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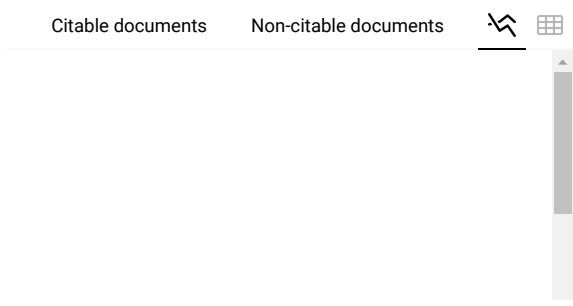
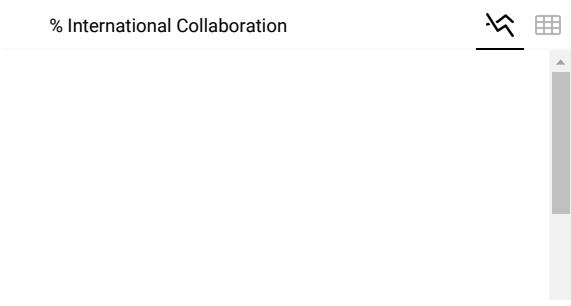
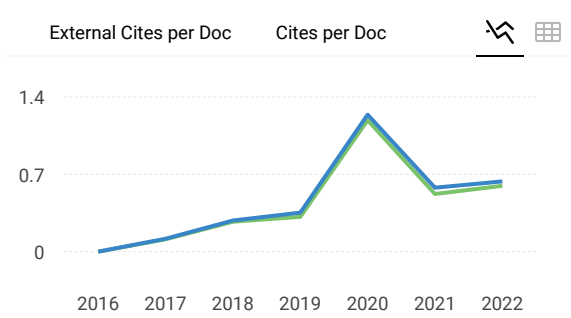
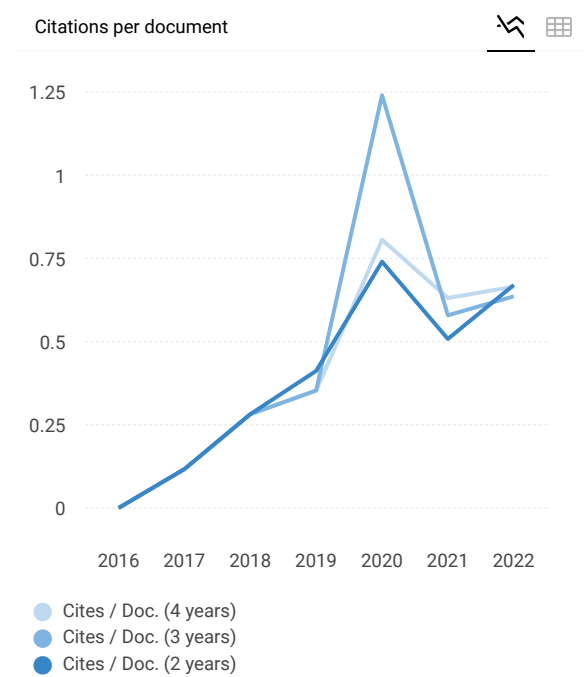
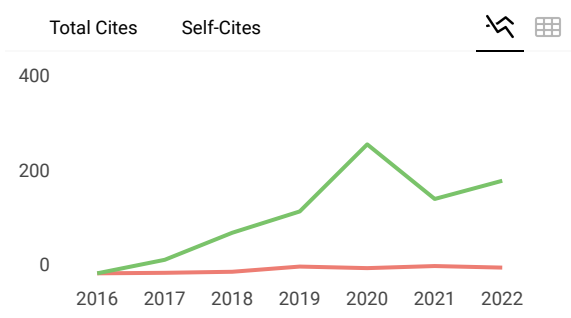
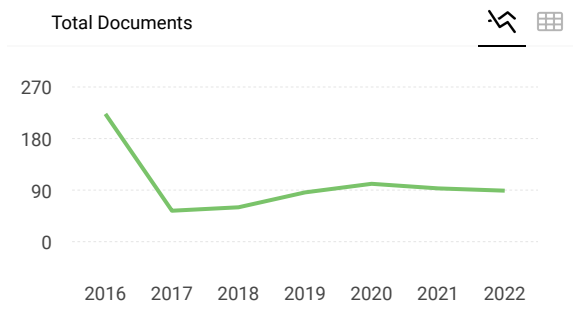
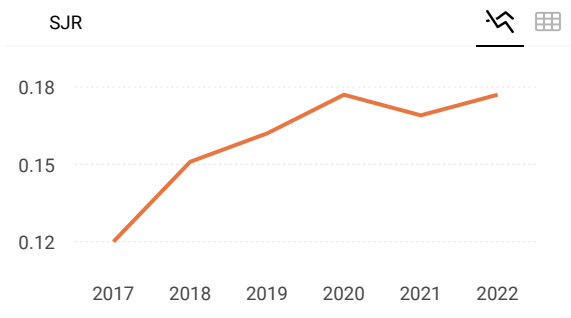
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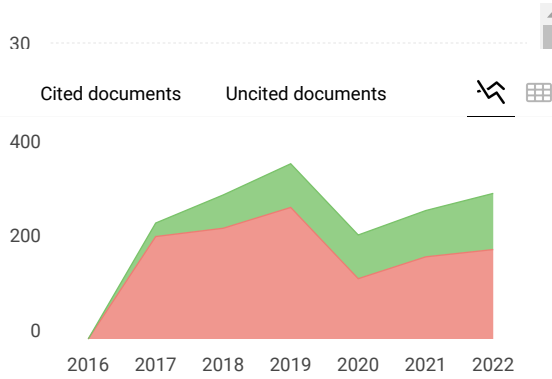
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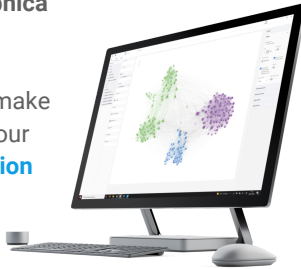
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# Journal of International Oral Health

