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The Differences of Plaque pH between Consumption of Sucrose Chocolate and Stevia Chocolate in Children aged 10-12 Years

Muhammad Rhaufly Dwi Arga Saputra[1*], Septriyani Kaswindiarti[1], Dendy Murdiyanto[1], Aprilia Yuanita Anwaristi[1]

[1]*Faculty of Dentistry, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia*

[*]Corresponding author:
Muhammad Rhaufly Dwi Arga Saputra
Faculty of Dentistry, Universitas Muhammadiyah Surakarta
Surakarta, Indonesia
Tel: (+62)81325702197; E-mail: j520200044@student.ums.ac.id

Abstract

Introduction: Chocolate is a popular sweet treat among children. The sugar used in chocolate can lead to the development of dental plaque and impact the process of teeth demineralization and remineralization. Until now, there has not been much research on how the acidity of dental plaque differs when children aged 10-12 consume chocolate sweetened with sucrose compared to chocolate sweetened with stevia. This study aims to explore and compare the effects of sucrose chocolate and stevia chocolate on the acidity of dental plaque in children aged 10-12 years.

***Methods*:** In this study, the researchers employed quasi-experimental research methods. The research received approval from the Ethical Committee of Health Research at Dr. Moewardi General Hospital, indicated by Ethical Clearance Number 1.884/X/HREC/2023. Initially, on the first day, the researchers measured the plaque pH of twenty-one children aged 10-12 years from SD Muhammadiyah 1 Surakarta. This measurement was done before any intervention by using an excavator on all surfaces of their back teeth – the outer, inner, and in-between surfaces. The researchers combined the collected plaque with 2.5 ml of deionized water in a properly labelled sterile container. The plaque pH was measured using a pH metre (Hanna, Romania), calibrated beforehand with a pH 7 solution. The subjects then consumed 30% sucrose chocolate for 60 seconds. Twenty minutes after this intervention, the researchers measured the plaque pH using the same method. On the second day, the same subjects were instructed to consume 1% stevia chocolate, again using the same method. The results, specifically the average difference in plaque pH before and after the interventions on the two days, were analysed using an independent T-test.

***Results*:** The findings revealed that the average plaque pH before eating sucrose chocolate was 6.6222 ± 0.13137, and after consumption, it decreased to 6.2794 ± 0.11425. Before consuming stevia chocolate, the average plaque pH was 6.6746 ± 0.13536, and after consumption, it decreased to 6.4794 ± 0.13059. The average difference in plaque pH before and after consuming sucrose chocolate was 0.3429 ± 0.08508, whereas for stevia, it was 0.1952 ± 0.08517. According to the independent t-test, there was a notable difference in plaque pH between children aged 10-12 years who consumed sucrose chocolate and those who consumed stevia chocolate ($p<0.05$).

***Conclusion*:** Based on the findings from the study comparing the plaque pH in 10-12-year-old children after consuming sucrose chocolate and stevia chocolate, it can be concluded that there is indeed a disparity in plaque pH between the two types of chocolate. Specifically, the plaque pH in children who consumed sucrose chocolate was lower than that in those who consumed stevia chocolate.

***Keywords*:** chocolate, sucrose, stevia, plaque pH

Introduction Section

Oral health refers to the condition where both the hard and soft tissues inside the mouth are in good shape, free from any diseases, and without aesthetic issues (Ayu et al., 2021). Among the common oral health problems faced by many Indonesians, dental caries is prevalent. As per the 2019 National Basic Health Research, 57.6% of Indonesians had oral health problems, with dental caries being the most significant concern at 45.3% (Skripsa et al., 2021). The primary factor contributing to dental caries is the accumulation of plaque (Karyadi & Roza, 2021).

The formation of dental plaque starts when *Staphylococcus aureus* bacteria accumulate in an organic matrix. As the bacteria accumulate, they create a transparent, soft deposit that transforms into a biofilm layer, firmly attached to teeth, gums, and other hard surfaces in the mouth. These bacteria can produce acid, and an increase in their numbers leads to a decrease in plaque pH (Sofrullah et al., 2021). Plaque pH refers to the acidic condition of the biofilm, capable of dissolving and potentially causing decay due to acid production by adherent bacteria on tooth surfaces. According to Pindobilowo et al. (2017), the decrease in plaque pH depends on plaque thickness, the types and combinations of bacteria present, saliva's ability to buffer, and the frequency of consuming carbohydrates. Plaque pH can range from 4 to 9.5, being most significant in the morning when carbohydrates from leftover food in the mouth are cleared. Typically, plaque pH hovers around 6-6.5, allowing acidogenic bacteria, particularly *Streptococcus* species, to be most active. *Streptococcus* and *Lactobacillus* bacteria can produce acid, with *Lactobacillus* capable of doing so at pH levels below 4.5 (Putri, 2013a). The consumption of sugary treats like candy, bread, biscuits, wafers, ice cream, and chocolate is known to lower plaque pH (Purbaningrum et al., 2017).

