

The Differences of Saliva pH between Consumption of Sucrose Chocolate and Stevia Chocolate in 10-12 years Old Children

Shafwan Rafif Widiyanto^{1*}, Septriyani Kaswindiarti¹, Nendika Dyah Ayu Murika Sari¹, Ariyani Faizah¹

¹Faculty of Dentistry, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia

*Corresponding author:

Shafwan Rafif Widiyanto

Faculty of Dentistry, Universitas Muhammadiyah Surakarta

Surakarta, Indonesia

Tel: (+62)8127689890; E-mail: j520200064@student.ums.ac.id

Abstract

Introduction: Children enjoy eating sweet treats like chocolate, which often contains sugar as a sweetener. This can influence the pH of saliva and affect the processes of demineralization and remineralization in teeth. Up until now, there has not been any research on the variation in saliva pH when consuming regular chocolate (with sucrose) compared to chocolate sweetened with stevia in children aged 10-12 years. This study aims to explore the differences in saliva pH between children in this age group who consume sucrose chocolate and those who consume stevia chocolate.

Methods: In this study, a Quasi-Experimental design was employed, and approval was granted by the Ethical Committee of Health Research at Dr. Moewardi General Hospital under Ethical Clearance Number 2.045/XI/HREC/2023. Initially, twenty-one children aged 10-12 years from SD Muhammadiyah 1 Surakarta had their saliva measured before any intervention. They were then given chocolate containing 30% sucrose for 60 seconds. Ten minutes after this intervention, the children were asked to collect saliva in their mouths and spit out 2ml into a sterile container. The collected saliva was then tested for pH using a pH metre (Hanna brand, Romania). On the following day, the subjects were instructed to consume chocolate sweetened with 1% stevia using the same method. The average difference in saliva pH before and two days after the intervention was analysed using an independent T-Test on the saliva pH difference.

Results: The research discovered that the average change in saliva pH before and after eating sucrose chocolate was 0.3413 ± 0.12557 , while for stevia chocolate, it was 0.1444 ± 0.07698 . Tests for normal distribution (Shapiro-wilk test) and data homogeneity (Levene's homogeneity test) both indicated acceptable conditions ($p > 0.05$). The independent T-Test revealed significant differences in saliva pH between children aged 10-12 years when consuming sucrose chocolate compared to stevia chocolate ($p < 0.05$).

Conclusion: In summary, the study on saliva pH differences in 10-12-year-old children after eating sucrose chocolate compared to stevia chocolate indicates that there is indeed a distinction in saliva pH between the two. Children who consumed sucrose chocolate showed a lower saliva pH compared to those who consumed stevia chocolate.

Keywords: Chocolate, Sucrose, Stevia, Saliva pH

Introduction

Maintaining proper dental and oral health is crucial to overall well-being. The 2019 National Basic Health Research (RISKESDASNAS) revealed that 57.6% of Indonesia's population faced dental and oral health issues, but only 10.2% sought medical care. Dental caries prevalence has risen by 9.8% from 43.4% in 2007 to 53.2% in 2013, particularly affecting vulnerable groups such as pre-schoolers, school children, pregnant women, and the elderly (Kemenkes RI, 2019). This underscores the importance of addressing dental and oral health concerns to promote better overall health.

Dental caries is a gradual process where the hard surface of the tooth breaks down due to organic acids from sugary foods like chocolate and candy. Various factors contribute to this, including bacteria in plaque, cariogenic foods, time, the teeth themselves, and saliva (Mardiati et al., 2017). Dental caries is a dental condition marked by damage starting on the tooth's surface and extending toward the pulp (Tarigan, 2013). The repeated drop in saliva pH over time leads to a gradual demineralization or softening of the teeth. If not addressed, this can result in the continuous growth of cavities in the teeth, which cannot return to their normal state (Mardiati & Prasko, 2017).

One of the factors that can increase the risk of dental caries is saliva. Saliva is an exocrine secretory fluid produced in the mouth by two main pairs of large salivary glands and smaller glands in the oral mucosa (Kasuma, 2015). Saliva's acidity level, measured using pH units (Power of Hydrogen), typically falls between 6.7 and 7.4, and it undergoes changes after eating (Savira et al., 2017).