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# Effect of Brushing the Teeth before and after Meals on Salivary pH: A Quasi-Experimental Study

Eko Fibryanto, Wienna Widyastuti

Department of Conservative Dentistry, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

## Abstract

**Aim:** To determine the difference in salivary pH before brushing the teeth, and 5, 30, and 60 minutes after eating and after brushing the teeth. **Materials and Methods:** This is a quasi-experimental study. The subjects were selected randomly with purposive sampling technique. Forty-five individuals were divided into three groups of 15 each, at test periods of 5, 30, and 60 min. Subjects had fulfilled the inclusion and exclusion criteria. The most common food consumed by the subjects was rice. Subjects were given instructions on how to brush their teeth, and collect and store saliva, and were instructed to use a toothpaste containing 1.12% sodium monofluorophosphate. The study was conducted in the morning, and after all the saliva sample tubes were collected, the saliva tubes were stored at 4°C. Salivary pH was measured with a pH meter (Mettler Toledo, Greifensee, Switzerland) before eating; before brushing the teeth at 5, 30, and 60 min after eating; and immediately after brushing the teeth. Data were analyzed with one-way analysis of variance (ANOVA) and General Linear Model repeated measure ANOVA ( $P < .05$ ). **Results:** The average salivary pH when brushing the teeth at 5, 30, and 60 min after meals was  $7.32 \pm 0.19$ ,  $7.40 \pm 0.16$ , and  $7.42 \pm 0.13$ , respectively. Based on the research conducted, there was no significant difference in salivary pH after brushing the teeth at 5, 30, and 60 min after meals ( $P < .05$ ). There were differences in pH between the time before and after eating and also after brushing the teeth in each group of 5, 30, and 60 min intervals ( $P < .05$ ). **Conclusion:** Brushing the teeth does not need to be delayed between 30 and 60 min after eating as the salivary pH will return to normal shortly after brushing.

**Keywords:** Mealtime, Salivary pH, Toothbrushing

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## INTRODUCTION

According to the 2018 Basic Health Research (RISKESDAS) report, which collected data on the dental and oral health of Indonesians, it was seen that 45.3% of the total 956,045 population complained of cavities. Furthermore, 88.8% of the 46,333 people studied in Indonesia suffered from dental caries, which is still the most common disease in the Indonesian society, among the many different dental and oral diseases.<sup>[1]</sup>

Dental caries is characterized by local damage to the hard tissues of the teeth due to bacterial acid products from carbohydrate fermentation. Bacteria utilize carbohydrates, mainly sucrose, from food waste as an ingredient to produce acid, which causes a demineralization process. Dental caries begins with decalcification of the inorganic

structure of the tooth, followed by the destruction of the organic matter. These conditions are formed through complex interactions between the host, microorganisms, time, and substrate.<sup>[2]</sup> *Streptococcus mutans* is a microorganism that causes dental caries because it can produce an extracellular matrix that is insoluble in water, thereby increasing the growth of bacteria on the tooth surface.<sup>[3]</sup> Furthermore, this bacteria can colonize in suitable zones, including the retentive areas of dental hard tissues, such as pits and fissures of posterior teeth.<sup>[4]</sup>

**Address for correspondence:** Eko Fibryanto,

Department of Conservative Dentistry, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia.  
E-mail: [eko.fibryanto@trisakti.ac.id](mailto:eko.fibryanto@trisakti.ac.id)

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Some types of carbohydrates, such as sucrose and glucose, are easily fermented by bacteria to produce acid, which causes a decrease in the pH (acidity) of saliva. Subsequently, if the acidic environment persists, the pH will drop to a critical level of 5.5, causing calcium hydroxyapatite demineralization of tooth enamel. However, pH changes caused by eating will return to normal after 15–20 min because the first 5–10 min after a meal is critical for pH stabilization (approximately 5.2–5.5).<sup>[5–7]</sup> A decrease in pH will cause demineralization of the vulnerable surface, which is the first stage in caries formation and can be worsened by reduced salivary secretion and acidic salivary pH.<sup>[5,8]</sup> Normally, the teeth are continually moistened by saliva, which acts as a buffer solution.<sup>[9]</sup> An increase in saliva corresponds to an increase in bicarbonate. Bicarbonate neutralizes acid from food residue fermented by bacteria in the mouth, thus keeping the salivary pH neutral.<sup>[10]</sup>

Knowledge of the maintenance of oral health and good oral hygiene is a major factor in preventing dental caries.<sup>[11]</sup> Such measures of caries prevention can be done by brushing the teeth, which will prevent plaque from forming on the teeth. Furthermore, plaque causes the pH of the saliva in the oral cavity to become acidic.<sup>[8]</sup> In addition to brushing the teeth, the frequency and time of cleaning the teeth are also very essential. Brushing the teeth is commonly done in the morning after eating and at night before going to bed. However, the right time to brush the teeth is 30 min after meals, which is after the pH returns to normal.<sup>[5]</sup>

Based on the background, this study was conducted to analyze the difference in salivary pH between the time before brushing the teeth; after eating at intervals of 5, 30, and 60 min; and after brushing the teeth.

