Plaque Acidogenicity Resulting from Dry Sugary Cereal and Beverages in Children

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THESIS

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HEB

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LIST OF ABBREVIATIONS

AAPD	American Academy of Pediatric Dentistry
CPP-ACP	Calcium phophopeptide- amporphous calcium phosphate
FL	Froot Loops
FL/J	Froot Loops/ Juice
FL/M	Froot Loops/ Milk
FL/W	Froot Loops/ Water
IRB	Institutional Review Board
PI	Principal Investigator
ΔpH	Delta pH

Summary

Dental caries is a chronic transmissible disease of microbial orgin. Bacteria is responsible for dental caries. Bacteria convert sucrose into by-products, one of which is acid. Glucose and fructose can also be converted to acid[3]. The most common acid produced during this process is lactic acid which is reponsible for pH drops in the plaque. If the pH falls below the critical pH of 5.5, demineralization of tooth enamel occurs, leading to carious lesions[4]. Cariogenicity in studies have been related to plaque acidogenicity. Acidogenicity has been studied by measuring dental plaque pH.

The role of food sequencing has been considered by the America Academy of Pediatric Dentistry. They recommend eating sugary snacks or juice with a meal which could avoid a separate event where plaque pH falls below the critical pH. They also recommend drinking water after a snack to attempt to buffer dental plaque acidity.

There has been an increase in consumption of dry ready-to-eat cereal as breakfast or a snack by children. Naval et al [1] have shown that in adults, the consumption of dry ready-to-eat cereal caused a decrease in dental plaque pH to below the critical pH of 5.5 in adults. However, they reported that drinking milk after the cereal reduced the fall in dental plaque pH and brought it back to neutral. They showed that the consumption of apple juice after the cereal further reduced dental plaque pH.

At present, there are limited studies that investigated the role of beverage consumption after consumption of sugary snacks on plaque acidogenicity in children. The purpose of this study is to investigate the effect of beverage consumed after a dry, ready-to-eat cereal on dental plaque pH in children. The beverages included water, apple juice, and fat free milk. We hope to identify a beverage that can promote a less acidic environment in the oral cavity after a sugary challenge.

1. Introduction

1.1 Caries

Dental caries is a chronic transmissible disease of microbial orgin. It is a "localized dissolution of tooth surface brought about by the metabolic activity of a microbial deposit present on the tooth surface".[2]

Bacteria is responsible for dental caries. Bacteria convert sucrose into by-products, one of which is acid. Glucose and fructose can also be converted to acid[3]. The most common acid produced during this process is lactic acid which is reponsible for pH drops in the plaque. If the pH falls below the critical pH of 5.5, demineralization of tooth enamel occurs[4]. If this continues, a carious process starts. However, the carious process can be halted or slowed by the introuduction of favourable conditions which would allow remineralization of the tooth enamel [5]. Remineralization can occur with the reuptake of calcium and phosphate from several sources, including host saliva, toothpaste and even the food that the host ingests [4].

The carious process depends on the balance between demineralization and remineralization [4]. Frequent consumption of sugary foods cause frequent falls in pH below the critical pH and can throw off this balance and push the carious process further[2].

Dental caries is a mutifactoral disease with a microbial basis. Caries progression is dependent on a number of factors that can affect its rate of progression. The factors that are needed for the formation of dental caries include a host (tooth structure), bacteria, substrate (carbohydrate), acidic environment and time[4]. Although other bacteria have been found to be involved in caries progression, *Streptococcus mutans* are responsible for starting the cariogenic process.

Dental caries is the most common chronic diseases affecting children [4]. It is five times more common than asthma which in itself affects over 10 % of the child population [6]. Although dental caries is a preventable disease, it remains a silent epidemic [7]. Dental caries can affect a child's eating, drinking and even a child's growth and development, in addition to causing dental pain [8] . Every year, "more than 51 million school hours are lost to dental related illness" [9]. Pain from a dental infection can affect a child's school performance [8] . Acute dental infections can affect a child's systemic health and compromise it.

This disease affects children in disadvantaged communities at disproportionately higher rates [6]. Children of low socioeconomic class are twice as likely to be affected by dental caries than children who are not from that class [4]. Their disease is also more likely to go untreated and progress [8]. Children born in poverty also are less likely to visit the dentist with 25 % of children born into poverty not having seen a dentist prior to kindergarten [8] .

1.2 Dental Plaque Bacteria

Biofilms of bacteria can be found ubiquitously [10]. They can be found on inanimate structures such as water pipelines or in biological structures such as heart valves. They can also be found on catheters and endotracheal tubes.

Bacteria, which exists in biofilms, become highly resistant to external environmental changes, host immune mechanisms and to chemotherapeutic agents [3]. Biofilms protect the bacteria via a polysaccharide extracellular matrix produced by the bacteria themselves and encases the bacteria [11]. Most host mechanisms and chemotherapeutic agents cannot easily penetrate this matrix. Bacteria in biofilm are highly organized. They exist in microcolonies which are connected by channels that allow exchange of nutrients and elimination of metabolic byproducts [11]. Bacteria within a biofilm can communicate with other microcolonies via quorum

sensing [3]. This allows bacteria to monitor other microcolonies and modulate their gene expression [11]. Bacteria on the surface of biofilms are easily detachable and can travel to form new biofilms in distant locations.

The bacteria responsible for dental caries exists on the tooth surface as a dental plaque biofilm, which is a "complex, communal, three-dimensional arrangement of bacteria" [10]. Bacteria can be found in microcolonies attached to teeth in the form of a plaque biofilm. They are protected from dislodgment and host defenses by a capusule of polysaccharides, proteins and DNA [4]. Plaque can be found in greater amounts in protected areas of the mouth such as interproximal regions and areas of cavitations for example in a carious lesion [12]

There are over 700 bacterial species, which make up oral biofilm [13] at densities of 10^{11} bacterial cells per gram of dental plaque [1]. Dental plaque biofilm is formed in four stages [11]. The first in the "Initial adherence stage". Salivary glycoproteins form on tooth surface and promote adhesion of bacteria to that surface. This first layer is known as acquired pellicle. The salivary mucins, statherin and proline rich proteins of this pellicle promote the adhesion of bacteria[13]. The acquired pellicle forms within minutes after a professional prophylaxis and bacteria adhere to the pellicle within an hour. Gram positive cocci are typically the first bacteria to adhere to the pellicle. *Streptococcus sanguis* and *Actinomyces viscosus* have been identified as early colonizers [3.] The second stage is the "Lag phase". This phase represents the initial lag in bacterial growth and division as the biofilm is being established [13]. The third stage is "Rapid growth" of the bacteria in the biofilm. Not only do they divide rapidly, additional species of bacteria are also incorporated into the biofilm by attaching to the early colonizing bacteria[13]. This process is known as co-aggregation. *Streptococcus mutan* is an example of this late colonizer [3.] At this stage, the bacteria produce insoluble extracellular polysaccharides. This polysaccharide makes

up the biofilm matrix. The fourth stage is the 'Steady state". At this stage, the growth is steady and outer bacteria even may detach and form new biofilms in distant areas [13].

There are three main theories of dental plaque and its relationship to dental caries. The first is the" specific plaque hypothesis" which states that out of the diverse types of oral bacteria that is harbored in the oral cavity, only a very specific group of bacteria can result in dental caries. This theory was proposed in 1976 [14]. This theory has been since disproven and modified into the "nonspecific plaque hypothesis" [15]. This theory was developed in 1986 and states that all the bacteria present in the plaque biofilm interact with each other and each have a role to play in dental caries. This theory was then further modified into the 'ecological plaque hypothesis in 1991[16]. This theory states that the environment in which the plaque can affect the progression of dental caries. It states that if key environmental factors are changed or the balance is tipped, the bacteria implicated in dental caries progression could thrive, and outcompete with the bulk of the other types of bacteria present in the plaque [17]. This could then tip the balance towards dental caries progression. This theory states that the bacteria that are involved in causing dental caries remains a minor percentage of dental plaque and is undergoing competition with the other bacteria. In dental health, the bacteria causing dental caries is weakly competitive and does not prevail in causing dental caries.

A variety of bacteria has been implicated in its role in dental caries. The two bacteria that have been highly implicated in dental caries are *Streptococcus mutans* and *Lactobacilli*. *S. mutans* are necessary for the initiation of the carious process. It is a facultative anaerobic gram positive coccus bacteria [3]. One byproduct of sucrose metabolism by *S mutans* is acid. It is this acid that causes dissolution of tooth structure, leading to dental caries [18].

Once the carious process starts, *Lactobacillus* levels increase and especially so when the caries enters the dentin-enamel junction. Lactobacillus levels also correlate well with host sugar intake. *S mutans* and *Lactobacillus* are acidogenic and aciduric, which means they produce acid and thrive in an acidic environment [3].

S mutans and *S sobrinus* produce extracellular polysaccharides from sucrose, glucose and fructose [3]. Glucose homopolysaacharide (glucans) and fructose homopolysaccharides are two of these extracellular polysaccharides produced by bacterial glucosyltransferase and fructosyl transferase (GFT) respectively. These polysaccharides aid in bacterial adhesion in the biofilm and provide energy sources for the biofilm.

1.3 Acidogenicity and Cariogenicity

Dental caries starts due to dissolution of enamel by acid produced by bacteria. Featherstone has stated that all acids produced by oral bacteria can cause dissolution of tooth enamel [18]. The acids include formic, acetic, propionic and lactic acid[18]. Lactic acid has been implicated in its extensive role in dental caries.

It is important to distinguish between acidogenicity of food versus cariogenicity of food. Acidogenic food is the ability of the food to cause a fall in plaque pH upon consumption, that is, the fact that it can be converted by plaque bacteria to an acid. Sucrose is a good example of this. However, in terms of cariogenicity, there are many factors that can modify the cariogenicity of an acidogenic food [19]. The cariogenicity of food is related to its acidogenicity, however, host factors, and properties of the food itself can modify this relationship. Host factors such as saliva buffering ability can change cariogenicity of an acidogenic food because the acid that is produced from consumption of that food can be buffered and a drastic fall in pH can essentially be prevented. Acidogenic food that is highly retentive on tooth surfaces can be more cariogenic

than acidogenic food that are easily cleared from the mouth. As such, it is important to understand that cariogenicity of food is dependent on many factors; just as dental caries, itself is multifactorial.

Studying food acidogenicity, by measuring changes in plaque pH with food consumption can give information about its cariogenicity; however, it cannot provide a representation of the true cariogenicity of food [19].

1.4 Determination of Plaque Acidity

There are a handful of methods avaliable to determine the acidogenic potential of foods and beverages. These rely on measuring acid production in dental plaque after consumption of these foods. This is supported by the *in vivo* plaque model [20] . The measurement of plaque pH after food ingestion is important in determining the acidogenic potential and cariogenic potential of foods.

1.4.1 Dental Plaque Sampling

In dental plaque sampling, the plaque biofilm is harvested and either the pH of the plaque mass is measured or the plaque is cultured for further testing. The plaque that is collected is the superficial plaque and typically from the buccal or lingual of a tooth surface which is most accessible. The pH value that is obtained is an average pH of the plaque mass. A disadvantage of this method is that if the research required sequential plaque pH to be obtained over a set time, harvesting plaque for subsequent data points would be difficult and inaccurate since the initial plaque mass has already been disrupted [21]. Another disadvantage is that the plaque that is collected is not from a caries prone area of the tooth, which typically is the occlusal and interproximal area [21].

1.4.2 Touch Electrode

The touch electrode has allowed plaque pH values to be collected more accurately. It was originally used by Stephan to determine plaque pH changes with glucose rinse [22]. The original electrode in the 1940s was made of antimony[23]. Stephan placed electrodes in contact with the labial surface of teeth for the measurement. They were later modified by Clement to glass electrodes with a 4.25mm diameter. These electrodes were too large to be practical but offered accurate pH measurements[24]. In the 1980s, Liu created Palladium-Palladium oxide minature pH electrodes which quickly became very popular in research due to its small size, ease of use and quick response time [25]. These electrodes, however were fragile. In this method, an electrode is placed in contact with dental plaque typically in the interproximal areas of teeth. This method allows repeated pH measurements to be obtained with minimal disruption of the plaque mass. The electrode measure pH at the plaque-saliva interface which could be prone to buffering by saliva and hence, a greater variation in pH could be seen over time and pH fluctuations could be observed[21]. Another disadvantage is the possibility of plaque disruption with repeated measurements, though not to the same extent as with the plaque sampling method.