Saliva with low acidity levels can lead to the removal of calcium, phosphate, and hydroxyl ions from the crystals on the teeth known as hydroxyapatite. When saliva reaches a critical pH of 5.5, it can cause the dissolution of hydroxyapatite, a process known as demineralization. The diffusion of calcium components from saliva into plaque can reduce the solubility of enamel and enhance the remineralization of teeth. Remineralization is possible under favorable oral conditions, including adequate levels of calcium and phosphate, high acidity levels in saliva, and the presence of a suitable organic and inorganic matrix for the formation of apatite crystals (Hidayati et al., 2014; Soesilo et al., 2006; Sulendra et al., 2013). Saliva pH tends to decrease when consuming sugary snacks like candy, bread, biscuits, wafers, ice cream, and chocolate (Purbaningrum et al., 2017).

Chocolate is extremely popular and regularly consumed. According to a survey conducted by Naveed et al. (2015), approximately 92.5% of children eat chocolate every day. This sweet treat is made from cocoa beans combined with chocolate liquor, sugar, cocoa butter, and some additives for flavour (Kelishadi, 2005). Cocoa beans contain *theobromine*, a substance that can enhance the formation and size of hydroxyapatite crystals, making tooth enamel surfaces harder and more resistant to demineralization (Vasanthakumar et al., 2016; Amaechi et al., 2013; Sadeghpour, 2007). *Theobromine* in chocolate has the potential to promote remineralization more effectively than toothpaste containing sodium fluoride (Kargul et al., 2012). The cariogenicity (ability to cause cavities) of chocolate depends on factors like its composition, texture, solubility, retentivity, and its ability to stimulate salivary flow. Increasing the cocoa concentration in chocolate may decrease the risk of causing cavities (Vasanthakumar et al., 2016). Despite its potential benefits, chocolate is often associated with dental caries because of its sweet taste, which comes from sugar, a type of carbohydrate known to contribute to tooth decay (Savira et al., 2017).

Sucrose, a sweetener added to processed chocolate products, is a simple sugar that can undergo fermentation. When salivary amylase breaks down sucrose, it provides a substrate for bacteria, leading to a reduction in saliva pH and initiating a demineralization process (Touger-Decker & van Loveren, 2003). This process involves the diffusion of calcium, phosphorus, and other minerals out of the enamel surface, causing dissolution on the surface of enamel crystals. Continuous demineralization may result in the gradual loss of the tooth enamel layer due to the reaction of acid ions with phosphate groups, leading to the breakdown of surface hydroxyapatite crystals and the formation of cavities (Casamassimo et al., 2013; Ren, 2011; Scheid & Weiss, 2012). Consuming excessive sucrose can diminish saliva's ability to neutralize acid, thereby increasing the risk of developing cavities (Kaswindarti et al., 2017). Acid production begins within 10 minutes after consuming carbohydrates through the glycolysis process, causing saliva pH to decrease to a critical level (5.2-5.5). It takes 30 to 60 minutes for saliva to return to a normal pH level (Hartini, 2005). An alternative sweetener for sucrose that can be used in processed chocolate products is stevia (Deviyanti, 2021).

Stevia is a natural sweetener that is 200 – 400 times sweeter than regular sugar (sucrose) and has a low number of calories (Ramadhan et al., 2023). Stevia's sweetness comes from three main components found in its leaves: *stevioside* (3-10% of the dry weight of leaves), *rebaudioside* (2-3%), and *dulcoside* (0.5-1%) (Wuryantoro & Susanto, 2014). Extracts from *Stevia rebaudiana Bertoni's* leaves not only serve as a natural sweetener without calories but also provide therapeutic benefits in dentistry. These advantages include its potential to reduce cariogenic bacteria like *Streptococcus mutans* in dental biofilms and saliva, enhance saliva pH and buffering capacity, improve the pH of dental biofilms, and decrease enamel demineralization levels (Usha et al., 2017; Brambilla et al., 2013; Shinde & Winnier, 2020; Palapati et al., 2017). Stevia also has a non-cariogenic effect, which helps prevent cavities, especially in children (Deviyanti, 2021).