Chocolate is made from cocoa powder obtained from cocoa beans and is known to have a positive effect on preventing tooth decay (Kaswindiarti et al., 2017). Cocoa beans contain *theobromine*, which promotes the formation and enlargement of hydroxyapatite crystals. This enhances the hardness of tooth enamel, making it more resistant to demineralization (OHagan-Wong et al., 2021; Amaechi et al., 2013)). The primary components of chocolate include *theobromine* (1.2%-2.4%) and minerals like iron (Fe), magnesium (Mg), zinc (Zn), copper (Cu), potassium (K), selenium (Se), phosphorus (P), and manganese (Mn). Chocolate also contains antioxidants such as *tannins*, *polyphenols*, and *flavonoids* (including monomers like epicatechin and catechin), which protect the body from free radicals (Nimbulkar et al., 2020). Theobromine is responsible for producing three types of glucosyltransferases (GTFs) - GTFB, GTFC, and GTFD, which can create water-insoluble glucans from sucrose. These glucans adhere firmly to the tooth surface, accumulate as acid, and contribute to dental plaque formation, leading to localised demineralization of the teeth (Nimbulkar et al., 2020). There are various types of chocolate available, such as milk chocolate, white chocolate, caramel chocolate, dark chocolate, and more (Vasanthakumar et al., 2016)

Dark chocolate is one of the edible chocolate types that are a source of antioxidants and may hold an important regulatory role in maintaining the immune system, as well as reducing blood pressure and enhancing blood flow. Dark chocolate has a stable decrease in plaque pH and less carcinogenicity compared to milk chocolate. This is due to the fact that the milk in the chocolate eliminates the benefits of the whole chocolate, making it more cariogenic. One of the most essential ingredients of dark chocolate is sweetener. The most commonly used sweetener in chocolate production is sucrose (Purbaningrum et al., 2017).

Sucrose is among the sweeteners added to chocolate products. Sucrose is a fermentable monosaccharide and once hydrolysed by the enzyme amylase, saliva will provide a substrate for cariogenic bacteria that can decrease plaque pH. (Janah et al., 2021). Consumption of sucrose in food can be digested by oral bacteria and form acids so that the plaque pH drops to below 5 within 1-3 minutes (Putri, 2013b). The addition of sucrose to food or beverage products can decrease the plaque pH around 5.6 (5.3 - 5.9) in adults (Siraj et al., 2019). According to Vasanthakumar et al. (2016) in his research stated that in just 20 minutes after carbohydrate consumption, acid will be formed through the process of glycolysis and plaque pH will decrease to a critical pH (4.72 - 5.22). Children frequently consume foods that contain high levels of sucrose, which leads to an acidic mouth condition so that the greater the possibility of enamel demineralisation occurs on the teeth and results in caries (Fuadah et al., 2023). Sucrose increases the incidence of caries the most, due to its leading to the formation of glucans that can facilitate bacterial adhesion on the teeth and limit the diffusion of acids and buffers in the plaque. This is due to the extracellular synthesis of sucrose faster compared to other sugars such as glucose, fructose, and lactose, which are rapidly converted by microorganisms in the oral cavity into acids. The adherence of sucrose to the enamel wall is more rigid than other types of sweeteners (Agung & Nurlitasari, 2017). An alternative sweetener which could be used in the production of processed chocolate is stevia sweetener (Deviyanti, 2021).