1.4.3 Indwelling Electrode

This technique involves placement of electrodes in the interproximal areas of teeth without removal during the experiment. It was originally developed by Graf and Muhelmann as an alternative plaque pH measurement which minimizes plaque disturbance [25]. In this method, disruption of the plaque mass is much reduced, and it has been shown to detect more pronounced and longer lasting pH falls than in the touch electrode method[21]. Both indewlling and touch electrodes provide a good method to measure plaque pH, however, in children who may not be

able to stay still and may not be tolerant of an electrode placed in between their teeth for the duration of the experiment, the touch electrode may offer a good alternative.

1.5 Diet and Dental Caries

The American Academy of Pediatric Dentistry (AAPD) recognizes diet and its impact on oral health. Dietary counselling is one of the topics discused during a dental exam, and is part of anticipatory guidance [AAPD]. Although dental caries is a multifactorial disease and diet alone is not the sole cause of dental caries, it is known that frequent snacking creates an oral environment conducive to dental caries.

Diet, especially those containing sugars, plays important role in causing tooth decay. When food is ingested, the plaque bacteria uses the carbohydrate content in the food to form acids, which cause the drop in plaque pH and creates an acidic environment in the mouth. This low pH then results in enamel dissolution which starts off the carious process. The pH of dental plaque reflects the acidogenic activity of microorganisms in the mouth. Many studies have shown association between low dental plaque pH and dental caries.

It is in the AAPD guidelines that the amount of juice consumption for children should be limited to 4-6oz a day. It is also advised that children do not snack more than three times a day and snacks should be limited to during meal times [9] . These guidelines are attempting to reduce the frequency of sugary challenges faced by the child, that is, the number of times the dental plaque pH falls below the critical pH of 5.5.

In children, snacking occurs in high frequencies. Most snacks for children are easy to obtain, high calorie, low nutrient foods such as chips, juice, and dried cereal eaten as snacks [9]. Most of these snacks may be highly cariogenic foods containing high levels of sugars. Children who consume snacks between meals that were low in sugar had 86% less smooth surface caries

and 68% less interproximal caries than children with high sugar snack consumption between meals [26]. Chlidren in the high caries risk group were also noted to have increased frequency of snacking[26].

The concept of "mouth friendly foods" was conjured to encourage children to snack on foods that are less likely to cause falls in plaque pH and so avoid a demineralization event[26]. Cheese, for example, has been extensively studied as a mouth friendly food. Cheese has been shown to be anticariogenic and in fact, consumption of cheese after a sugary challenge can help reduce the pH fall that would occur after a sugary food intake alone[27]. It is hypothesized that cheese could aid in remineralization of tooth structure by increasing salivary flow and hence buffering action on consumption. It also delivers calcium phophopeptide- amporphous calcium phosphate (CPP-ACP) which could further aid in remineralization of tooth structure[28]. Cheese can have a direct antimicrobial affect on dental plaque thereby decreasing the bacteria which produce the acid responsible for acidic challenges on tooth [27]. Foods that contain calcium, phosphate and casein have also shown to be mouth friendly foods [27]. Foods containing polyphenols can reduce acidic challenges on tooth because of its interference with glucosyltransferase which is an enzyme found in *S. mutans* and is involved in the production of lactic acid[27]. Foods such as peanuts, which can stimulate salivary flow, have been shown to be protective for the oral cavity as well[28]. Foods such as apples and cranberries contain flavanoids which have antibacterial properties and can reduce bacterial plaque adhesion [28]. Touger-Decker recommended eating foods that reduce the risk of formation of a carious lesion such as dairy products with a meal to help reduce acid production from plaque.

Caries is still a silent epidemic to this day and affects low income children substaintially more. Research effort has been placed into determining dietary practices which are conducive to

caries prevention. It is important to provide dietary advice that is realistic and easy to implement since they are more likely to be followed that way.

1.5.1 Sugary Foods

According to the US Food Supply Data paper, consumption of sugar has increased 23% between 1970 and 1996 with a large increase in consumption of high fructose corn syrup and other corn sweeteners [29].

The consumption of high sugar foods have been strongly associated with dental caries, although the frequency of ingestion and the form of the sugar is an important factor. Sugars which are sticky are retained longer in the mouth and are considered more cariogenic than food which are easily cleared in the oral cavity.

The link between dental caries and sugars has been proven and well established. Chemically, sugars can be catergorized based on the number of monomers it is made up of. The sugar monomers are glucose, galactose, fructose and invert sugar which is a 1:1 form of glucose and fructose. These monomers are also known as monosaccharides and can build up into disaccharides or polysaccharides. Disaccharides are sucrose, maltose, lactose, trehalose. Examples of polysaccaharides are starch, cellulose and glycogen. Sucrose has been highly implicated in the carious process.

Stephan found that the consumption of sugar has a direct relationship with dental plaque pH. He did so by collecting dental plaque from teeth after sucrose consumption. He found that upon consumption of sucrose, the dental plaque pH fell which was related to demineralization of tooth structure[30]. Stephan also created the concept of critical pH. He found that at pH below 5.5, tooth enamel demineralized which started off the carious process [30]. He found that the area under the pH vs time curve was also an important factor. It is correlated with the time spent under the

critical pH of 5.5[30]. The longer the time spent under the area under the curve, the longer the tooth enamel remained under conditions favourable for demineralization.

Plaque pH after a sucrose exposure did return to physiologic values over time [31]. Stephan attributed this to many mechanisms, one of which is the intrinsic ability of saliva to buffer the acid that is produced[31]. Although the pH changes can follow a typical Stephan curve, Dong et al found that the dental plaque pH of caries free children returned to their original pH values much faster after a sugary challenge than children with dental caries [32]. Caries free children had a less pronounced fall in pH than children with caries lesions [32].

The increased frequency of sugar consumption has been related to a decrease in dental pH explained in part by the Stephan curve. Every sugar challenge results in a Stephan curve and a time spent under the curve. Frequent sugary challenges increase the number of Stephan curves experienced inside the oral cavity and hence increasing the frequency and total time spent under the curve. This then increases the risk of dental caries.

Unprocessed starch is considered a low cariogenic carbohydrate [5]. However, if the starch is processed, cooked or converted into a soluble starch and eaten frequently, it can become cariogenic. In addition, if these starchy foods are retained in the oral cavity, it can be digested by salivary amylase into simple sugars which can then be metabolized by oral bacteria and thus become cariogenic.

1.5.2 Ready-to-eat Dry Cereal

The consumption of breakast contributes to a well balanced diet [33]. Cereals are the most commonly consumed breakfast in the United States. By 2022, sales from breakfast cereal is expected to exceed US$43 billion[34]. Children who consume breakfast cereal regularly are less likely to have low vitamin and mineral intake and even have better overall diets. High nutrient

dense cereal is often the main source of calories, protein and vitamins [35]. It provides a good source of calcium, vitamin A, magnesium and folate [35]. Cereal is highly popular in children and is a viable food option for children from low socioeconomic background. The increase in cereal consumption is thought to be related to a busy lifestyle and little time for a cooked breakfast in this day and age [34].

Dry ready-to-eat cereals are often packed with high levels of sugar. Cariogenic potential of cereal depends upon their sugar content, and their retentiveness on the tooth surfaces. The amount of sugar in cereal varies. Sugar coated cereal can have as much as 44 g of added sugar per 100 g, which is more than cake [36]. Many of the marketed cereals such as Froot Loops contain added sugar and are targeted to appeal to children[37]. Some studies have shown that honey flavoured cereals showed the most plaque pH falls after consumption followed by chocolate containing cereal and finally fruit containing cereal[33]. Many of these cereals are consumed by children as snacks throughout the day rather than just as at breakfast. This dietary habit increases the frequency of sugar consumption thoughout the day, thus increasing the frequency of low dental plaque pH and hence caries risk.

When the consumption of sweetened dry cereals is followed by consumption of beverages such as milk, or juice, the acidogenicity of plaque may be altered depending on the inherent properties of the beverages[37]. Consuming milk with cereal can often affect the cariogenic potential of cereal due to the ability of milk to buffer the acid that may be produced [37, 1]. Very few studies investigated the effects of consuming dry breakfast cereal followed by different beverages on oral health.

1.5.3 Beverages

In the past years, beverage consumption patterns in children have changed drastically. Children are consuming less milk and have increased intake of juice and soda. These changes have an effect on a child's health including a reduction in calcium intake and increase risk of childhood obesity [38]. In addition, sweetened beverages are an additional source of sugar in a child's diet and it has an impact on a child's oral health[38].

Different types of beverages have been studied in order to determine the impact each has on a child's oral health and hence attempt to identify mouth friendly beverages which can be recommended as part of a child's balanced diet.

1.5.3.1 Milk

The American Academy of Pediatrics recommend that children be wholly breast fed for the first six months of life and after that, solid foods can be introduced while still breast feeding until 1 year old. After that, the child should have dairy as part of a balanced diet. The American Academy of Pediatric Dentistry has stated that prolonged night time ad-lib breast feeding should be avoided because it has been implicated in the carious process. Breast milk alone is known to be non cariogenic, but when given in combination with other foods and when given at high frequencies, it can support bacterial growth.

It is known that whole milk alone is non cariogenic and is considered "mouth friendly", however, milk advertised for children often have sugar added and infant formulas have high sugar levels also. It is important to distinguish between cow's milk which is "mouth friendly" and infant formulas or flavoured milk which have high sugar concentrations and therefore contribute to the carious process.

Much research has been done in the past 60 years to understand the properties of milk that make it "mouth friendly". Milk consists of the sugar lactose, which is the least cariogenic of

all monosaccharides and disaccharides[39]. Milk is labelled non-cariogenic due to its ability to remineralize tooth, its buffering capacity and its ability to inhibit plaque biofilm metabolism and replication. Milk contains calcium and phosphate which can be used to remineralise tooth structure. Milk contains lactoferrin, lysozymes and antibodies which are responsible for its ability to inhibt plaque biolim metabolism and replication. It also has casein as its primary milk protein. Casein from milk is incooperated into dental plaque and prevent sub surface demineralization of tooth enamel [40]. Bowen et al used rats with salivary glands removed, and hence were of high caries risk since they are lacking the protective effects of saliva, to show that consumption of milk caused no carious lesions in these rats [41].

1.5.3.2 Fruit Juice

The National Diet and Nutrition Survey of Young People (aged 4-18 years) found that soft drinks, which included fruit juices and confectionery contributed to more than 50% of non-milk extrinsic sugars [42]. There has been a rise in juice consumption in the recent years. Juices are offered to children due to its low cost, easy accesbility, favourable taste and the perception that they are good for the child's health[43]. There are a variety of different sources of juice, including fresh juice, fruit flavoured drinks and sweetened juice. Most have high sugar concentrations.

A variety of factors determine the cariogenicity of juice including pH of the juice beverage itself, buffering capacity of the beverage, sugar content of the beverage and the way in which the beverage is consumed[44]. Preethi et al have shown that after consumption of various types of fruit juices, the mean pH fell below the critical pH (5.5) and failed to rise back to baseline values even after 30min [45]. As such, the consumption of juices can cause a shift in the balance between demineralisation and remineralisation.

Pure fruit juices contain the sugars fructose and glucose, while fruit juices with added sugar contain a blend of sucrose and high fructose corn syrup. Therefore, fruit juices with added sugar are more cariogenic than pure fruit juices [38]. Even then, the consumption of pure fruit juice has been shown to be associated with increased caries risk [38]. Even the consumption of diluted apple juice has been shown to decrease plaque pH[46]. Orange and apple juice could lower plaque pH to levels comparable to the consumption of 10% sucrose solution[47].

These jucies are often placed into sippy cups or baby bottles, which divides the juice intake into many small intervals thus increase the frequencies of acid challenges on tooth surface [43]. Taking this into account, the AAPD established a guideline that juice intake in children should be limited to 4-6 oz a day and consumed in one sitting, preferable with a meal[9].

Most fruit juices are acidic with some apple juices found to be of pH 2.9. Fruit juices contain citric acid and ascorbic acid,which in addition to their high sugar levels, cause a fall in plaque pH to 4.89 after consumption [48]. Dental erosion is the loss of the outermost layer of enamel due to dissolution by acid during a pH fall in the oral cavity. The acid can be extrinsic or intrinsic. Intrinsic acid can be the hydrochloric acid in gastric contents which could be regurgitated into the oral cavity in a pathological process known as Gastroesophageal reflux disease. Extrinsic acid is acid which can come from external sources for example food and beverages. Many of this extrinsic acid comes from fruits and fruit juices. A correlation between dental erosion and frequent consumption of cola drinks and apple juice has been reported by Marshall et al [44,49].