Children are prone to developing cavities because plaque builds up soon after their teeth come in, with a peak risk between ages 2-5 for baby teeth and around ages 10-12 for permanent teeth (Moynihan and Petersen, 2004). Kids in the 10-12 age range are often fond of sweet foods like chocolate and candy, but they may not fully grasp the impact of consuming sugary, sticky treats. Their awareness of proper tooth-brushing timing is limited, allowing food remnants to linger in their mouths for extended periods, raising the risk of cavities (Sulendra et al., 2013). A suitable alternative sweetener for children, such as stevia, is highly necessary. Stevia is a readily available natural option (Deviyanti, 2021).

Choosing stevia as a natural sweetener comes with minimal risks, and Indonesia's rich biodiversity provides ample resources for its processing. As noted by Brambilla et al. (2014), using 1% stevia in mouthwash formulations causes a smaller pH decrease in dental biofilms compared to a 1% sucrose solution. Stevia is also incredibly sweet, 100-300 times more than sucrose, making it widely accepted by the public even in small concentrations. The decision to use a 30% sucrose sweetener in this study is based on popular choices, as indicated by a survey from Rumah Cokelat Bodag (Madiun), Ndalem (Yogyakarta), and Monggo (Yogyakarta), where chocolate with 30% sucrose sweetener is most favoured. According to

Pecharki et al. (2005), biofilms formed with 30% sucrose lead to much lower salivary pH compared to other concentrations. SD Muhammadiyah 1 Surakarta was selected due to the children's overall low caries levels from preliminary screenings. The school's diverse population provides flexibility in selecting subjects based on specific criteria. Notably, prior research on the differences in saliva pH between consuming sucrose chocolate and stevia chocolate in children aged 10-12 years has not been conducted. This study aims to uncover the variance in saliva pH between consuming sucrose chocolate and stevia chocolate in children aged 10-12 years.

Methods

In this study, a quasi-experimental research design is employed, and ethical approval has been obtained from the Ethical Committee of Health Research at Dr. Moewardi General Hospital under Ethical Clearance Number 2.045/XI/HREC/2023. The participants in this study are children aged 10-12 years from SD Muhammadiyah 1 Surakarta. Each child in the same group receives both sucrose chocolate and stevia, both weighing 3 grams and having the same shape. The children measure the pH of their saliva. To be eligible for participation, children must be between 10-12 years old, have a caries classification according to ICDAS (category 0-2), be cooperative, and have obtained consent from their parents or guardians. Exclusion criteria include a history of chocolate allergy and the presence of systemic diseases. The purposive sampling technique is used, with a sample size guided by the acceptable range of 10-15 subjects per group, based on Gay in Umar (2011). The study will be conducted with two sample groups, each consisting of 21 subjects.

The Process of Making Chocolate

The chocolate was divided into two groups: Group I: chocolate with 30% sucrose, Group II: chocolate with 1% stevia. The chocolate was produced at Rumah Cokelat Bodag, Madiun. The cacao beans selected were fermented cacao beans. The beans were roasted at 121°C for 60 minutes until they were sufficiently mature, dark brown in colour, and strong in aroma, then the skin was stripped from the beans using a desheller machine for 30-60 minutes to obtain cocoa nibs.

Nibs are processed into paste through a casting process using a casting machine to obtain semi-liquid or smooth chocolate paste. The finished paste is then subjected to a ball mill process. The ball mill is a chocolate refining machine in the form of a cylindrical tube containing iron balls that are rotated to crush each other so as to reduce the chocolate ingredients. The chocolate making process requires ingredients such as chocolate liquor, butter, sugar, and inulin to be mixed in a ball mill or mixer to form a dough. The capacity of the ball mill machine is 5 kg with 10 kg iron balls. The ball mill is a cocoa processing machine that functions to refine the chocolate formula with the help of stainless-steel iron balls contained in the refining tube.