Stevia is among the other sweeteners that are able to be used in the production of processed chocolate. The stevia plant is potentially developed as a raw material for natural sugar (sweetener) extracts from stevia leaves that can contribute as a companion to cane sugar and a substitute for synthetic sugar. Stevia is considered as a source of non-cane sweetener that has advantages over sugar cane. The advantages of stevia are particularly the leaves that contain *diterpene glycoside* with a sweetness level between 200-300 times that of cane sugar (Rochmah et al., 2022). Stevia possesses antimicrobial effects on fungi and various bacteria, including *Streptococcus mutants*, the bacteria that cause caries. Stevia has minimal adhesion, so it does not damage the surface of the enamel wall (Ajagannanavar et al., 2014). Based on research by Harismah et al. 2014) hedonic test comparing the addition of sucrose and stevia sweeteners to yoghurt, the results showed: sucrose > sucrose + stevia > stevia. Stevia is considered less desirable because it gives a concentrated after taste. The

addition of stevia sweetener in food or beverage products can decrease plaque pH around 7.03 (6.9 - 7.3) in adults (Siraj et al., 2019). Stevia also has a non-cariogenic effect that prevents caries, especially in children (Deviyanti, 2021)

Children face a risk of developing cavities due to plaque build-up shortly after their teeth emerge. The peak age for cavities is between 2-5 years for baby teeth and during pre-adolescence, typically between 10-12 years for permanent teeth (Moynihan and Petersen, 2004). According to the 2019 National Basic Health Research, the average rate of cavities in children aged 10-12 years was 1.89% (Skripsa et al., 2021). School-aged children, especially those in primary school, are particularly prone to oral and dental issues. This susceptibility arises from poor behaviours and habits, a lack of knowledge, and inadequate tooth-brushing practices, allowing food particles to linger in the mouth and potentially lead to cavities (Safela & Purwaningsih, 2021; Fatimatuzzahro et al., 2016). It is crucial to find alternatives to traditional sweeteners that are suitable for children, and stevia is considered a promising option. Stevia, derived from a natural source, is widely available (Deviyanti, 2021).

The decision to use stevia as a natural sweetener has a minimum risk and Indonesia has an extensive biodiversity of natural resources, so it is necessary to be processed. According to Brambilla et al., (2014) the use of 1% stevia is able to lead to a smaller decrease in the plaque pH of dental biofilm than a 1% sucrose solution in mouthwash preparations. Stevia sweetener also has a sweetness level 100-300 times higher than sucrose, therefore with a small concentration it already tastes sweet and can be received by the community. The use of 30% sucrose sweetener in this research is due to a survey from Rumah Cokelat Bodag (Madiun), Ndalem (Yogyakarta), and Monggo (Yogyakarta) which are most in demand and accepted by the community is chocolate with 30% sucrose sweetener. According to Pecharki et al. (2005) stated that biofilm formed in the presence of 30% sucrose showed a much lower pH of plaque compared to other concentrations. This study seeks to find out how plaque pH differs when children aged 10-12 years consume sucrose chocolate compared to stevia chocolate. The main goal is to compare the plaque pH levels resulting from the consumption of sucrose chocolate and stevia chocolate in this age group.

Methods

This study employs quasi-experimental research methods and has received approval from the Ethical Committee of Health Research at Dr. Moewardi General Hospital under Ethical Clearance Number 1.884/X/HREC/2023. The participants in this research are children aged 10-12 years from SD Muhammadiyah 1 Surakarta. Each child in the same group receives both sucrose and stevia chocolate in equal amounts (3 grams) and shapes. The plaque pH of each child is measured. To be included in the study, children must be between 10-12 years old, have cavity-free teeth, no tartar, be cooperative, and have parental/guardian consent. Exclusion criteria include a history of chocolate allergy and systemic diseases. The sampling technique used is purposive sampling, and the number of samples aligns with the acceptable range recommended for experimental research, with 21 subjects in each of the two groups. The results, focusing on differences in mean plaque pH before and after the intervention on two days, will be analysed using an independent t-test.

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 121^0C 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 castering process using a castering 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 paste, fat, 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 paste	Main materials	59,65	59,65
Sugar	Sweeteners	30	1
Lecitin	Emulsifiers	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 40^0C. Conching process is a refining process using a conching machine

for 2 hours at 40^0C. 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 40^0C to 27^0C which is essential for the success of the moulding process. The tempering process includes three stages. In the first stage, the temperature at 40^0C in the conching machine is increased in order to ensure that all the fat crystals have melted. The second stage the temperature lowered at 32^0C and then lowered again at 27^0C. Chocolate without a proper tempering process will result in chocolate with a poor melting point, and a proper tempering temperature in the range of 18-32^0C.