1.6 Food Sequencing

Sequencing of food has recently gained interest, mainly in aiding with weight loss and glucose control in diabetics. For example, it has been shown that consuming a non-glucidic

nutrient foods before a high carbohydrate containing food helps with glucose tolerance by delaying glucose absorption [50]. This has been termed preload [51].

Food sequencing can also play a role in maintaining oral health. A previous study in Wu's laboratory has shown that the consumption of the appropriate beverage after eating dry ready to eat cereal can raise the pH of plaque in adults[1]. They showed that consumption of dry cereal alone or cereal mixed with milk caused the plaque pH to decrease. However, consumption of milk following sugary cereal prevented the decrease in plaque pH [1]. Consuming juice after cereal did not prevent the pH from falling and in fact, caused the pH to decrease further [1]. These findings have not been reported in children. If this is found to be true, children can be encouraged to consume a glass of milk rather than juice to help increase plaque pH.

Recognizing the importance of food sequencing on oral health, the American Academy of Pediatric Dentistry recommend eating sugary snacks or juice with a meal which could avoid a separate event where plaque pH falls below the critical pH[9]. They also recommend drinking water after a snack to attempt to buffer dental plaque acidity.

1.7 Hypothesis

We hypothesize that the consumption of milk after eating ready-to-eat sugar added cereal will result in a reduced drop in dental plaque pH compared to consumption of fruit juice.

1.8 Purpose of This Study

The purpose of this study is to investigate the effect of plaque acidogenicity in children after consuming a dry, ready-to-eat cereal followed by beverages including water, apple juice, and fat free milk. We hope to identify a beverage that can promote a less acidic environment in the oral cavity after consumption of a sugary cereal in children.

2. Methods

2.1 Study Subjects and Recruitment

This was a randomized controlled crossover study consisting of 10 healthy participants between the ages of 7 and 12 year olds. More than 20 children regardless of race and gener were recruited using word of mouth or 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 with the use of a phone script. The study procedures, criteria and purpose of the study were explained to them. Once verbally screened using the phone script and a set of inclusion and exclusions criterias, children with their legal guardians were invited to the UIC College of Dentistry buiding for a screening study. Legal guardians of prospective subjects were instructed that as part of the test protocol, subjects were to refrain from oral hygiene practices at home for at least 24hrs prior to the study. They were also advised not to eat anything for 2 hrs prior to the start of the study. At the screening visit, the participants were given a limited oral examination to determine if they were eligible for the study based on the inclusion and exclusion criteria. The inclusion criteria of plaque pH falling to lower than 5.5 after rinsing with 10% sucrose solution is used to ensure the study participants can show the expected Stephans curve for that control.

Inclusion Criteria:

- Give written informed consent and assent to participate
- Be between the ages of 7-12 year old
- Be in good general health
- Ability to refrain from oral hygiene practices for 24 hours and eating/drinking (with the exception of water) for 2 hours prior to visits

- Plaque pH should lower to less than 5.5 after rinsing with 10% sucrose solution.

Exclusion Criteria:

- Obvious periodontal disease with signs of purulent exudates, abscesses or tooth mobility
- Taking systemic antibiotics or have any respiratory disease such as coughing as this would affect the plaque pH measurement
- Having fixed orthodontic appliances
- Presence of clinically detectable caries or crowns in the testing site
- Allergic to any of the cereals/snack foods or beverages being used for the study

2.2 Test Foods

The test foods included commercially marketed dry ready-to-eat Froot Loops (FL) cereal, Dean's fat free milk, Minute Maids 100% apple juice (24 g of sugar in 240 g) and fountain water. All the test products were purchased from the Jewel Osco Grocery Store (Jewel Osco, Chicago IL 60612).

The test food groups were:

- Dry FL (10 g) followed by 30 ml of non-fat milk (FL/M)
- Dry FL (10 g) followed by 30 ml of apple juice (FL/J)
- Dry FL (10 g) followed by 30 ml of fountain water (FL/W)

- 10 ml sucrose solution (10%)
- 10 ml sorbitol solution (10%)

10% sucrose and sorbitol solutions were used as positive and negative controls respectively, as identified by Human Plaque Acidity Model Working Group [52].

All data are de-identified by giving each subject a numeric identification number. The master list that is used to track subjects is locked in a cabinet in our lab throughout the study. Data is collected in spreadsheets specifically created for the study.

2.3 Plaque pH measurement

The dental plaque pH is measured *in vivo* using a touch microelectrode (model NMPH3 dental beetrode, 45^0 bend, 2 mm receptacle, World Precision Instruments (WPI), FL USA) and glass reference electrode (DRIREF-5, WPI) in contact with a 3M KCL solution salt solution. The subjects are asked to place their finger in that salt solution to create a salt bridge. This salt bridge completes the circuit.

Before each study, a standard graph is created. The standard graph is used to convert the mV readings from the pH meter into pH values. The mV values for standard solution of pH 4, 7 and 10 are obtained. These pH and mV values are utilized to create a pH standard calibration curve.

For measuring plaque pH, the participants lie in a dental chair at a 45 degree angle. They are not moved from this position throughout the study. The microelectrode is then inserted into the interproximal space below the contact area between the maxillary premolars or maxillary primary molars on both the left and right sides. Readings are taken at the designated time

intervals (as described in study design) and subjects are advised to refrain from talking or moving excessively throughout the study period.

2.4 Calibration Curve

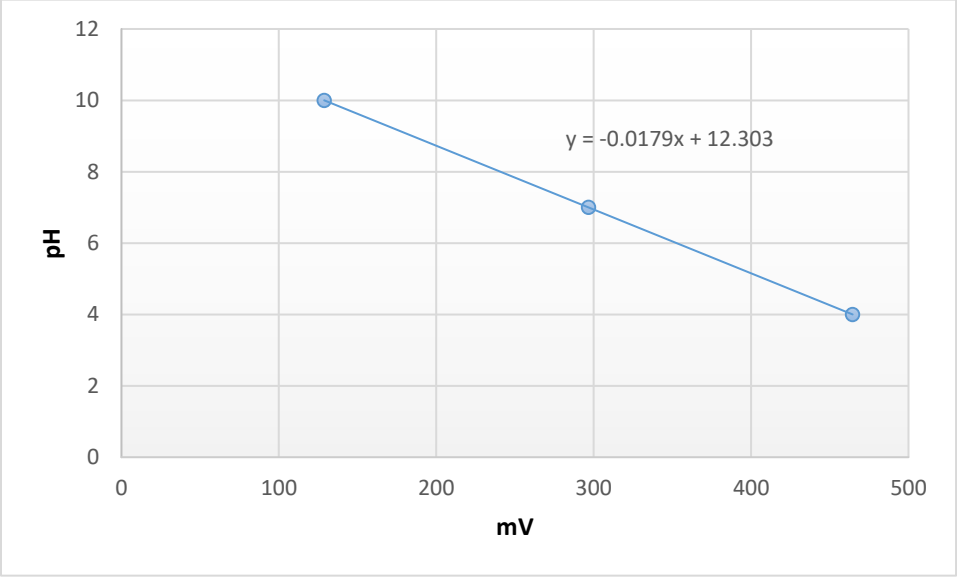
Following the manufacturer's directions, before each study, a pH standard calibration curve is created by relating mV values for standard solution of pH 4, 7 and 10. This graph is used to convert the mV readings from the pH meter into pH values during the study.

TABLE I- SAMPLE CALIBRATION VALUES

mV[a]	**pH[b]**
464.5	4
296.8	7
128.8	10

a- The mV values presented are the values recorded directly from the electrode.
b- The values presented are the corresponding pH values of the standard solutions used to obtain the mV values and eventually used to construc a calibration curve

FIGURE 1- pH STANDARD CALIBRATION CURVE



During the experiment, readings are read from the pH meter and recorded in mV. The standard calibration graph is used to convert these mV readings into pH values using the equation obtained from the standard calibration curve in the method as shown below:

$$Y= -0.0179x+12.30$$

In this example, x, which is the mV reading recorded from the electrode is 283.2mV so we have:

$$Y=-0.0179*(283.2)+12.30$$

$$Y= 7.23072$$

TABLE II- CONVERSION OF mV VALUES TO PH

Time/min	mV[a]	pH[b]
0	283.2	7.23072

a- The mV values presented are the values recorded directly from the electrode.
b- The values presented are the corresponding pH values for each mV reading as converted using the calibration curve

2.5 Study Design

Legal guardians of prospective subjects are instructed that as part of the test protocol, subjects are to refrain from oral hygiene practices at home for at least 24hrs prior to the study. They are also advised not to eat anything for 2 hrs prior to the start of the study. The subjects and the guardians read over and sign consent and assent forms. The subjects are then given numerical identification numbers which are then used for record keeping throughout the study.

The initial screening involves measuring the plaque pH over time as the subjects rinse with 10% sucrose. The resting pH of plaque is first measured at the beginning of the study by placing the electrode in the interproximal area between the upper premolar teeth (upper primary molar teeth) on each side of the subject's mouth. Subjects then rinse with 10mL of 10% sucrose

solution for 1min. The pH is then measured at 2, 5, 10, 15, 20, and 30 min. The subject will qualify for the study if their plaque pH falls below pH 5.5.

For each subsequent study session, participants are to refrain from oral hygiene practices at home for at least 24hrs prior to the study and not to eat anything for 2 hrs prior to the start of the study. The study involves the subjects with their legal guardian to attend 4-5 visits (including screening visit) to complete the full study. If a subject drops out of the study, their data is not used.

The test groups are listed in the previous section "test food". These include FL, FL/M, FL/J, and FL/W. Sucrose and sorbitol solutions serve as negative controls.

To determine which test food the subject will be consuming, a randomized selection is done at each visit. Participants will draw a paper blindly with the name of the test food group from a bowl.

At each subsequent test food group study, the resting plaque pH is first measured at the beginning of the study by placing the electrode in the interproximal area between the upper premolar teeth (upper primary molar teeth) on each side of the subject's mouth. This is the baseline pH. For the test food groups which involve rinsing with 10 ml of 10% sucrose or 10% sorbitol solution, plaque pH reading is recorded at 2, 5, 10, 15, 20, and 30 minutes after rinsing. For the test food groups which involve consuming FL followed by fat free milk, apple juice or water, plaque pH readings are recorded at 2 and 5 minutes after eating FL. At the end of 5minutes, the subjects will consume 30mL of fat free milk, apple juice or water. The plaque pH is then measured at 2, 5, 10, 15, 20 and 30 min after consumption. The total time for each study session is 45—60min. There is a washout period of at least 2 days between each session. A schematic of the study is presented in Figure 2 and 3. For each study, two personnel are

involved. One dentist places the electrode in the subject's mouth, specifically in the interproximal area between the upper premolar teeth (upper primary molar teeth) and ensuring the electrode is immersed in plaque. The dentist keeps the electrode in a steady position. The other staff records the pH readings.

