………………………………………..4-5
* Reseach Objective………………………………………………………………5
* Method……………………………………………………………………….6-7

1. Data Analysis………………………………………………………...7-8
2. Data Collection………………………………………………………...8

* Expected Results……………………………………………………………....9
* Time Table and Project Management…………………………………………9
* Equipment……………………………………………………………………..9

References Cited……………………………………………………………………………..10

Curriculum Vitae…………………………………………………………………………..11-12

**Introduction**

**Dental plaque** is a colorless biofilm that forms around the tooth surfaces (Leme, 2006).It is also able to attach on every tooth surface, one of which is the irregular surface of the tooth enamel (Leme, 2006). It develops naturallyon teeth, and forms part of the defense systems of the host by helping to prevent colonization of enamel by exogenous and often pathogenic microorganisms (Wu, 2008). The teeth are covered by a film of salivary mucoproteins which is colonized by 350 different kinds of bacteria. One of the most important is Streptococcus mutans, a normal flora, Gram-positive, facultative anaerobic, acid-producing, coccus-shaped bacterium (Wu, 2008). Dental plaque can give rise to dental caries (tooth decay), a multi-factorial infectious disease resulted by the localized destruction of the tissues of the tooth by acid-producing bacteria that cause damage in the presence of fermentable carbohydrates such as sucrose, fructose and glucose (Wu, 2008). The repeated intake of fermentable sugar in the diet produces frequent conditions of low pH in plaque which inhibits the growth of many of the species associated with dental health, and selects for the highly acidogenic (acid-producing) and aciduric(acid-loving) species, such as mutans streptococci (Leme, 2006). Plaque bacteria, such as *Streptococcus mutans*, utilize the readily fermentable carbohydrates on tooth surfaces to produce acids that penetrate the solid tooth surface, leading to enamel demineralization and tooth decay (Wu, 2008). The majority of microbial organisms prefer neutral pH levels (pH 7) (Leme, 2006). The low pH induced by sucrose fermentation triggers a shift in the balance of resident plaque microflora to a more cariogenic one, according to the ecological plaque hypothesis (Marsh, 1991).

Many research showed that Seedless Raisins (Vitis vinifera) is beneficial to oral health and have been consumed as a nutritional healthy food.  One of its benefits is its capability to prevent dental caries by fighting bacteria that cause cavities and gum disease, due to its antimicrobial activity of various phytochemicals, including triterpenoids(Wolska,2010), flavonoids(Shim, 2002) and polyphenols (Karadeniz, 2000).

Flavonoids and triterpenoids have been reported to be antimicrobial against both Gram-positive and Gram-negative species (Pupponen-Pimiä, 2005).).  In fact, several triterpenoid compounds, isolated from a methanol extract of Thompson seedless raisins were found to inhibit bacteria associated with dental caries and periodontal disease (Fausto Rivero-Cruz et al., 2008). Other published studies demonstrated the interaction of pH and the activity of antimicrobial compounds in raisins. For example, phenolic acids have increased antimicrobial properties at the pH range of raisin and raisin products (*Wen* et al., 2003). These acids are more effective against bacteria in the undissociated state, at acidic pH, and thus more effective at preventing outgrowth or eliminating bacterial populations (Puupponen-Pimiä, 2005). Concentrations of gallic acid and protocatechuic acid, both phenolic acids, found in raisins, are also at sufficient levels to inactivate bacteria (Wen, 2003). However, due to Raisins high sugar concentration level, knowing the right amount of consumption is necessary. How much is too much or enough? The purpose of this paper is to determine the amount of raisins consumption that is enough to suppress the growth and reduced the acidity level produced by *streptococcus mutans*

**Research Objectives**

Raisins are perceived as sweet and sticky, food with those characteristics is assumed to cause cavities. However, many researches have shown that raisins containing phytochemicals prevent cavities.  But because our sugar concentration level should also be monitored, we presume knowing the smallest amount consumption of raisins to produce an effect on dental cavity, is crucial.  We will accomplish this by examining the level of dental plaque pH at different intervals, using touch microelectrode methodology. We hypothesized that the higher the amount of raisin consumption, the lower the dental pH level.

**METHODS**

The same methodology that was used by Dr. Wu and her colleagues (Achint, 2009) will also be used for this experiment with a few modifications.

**Study Design**

Thirty subjects (adolescents of 7-11 years of age), who consent were given by their parents, will participate in this randomized controlled study. Each participant will visit the FGCU Microbiology laboratory room between 8 a.m. and 12 p.m; prior to the visit, they will be instructed not to clean their teeth proximally for 3 days, not to brush their teeth the same day, and not to eat or drink for at least 2 hrs prior to the test.