## MATERIALS AND METHODS

### Setting and design

This type of research is a quasi-experimental research with the pretest–posttest control group design. The population of this study consisted of students from the Faculty of Dentistry, Universitas Trisakti, who were selected randomly using the purposive sampling technique.

### Sampling criteria

The sample size calculation used G\*Power. The sample of this study was saliva obtained from 45 individuals. The inclusion criteria for this study included individuals aged 19–22 years who had no systemic disease, were not on any drugs, and were not receiving radiation treatment. Furthermore, they were also caries free, not fasting, and willing to participate in the study. These inclusion criteria were observed through intraoral examination and questionnaires. Exclusion criteria for this study included poor oral hygiene, systemic disease, gingival recession, and lastly, history of bleeding when brushing teeth.

### Ethical approval and informed consents

The study is conducted in accordance with the Declaration of Helsinki regarding the ethical principles for medical research involving human subjects. Subjects who were willing to participate in the study were asked to provide informed consent. This research has received ethical approval from the Health Research Ethics Commission, Faculty of Dentistry, Universitas Trisakti, with number 211/KE/FKG/10/2015 on October 20, 2015.

### Methodology

This study was conducted in Faculty of Dentistry, Universitas Trisakti. Subjects were given instructions on how to brush their teeth, collect saliva, and store saliva, as well as the toothpaste containing 1.12% sodium monofluorophosphate (Pepsodent, Unilever, Indonesia). In addition, the most common food consumed by the subjects was rice. The saliva was collected by individuals spitting on sterile storage tubes (Biologix, Shandong, China). The study was conducted in the morning, and after all the saliva sample tubes were collected, the saliva tubes were stored at 4°C.

The research sample was divided into three groups based on the time interval of brushing the teeth after breakfast. The first group was 5 min after eating, and the second and third groups were 30 and 60 min after eating, respectively. In the first group, each subject was instructed to spit in a storage tube before breakfast and drinking water. Then, the same subjects were asked to spit back into the storage tube at 5 min after having breakfast and were instructed to brush their teeth. After brushing their teeth, the subjects returned to spitting. After completion, the saliva contained in the container was examined using a pH meter (Mettler Toledo, Greifensee, Switzerland). Meanwhile, the same experimental procedure was done on the second and third groups.

### Statistical analysis

Data analysis was carried out to determine the difference in salivary pH between the times of brushing the teeth, which were 5, 30, and 60 min after eating using the one-way analysis of variance (ANOVA) parametric test ( $P < .05$ ). On the other hand, data analysis determined the difference in salivary pH between the time before eating and after eating from each group for 5, 30, and 60 min using the parametric test General Linear Model (GLM) repeated measure ANOVA ( $P < .05$ ). Data were analyzed using IBM SPSS 20 software (SPSS Inc., Chicago, Illinois).

## RESULTS

The data were found to be normally distributed and homogeneous ( $P > .05$ ) based on the normality test using the Shapiro–Wilk test and the homogeneity test using the Levene’s test. Furthermore, the results of a one-way ANOVA test showed no significant difference in the degree of acidity (pH) between groups that brushed their

teeth at 5, 30, and 60 min after eating ( $P > .05$ ). Table 1 shows the results of the data analysis.

The results of the GLM repeated measure ANOVA test analysis showed that there were differences in the degree of acidity (pH) between the time before and after eating and also after brushing the teeth in each group of 5, 30, and 60 min intervals ( $P < .05$ ). In the 5-min time group, the average initial pH of saliva before breakfast and after breakfast was  $6.62 \pm 0.18$  and  $5.54 \pm 0.45$ , respectively, whereas the pH after brushing the teeth was  $7.32 \pm 0.19$ . However, in the 30-min time group, the average initial pH of saliva before and after breakfast was  $6.52 \pm 0.16$  and  $7.10 \pm 0.16$ , respectively, whereas after brushing the teeth, it was  $7.40 \pm 0.16$ . Lastly, in the 60-min time group, the initial pH of saliva before and after breakfast was  $6.73 \pm 0.22$  and  $7.22 \pm 0.16$ , respectively, whereas after brushing the teeth, it was  $7.42 \pm 0.13$ , as represented in Table 2.

## DISCUSSION

This study proves that the pH of saliva tends to be acidic in the morning before eating ( $6.52 \pm 0.16$  to  $6.73 \pm 0.22$ ). Furthermore, the result indicates that the salivary buffer system is not working optimally. Salivary flow is influenced by the parasympathetic nerves that work irrespective of a stimulus. However, salivary flow during sleep tends to decrease so that it impacts the condition of the oral cavity.