FIGURE 2 SCHEMATIC OF STUDY DESIGN

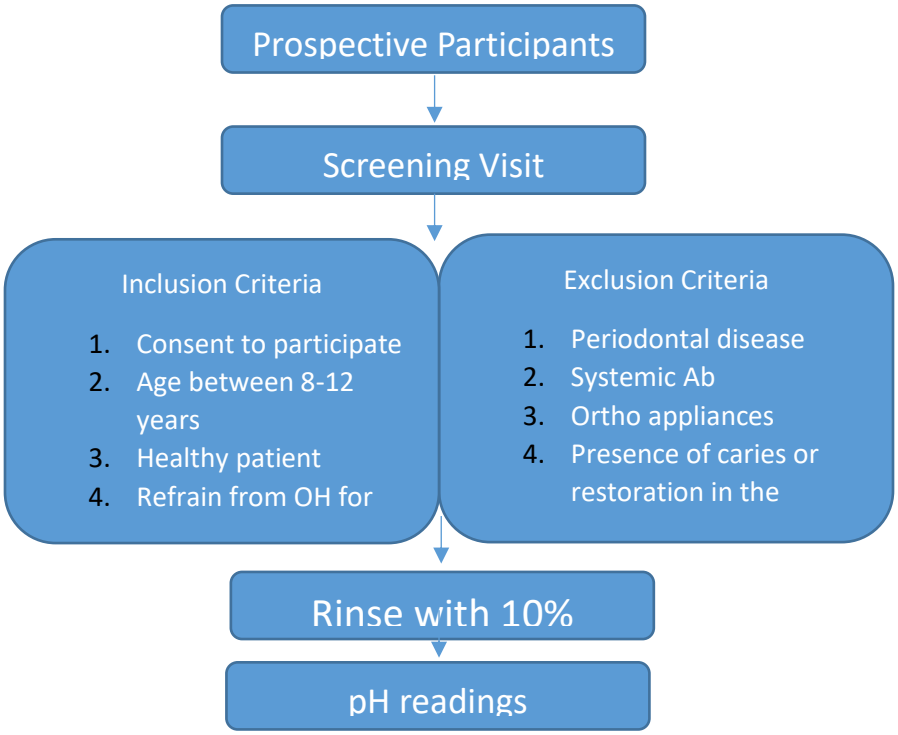
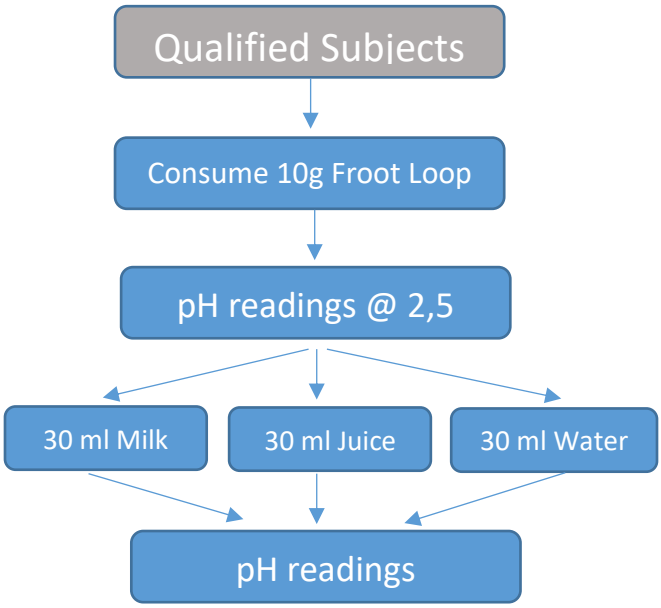


FIGURE 3 STUDY DESIGN



2.6 Statistical Data Analysis

The data is analyzed in two ways:

1) An average of data from all subjects for one test food group is calculated. A graph is plotted with the pH values over time for each test group. The pH readings utilized for analysis are those from the right side only. A graph is also fabricated using Microsoft Excel for visual comparison of the three test group data.

2) Values for area under the graph and delta pH are calculated for each test group. Delta pH is the pH at a selected time point during the test minus the baseline pH value.

Statistical analysis is performed using SPSS version 21. We performed a paired samples T test in two different ways. The first paired samples T test is performed to determine if the end

point pH (at time 35min) is of statistically significant difference from the baseline pH. The other paired sample T test is performed to determine if the minimum pH values obtained for each test groups were statistically significant when compared with baseline pH.

Area under the curve is calculated for area under pH of 5.5 or pH of 5.9 using Microsoft Excel.

Figure 4. NMPH3 Microelectrode. Dental Beetrode with 450 bend and 2 mm receptacle

Figure 5 Jenco electronics, JE67IP pH meter

Figure 6 *In vivo* Plaque pH determination in interproximal space of maxillary premolars in subject's oral cavity

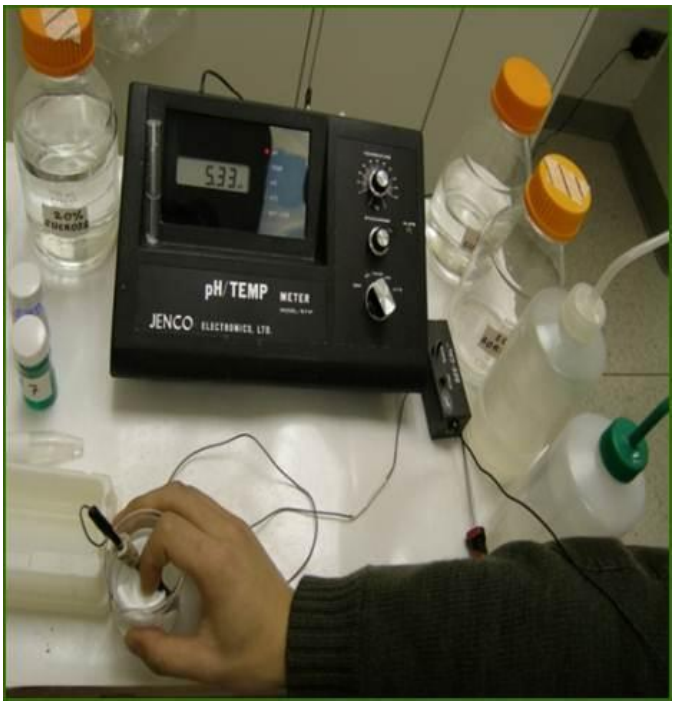


Figure 7 Creation of salt bridge in 3M KCL solution

3. Results

This randomized controlled cross over study included ten healthy subjects between the ages of 7 and 12 (Table III). The mean plaque pH values at baseline and at specific time points during the study are presented in Table IV and Table V.

The control solution and beverages were rinsed for 1min and the Froot Loop cereal was eaten within 2min. The plaque pH readings were taken at different time intervals as shown in figure 2 and 3 (in Materials and Methods section). The plaque pH readings over the 30-35min testing period after rinsing with the control solutions and after eating the cereal followed by rinsing with the test beverage are presented in Table IV and V. Except for 10% Sorbitol solution, which serves as a negative control, the plaque pH fell within 2min of the consumption of the cereal and 10% Sucrose.

For our study, 19 children (eligible subjects) were screened. Of the 19, only 10 qualified to become subjects (qualified subjects). The pH values were read for the right and left upper side of the subjects' mouth, however, only the values for the right side of the mouth were utilized.

In our analysis, the time point of 0min, which is the time immediately after cereal consumption is used as our baseline. There is also a pH measurement at time -2min, which represents the value just prior to cereal consumption and this is recorded as our resting pH. When each subject's individual baselines were compared against the mean baseline for each test group, it was found that none of the readings were more than 2 standard deviations from the mean, which means that the readings taken and instruments used were able to provide an accurate reading (6.92 ± 0.73 for FL/ J, 6.69 ± 0.82 for FL/ W, 6.87 ± 0.76 for FL/ M).

3.1 Sucrose

The sucrose control test group demonstrated a typical Stephen curve (Figure 9). It caused a fall in the plaque pH from 6.36 ± 0.14 to 5.72 ± 0.40 at the end of 2 min after rinsing with it. After this 2min time period, the plaque pH continued to decrease down to a minimum pH of 5.45 ± 0.84 at 5 min after rinsing. After this, the plaque pH gradually recovered. At the end of the 30min study, the plaque pH was 6.58 ± 0.55 (Table IV).

3.2 Sorbitol

Rinsing with sorbitol should not cause any pH changes, that is, Sorbitol is used as a negative control. The pH over time curve obtained for sorbitol showed the pH value was maintained at a stable value (Figure 9). Rinsing with sorbitol solution demonstrated the small fall in plaque pH from 6.75 ± 0.72 to 6.52 ± 0.80 at 2 min time interval. The lowest pH value observed is 6.289 ± 0.93. Sorbitol did not demonstrate an appreciable fall in plaque pH over 30 minute study. As such, the sorbitol study is done for 4 of the test subjects only. The plaque pH at the end of the 30 minute study returned to and exceeded the baseline pH at a value of 7.60 ± 1.20 (Table IV).

For the test group of 10ml of 10% sorbitol solution, which serves as a negative control, pH changes are not expected with this test group and is done only for 4 subjects. This test group was ended after it was shown from the 4 subjects that sorbitol did not result in a change in plaque pH.

3.3 Froot Loops/ Milk

In the test group involving dry FL followed by milk, the plaque pH demonstrated a drop from baseline value of 6.87±0.76 to 6.17 ± 0.63 at the 5 min mark. Upon consuming milk, the plaque pH gradually increased to 6.63 ± 0.61 at the end of 30 min. This end point pH is close to

the initial baseline pH of 6.87±0.76 (Table VI). The minimum pH recorded for the milk test group is 6.17 at the 5 min mark.

Thus consumption of milk after dry FL did not promote any further drop in plaque pH and helped to bring the plaque pH back to initial baseline pH. This can be visualized in our pH over time curve for milk (Figure 8).

The paired samples T test (run using SPSS) for baseline pH values and endpoint pH values for milk is found to be 0.361 which is not statistically significant (Table VII). The paired samples T test for baseline pH values and minimum pH value for the duration of the study is found to be 0.001 which is a statistically significant difference (Table VII).

3.4 Froot Loops/ Water

In the test group involving dry FL followed by water, the plaque pH demonstrated a drop from baseline value of 6.69±0.82 to 6.02 ± 0.51 at the 5 min mark. Upon consuming water, the plaque pH gradually increased to 6.27 ± 0.41 at the end of 30 min. This end point pH is close to the initial baseline pH of 6.69±0.82, though not as close as the milk group was (Table VI). The minimum pH recorded for the water test group is 5.63 at the 10 min mark.

Thus consumption of water after dry FL did aid in bringing the plaque pH back to close to the baseline, though not as much as milk did. This can be visualized on our pH over time curve for water (Figure 8).

The paired samples T test (run using SPSS) for baseline pH values and endpoint pH values for water is found to be 0.200 which is not statistically significant (Table VII). The paired samples T test for baseline pH values and minimum pH value for the duration of the study is found to be 0.004 which is a statistically significant difference (Table VII).

3.4 Froot Loops/ Juice

In the test group involving dry FL followed by juice, the plaque pH demonstrated a drop from baseline value of 6.93±0.73 to 5.53 ± 0.79 at the 5 min mark. Upon consuming juice, the plaque pH gradually ended at 5.90 ± 0.75 at the 30 min time point. This end point pH is not close to the initial baseline pH of 6.93 ± 0.73. This can be visualized in our pH over time curve for juice (Figure 8).

The paired samples T test (run using SPSS) for baseline pH values and endpoint pH values for juice is found to be 0.002 which is statistically significant (Table VII). The paired samples T test for baseline pH values and minimum pH value for the duration of the study is found to be 0.000 which is a statistically significant difference (Table VII).

3.5 Comparison of FL/M, FL/J and FL/W

After consumption of the FL, at the 5 min mark, the plaque pH for all 3 test groups fell to between 5.53 ± 0.79 and 6.17 ± 0.63. After consumption of the beverage, the pH changes relay the changes due to the beverage.

FL/Milk group resulted in a plaque pH value at the end of the study that is close to the baseline pH. The FL/ Water groups also a reached pH value at the end of the study which is close to the baseline pH.

The minimum pH reached during the FL/Juice test was 5.25 which was ± 1.04 which was much lower than the minimum pH reached during the FL/ Milk and FL/ Water groups which were 6.17 ± 0.63 and 5.63 ± 0.59 respectively. The FL/Milk group, however, showed the least minimum pH values.

Delta pH was also calculated as the difference between the minimum pH value and the baseline pH value. This was done to compare the greatest drops in pH for each test group. The maximum fall in pH (Delta pH) for the FL/Juice group was 1.65 which was the greatest delta pH

recorded compared with other test groups. The FL/ Milk and FL/ Water groups had maximum delta pH values of 0.7 and 1.06 respectively. The FL/Milk group showed the smallest maximum delta pH values.

The paired samples T test (run using SPSS) for baseline pH values and endpoint pH values for milk and water are found to be 0.361 and 0.2 respectively which are not statistically significant differences. While that of juice was found to be 0.002, which was found to be significantly different (Table VII). This indicates that juice ended at a pH values that was statistically different from its start pH value and hence, juice was a poor buffer of plaque pH falls. The values for milk and water, however, indicate that milk and water were good buffers of plaque pH falls since the end point pH values were not statistically different from the start points.

The paired samples T test for baseline pH values and minimum pH value for the duration of the study is found to be 0.001, 0.001 and 0.004 for milk, water and juice respectively. These values indicate statistically significant differences (Table VII). We can conclude that during the course of the study, the pH values did fall to a point that was statistically different from its start point.

3.6 Area under curve

The area under the curve was calculated for area under the pH of 5.5 and 5.9.

The calculation for area under the curve is done in excel. First, the specific time points where the pH fell below 5.5 was derived by extrapolation. The following formula was then used to calculate the area under the curve on excel. The area under the curve for juice was found to be 51.67 (Table VIII). The area under the curve was also calculated for 10% sucrose, which is known to be cariogenic and found to be 7.85 (Table VIII. The area under the curve for pH 5.9 for sucrose, juice and water are found to be 100.96, 184.13 and 48.98 respectively.