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 16^0C 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 Plaque 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 and not to eat or drink anything except water for 30 minutes before measurement.

Subjects were measured for plaque pH before consume sucrose and stevia chocolate by collecting plaque samples using an excavator on all buccal, palatal or lingual and proximal surfaces of posterior teeth, then mixed with 2.5 ml of deionized water in a sterile container that has been labelled. Plaque pH was measured using a pH meter (Hanna, Romania) that had previously been calibrated using pH 7 buffer solution. The pH number will immediately appear after a few moments from the pH meter monitor reading. Measurement of plaque pH was carried out three times and the results obtained were the mean of the measurement results (Sofrullah et al., 2021).

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. Subjects were measured using a pH meter (Hanna, Romania). Day II, subjects were treated with stevia chocolate with the same procedure.

Plaque pH Measurement After Intervention

Measurement of plaque pH post-intervention after 60 seconds of chocolate consumption, then measured by plaque pH at 20 minutes after chocolate consumption was measured by collecting plaque samples using an excavator on all buccal, palatal or lingual surfaces and proximal posterior teeth, then mixed with 2.5 ml of deionized water in a sterile container that has been labelled. Plaque pH was measured using a pH meter (Hanna, Romania) that had previously been calibrated using pH 7 buffer solution. The pH number will immediately appear after a few moments from the pH meter monitor reading. Measurement of plaque pH was taken three times and the results obtained were the mean of the measurement results (Sofrullah et al., 2021). The mean differences in plaque pH between the sucrose and stevia chocolate groups were compared and analysed.

Results

The information for this study was gathered from 21 participants, children aged 10-12 years attending SD Muhammadiyah 1 Surakarta. The data underwent the Shapiro-Wilk normality test, revealing values of 0.547 for the group consuming sucrose chocolate and 0.289 for the group consuming stevia chocolate, both exceeding 0.05. This suggests that the data is normally distributed ($p>0.05$). The homogeneity test using Lavene's test resulted in a value of 0.813, also greater than 0.05, indicating homogeneity. Parametric independent t-tests were then applied to analyse the plaque pH in the two intervention groups, as the data showed normal distribution ($p>0.05$). The aim was to identify differences in the mean values before and after intervention in the groups consuming sucrose chocolate and stevia chocolate. The findings revealed a significant distinction in the mean plaque pH between those who consumed sucrose and stevia chocolate among children aged 10-12 years. Specifically, the group consuming sucrose chocolate showed a decrease in plaque pH compared to the group consuming stevia chocolate.

The outcomes of the independent t-test revealed a noteworthy distinction in plaque pH between the consumption of sucrose chocolate and stevia chocolate in children aged 10-12 years ($p<0.05$). Before having sucrose chocolate, the average plaque pH was 6.6222 ± 0.13137, and after consumption, it decreased to 6.2794 ± 0.11425, resulting in a mean difference of 0.3429 ± 0.08508 (as shown in Table 2). Similarly, before consuming stevia chocolate, the mean plaque pH was 6.6746 ± 0.13536, and after consumption, it decreased to 6.4794 ± 0.13059, with a mean difference of 0.1952 ± 0.08517 (as indicated in Table 2). The research findings also indicated a significant difference in plaque pH between consuming sucrose chocolate (0.3429 ± 0.08508) and stevia chocolate (0.1952 ± 0.08517), as detailed in Table 2.

Table 2. Mean, Standard Deviation, and Independent t-test of Difference in Plaque 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	Plaque pH	21	6,6222 ± 0,13137	6,2794 ± 0,11425	0,3429 ± 0,08508	0,000
Stevia Chocolate		21	6,6746 ± 0,13536	6,4794 ± 0,13059	0,1952 ± 0,08517	

Discussion

The research findings indicated a notable difference in the average change of plaque pH between those who consumed sucrose chocolate and stevia chocolate. In the group that had sucrose chocolate, there was a decrease in plaque pH from before to after the intervention, while in the group consuming stevia chocolate, the decrease in plaque pH was smaller. These results align with earlier studies suggesting that using a mouthwash with stevia can result in a less pronounced decrease in the pH of dental biofilm compared to sucrose. Additionally, it reinforces the idea that stevia possesses a non-cariogenic effect, offering protection against cavities, particularly in children (Deviyanti, 2021).