The degree of salivary acidity after eating in this study also showed a significant decrease when compared to the acidity before eating in the 5-min time group. Subsequently, this confirms the presence of acid formed 5 min after eating, which can occur due to the fermentation of carbohydrates by *S. mutans* and *Lactobacillus*. This study confirmed different results in the 30- and 60-min time groups. From the results, the pH of the saliva after eating without brushing the teeth was neutral ranging from  $7.10 \pm 0.16$  to  $7.22 \pm 0.16$ , which is possibly caused by the buffering capacity of the saliva in neutralizing the acid formed, which can be observed within 30 min after eating. Previous studies stated that the decrease in salivary pH due to food consumption is neutralized within 15–60 min by the saliva in the oral cavity.<sup>[7]</sup>

According to the data in Table 2, it is proven that the average pH of saliva before eating and before brushing the teeth in the 5-min group tends to be acidic at  $6.62 \pm 0.18$ ,

which decreased significantly after eating at  $5.54 \pm 0.45$ . Furthermore, brushing the teeth appears to affect the acidity of saliva, which is confirmed by the results of this study showing that the salivary pH returned to normal at  $7.32 \pm 0.19$  after brushing the teeth. Therefore, it was confirmed that the time it takes for the pH to return to normal is 5 min after eating and brushing the teeth.

This study also shows that there is no need to wait 30 min or even 60 min after eating to brush the teeth because within 5 minutes, the salivary pH can return to normal (pH > 7) after brushing your teeth. This can be seen from the results of the one-way ANOVA test and the average pH of saliva obtained after brushing the teeth following meals, showing no significant difference as represented in Table 1. Furthermore, brushing the teeth can trigger salivary secretion, and it is observed after brushing the teeth that the salivary flow rate increases.<sup>[12]</sup> Subsequently, high salivary secretion increases the amount of saliva in the oral cavity, which increases the salivary buffer capacity returning the pH to neutral.<sup>[8]</sup> Toothbrushing prolongs remineralization effectively, which impacts salivary pH and decreases caries risk for 60 min after brushing the teeth.<sup>[13]</sup> In addition, the composition of toothpaste ingredients may also contribute to the increase in the pH value. The toothpaste in this study contains 1.12% sodium monofluorophosphate and calcium carbonate, and sufficient longitudinal clinical data support the efficacy of sodium monofluorophosphate and ionic fluorides as an anti-eroding agent.<sup>[14]</sup>

Sodium monofluorophosphate, chemically known as  $\text{Na}_2\text{PFO}_3$ , is an anticariogenic compound in the toothpaste. Its mechanism of action is the inhibition of *S. mutans* adenosine diphosphate (ADP)-glucose pyrophosphorylation, which catalyzes a key step of internal polysaccharide (IPS) biosynthesis. This inhibition is correlated with a decrease in acidogenesis and IPS accumulation in *S. mutans* cells. Furthermore, ADP-glucose pyrophosphorylation can be seen as a molecular target for controlling the virulence of *S. mutans*.<sup>[15]</sup>

A toothpaste containing sodium monofluorophosphate can restore lost minerals in the teeth or remineralize and maintain the strength of the enamel.<sup>[16]</sup> Other studies have shown that sodium monofluorophosphate can reduce plaque accumulation and inflammation in the gingiva.<sup>[17]</sup>

Calcium carbonate ( $\text{CaCO}_3$ ) is one of the ingredients found in the toothpaste.<sup>[18]</sup> Furthermore, the use of toothpaste with nano calcium carbonate content can restore the hardness of the demineralized enamel and provide superior caries protection compared to Miswak toothpaste.<sup>[19]</sup> Other studies have also shown that this toothpaste can also help to remineralize the teeth.<sup>[20]</sup> This is because when calcium carbonate dissolves, the calcium released can aid remineralization, whereas carbonate release can provide an increase in pH in the mouth.<sup>[21]</sup>

**Table 1: Differences in brushing time and salivary pH**

Brushing time	n	Average ± SD (pH)	P
5 min after eating	15	7.32 ± 0.19	.222
30 min after eating	15	7.40 ± 0.16	
60 min after eating	15	7.42 ± 0.13	

SD = standard deviation, ANOVA = analysis of variance  
One-way ANOVA ( $P < .05$ )

**Table 2: Differences in the degree of acidity before eating, after eating, and after brushing the teeth**

Time	n	Average ± SD (pH)	P
Before eating	15	6.62 ± 0.18	.001*
5 min after meals, before brushing the teeth		5.54 ± 0.45	
5 min after eating, after brushing the teeth		7.32 ± 0.19	
Before eating	15	6.52 ± 0.16	.001*
30 min after eating, before brushing the teeth		7.10 ± 0.16	
30 min after eating, after brushing the teeth		7.40 ± 0.16	
Before eating	15	6.73 ± 0.22	.001*
60 min after eating, before brushing the teeth		7.22 ± 0.16	
60 min after eating, after brushing the teeth		7.42 ± 0.13	

SD = standard deviation, ANOVA = analysis of variance

\*General Linear Model (GLM) repeated measure ANOVA ( $P < .05$ )

Subsequently, increased enamel hardness can occur from the use of toothpaste containing calcium carbonate and sodium monofluorophosphate, which can assist the remineralization process under normal pH conditions. The remineralization process will affect the mineral content of the enamel, especially calcium and phosphate ions, which bind to the tooth structure.<sup>[22]</sup>

Examination of the pH of the oral cavity is important because the acidic environment is a predisposing factor for demineralization in the caries process. Furthermore, the low pH atmosphere facilitates the growth of acidogenic bacteria, such as *S. mutans* and *Lactobacillus*.<sup>[23]</sup> Therefore, the right time to brush the teeth after eating is necessary to maintain healthy teeth and mouth.