TABLE III- STUDY PARTICIPANT DEMOGRAPHICS

Gender	Female	6
	Male	4
Ethnicity	Asian	3
	Caucasian	1
	African American	3
	Hispanic	3
Age	Age 6-7	3
	Age 8-10	5
	Age 11-12	2

TABLE IV- IN VIVO DENTAL PLAQUE PH OF STUDY PARTICIPANTS AFTER RINSING WITH 10% SUCROSE AND SORBITOL SOLUTIONS

Time (min)	10% Sucrose	10% Sorbitol
Resting	6.36 ± 0.14	6.75 ± 0.72
Baseline	6.18 ± 0.38	6.53 ± 0.81
2**	5.72 ± 0.40	6.52 ± 0.80
5	5.45 ± 0.84	6.29 ± 0.93
10	5.75 ± 1.07	6.33 ± 0.80
15	5.57 ± 0.69	6.38 ± 0.92
20	6.07 ± 0.60	6.43 ±0.80
30	6.58 ± 0.55	7.60 ± 1.20

*Value represents mean ± SD

** Two minutes after rinsing with solution

TABLE V- IN VIVO DENTAL PLAQUE PH OF STUDY PARTICIPANTS AFTER CONSUMPTION OF DRY READY TO EAT CEREAL (FL) FOLLOWED BY NON-FAT MILK, APPLE JUICE OR WATER

Time (min)	FL/Milk	FL/Juice	FL/Water
Resting	6.93±0.79[a]	6.91±1.77	6.51±0.74
Baseline[b]	6.86±0.76	6.93±0.73	6.69±0.82
2	6.75±0.84	6.24±0.79	6.14±0.62
5	6.17±0.63	5.53±0.79	6.02±0.51
7[c]	6.45±0.67	5.25±1.04	5.81±0.64
10	6.24±0.62	5.29±0.94	5.63±0.59
15	6.17±0.67	5.52±0.74	5.93±0.62
20	6.44±0.60	5.55±0.86	6.10±0.34
25	6.54±0.81	5.79±0.73	6.05±0.60
35	6.63±0.61	5.90±0.75	6.27±0.41

a- Value represents mean ± SD
b- Zero minutes after consuming dry FL
c- Two minutes after consuming the beverage

TABLE VI- BASELINE PH, MINIMUM PH, TIME OF MINIMUM PH AND MAXIMUM DELTA pH (ΔpH) RECORDED FOR EACH TEST GROUP

Test Food	Baseline pH	Minimum pH	Time of minimum pH (min)	Maximum delta pH[a] (ΔpH)
10% Sucrose	6.18	5.45	5	0.75
10% Sorbitol	6.53	6.29	5	0.24
FL/ Milk	6.87	6.17	5	0.7
FL/ Juice	6.93	5.25	7	1.65
FL/ Water	6.69	5.63	10	1.06

a- Maximum delta pH is defined as baseline pH minus minimum pH recorded during the study for each test group

TABLE VII- p VALUES FROM PAIRED SAMPLES T TESTS PERFORMED TO COMPARE BASELINE PH VALUES WITH THE MINIMUM PH AND END POINT PH WITHIN EACH TEST GROUP

Test Food	Baseline Vs Minimum pH p values[a]	Baseline Vs End Point p values[b]
FL/Milk	0.001	0.361
FL/Juice	0	0.002
FL/Water	0.004	0.2

a- This value represents the p value obtained when performing paired samples t test to compare the minimum pH value and the baseline pH value for each test group.
b- This value represents the p value obtained when performing paired samples t test to compare the end point pH value and the baseline pH value for each test group. End point is defined as time=30min

TABLE VIII- AREA UNDER THE CURVE FOR PH 5.5 AND 5.9 FOR EACH TEST GROUP

Test Food	$AUC_{5.5}$[a]	$AUC_{5.9}$[b]
Sucrose	7.85	100.96
FL/Milk	N/A	N/A
FL/Juice	51.67	184.13
FL/Water	N/A	48.98

a- Values represent area under the curve of pH 5.5 for each test group
b- Values represent area under the curve of pH 5.9 for each test group

FIGURE 8- *IN VIVO* DENTAL PLAQUE pH AFTER CONSUMPTION OF TEST FOODS FL/MILK, FL/JUICE, FL/WATER

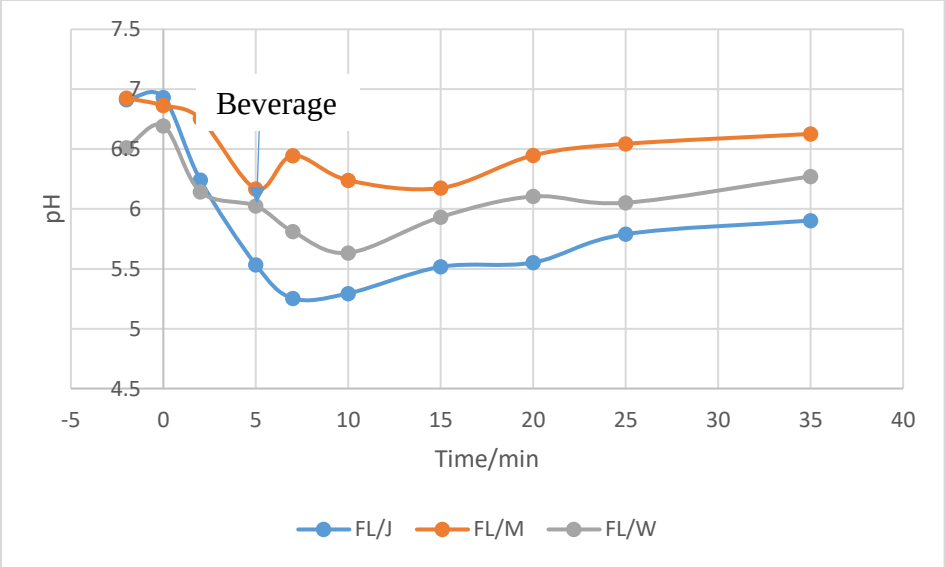
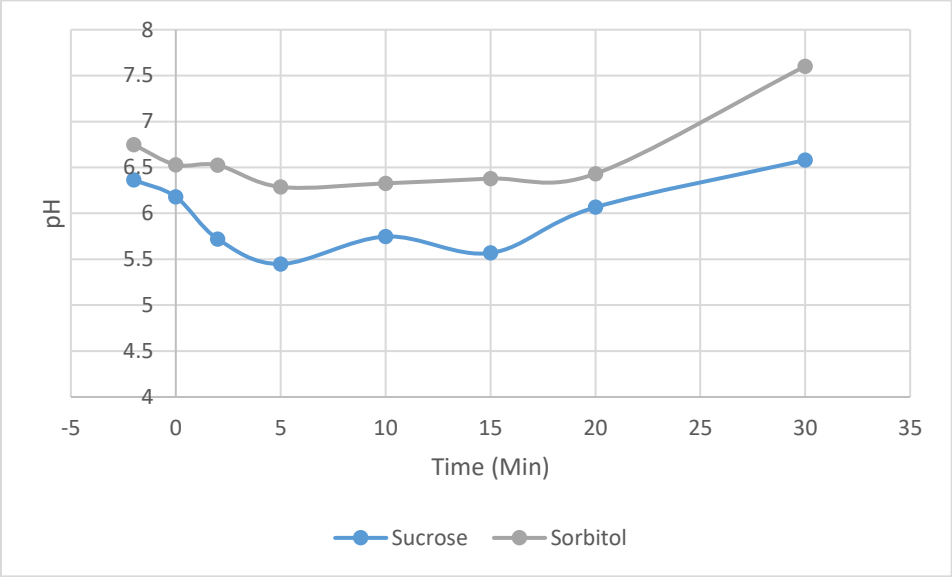


FIGURE 9- *IN VIVO* DENTAL PLAQUE pH AFTER RINSING WITH 10% SUCROSE, AND 10% SORBITOL



4. Discussion

Dental plaque biofilm is complex community consisting of a diverse number of bacterial species embedded in a polymer matrix such as glucan that are protected from the environment. Dental plaque biofilm adheres strongly to tooth surfaces and resists dislodgment. Acid produced by plaque bacteria after a sugary challenge supports research into how dental plaque pH changes with food intake in determining the cariogenic potential of these foods. The ability to accurately measure dental plaque pH *in vivo* remains important. There are three methods for measuring plaque pH intraorally: touch electrode, indwelling electrode and plaque sampling. A touch electrode was used in this study, specifically we used the Beetrode electrode. This method was chosen because of its small size making it possible to insert in interproximal spaces (tip was 0.1mm [53]). The ability to obtain pH readings promptly is a benefit when performing plaque studies in children. The additional advantage of this electrode is that it causes minimal plaque disturbance. This allowed repeated measurements of pH values with minimal plaque dislodgment and maximized accuracy. The disadvantage is that the electrode tips are very fragile, and easy to break with the slightest bend or pressure. If the subject moved during the measurement, the electrode tip could be compromised.

This study involves using pH electrodes to measure plaque pH intraorally in order to determine the effect of three different beverages on plaque pH. The Beetrode electrode allowed repeated measurements of plaque pH with minimal disturbance of the plaque and at short intervals, which is preferential in children. Baseline pH values were measured at the start of each session and these baseline values were not statistically different from each other, because none were more than 2 standard deviations from the mean, indicating their reproducibility.

The fact that different types of food can have varying effects on plaque acidogenicity has long been investigated. Researchers have stressed that the sugar content, retentiveness, solubility and rate of clearance by saliva are factors that affect the cariogenicity of a food. Stephan has shown that the consumption of sucrose causes the plaque pH to fall in a pattern as described by the Stephan curve. He recognized that if the pH falls below 5.5, which is the critical pH, tooth demineralization would occur. He also stated that the higher the frequency of this fall in pH, the more the caries balance would be pushed towards the carious process. Since this theory was developed, many researchers have added modifications to this. The theory of mouth friendly foods has also been discussed [54]. Cheese and milk are just two of the identified mouth friendly foods [55,56]. They have properties which prevent the plaque pH fall and hence are deemed mouth friendly since they do not contribute to the carious process. Food sequencing is also another recent development. It involves the theory that the consumption of certain types of foods after sugary food could offer protective properties to the mouth by preventing the fall in plaque pH. This theory has been applied to weight loss and diabetic control. It is found that consumption of certain types of food before a high carbohydrate meal could aid in controlling the effect of that high carbohydrate meal on the glycemic index.

Cereals have become a common breakfast food. This is because of its ease of consumption, preparation and accessibility. Recently, more and more people are consuming dry cereal as snacks. More and more so, cereal is being consumed as a dry snack. Children especially are taking up this dietary habit.

Sucrose and sorbitol were used as positive and negative controls in this study. The pH curve obtained for sucrose was the typical Stephan curve with a steady fall in pH to below the

critical pH of 5.5 and then a slow rise in pH back to baseline at the end of the study. Sorbitol which is a sugar that is not metabolized by cariogenic bacteria did not show a significant change in pH, as expected. This test showed that our pH measurement method is a reliable method.

Among the 19 eligible subjects, only ten subjects were qualified. Some eligible subjects did not meet the criteria of pH falling below 5.5 during the sucrose screening visit. Some were unable to return for further study visits and so, the readings were not utilized in the study.

The pH values were measured for the right and left upper side of the subjects' mouth, however, only the values for the right side of the mouth were utilized. This was because consistently, the participants had greater amounts of plaque on the right side of their mouth than the left. This may be because children who are right handed may brush the left side of the mouth more vigorously and hence the left side became less susceptible to plaque accumulation.

Consumption of FL in all three test groups showed plaque pH falls over the 5 minute period. With the consumption of juice at the 5min mark, the plaque pH continued to decrease and then showed a slow increase over the 30min to an end point pH of 5.90 and was unable reach baseline values. The paired T test showed that the end point pH (at 35 min mark) was significantly different from the baseline pH (at time 0 min). This showed the conclusion that juice consumption after FL did not aid in regaining plaque pH values to pre-FL consumption levels. In addition, juice resulted in the lowest minimum pH of 5.24. While consumption of water after FL showed a modest fall in pH to a minimum pH of 5.63. The consumption of milk after FL showed the least fall in pH with a minimum pH of 6.17 (Table V).

From the calculations of Δ pH, the maximum Δ pH value obtained for juice at 1.65 which is the largest Δ pH of all three beverages. Water showed a modest maximum Δ pH of 1.06 and milk had the smallest maximum Δ pH value of 0.7 (Table VI).