Table 1. Sucrose and stevia chocolate composition formula

Materials	Function	Formulation (in %)	
		Sucrose	Stevia
Chocolate Liquor	Main materials	59,65	59,65
Sugar	Sweeteners	30	1
Lesitin	Emulsifier	0,35	0,35
Cocoa butter	Chocolate consistency and creates a better taste	10	10
Inulin	Fat replacer	0	29
	Total	100	100

Conching process is a refining process using a conching machine for 2 hours at 40°C. Conching process is a refining process using a conching machine for 2 hours at 40°C. The conching process is the process of smoothing and homogenising the resulting paste so as to obtain smooth and tender chocolate. The addition of lecithin to the chocolate formula is to increase the viscosity thus making the paste dough more flowable and easy to pour during moulding. Sweeteners are also added during the conching process.

Tempering is the creation of a crystal core in chocolate in order to produce chocolate which is solid at room temperature and melts at body temperature. After the chocolate paste mixture is homogeneous, it is taken out of the conching machine and continued with the tempering process. This is the process of lowering the temperature using a tempering machine, marble table and in a low temperature room. The temperature is lowered from 40°C to 27°C which is essential for the success of the moulding process. The tempering process includes three stages. In the first stage, the temperature at 40°C in the conching machine is increased in order to ensure that all the fat crystals have melted. The second stage the temperature lowered at 32°C and then lowered again at 27°C. Chocolate without a proper tempering process will result in chocolate with a poor melting point, and a proper tempering temperature in the range of 18-32°C.

The moulding process is the process performed after tempering. It involves moulding chocolate on a marble table and stomping the mould to ensure there is no oxygen in the mould. After moulding, it is immediately cooled at 16°C for 2 hours and then packed. The moulding process is carried out to solidify the mixture. The chocolate is moulded in silicone moulds and cooled in the refrigerator. Once solidified, the chocolate is removed from the moulds and covered in aluminium foil and stored in the refrigerator. The chocolate is moulded into 3 grams per bean and cooled in the refrigerator. The chocolate is removed from the moulds when hardened and wrapped in aluminium foil and stored in the refrigerator.

Research Methods

Measurement of Saliva pH Before Intervention

Preparation of the research subjects was completed by screening to see the condition of the child's mouth first according to the inclusion criteria. The research subjects were examined using a diagnostic set (mouth mirror, nierbeken, and excavator). Subjects were also instructed to brush their teeth using non-fluoride toothpaste with Fones technique and not to eat or drink anything except water for 30 minutes before measurement.

Subjects had their saliva pH measured before consuming chocolate sucrose and stevia using a pH meter (Hanna brand, Romania). Before measuring the saliva pH, the pH meter is calibrated first with buffer solution (pH 7) alternately. Subjects were asked to collect saliva in the oral cavity and spit it into a sterile container amounting to 2 ml. Saliva that has been collected in the sterile container, then measured with a pH meter and reading the results of the saliva pH obtained from the measurement results before intervention. Measurement of saliva pH was taken three times and the results obtained were the mean of the measurement results

Consumption of Sucrose and Stevia Chocolate

On Day 1, subjects were given the intervention to consume sucrose chocolate. Subjects were instructed to consume chocolate with a mass of 3 grams for 60 seconds. Subjects were instructed to remain seated calmly and upright leaning on a chair. Ten minutes later, the subjects were asked to collect saliva in the oral cavity and spit it into sterile container amounting 2 ml. Day II, the same subjects were instructed to consume stevia chocolate using the same method

Measurement of Saliva pH After Intervention

Before measuring the saliva pH after intervention, the pH meter is calibrated first with buffer solution (pH 7) alternately. Saliva that has been collected in the sterile container after the intervention is then measured with a pH meter and readings of the saliva pH were recorded from the measurements taken. Measurement of saliva pH was taken three times and the results obtained were the mean of the measurement results. The mean differences in saliva pH between the sucrose and stevia chocolate groups were compared and analysed.