**Plaque pH Measurements**

Plaque pH will be measured by the touch microelectrode method ([Lingström, 1993](http://jdr.sagepub.com/content/85/4/334.full#ref-18)). A salt bridge was established in a 3 M KCl solution between the reference electrode and a finger of the test participant. The electrode was calibrated against standard buffers before each reading ([Scheie, 1992](http://jdr.sagepub.com/content/85/4/334.full#ref-26)). Ten percent sucrose and sorbitol will be used as positive and negative controls. Each subject will randomly assign to one of the four groups. The first group (consisting of 10 subjects) will be asked to consume 20 grams of raisins from Thompson Seedless Grapes (TSG), the second, a group of 5, will be asked to eat 25 grams, and the third (also a group of 5), will consume 30 grams, and the fourth group (which will also be use as a control: subjects with no sign of cavity) will consume 20 grams of raisins. Measurements will perform at two proximal dental sites: in the anterior and in the premolar/molar regions. One site will be located in the lower and the other in the upper jaw. No metal fillings were present at the sites of measurements. After registering the resting pH (baseline = 0 min), a mouthrinse will be performed with 20 mL of 10% sucrose for 1 min. Plaque pH will then be recorded 2, 5, 10, 15, 20 and 30 min after the start of the experiment. During this period, the participants will be told to remain still and to avoid talking. This procedure will then be repeated the following week using Sorbitol as the mouthrinse control.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Controls | Group I | Group II | Group III | Group IV |
| Sorbitol | 10% | 5% | 15% | 10% |
| Sucrose | 10% | 5% | 15% | 10% |
| Food Group |  |  |  |  |
| Raisins | 10% | 5% | 15% | 10% |

Table 1

**Data Analysis**

The data in this proposal will be expressed as means by calculating the means of the results of each group. The linear regression and the Pearson Correlation Coefficient will then be used to compare the strengths and relationships between the two variables by fitting a linear equation to observe the data. One variable (Raisins) is considered to be an explanatory variable, while the other (plaque pH level) is considered to be a dependent. The equation is in the form of ***Y = a + bX***, where ***X*** is the explanatory variable and ***Y*** is the dependent variable. The slope of the line ***b***, and ***a*** is the intercept (the value of ***y*** when ***x*** = 0).

The [correlation coefficient](http://www.stat.yale.edu/Courses/1997-98/101/correl.htm) is a valuable numerical measure of association between the two variables, which is a value between -1 and 1 indicating the strength of the association of the observed data for the two variables. One-way or one-factor ANOVA test will also be used to assess the significance of the data by analyzing the variance in order to derive conclusions about the means of the different groups. In the above experiment the yields obtained from the groups may be different and we may be tempted to conclude that the differences exist due to the amount of consumptions. But this difference may also be the result of certain other factors, such as the pH level of Saliva, number of acidogenic microorganisms that are present in the plaque, which are attributed to chance and which are beyond human control. This factor is termed as “[error](http://www.experiment-resources.com/experimental-error.html)”. Thus, the differences or variations that exist within the groups may be attributed to error. Thus, estimates of the amount of variation due to assignable causes (or [variance](http://www.experiment-resources.com/statistical-variance.html) between the samples) as well as due to chance causes (or variance within the samples) are obtained separately and compared using an F-test and conclusions are drawn using the value of F. If the p-value obtained is greater than the actual p-value then the hypothesis that the group that consumes the greater amount of raisins will produce the same effect on growth and acid production by Streptococcus mutans on dental plaque as the other groups will be rejected and the null hypothesis that there will be a greater effect produce by the group that consumes the greater amount of raisins.

**Data Collection**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Group I | Group II | Group III | Group IV |
| Sorbitol | x1>group II | All>x2 | x3>group I, II | x4>All |

Table 2: each number (X) would represent the mean of each group for the first trial, using sorbital as the control.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Group I | Group II | Group III | Group IV |
| Glucose | x2>L1>groupII | All>L2<All | x3>L3>group I, II | x4>L4>All |

Table 3: “L” represents the numbers obtain for each group using glucose as a control and the time interval for the second trial.

**Expected Results**

Since Sorbitol has no effect on acid production, I would expect all the groups’ data to be numerically close to each other when using sorbitol as a control and the plaque pH level to be higher than before. However, I would expect group IV to give the highest number due to the fact that the subjects would have no cavity, and group II to produce the lower number. Using Sucrose as a control, I would expect the results to be lower in number than the first trial for each group because sucrose would decrease the dental pH level; therefore greater amount of raisins consumption will be needed to bring the plaque pH lever higher.

**Time Table & Project Management**

Five hours, three times a week for about 4 months would be put aside for this project. Dr. Michael Lagier, a professor at Florida Gulf Coast University, who is an expert in this field, would be asked to supervise and manage the experiment.

**Equipment**

* Touch Microelectrode $200

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**CV**

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**GOAL**

I’m currently working on my Bachelor in Biology, once I achieved that I would like to get a Master in microbiology or Psychology, then go to medical school and become a Neurosurgeon.

**EDUCATION**

BA: Florida Gulf Coast University (2009-Present)

Major: Biology

University of North Florida (2008-2009)

1 UNF Drive, Jacksonville, FL 32224

Major: Biology; Minor: Behavior Medecine

AA degree: Manatee Community College (2006-2008)

5840 26th St. W., Bradenton, FL 34207 (941-752-5000)

**ASSOCIATED COURSES**

* General microbiology (2006)
* Biology (2007)
* Chemistry (2007)
* Physiology (2009)
* Genetics (2010)
* Pathogenic microbiology (2010)

**WORK**

MCC’s library: Student Assistant

* Responsibilities include: check in/out books to students, worked at the front desk and helped them with whatever they needed. I answered phone calls, run errands, and shelved books. Behind the desk, I processed books/magazines and do other little projects that I was asked to do

**VOLUNTEER**

UNF Premed Club

Hope Outreach International

GT Bray Park

**SCIENTIFC SKILLS**

* Gel Chromatography
* Thin Layer Chromatography
* Spectrophotometer Reading
* Centrifuge Handling

**OTHER SKILLS**

Computer

* Microsoft word (Expert)
* Excel (intermediate)
* Internet (Expert)
* Typing (fast)
* PowerPoint (intermediate)