Several factors in this study, such as storing saliva samples, may affect the results obtained. Saliva samples are stored in a storage tube with ice cubes wrapped around them. The method follows the UK Biobank standards regarding the collection, storage, and transportation of saliva.<sup>[24]</sup> In addition, another factor that may have influenced this research is the availability of other food alternatives or side dishes as an accompaniment to rice.

Saliva collection in this research was conducted in the morning so that the pH of the saliva before brushing the teeth prior to meals tends to be acidic. However, the pH of unstimulated saliva is usually acidic. The presence of food debris left between the teeth of individuals who do not brush at night also affects the acidity of the oral cavity since microorganisms can ferment the carbohydrates present and result in a decrease in salivary pH to become acidic.<sup>[25]</sup> Owing to the fermentation by organic acid bacteria, the pH of the saliva becomes acidic, resulting in the formation of a biofilm that coats the tooth enamel. For a limited time under acidic conditions, this can increase the risk of dental caries depending on the levels of *S. mutans* and *Lactobacilli* in the saliva.<sup>[26]</sup> The saliva maintains pH by two mechanisms, namely salivary flow, which will remove residual carbohydrates from food that bacteria can metabolize, and second, by eliminating acid production by bacteria, which is the mechanism for protecting the salivary pH against dental caries.<sup>[7]</sup>

This study was limited to participants aged 19–22 years old with no systemic disorders. This study also controlled the type of dietary staple (rice) that was consumed in the morning. Further studies are needed for elderly people who are designed to use easily fermented carbohydrates such as sucrose and glucose.

Based on this study, healthy people in general can brush their teeth immediately after eating. We do not need to wait for 30 or 60 min prior to brushing. The salivary pH can return to normal after brushing the teeth. Delaying brushing time for 30 or 60 min after eating is also unnecessary because the pH of saliva returns to neutral after that time. For public health implication, we can prevent the demineralization process of caries that occurs in the acidic environment by recognizing the right time for brushing the teeth.

## CONCLUSION

The degree of acidity (pH) tends to be acid in the morning before meals and decreases in 5 min after meals, but the pH increases to normal 30 and 60 min after meals. Brushing teeth within 5, 30, and 60 min after meals can cause the pH of the saliva to return to normal.

## Acknowledgment

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## Conflicts of interest

The authors have declared no conflicts of interest.

## Author contributions

EF contributed to concepts, design, definition of intellectual content, literature search, data acquisition, statistical analysis, article preparation, and article editing. WW contributed to concepts, design, definition of intellectual content, literature search, data acquisition, article preparation, and article review.

### Ethical policy and institutional review board statement

This research has received ethical approval from the Health Research Ethics Commission, Faculty of Dentistry, Universitas Trisakti, with number 211/KE/FKG/10/2015.

### Patient declaration of consent

All necessary participant consent has been obtained and the appropriate institutional forms have been archived.

### Data availability statement

Data are available on valid request by contacting the corresponding author.

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# Effect of Brushing the Teeth before and after Meals on Salivary pH: A Quasi- Experimental Study

*by* Wiena Widyastuti FKG

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# Effect of Brushing the Teeth before and after Meals on Salivary pH: A Quasi-Experimental Study

Eko Fibryanto, Wiena Widyastuti

Department of Conservative Dentistry, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

## Abstract

**Aim:** To determine the difference in salivary pH before brushing the teeth, and 5, 30, and 60 minutes after eating and after brushing the teeth. **Materials and Methods:** This is a quasi-experimental study. The subjects were selected randomly with purposive sampling technique. Forty-five individuals were divided into three groups of 15 each, at test periods of 5, 30, and 60 min. Subjects had fulfilled the inclusion and exclusion criteria. The most common food consumed by the subjects was rice. Subjects were given instructions on how to brush their teeth, and collect and store saliva, and were instructed to use a toothpaste containing 1.12% sodium monofluorophosphate. The study was conducted in the morning, and after all the saliva sample tubes were collected, the saliva tubes were stored at 4°C. Salivary pH was measured with a pH meter (Mettler Toledo, Greifensee, Switzerland) before eating; before brushing the teeth at 5, 30, and 60 min after eating; and immediately after brushing the teeth. Data were analyzed with one-way analysis of variance (ANOVA) and General Linear Model repeated measure ANOVA ( $P < .05$ ). **Results:** The average salivary pH when brushing the teeth at 5, 30, and 60 min after meals was  $7.32 \pm 0.19$ ,  $7.40 \pm 0.16$ , and  $7.42 \pm 0.13$ , respectively. Based on the research conducted, there was no significant difference in salivary pH after brushing the teeth at 5, 30, and 60 min after meals ( $P < .05$ ). There were differences in pH between the time before and after eating and also after brushing the teeth in each group of 5, 30, and 60 min intervals ( $P < .05$ ). **Conclusion:** Brushing the teeth does not need to be delayed between 30 and 60 min after eating as the salivary pH will return to normal shortly after brushing.