In our data analysis, we also calculated the area under the curve for pH 5.5. This value of area under the curve is significant because it represents the extent of area that the pH values were below the critical pH of 5.5. At pH values below 5.5, enamel dissolution and demineralization occur and the longer the pH remains below 5.5, the longer the time of the demineralization. Area under the curve for pH 5.5 would allow comparison of the extent of area that the pH values were below the critical pH of 5.5 for each test group.

Based on our results, only juice showed plaque pH falls below 5.5 and the area under curve (for pH 5.5) was calculated to be 51.67 (Table VIII). The same area under the curve of pH 5.5 was calculated for sucrose and found to be a much smaller value of 7.85. Sucrose is a known acidogenic substance due to its ability to lower plaque pH below the critical pH of 5.5. Our data indicating that juice resulted in a much larger area under the curve pH 5.5, further confirmed a higher acidogenic potential, more than that of 10% sucrose solution. In our study, milk and water did not show pH values lower than pH 5.5.

The area under the curve is also calculated for pH 5.9. FL/J, FL/W and 10% sucrose all showed area under the curve for pH 5.9. FL/M did not show pH values under 5.9 and so area under the curve was not calculated. The area under the curve of pH 5.9 was much larger for juice at 184.13, while that of sucrose was less at 100.93. This shows that the FL/J group had a much longer time spent under the pH of 5.9 than even 10% sucrose. Water had an area under curve for pH 5.9 of 48.98, which is the smallest of all three groups compared. This shows that water was able to buffer any falls in pH after cereal consumption to a certain extent, more than juice was

able to. It is important to note that the FL/M group did not have any pH values recorded below 5.9, indicating that milk was the best at buffering the low plaque pH among of all three food groups.

Age appropriate dietary advice is a part of anticipatory guidance during a child's dental exam. The AAPD has published guidelines regarding limiting sugar intake in children and reducing the frequency of sugary intake. The AAPD has also advised on the benefit of drinking water after a sugary food consumption to reduce pH falls after sugar challenge. Although there is an extensive amount of research into food sequencing for weight loss and control of diabetes, food sequencing and its role in caries prevention has been less frequently studied. Wu's laboratory has reported that consumption of dry cereal alone causes pH falls to 5.52 after 10min in adults and ending at 5.83 after a 30min time period [Naval]. In the current research, we have shown that, in children, consumption of milk or water after consumption of dry ready-to-eat cereal could be reduce and buffer the pH falls that can be expected after consumption of dry ready-to-eat cereal alone. Similar to findings in adults, children who consumed juice after cereal did not aid in buffering but caused plaque pH values to decrease further and was unable to re-establish to baseline values.

4.1 Limitation of Study

In this study, we were able to recruit twenty study participants. However after screening for exclusion, inclusion criteria and plaque acidogenicity, there were ten participants who completed this stuy. In future studies, we could aim to recruit more participants.

As described in the methods, plaque pH readings from the left and right maxillary arches interproximal spaces. But in our data analysis, we used the pH readings from the right side only.

This was because the left side of the maxillary arch of our study participants often had less amounts of dental plaque when compared with the right side. We suggest that this was because children often chew on one side of the mouth and often have better oral hygiene on one side of the mouth. We believe this may have caused the plaque to not be within the quantity needed for plaque measurement. In addition, the right handed dentist would have better access to the right side of the mouth compared to the left side. Perhaps in future studies, a left and right handed dentist could perform the study. The equipment set up could also be adjusted such that it would allow the dentist to move from one side of the dental chair to the other.

5. Conclusions

1. Consumption of dry ready-to-eat Froot Loop cereal (FL) resulted in plaque pH falls over a 5min period
2. Drinking juice after eating FL (FL/J) caused the plaque pH to decrease to below the critical pH of 5.5 and stayed below 5.5 longer than for 10% sucrose. The plaque pH for FL/J did not regain close to baseline pH and was statistically different between the end point pH and baseline pH. Juice consumption after FL also resulted in the lowest minimum plaque pH compared to water and milk.
3. Consumption of milk after eating FL caused the plaque pH to reach a minimum value that was the higher when compared to water and juice. Milk also brought the plaque pH close to baseline values at the end of 30minutes, such that the difference between the two values were not statistically significant from each other.
4. Consumption of water after eating dry Froot Loop cereal (FL/W) caused plaque pH to decrease to a value intermediate between juice and milk. Water did bring the plaque pH close to baseline values at the end of the study, such that the difference between the two values were not statistically significant from each other.
5. Drinking milk after consumption of a sugary Froot Loop cereal reduces *in vivo* plaque acidogenicity, as did water.
6. Milk and water can be consumed after consumption of sugary foods or snacks to help reduce plaque acidogenicity and have a positive effect on the oral

cavity. In an effort to prevent a negative effect on the oral cavity, this type of food sequencing can be considered in children who actively and frequently consume sugary snacks.

7. Further research is needed into the role of food sequencing on plaque acidogenicity.

Cited Literature

1. Naval S, Koerber A, Salzmann L, Punwani I, Johnson B, Wu C. The effects of beverages on plaque acidogenicity after a sugary challenge. *JADA*. 2013;144.

2. Edwina Kidd and Ole Fejerskov. **Essentials of dental caries**. . July 2016.

3. Costerton JW, Lewandowski Z, DeBeer D, Caldwell D, Korber D, James G. **Biofilms, the customized microniche.**. *J Bacteriol.* 1994;176(8):2137-42.

4. Selwitz RH, Ismail AI, Pitts NB. Dental caries. *Lancet*. 2007;369(9555):51-59. doi: S0140-6736(07)60031-2 [pii].

5. Norman Tinanoff. Association of diet with dental caries in preschool children. *Dent Clin N Am*. 2005;49 (727-737).

6. Regina B. Surgeon general's perspectives. *Public Health Reports*. 2010;Volume 125(March–April 2010).

7. Center for Disease Control and Prevention. **Protecting your child's teeth**. https://www.cdc.gov/fluoridation/factsheets/fl_caries.htm. Updated 20152017.

8. Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. *Am J Dent*. 2009;22(1):3-8.

9. Council on Clinical Affairs. Guideline on caries-risk assessment and management for infants, children, and adolescents. *AAPD REFERENCE MANUAL*. 2014.

10. Raner E, Lindqvist L, Johansson S, et al. pH and bacterial profile of dental plaque in children and adults of a low caries population. *Anaerobe*. 2014;27:64-70. doi: 10.1016/j.anaerobe.2014.03.010 [doi].

11. Pollard, Higliam, Curzon, Edgcn. Acid anion profiles in dental plaque following consumption of cereal-based foods and fruits. *European journal of ORAL SCIENCES*. 1996;104.

12. Pretty IA, Edgar WM, Smith PW, Higham SM. Quantification of dental plaque in the research environment. *J Dent*. 2005;33(3):193-207. doi: S0300-5712(04)00163-0 [pii].

13. Rajiv Saini, Santosh Saini and Sugandha Sharma. **Biofilm: A dental microbial infection**. *J Nat Sci Biol Med*. 2011;Jan-Jun.

14. Loesche WJ. Chemotherapy of dental plaque infections. *Oral science reviews*. 1976;(9): 65–107.

15. Theilade E. *The non-specific theory in microbial etiology of inflammatory periodontal diseases*. *J Clin Periodontol*. 1986;13, 905-911.

16. Rosier BT, De Jager M, Zaura E, Krom B. Historical and contemporary hypotheses on the development of oral diseases: Are we there yet? *Frontiers in Cellular and Infection Microbiolog*. 2014.

17. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. *Adv Dent Res*. 1994;8(2):263-271. doi: 10.1177/08959374940080022001 [doi].

18. JDB Featherstone. **Dental caries: A dynamic disease process**. *Australian Dental Journal*. 2008.

19. M. Wilson. Food constituents and oral health current status. *Woodhead Publishing*. 2009;1st Edition.

20. Curzon ME, Hefferren JJ. Modern methods for assessing the cariogenic and erosive potential of foods. *Br Dent J*. 2001;191(1):41-46. doi: 10.1038/sj.bdj.4801087a [doi].

21. Lingstrom P, Imfeld T, Birkhed D. Comparison of three different methods for measurement of plaque-pH in humans after consumption of soft bread and potato chips. *J Dent Res*. 1993;72(5):865-870. doi: 10.1177/00220345930720050601 [doi].

22. Stephan RM. **Changes in hydrogen-ion concentration on tooth surfaces and in carious lesions**. *The Journal of the American Dental Association*. Volume 27 , Issue 5 , 718 - 723.

23. GUAY-FEN HUANG MG. Resting dental plaque pH values after repeated measurements at different sites in the oral cavity. *Proc. Natl. Sci. Counc.* 2000;24.

24. Preston AJ, Edgar WM. Developments in dental plaque pH modelling. *J Dent*. 2005;33(3):209-222. doi: S0300-5712(04)00171-X [pii].

25. Liu CC, Bocchicchio DB, Overmyer PA, Neuman MR. A palladium-palladium oxide miniature pH electrode. *Science*. 1980;207(4427):188-189.

26. Paula Moynihan and Poul Erik Petersen. Diet, nutrition and the prevention of dental diseases. *Public Health Nutrition*. 2004;7(1A), 201–226.

27. Riva Touger-Decker and Cor van Loveren. Sugars and dental caries. *Am J Clin Nutr*. 2003;78.

28. David J. Bradshaw and Richard J. M. Lynch. Diet and the microbial aetiology of dental caries: New paradigms. *International Dental Journal*. 2013;63 (Suppl. 2): 64–72.

29. Linda Scott Kantor. A comparison of the U.S. food supply with the food guide pyramid recommendations. *The Food Supply*. 2005.

30. Stephan RM. Two factors of possible importance in relation to the etiology and treatment of dental caries and other dental diseases. *Science*. 1940;92(2399):578-579. doi: 92/2399/578 [pii].

31. William H. Bowen. The stephan curve revisited. *Odontology*. 2013;101:2–8.

32. Dong YM, Pearce EI, Yue L, Larsen MJ, Gao XJ, Wang JD. Plaque pH and associated parameters in relation to caries. *Caries Res*. 1999;33(6):428-436. doi: 16547 [pii].

33. Katz S, Olson BL, Park KC. Factors related to the cariogenic potential of breakfast cereals. *Pharmacol Ther Dent*. 1975;2(2):109-131.

34. Market research report, ed. ***Breakfast cereals market analysis, market size, application analysis, regional outlook, competitive strategies and forecasts, 2015 to 2022***. ; 2016.

35. Peter G. Williams. The benefits of breakfast cereal consumption: A systematic review of the evidence base. *American Society for Nutrition. Adv. Nutr*. 2014;636S–673S,.

36. Louie JC, Moshtaghian H, Boylan S, et al. A systematic methodology to estimate added sugar content of foods. *Eur J Clin Nutr*. 2015;69(2):154-161. doi: 10.1038/ejcn.2014.256 [doi].

37. Sigrid A Gibson. Breakfast cereal consumption in young children: Associations with non-milk extrinsic sugars and caries experience: Further analysis of data from the UK national diet and nutrition survey of children aged 1.5±4.5 years. *Public Health Nutrition:*. 2000;3(2), 227±232.

38. Marshall TA, Levy SM, Broffitt B, et al. Dental caries and beverage consumption in young children. *Pediatrics*. 2003;112(3 Pt 1):e184-91.

39. Moynihan PJ. Dietary advice in dental practice. *Br Dent J*. 2002;193(10):563-568. doi: 10.1038/sj.bdj.4801628 [doi].

40. Reynolds EC. **The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model.**. *J Dent Res.* 1987.

41. Bowen WH, Pearson SK, VanWuyckhuyse BC, Tabak LA. **Influence of milk, lactose-reduced milk, and lactose on caries in desalivated rats.**. *Caries Res.* 1991.

42. Gregory J, Lowe S, Bates C. National diet and nutrition survey: Young people aged 4 to 18 years. *National diet and nutritional survey*. 2000.

43. Norman Tinanoff, Carol A. Palmer. Dietary determinants of dental caries and dietary recommendations for preschool children. *Journal of Public Health Dentistry*. 2000;60.

44. PREETHI B P, MAITREYEE D S, DODAWAD R. **The effect of four fruit juices on the ph of dental plaque - A four period cross-over study**. *Journal of clinical and diagnostic research*. 2010.

45. Preethi B P, Maitreyee D S,et al. The effect of four fruit juices on the ph of dental plaque - A four period cross-over study. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*. 2010.

46. Azrak B, Callaway A, Knözinger S, Willershausen B. **Reduction of the pH-values of whole saliva after the intake of apple juice containing beverages in children and adults**. *Oral Health Prev Dent.* 2003.