Results

Data for this research were gathered from 21 participants, specifically children aged 10-12 years at SD Muhammadiyah 1 Surakarta. The researchers assessed the normality of the data using the Shapiro-Wilk test. The results showed values of 0.501 for the group that consumed sucrose chocolate and 0.903 for the group that consumed stevia chocolate, both of which were greater than 0.05, suggesting that the data follows a normal distribution. To ensure homogeneity, Levene's test was conducted, yielding a value of 0.060, which is also greater than 0.05, indicating homogeneity. The researchers employed parametric independent t-tests to analyse the saliva pH of the two intervention groups due to the normal distribution of the data ($p > 0.05$). The researchers aim was to detect differences in the average change in saliva pH before and after intervention between the sucrose chocolate and stevia chocolate groups. The results indicated a significant distinction in the mean change of saliva pH among children aged 10-12 years who consumed sucrose chocolate compared to those who consumed stevia chocolate. Specifically, the group that consumed sucrose chocolate exhibited a decrease in saliva pH in contrast to the group that consumed stevia chocolate.

The outcomes of the independent t-test showed a noteworthy distinction in saliva pH levels when comparing the consumption of sucrose chocolate and stevia chocolate among children aged 10-12 years ($p < 0.05$). Before consuming sucrose chocolate, the average saliva pH was 6.9444 ± 0.18599 , and after consumption, it dropped to 6.6032 ± 0.15272 , resulting in a mean difference of 0.3413 ± 0.12557 (refer to table 2). Conversely, for stevia chocolate, the average saliva pH before consumption was 7.0127 ± 0.11808 , and after consumption, it was 6.8683 ± 0.11570 , with a mean difference of 0.1444 ± 0.07698 (refer to table 2). Furthermore, the study findings indicated a significant difference in saliva pH between those who consumed sucrose chocolate (0.3413 ± 0.12557) and those who consumed stevia chocolate (0.1444 ± 0.07698) (refer to table 2).

Table 2. Mean, Standard Deviation, and Independent t-test of Difference in Saliva pH between Sucrose Chocolate and Stevia Chocolate Consumption in 10-12 Years Old Children.

Intervention groups	Variable (unit)	N	Mean before (x ± SD)	Mean after (x ± SD)	Difference (x ± SD)	Sig.
Sucrose Chocolate	Saliva pH	21	6,9444 ± 0,18599	6,6032 ± 0,15272	0,3413 ± 0,12557	0,000
Stevia Chocolate		21	7,0127 ± 0,11808	6,8683 ± 0,11570	0,1444 ± 0,07698	

Discussion

The study's discoveries show a difference in saliva pH between those who ate sucrose chocolate and those who opted for stevia chocolate. The group consuming sucrose chocolate experienced a drop-in saliva pH after the intervention, while the decrease was smaller in the stevia chocolate group. This distinction between the two groups was proven to be meaningful. These findings support previous research, indicating that *Stevia rebaudiana Bertoni*, used as a natural substitute for sugar, has significant non-cariogenic properties. It can effectively reduce the presence of cariogenic bacteria, *Streptococcus mutans*, in both dental biofilm and saliva (Deviyanti, 2021).

Chocolate contains *theobromine*, which plays a role in strengthening tooth enamel and reducing the risk of dental cavities. *Theobromine* stimulates the production of three types of glucosyltransferase (GTF) - GTFB, GTFC, and GTFD. These enzymes are capable of synthesising adhesive and water-insoluble glucans from sucrose, creating a firm attachment to the tooth surface. The accumulated glucans transform into acid, contributing to a decrease in saliva pH and resulting in localised demineralization of the teeth (Nimbulkar et al., 2020).