**Keywords:** Mealtime, Salivary pH, Toothbrushing

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## INTRODUCTION

According to the 2018 Basic Health Research (RISKESDAS) report, which collected data on the dental and oral health of Indonesians, it was seen that 45.3% of the total 956,045 population complained of cavities. Furthermore, 88.8% of the 46,333 people studied in Indonesia suffered from dental caries, which is still the most common disease in the Indonesian society, among the many different dental and oral diseases.<sup>[1]</sup>

Dental caries is characterized by local damage to the hard tissues of the teeth due to bacterial acid products from carbohydrate fermentation. Bacteria utilize carbohydrates, mainly sucrose, from food waste as an ingredient to produce acid, which causes a demineralization process. Dental caries begins with decalcification of the inorganic

structure of the tooth, followed by the destruction of the organic matter. These conditions are formed through complex interactions between the host, microorganisms, time, and substrate.<sup>[2]</sup> *Streptococcus mutans* is a microorganism that causes dental caries because it can produce an extracellular matrix that is insoluble in water, thereby increasing the growth of bacteria on the tooth surface.<sup>[3]</sup> Furthermore, this bacteria can colonize in suitable zones, including the retentive areas of dental hard tissues, such as pits and fissures of posterior teeth.<sup>[4]</sup>


**Address for correspondence:** Eko Fibryanto,  
Department of Conservative Dentistry, Faculty of Dentistry, Universitas  
Trisakti, Jakarta, Indonesia.  
E-mail: [eko.fibryanto@trisakti.ac.id](mailto:eko.fibryanto@trisakti.ac.id)

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1 Some types of carbohydrates, such as sucrose and glucose, are easily fermented by bacteria to produce acid, which causes a decrease in the pH (acidity) of saliva. Subsequently, if the acidic environment persists, the pH will drop to a critical level of 5.5, causing calcium hydroxyapatite demineralization of tooth enamel. However, pH changes caused by eating will return to normal after 15–20 min because the first 5–10 min after a meal is critical for pH stabilization (approximately 5.2–5.5).<sup>[5–7]</sup> A decrease in pH will cause demineralization of the vulnerable surface, which is the first stage in caries formation and can be worsened by reduced salivary secretion and acidic salivary pH.<sup>[5,8]</sup> Normally, the teeth are continually moistened by saliva, which acts as a buffer solution.<sup>[9]</sup> An increase in saliva corresponds to an increase in bicarbonate. Bicarbonate neutralizes acid from food residue fermented by bacteria in the mouth, thus keeping the salivary pH neutral.<sup>[10]</sup>

Knowledge of the maintenance of oral health and good oral hygiene is a major factor in preventing dental caries.<sup>[11]</sup> Such measures of caries prevention can be done by brushing the teeth, which will prevent plaque from forming on the teeth. Furthermore, plaque causes the pH of the saliva in the oral cavity to become acidic.<sup>[8]</sup> In addition to brushing the teeth, the frequency and time of cleaning the teeth are also very essential. Brushing the teeth is commonly done in the morning after eating and at night before going to bed. However, the right time to brush the teeth is 30 min after meals, which is after the pH returns to normal.<sup>[5]</sup>

Based on the background, this study was conducted to analyze the difference in salivary pH between the time before brushing the teeth; after eating at intervals of 5, 30, and 60 min; and after brushing the teeth.

## MATERIALS AND METHODS

### Setting and design

This type of research is a quasi-experimental research with the pretest–posttest control group design. The population of this study consisted of students from the Faculty of Dentistry, Universitas Trisakti, who were selected randomly using the purposive sampling technique.

### Sampling criteria

The sample size calculation used G\*Power. The sample of this study was saliva obtained from 45 individuals. The inclusion criteria for this study included individuals aged 19–22 years who had no systemic disease, were not on any drugs, and were not receiving radiation treatment. Furthermore, they were also caries free, not fasting, and willing to participate in the study. These inclusion criteria were observed through intraoral examination and questionnaires. Exclusion criteria for this study included poor oral hygiene, systemic disease, gingival recession, and lastly, history of bleeding when brushing teeth.

### Ethical approval and informed consents

The study is conducted in accordance with the Declaration of Helsinki regarding the ethical principles for medical research involving human subjects. Subjects who were willing to participate in the study were asked to provide informed consent. This research has received ethical approval from the Health Research Ethics Commission, Faculty of Dentistry, Universitas Trisakti, with number 211/KE/FKG/10/2015 on October 20, 2015.

### Methodology

This study was conducted in Faculty of Dentistry, Universitas Trisakti. Subjects were given instructions on how to brush their teeth, collect saliva, and store saliva, as well as the toothpaste containing 1.12% sodium monofluorophosphate (Pepsodent, Unilever, Indonesia). In addition, the most common food consumed by the subjects was rice. The saliva was collected by individuals spitting on sterile storage tubes (Biologix, Shandong, China). The study was conducted in the morning, and after all the saliva sample tubes were collected, the saliva tubes were stored at 4°C.

15 The research sample was divided into three groups based on the time interval of brushing the teeth after breakfast. The first group was 5 min after eating, and the second and third groups were 30 and 60 min after eating, respectively. In the first group, each subject was instructed to spit in a storage tube before breakfast and drinking water. Then, the same subjects were asked to spit back into the storage tube at 5 min after having breakfast and were instructed to brush their teeth. After brushing their teeth, the subjects returned to spitting. After completion, the saliva contained in the container was examined using a pH meter (Mettler Toledo, Greifensee, Switzerland). Meanwhile, the same experimental procedure was done on the second and third groups.