47. I. HUSSEIN, M. A. POLLARD & M. E. J. CURZON. A comparison of the effects of some extrinsic and intrinsic sugars on dental plaque pH. *International Journal of Paediatric Dentistry*. 1996.

48. Isha Goel, S. Navit , Sandeep Singh Mayall, Mandeep Rallan, Pragati Navit, Sneha Chandra. Effects of carbonated drink & fruit juice on salivary pH of children: An in vivo study. *International Journal of Scientific Study*. 2013.

49. Y. H. Al-Dlaigan, Shaw, A. Smith. **Dental erosion in a group of british 14-year-old school children. part II: Influence of dietary intake**. *BRITISH DENTAL JOURNAL*. 2001;190.

50. D Tricò, E Filice, S Trifirò and A Natali. Manipulating the sequence of food ingestion improves glycemic control in type 2 diabetic patients under free-living conditions. *Nutrition & Diabetes*. 2016.

51. D. Tricò , E.Filice, S.Baldi, S.Frascerra, A.Mari, A.Natali. Sustained effects of a protein and lipid preload on glucose tolerance in type2 diabetes patients. *ScienceDirect*. 2016.

52. Schachtele Ch.F. et al. Human plaque acidity models - working group consensus repor. *J. Dent. Res*. 1986.

53. I. Şaroğlu Sönmez, Ş. Aras. Effect of white cheese and sugarless yoghurt on dental plaque acidogenicity. *Caries Res.* 2007;41:208–211.

54. Moynihan P PP. **Diet and health: Implications for reducing chronic disease risk**. *Public Health Nutr.* 2004;7(1A):201-26.

55. Papas A, Joshi A, Belanger AJ,. Dietary models for root caries. . *Am J Clin Nutr*. 1995;61(suppl):417S–22S.

56. Jensen ME WJ. Effects of processed cheese on human plaque pH and demineralization and remineralization. *Am J Dent*. 1990;3: 217–23.

APPENDIX A
Initial IRB Approval

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)

November 25, 2015

Christine D. Wu, MS, PhD
Pediatric Dentistry
801 S. Paulina St., 469J Dent. Bldg.
M/C 850
Chicago, IL 60612
Phone: (312) 355-1990 / Fax: (312) 996-1981

RE: **Protocol # 2015-1046**
"Plaque Acidogenicity Resulting from Dry Sugary Cereal and Beverages in Children"

Dear Dr. Wu:

Your Initial Review (Response To Modifications) was reviewed and approved by the Expedited review process on November 25, 2015. You may now begin your research

Please note the following information about your approved research protocol:

Protocol Approval Period: November 25, 2015 - November 24, 2016
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: National Dairy Research Council
PAF#: 2015-05733
Grant/Contract No: 553192 628000 191100 G3935
Grant/Contract Title: Consumption of milk after sugar snacks reduces dental plaque acid production and benefits oral health in children

Research Protocol(s):

a) Plaque Acidogenicity Resulting from Dry Sugary Cereal and Beverages in Children, Version 1.1; 11/11/2015

Recruitment Material(s):

a) Telephone Script, Version 1.1; 11/24/2015
b) Flyer: "Volunteers needed for Research Study", Version 1; 09/29/2015

Informed Consent(s):

a) Alteration of Informed Consent granted for the use of the telephone screening 45 CFR 46.116(d)
b) Waiver of Documentation of Informed Consent granted for the use of the telephone screening 45 CFR 46.117(c)(2)

Assent(s):

a) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.1; 11/24/2015

Parental Permission(s):

a) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.1; 11/24/2015

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

(4) Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving X-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)
Examples: (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action

10/12/2015	Initial Review	Expedited	10/22/2015	Modifications Required
11/13/2015	Response To Modifications	Expedited	11/25/2015	Approved

Please remember to:
→ Use your **research protocol number** (2015-1046) 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) 996-0548. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Brandi L. Drumgole, B.S.
Assistant Director
Office for the Protection of Research Subjects

Enclosure(s):

1. **Assent Document(s):**
   a) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.1; 11/24/2015
2. **Parental Permission(s):**
   a) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.1; 11/24/2015
3. **Recruiting Material(s):**
   a) Telephone Script, Version 1.1; 11/24/2015
   b) Flyer: "Volunteers needed for Research Study", Version 1; 09/29/2015

cc: Marcio Da. Fonseca, Pediatric Dentistry, M/C 850
OVCR Administration, M/C 672

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
Continuing Review

November 15, 2016

Christine D. Wu, MS, PhD
Pediatric Dentistry
801 S. Paulina St., 469J Dent. Bldg.
M/C 850
Chicago, IL 60612
Phone: (312) 355-1990 / Fax: (312) 996-1981

RE: Protocol # 2015-1046
"Plaque Acidogenicity Resulting from Dry Sugary Cereal and Beverages in Children"

Dear Dr. Wu:

Your Continuing Review was reviewed and approved by the Expedited review process on November 13, 2016. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: November 24, 2016 - November 24, 2017
Approved Subject Enrollment #: 30 Total (9 enrolled to date)
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: National Dairy Research Council
PAF#: 00025613
Grant/Contract No: 553192 628000 191100 G3935

Grant/Contract Title: Consumption of milk after sugar snacks reduces dental plaque acid production and benefits oral health in children

Research Protocol(s):

b) Plaque Acidogenicity Resulting from Dry Sugary Cereal and Beverages in Children, version 2.1; 03/08/2016

Recruitment Material(s):

c) Flyer: "Volunteers needed for Research Study", Version 2; 04/28/2016

d) Telephone Script, Version 1.2; 03/24/2016

Informed Consent(s):

c) Alteration of Informed Consent granted for the use of the telephone screening 45 CFR 46.116(d)

d) Waiver of Documentation of Informed Consent granted for the use of the telephone screening 45 CFR 46.117(c)(2)

Assent(s):

b) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.1; 11/24/2015

Parental Permission(s):

b) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.2; 03/24/2016

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

(**4) Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving X-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)**
Examples: (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
11/03/2016	Continuing Review	Expedited	11/13/2016	Approved

Please remember to:

→ Use your **research protocol number** (2015-1046) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements in the guidance document,
"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) 355-1404. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,
Sheilah R. Graham, MPH
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

Enclosure(s):

4. **Assent Document(s):**
   b) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.1; 11/24/2015
5. **Parental Permission(s):**
   b) Plaque Acidogenicity Resulting from Dry Sugary Cereal Beverages in Children, Version 1.2; 03/24/2016
6. **Recruiting Material(s):**
   c) Flyer: "Volunteers needed for Research Study", Version 2; 04/28/2016
   d) Telephone Script, Version 1.2; 03/24/2016

cc: Marcio Da. Fonseca, Pediatric Dentistry, M/C 850
OVCR Administration, M/C 672

Appendix C
Consent

University of Illinois at Chicago
Research Information and Parental Permission for Participation in Biomedical Research
Plaque Acidogenicity Resulting from Dry Sugary Cereal and Beverages in Children

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. Christine D. Wu, Professor
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 of dry sugary cereals and beverages on the dental tartar pH because you 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. This acid destroys the tooth structure and produces dental caries. Different bacterial species are present on the surfaces of teeth in the form of dental bacterial deposit on tooth surface. Some of these species break down sugars and other food components to produce acid when foods rich in carbohydrates are consumed. As acid production by bacteria in dental bacterial deposit on tooth surface is an important factor leading to the development of dental caries, we wish to measure dental bacterial deposit on tooth surface acidogenicity after consumption of dry cereal, dry cereal combined with milk and dry cereal followed by drinking milk or fruit juice or water

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 at any time without affecting that relationship.**

Approximately 30 subjects may be involved in this research at UIC.

What is the purpose of this research?

This research is being done to better understand the effect of sugary dry cereal and beverages on the dental tartar pH in children.

What procedures are involved?

This research will be performed at the College of Dentistry at 801 S. Paulina Street, Chicago, IL, 60612.
You will need to come to the study site seven times including this visit over the next 2 months period (1 visit per week). Each of those visits will take about 45-60 minutes.

Study procedures:

- Before each visit, we request that your child does not eat or drink for 2 hours prior to the test or brush, floss or use a mouth rinse for 24 hours prior to the test.
- The test will involve placing a small metal wire between your child's teeth to measure bacterial acid production.
- First a screening test will be performed to make sure that your child demonstrates an adequate drop in bacterial deposit on tooth surface pH.pH is a measurement of the amount of acid is in the bacterial deposit on tooth surface. During the initial screening, the resting bacterial deposit on tooth surface pH, that is the bacterial deposit on tooth surface pH taken prior to eating or drinking anything, will be measured by placing a small metal wire in the area between the upper posterior teeth on each side of your child's mouth. Your child will then be asked to rinse with 10 ml sugar solution (10% sucrose) for 1 minute, following which tartar pH will be measured at 2, 5, 10 , 15, 20 and 30min. A drop in plaque pH value to ≤ 5.5 will qualify him/her to participate in the research.
- In addition, your child will be given an oral exam to check for any dental problems that may affect the study results. If your child has dental problems, such as cavities in the area of bacterial deposit on tooth surface testing, he or she may not be able to participate in the study. If you agree to let your child participate and your child is accepted based on the criteria previously listed, he/she will have to come to the College of Dentistry for six more visits in next six weeks (one visit per week).

At each visit, we will give your child one test food to eat. The test food groups will be randomly assigned for each visit (that is, random just like flipping a coin). The seven different food groups are a) commercially marketed dry Froot Loops (FL) cereal, b) FL followed by drinking whole milk, c) FL followed by drinking apple juice, d) FL followed by drinking water, e) FL combined with whole milk and g) 10% sorbitol solution. Sucrose and sorbitol solutions are purified water sugar solutions.

- On the day of testing, resting bacterial deposit on tooth surface pH will be first measured by placing the thin metal wire in the same areas as the previous visit. Your child will be then asked to eat or rinse one of the randomly assigned test food groups. The bacterial deposit on tooth surface pH readings will then be recorded at various time intervals up to 30-35 minutes.
- The estimated time for each visit is 45-60 minutes.

What are the potential risks and discomforts?

The likely risk expected in this study is slight discomfort during the pH measurement. The risk of loss of confidentiality is possible. A possible risk of research is that your child' participation in the research or information about your child and your child' health might become known to individuals outside the research. To protect confidentiality, your child' 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 others to understand the effect of sugary cereal and beverages on tartar pH in children in the future.

What about privacy and confidentiality?

A possible risk of research is that your child' participation in the research or information about your child and your child' health might become known to individuals outside the research. To protect confidentiality, your child' information will only be identified by a numeric code. No information about your child, or 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.

A possible risk of the research is that your participation in the research or information about you and your health might become known to individuals outside the research.

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. All data will be stored in a locked file cabinet within a locked room.

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 and $10 for the screening 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 $90 for completing the six study visits at the end of the study. The total amount you can get for completing the screening and six study visits is $100

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 participation in this study without your consent if: They believe it is in your best interests.

In the event you withdraw or are asked to leave the study, you will still be compensated as described above.

Who should I contact if I have questions?

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.

What are my rights as a research subject?

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.

Remember:

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.

Signature of Parent or Legal Guardian

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

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

_____

Printed Name of Child

APPENDIX D
ASSENT

University of Illinois at Chicago

ASSENT TO PARTICIPATE IN RESEARCH

Form Template – 09/29/2015

Plaque Acidogenicity Resulting from Beverages Consumed after Sugary Cereal in Children

- My name is Dr. Larry Salzmann and my research partner is named Dr. Christine Wu.. We are asking you to take part in a research study because we are trying to learn more about the effect of sugary breakfast cereal and beverages on oral health. If you agree to be in this study you will visit College of Dentistry for total of 7 times including this visit. At two of the visits, you will be asked to rinse with the sweetened water solution and at the other five visits, you will be asked to eat one of the test foods such as cereal, or cereal combined with milk or eating cereal and following it by drinking milk or juice or water. At every visit we will measure the amount of acid in your mouth by holding a small thin metal wire on your upper teeth at 0, 2, 5, 10, 15, 20, and 30 minutes time intervals. This will help us to measure the tartar pH which relates to the amount of acid in your mouth.

- There is no risk or harm involved in letting us touch your tooth surface and gum area with the thin metal wire. It does not cause any pain but you might experience slight discomfort.