Sucrose, on the other hand, is a disaccharide formed by the chemical bonding of two monosaccharides. It is a sweetener commonly added to processed chocolate products and is a type of simple sugar that can undergo fermentation. When sucrose is broken down by the amylase enzyme in saliva, it provides a substrate for bacteria, leading to a reduction in saliva pH and initiating a demineralization process (Touger-Decker & van Loveren, 2003). This process involves the diffusion of calcium, phosphorus, and other minerals out of the enamel surface, causing dissolution on the surface of enamel crystals. If this process continues, the gradual loss of the tooth enamel layer may occur due to the reaction of acid ions with phosphate groups, leading to the decomposition of surface hydroxyapatite crystals and the potential formation of cavities (Casamassimo et al., 2013; Ren, 2011; Scheid & Weiss, 2012).

Consuming excessive amounts of sucrose can diminish saliva's ability to neutralise acid, thereby increasing the risk of cavities by reducing saliva pH (Soesilo et al., 2006). Acid production begins within 10 minutes after consuming carbohydrates through the glycolysis process, causing saliva pH to decrease to a critical level (5.2-5.5). It takes 30 to 60 minutes for saliva to return to a normal pH (Hartini, 2005). Glucans play a crucial role in the bacterial glycolysis process, generating energy and lactic acid, leading to a rapid decrease in saliva pH within 1-3 minutes until it reaches a pH of 4.5-5.0, and then gradually returning to normal within 30-60 minutes (Rodian et al., 2011).

The study results show that the average saliva pH decreases after eating sucrose chocolate. This corresponds with a study by Praptiningsih & Ningtyas (2020) which suggests that bacteria grow best within a saliva pH range of 6.5-7.5. When the saliva pH in the mouth is low (4.5-5.5), it creates a favourable environment for the growth of acidogenic bacteria like *Streptococcus mutans* and *Lactobacillus*. This drop in pH can lead to increased bacterial growth in the mouth, contributing to tooth demineralization (Praptiningsih & Ningtyas, 2020). According to Tanabe et al. (2013), the normal saliva pH level in the mouth is 7. If the saliva pH value falls to ≤ 5.5, it indicates a critical situation. The pH of saliva operates on an inverse relationship, where a lower pH means more acidity, and a higher pH signifies increased alkalinity. A pH of 7 indicates a neutral state, meaning the saliva is neither acidic nor alkaline (Tanabe et al., 2013)

Stevia is a natural sweetener that is much sweeter than regular sugar (sucrose) – around 200 to 400 times – and it has a lowcalorie content (Ramadhan et al., 2023). The sweetness of stevia comes from three main components present in its leaves: *stevioside* (3-10% of the dry weight of leaves), *rebaudioside* (2-3%), and *dulcoside* (0.5-1%) (Wuryantoro & Susanto, 2014). Extracts obtained from the leaves of *Stevia rebaudiana Bertoni* not only act as a sweetener without calories but also provide therapeutic benefits in dentistry. These advantages include a non-cariogenic potential, meaning it can reduce harmful bacteria like *Streptococcus mutans* in dental biofilms and saliva, enhance saliva pH and buffering capacity, improve the pH of dental biofilms, and decrease levels of enamel demineralization (Usha et al., 2017; Brambilla et al., 2013; Shinde & Winnier, 2020; Pallepati et al., 2017). Stevia also shows a non-cariogenic effect, helping to prevent cavities, especially in children (Deviyanti, 2021).

The reduction in saliva pH caused by the use of stevia sweetener was significantly smaller compared to sucrose. This aligns with a study by Usha et al. 92017), which conducted in vivo research demonstrating that the pH values of saliva before and after using a mouthwash containing 0.5% extract from *Stevia Rebaudiana Bertoni* leaves were 6.38 and 6.9, respectively. The research concluded that a 0.5% extract of *Stevia rebaudiana Bertoni* leaves could enhance saliva pH, increase saliva buffering capacity, and decrease the presence of cariogenic bacteria in saliva, particularly in individuals with a high risk of cavities (Usha et al., 2017).

Conclusions

According to the results of the research on the differences in saliva pH between the consumption of sucrose chocolate and stevia chocolate in children 10-12 years old, it can be concluded that there are differences in saliva pH between the consumption of sucrose chocolate and stevia chocolate. The decrease of saliva pH in children who consumed sucrose chocolate is lower than children who consumed stevia chocolate.

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