### Statistical analysis

Data analysis was carried out to determine the difference in salivary pH between the times of brushing the teeth, which were 5, 30, and 60 min after eating using the one-way analysis of variance (ANOVA) parametric test ( $P < .05$ ). On the other hand, data analysis determined the difference in salivary pH between the time before eating and after eating from each group for 5, 30, and 60 min using the parametric test General Linear Model (GLM) repeated measure ANOVA ( $P < .05$ ). Data were analyzed using IBM SPSS 20 software (SPSS Inc., Chicago, Illinois).

## RESULTS

The data were found to be normally distributed and homogeneous ( $P > .05$ ) based on the normality test using the Shapiro–Wilk test and the homogeneity test using the Levene's test. Furthermore, the results of a one-way ANOVA test showed no significant difference in the degree of acidity (pH) between groups that brushed their

teeth at 5, 30, and 60 min after eating ( $P > .05$ ). Table 1 shows the results of the data analysis.

The results of the GLM repeated measure ANOVA test analysis showed that there were differences in the degree of acidity (pH) between the time before and after eating and also after brushing the teeth in each group of 5, 30, and 60min intervals ( $P < .05$ ). In the 5-min time group, the average initial pH of saliva before breakfast and after breakfast was  $6.62 \pm 0.18$  and  $5.54 \pm 0.45$ , respectively, whereas the pH after brushing the teeth was  $7.32 \pm 0.19$ . However, in the 30-min time group, the average initial pH of saliva before and after breakfast was  $6.52 \pm 0.16$  and  $7.10 \pm 0.16$ , respectively, whereas after brushing the teeth, it was  $7.40 \pm 0.16$ . Lastly, in the 60-min time group, the initial pH of saliva before and after breakfast was  $6.73 \pm 0.22$  and  $7.22 \pm 0.16$ , respectively, whereas after brushing the teeth, it was  $7.42 \pm 0.13$ , as represented in Table 2.

## DISCUSSION

This study proves that the pH of saliva tends to be acidic in the morning before eating ( $6.52 \pm 0.16$  to  $6.73 \pm 0.22$ ). Furthermore, the result indicates that the salivary buffer system is not working optimally. Salivary flow is influenced by the parasympathetic nerves that work irrespective of a stimulus. However, salivary flow during sleep tends to decrease so that it impacts the condition of the oral cavity.

The degree of salivary acidity after eating in this study also showed a significant decrease when compared to the acidity before eating in the 5-min time group. Subsequently, this confirms the presence of acid formed 5 min after eating, which can occur due to the fermentation of carbohydrates by *S. mutans* and *Lactobacillus*. This study confirmed different results in the 30- and 60-min time groups. From the results, the pH of the saliva after eating without brushing the teeth was neutral ranging from  $7.10 \pm 0.16$  to  $7.22 \pm 0.16$ , which is possibly caused by the buffering capacity of the saliva in neutralizing the acid formed, which can be observed within 30 min after eating. Previous studies stated that the decrease in salivary pH due to food consumption is neutralized within 15–60 min by the saliva in the oral cavity.<sup>[7]</sup>

According to the data in Table 2, it is proven that the average pH of saliva before eating and before brushing the teeth in the 5-min group tends to be acidic at  $6.62 \pm 0.18$ ,

which decreased significantly after eating at  $5.54 \pm 0.45$ . Furthermore, brushing the teeth appears to affect the acidity of saliva, which is confirmed by the results of this study showing that the salivary pH returned to normal at  $7.32 \pm 0.19$  after brushing the teeth. Therefore, it was confirmed that the time it takes for the pH to return to normal is 5 min after eating and brushing the teeth.

This study also shows that there is no need to wait 30 min or even 60 min after eating to brush the teeth because within 5 minutes, the salivary pH can return to normal ( $\text{pH} > 7$ ) after brushing your teeth. This can be seen from the results of the one-way ANOVA test and the average pH of saliva obtained after brushing the teeth following meals, showing no significant difference as represented in Table 1. Furthermore, brushing the teeth can trigger salivary secretion, and it is observed after brushing the teeth that the salivary flow rate increases.<sup>[12]</sup> Subsequently, high salivary secretion increases the amount of saliva in the oral cavity, which increases the salivary buffer capacity returning the pH to neutral.<sup>[8]</sup> Toothbrushing prolongs remineralization effectively, which impacts salivary pH and decreases caries risk for 60min after brushing the teeth.<sup>[13]</sup> In addition, the composition of toothpaste ingredients may also contribute to the increase in the pH value. The toothpaste in this study contains 1.12% sodium monofluorophosphate and calcium carbonate, and sufficient longitudinal clinical data support the efficacy of sodium monofluorophosphate and ionic fluorides as an antieroding agent.<sup>[14]</sup>