- You may learn more about teeth and oral health. There is no direct benefit yo you but it will allow us to learn more about the effects of certain foods and beverages 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 312-996-6661 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

APPENDIX E
Sample Data Collection Chart for Controls

Time	Mv	Ph				
		4				
		7				
		10				
Base						
Sucrose 0min						
2min						
5min						
10min						
15min						
20min						
30min						
	R			L		
	mV	pH		mV	pH	Time/min
Base						0
Sucrose 0min						Sucrose 0min
2min						2
5min						5
10min						10
15min						15
20min						20
30min						30

APPENDIX F
Sample Data Collection Chart for Froot Loops and Beverage Test Groups

Time	Mv	Ph					
		4					
		7					
		10					
Base							
L							
Cereal 0min							
2min							
5min							
DRINK							
2min							
5min							
10min							
15min							
20min							
30min							
	R	pH		L	pH	Time/min	
Base						Base	
0						0	Cereal
2						2	
5						5	
7						7	DRINK
10						10	
15						15	
20						20	
25						25	
35						35	

		Right- Froot Loop/ Juice									
Time	Time	1	2	3	4	5	6	7	8	9	10
Base	-2	6.012	5.40108	6.62736	6.85745	11.96705	5.8437	6.5689	6.60188	6.07556	7.17495
0	0	6.65356	6.14068	6.75192	7.29514	6.14412	7.17015	8.4411	7.47908	5.90356	7.32027
2	2	6.12896	5.55244	4.99251	6.72943	6.00824	5.24245	7.7233	6.55544	6.94416	6.54004
5	5	5.90364	4.50152	4.84546	5.93363	6.151	4.0196	6.7539	5.5974	5.71264	5.92416
7	7	5.64048	4.41724	4.28148	6.10317	5.12588	3.56635	7.56605	5.40132	5.02464	5.41208
10	10	5.25004	4.77156	4.36625	5.43539	5.89816	3.71065	7.4273	5.19664	5.12956	5.75808
15	15	6.11176	4.65976	4.38701	5.52362	5.33056	5.29425	7.20345	5.93968	5.29124	5.42419
20	20	6.68796	4.55312	4.91466	5.84021	6.0048	4.4007	7.1239	4.76148	5.7608	5.47782
25	25	6.16336	5.24972	4.84027	5.66894	5.5318	5.7364	7.4384	6.60532	4.99024	5.66812
35	35	6.57272	5.11212	5.03403	6.17064	6.21808	4.72075	7.32555	6.01364	5.6146	6.24767

		Left- FL/J									
Time	Time	1	2	3	4	5	6	7	8	9	10
Base	-2	5.24488	5.74164	7.26573	7.25535	5.78464	6.14895	6.68175	7.46016	5.55268	6.74245
0	0	7.18504	7.41176	7.49409	7.73975	6.52768	8.0748	8.81295	8.487	6.767	8.02957
2	2	6.64496	6.53284	6.96644	7.53388	5.92568	6.9315	7.83245	7.025	6.06008	7.48289
5	5	7.09904	6.46404	6.67061	6.28482	6.59304	7.1091	7.7307	5.83476	5.18804	6.78397
7	7	5.2896	5.94116	4.51676	5.4994	5.69004	4.74665	7.96565	6.51932	4.95928	5.54183
10	10	5.89848	5.86032	5.06171	6.22427	5.67284	4.86875	7.6678	6.64316	5.24136	6.34974
15	15	6.51424	5.04676	5.52189	6.27271	5.48708	5.53845	7.17385	5.83132	5.67996	5.74943
20	20	6.59336	1.58096	5.98034	5.98899	5.70036	5.2295	7.1646	6.04632	5.48044	6.2321
25	25	6.4936	5.52492	5.59455	6.02013	5.84484	5.5884	7.4754	7.326	5.83304	6.66287
35	35	6.68452	6.61712	5.21222	6.39554	5.77088	5.8067	7.29225	6.40236	5.75564	7.18533

		Right side – Froot Loop/ Water									
Time	Time	1	2	3	4	5	6	7	8	9	10
Base	-2	5.43936	5.69952	6.11676	6.47415	5.4792	7.30632	6.70053	7.26148	7.33918	7.290547
0	0	6.01026	6.04524	6.53742	7.36848	5.42046	6.28032	6.30955	8.06128	7.88728	7.019295
2	2	5.88051	5.78552	6.71355	6.01587	7.07052	5.03544	6.73513	5.5518	6.76846	5.879694
5	5	4.79061	5.79068	6.65883	6.60411	6.35318	6.01527	5.80958	5.85452	6.24646	6.125356
7	7	5.38227	4.99776	6.5682	6.71868	6.83912	5.3022	5.52067	5.18716	5.71576	5.879694
10	10	4.94631	5.03044	5.68584	6.24843	6.1378	4.77039	5.81131	5.225	6.65014	5.826808
15	15	4.98264	5.22996	5.50629	6.97005	6.94414	5.98107	6.03967	5.93192	5.60092	6.137298
20	20	6.00507	5.2334	6.22278	6.48441	6.70918	6.1401	5.99642	6.085	5.97154	6.195302
25	25	5.13488	5.28844	6.53742	6.33906	7.04204	6.01527	6.13655	5.33508	6.64666	6.043469
35	35	6.20921	5.62384	6.17319	6.48099	6.67358	6.04434	6.79049	5.77196	6.90592	6.046881

		Left Side – FL/W									
Time	Time	1	2	3	4	5	6	7	8	9	10
Base	-2	5.43244	6.6696	7.10172	7.42149	6.71274	7.16439	6.87872	7.43864	7.4053	7.227425
0	0	6.96003	7.14088	7.94475	8.62704	7.72022	6.79161	7.35793	7.40252	9.23404	7.841582
2	2	6.33031	7.306	8.00802	8.16705	6.89786	7.06521	7.26451	7.11012	6.88852	7.118242
5	5	5.49126	6.54404	7.16841	7.84386	6.6362	6.71124	6.82855	6.07812	7.05904	6.652507
7	7	5.51375	6.68336	7.08291	7.79427	5.64652	8.39217	6.88391	5.6464	6.61012	6.22601
10	10	5.43763	6.4546	6.36642	7.54974	5.53438	7.29435	5.87013	7.68804	6.61534	7.154068
15	15	6.36145	6.37548	6.75801	7.04529	6.97974	7.55085	6.28533	6.31548	6.67276	6.77022
20	20	6.20229	6.35484	6.91362	7.16157	11.51052	7.53546	5.92203	6.40664	7.68022	6.824812
25	25	5.77498	6.15876	7.16328	7.10343	6.52762	7.18149	6.24727	6.31204	7.0399	7.247897
35	35	5.82861	6.2224	6.63147	6.79563	6.55788	7.02588	6.95311	6.93296	7.64716	6.923759

		Right side Froot Loop/ Milk									
Time	Time	1	2	3	4	5	6	7	8	9	10
Base	Base	5.871	7.71675	7.69123	7.24143	6.21033	7.32812	6.66555	6.30386	5.95402	8.2725
0	0	6.95116	7.07037	6.86948	6.19478	7.15254	7.34876	7.60519	5.6096	5.7313	8.1186
2	2	7.06296	7.09602	6.392	7.05805	6.55233	6.76912	8.55159	5.55566	5.41114	7.08747
5	5	5.8194	6.80532	5.85397	5.72768	6.39672	6.1224	7.27057	5.16242	5.62342	6.89766
7	7	5.99312	7.16784	6.72762	6.53559	5.96751	6.13444	7.54942	5.44778	5.73304	7.19862
10	10	5.97764	7.10115	6.87813	6.40584	5.77257	6.475	6.04363	5.49824	5.18494	7.05669
15	15	5.38252	7.30806	6.23976	6.61517	5.70588	6.49048	6.75343	5.19896	5.40418	6.64629
20	20	5.95012	7.40382	6.58576	6.27263	5.69562	6.56272	7.34324	5.6531	6.05842	6.94554
25	25	6.10664	8.26737	5.76574	6.41276	5.51094	6.5266	7.7049	6.04112	6.26374	6.83439
35	35	6.13932	7.87065	6.49061	6.57019	5.92647	7.19052	7.31451	5.90018	6.32116	6.53856

		Left side FL/M									
Time	Time	1	2	3	4	5	6	7	8	9	10
Base	Base	5.97076	8.21265	7.31236	6.99058	6.50445	7.32812	7.32803	6.05156	7.06414	7.79883
0	0	6.61232	7.63125	7.64798	7.64971	7.72197	7.34876	7.05256	6.61184	6.51082	8.67264
2	2	7.30204	7.81764	7.28122	7.48882	7.91691	6.76912	7.59167	5.93672	6.15412	8.03481
5	5	6.989	6.56421	5.88511	6.55808	6.62073	6.1224	6.75681	5.49128	6.29332	7.43289
7	7	6.0688	7.69452	6.17748	6.68091	6.41553	6.13444	7.35338	5.96978	6.78052	7.56114
10	10	5.95528	7.21572	6.23457	6.60652	5.97606	6.475	7.12523	6.3839	6.99628	7.14048
15	15	6.62436	7.24992	6.22938	6.28474	5.71614	6.49048	6.80244	6.27776	6.39076	6.79848
20	20	6.84624	7.80909	6.00967	6.15672	6.03762	6.56272	7.11847	5.68964	7.15114	7.16442
25	25	7.08704	7.64493	5.84878	6.24668	5.44254	6.5266	7.19114	6.44828	6.94582	7.17126
35	35	7.29344	8.04678	6.03562	6.69648	6.01197	7.19052	7.24015	6.30386	7.0537	6.90279

Right	1	2	3	4
Base	6.14135	7.8129	6.03716	6.99485
Sorbitol				
0min	5.83341	7.9166	6.1781	6.18773
2min	5.71923	7.8656	6.26858	6.24416
5min	5.46319	7.7551	5.51516	6.41516
10min	5.35247	7.5681	6.14504	6.24245
15min	5.44762	7.7398	5.63348	6.69218
20min	5.80573	7.7313	5.7605	6.42713
30min	6.95791	7.6089	9.52064	6.32282

Left	1	2	3	4
Base	6.23477	8.1631	5.84576	6.71612
Sorbitol				
0min	5.94586	7.9999	6.50174	7.02563
2min	5.95451	8.2005	6.07892	6.41687
5min	6.02198	7.9268	6.01106	6.28862
10min	5.85763	8.1427	6.152	6.48356
15min	7.01327	8.3331	5.91188	6.54341
20min	4.58781	8.4334	5.82662	6.68363
30min	6.97175	8.0203	9.03344	6.15866

Right	1	2	3	4	5	6	7	8	9	10
Base Sucrose					6.40956		6.14135	6.39482	6.5111	
0min	6.0808	6.61306	6.10754	6.3603	5.45471	6.28904	5.83341	6.29051	5.9107	6.85384
2min	5.85552	5.48632	6.58102	5.52726	5.38542	5.89566	5.71923	5.21663	6.1623	5.37636
5min	5.59152	5.66076	6.38344	5.54684	3.33038	6.25522	5.46319	4.78229	6.1793	5.2938
10min	5.72	5.14812	5.64118	5.26738	8.74852	6.07722	5.35247	4.66259	5.7033	5.15276
15min	6.43456	6.41548	4.97902	5.12142	4.52183	6.37982	5.44762	4.79084	6.1878	5.40904
20min	7.09808	6.56678	5.8797	6.16272	5.82482	6.53468	5.80573	4.72757	6.2235	5.83904
30min	7.97104	6.80174	6.07016	6.18764	6.58025	6.47238	6.95791	6.46322	6.4037	5.89752

Left	1	2	3	4	5	6	7	8	9	10
Base Sucrose					6.11212		6.23477	6.95912	7.1432	
0min		6.98864	6.49024	6.25528	5.81806	6.28904	5.94586	6.89414	6.1538	7.09808
2min		6.36564	7.38024	6.03634	4.97982	5.32784	5.95451	6.9899	5.9243	6.86072
5min		5.68924	6.70384	5.78536	8.62515	5.47024	6.02198	6.12635	5.4891	6.11596
10min		6.51694	6.60772	5.36528	8.64712	6.32642	5.85763	6.20501	5.5639	5.40044
15min		7.0456	5.42402	5.65186	5.82482	5.55924	7.01327	5.45774	5.5316	5.50536
20min		6.92812	5.68212	6.04524		6.36914	4.58781	6.19646	6.0416	5.97664
30min		6.89786	6.1645	6.38522	7.81226	5.9455	6.97175	6.72485	6.9103	6.19164

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Nova Southeastern University (NSU)
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Special Olympics: *Fort Lauderdale, FL*

Give Kids a Smile (GKAS): *Fort Lauderdale, FL*

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