Plaque pH during rest refers to the plaque pH 2-2.5 hours after the last intake of dietary carbohydrates and usually ranges from 6-7. Following fermentable carbohydrates, plaque pH decreases rapidly during the first 5 minutes and reaches a minimum after about 5-20 minutes, unless there is additional consumption of fermentable carbohydrates. As time passes, plaque pH slowly rises back to the initial value over 30-60 minutes (Xuedong, 2016).

Sucrose is among the sweeteners added in processed chocolate products. Sucrose is a fermentable monosaccharide and when hydrolysed by the enzyme amylase, saliva will provide a substrate for cariogenic bacteria that can reduce plaque pH, leading to demineralisation (Janah et al., 2021). Mineral content such as calcium, phosphorus or others will diffuse out of the enamel surface in the process and dissolution occurs on the surface of the enamel crystal. The process that continues will result in progressive loss of tooth enamel due to the interaction of acid ions with phosphate groups resulting in partial or complete decomposition of surface hydroxyapatite crystals and ultimately the formation of a cavity. Consumption of sucrose in food can be broken down by oral bacteria and form acids so that the plaque pH drops to below 5 within 1-3 minutes (Putri, 2013b).

This study found that the average plaque pH decreased after consuming sucrose chocolate. This aligns with the findings of Siraj et al. (2019), who observed that adding sucrose sweeteners to food or drinks can lower plaque pH to approximately 5.6 (within the range of 5.3 - 5.9). Vasanthakumar et al. (2016) also mentioned that within just 20 minutes of consuming carbohydrates, acid is formed through glycolysis, causing plaque pH to drop to a critical level (between 4.72 and 5.22)..

The reduction in plaque pH is notably less in stevia sweetener compared to sucrose, and this aligns significantly with Brambilla et al.'s (2013) research. Their study highlights that natural sweeteners derived from *Stevia rebaudiana Bertoni* leaf extract are considered nonacidogenic sweeteners. In practical terms, this means that in real-life situations, these sweeteners cause a smaller drop in the pH of dental biofilm compared to sucrose when used in mouthwash preparations. The research by Brambilla et al. (2013) also demonstrated that *Stevia rebaudiana Bertoni* leaf extract, being a natural sweetener, is noncariogenic. This is because it leads to a lower growth of Streptococcus mutants' bacteria in biofilms compared to sucrose in mouthwash. The subtle decrease observed in stevia aligns with the theory proposed by Brambilla et al. (2013) suggesting that stevia plays a role in preventing the attachment of chocolate to the surface of tooth enamel.

This research showed a slight decrease of plaque pH between sucrose chocolate and stevia chocolate. This is in accordance with the research of Lakshmi et al. (2019) *theobromine* content in chocolate has the ability to significantly reduce the attachment of dental plaque deposition. *Theobromine* also has the capacity to reduce plaque deposition and prevent caries. In addition, theobromine also has significant antimicrobial properties that prevent bacteria from adhering to tooth enamel. *Theobromine* helps strengthen tooth enamel, and makes teeth less prone to decay, because chocolate also contains *theobromine* compounds that play a role in demineralizing tooth enamel, making it noncariogenic (OHagan-Wong et al., 2021; Amaechi et al., 2013). Based on this theory, it can be concluded that the content of *theobromine* compounds in chocolate is one of the factors in reducing plaque pH that does not drop drastically and does not adhere to the surface of tooth enamel.

This research has disadvantages, including the lack of variation in the concentration of sucrose and stevia chocolate, which still focuses on one concentration. Another factor that needs to be considered is that with children as consumers, the chocolate that is produced has to be considered in terms of colour, taste, and texture, so it is necessary to do an organoleptic test. The disadvantages of this research include that there has never been any previous research on the use of stevia. Recommendations for future research are that experimental measurements be carried out several times to better identify changes in plaque pH both before and after consuming chocolate. In addition, stevia chocolate is better than sucrose chocolate, so stevia chocolate has the potential as a sweet food that is good for consumption by children because it contains stevia as a non-cariogenic sweetener. It is proven from this research that the pH of sucrose plaque is lower than that of stevia chocolate.

Conclusion

According to the results of the research on the differences in plaque pH between the consumption of sucrose chocolate and stevia chocolate in children aged 10-12 years, it can be concluded that there are differences in plaque pH between the consumption of sucrose chocolate and stevia chocolate. Plaque pH of children who consume sucrose chocolate is lower than that of stevia chocolate.

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