Sodium monofluorophosphate, chemically known as  $\text{Na}_2\text{PFO}_3$ , is an anticariogenic compound in the toothpaste. Its mechanism of action is the inhibition of *S. mutans* adenosine diphosphate (ADP)-glucose pyrophosphorylation, which catalyzes a key step of internal polysaccharide (IPS) biosynthesis. This inhibition is correlated with a decrease in acidogenesis and IPS accumulation in *S. mutans* cells. Furthermore, ADP-glucose pyrophosphorylation can be seen as a molecular target for controlling the virulence of *S. mutans*.<sup>[15]</sup>

A toothpaste containing sodium monofluorophosphate can restore lost minerals in the teeth or remineralize and maintain the strength of the enamel.<sup>[16]</sup> Other studies have shown that sodium monofluorophosphate can reduce plaque accumulation and inflammation in the gingiva.<sup>[17]</sup>

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Brushing time	n	Average $\pm$ SD (pH)	P
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60 min after eating	15	$7.42 \pm 0.13$	

SD = standard deviation, ANOVA = analysis of variance  
One-way ANOVA ( $P < .05$ )

**Table 2: Differences in the degree of acidity before eating, after eating, and after brushing the teeth**

Time	n	Average ± SD (pH)	P
Before eating	15	6.62 ± 0.18	.001*
5 min after meals, before brushing the teeth		5.54 ± 0.45	
5 min after eating, after brushing the teeth		7.32 ± 0.19	
Before eating	15	6.52 ± 0.16	.001*
30 min after eating, before brushing the teeth		7.10 ± 0.16	
30 min after eating, after brushing the teeth		7.40 ± 0.16	
Before eating	15	6.73 ± 0.22	.001*
60 min after eating, before brushing the teeth		7.22 ± 0.16	
60 min after eating, after brushing the teeth		7.42 ± 0.13	

SD = standard deviation, ANOVA = analysis of variance

\*General Linear Model (GLM) repeated measure ANOVA ( $P < .05$ )

Subsequently, increased enamel hardness can occur from the use of toothpaste containing calcium carbonate and sodium monofluorophosphate, which can assist the remineralization process under normal pH conditions. The remineralization process will affect the mineral content of the enamel, especially calcium and phosphate ions, which bind to the tooth structure.<sup>[22]</sup>

Examination of the pH of the oral cavity is important because the acidic environment is a predisposing factor for demineralization in the caries process. Furthermore, the low pH atmosphere facilitates the growth of acidogenic bacteria, such as *S. mutans* and *Lactobacillus*.<sup>[23]</sup> Therefore, the right time to brush the teeth after eating is necessary to maintain healthy teeth and mouth.

Several factors in this study, such as storing saliva samples, may affect the results obtained. Saliva samples are stored in a storage tube with ice cubes wrapped around them. The method follows the UK Biobank standards regarding the collection, storage, and transportation of saliva.<sup>[24]</sup> In addition, another factor that may have influenced this research is the availability of other food alternatives or side dishes as an accompaniment to rice.

Saliva collection in this research was conducted in the morning so that the pH of the saliva before brushing the teeth prior to meals tends to be acidic. However, the pH of unstimulated saliva is usually acidic. The presence of food debris left between the teeth of individuals who do not brush at night also affects the acidity of the oral cavity since microorganisms can ferment the carbohydrates present and result in a decrease in salivary pH to become acidic.<sup>[25]</sup> Owing to the fermentation by organic acid bacteria, the pH of the saliva becomes acidic, resulting in the formation of a biofilm that coats the tooth enamel. For a limited time under acidic conditions, this can increase the risk of dental caries depending on the levels of *S. mutans* and *Lactobacilli* in the saliva.<sup>[26]</sup> The saliva maintains pH by two mechanisms, namely salivary flow, which will remove residual carbohydrates from food that bacteria can metabolize, and second, by eliminating acid production by bacteria, which is the mechanism for protecting the salivary pH against dental caries.<sup>[7]</sup>

This study was limited to participants aged 19–22 years old with no systemic disorders. This study also controlled the type of dietary staple (rice) that was consumed in the morning. Further studies are needed for elderly people who are designed to use easily fermented carbohydrates such as sucrose and glucose.

Based on this study, healthy people in general can brush their teeth immediately after eating. We do not need to wait for 30 or 60 min prior to brushing. The salivary pH can return to normal after brushing the teeth. Delaying brushing time for 30 or 60 min after eating is also unnecessary because the pH of saliva returns to neutral after that time. For public health implication, we can prevent the demineralization process of caries that occurs in the acidic environment by recognizing the right time for brushing the teeth.

## CONCLUSION

The degree of acidity (pH) tends to be acid in the morning before meals and decreases in 5 min after meals, but the pH increases to normal 30 and 60 min after meals. Brushing teeth within 5, 30, and 60 min after meals can cause the pH of the saliva to return to normal.

## Acknowledgment

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## Conflicts of interest

The authors have declared no conflicts of interest.

## Author contributions

EF contributed to concepts, design, definition of intellectual content, literature search, data acquisition, statistical analysis, article preparation, and article editing. WW contributed to concepts, design, definition of intellectual content, literature search, data acquisition, article preparation, and article review.

### Ethical policy and institutional review board statement

This research has received ethical approval from the Health Research Ethics Commission, Faculty of Dentistry, Universitas Trisakti, with number 211/KE/FKG/10/2015.

### Patient declaration of consent

All necessary participant consent has been obtained and the appropriate institutional forms have been archived.

### Data availability statement

Data are available on valid request by contacting the corresponding author.

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