

Effect of rinsing with 20% pineapple core extract solution on salivary flow rate, ph, and total protein: a quasi-experimental study

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ABSTRACT

Introduction: Saliva plays an important role in maintaining oral health by maintaining an optimal oral PH. A decrease in salivary pH causes enamel demineralization. Pineapple (*Ananas comosus*) has beneficial effects on the oral cavity, including increased salivary secretion. Its bromelain enzyme, which is abundant in the pineapple core, can inhibit bacteria and thereby prevent dental caries. A concentration of 20% was selected based on previous research and is lower than the previously reported concentrations of 25%, 50%, and 75%. This study aimed to analyze the effect of rinsing with 20% pineapple core extract solution on salivary flow rate, pH, and total protein.

Methods: This was a quasi-experimental study using a pretest-posttest control group design. The population comprised Faculty of Dentistry, Universitas Sumatera Utara students aged 18–22 years. Samples were selected using purposive sampling according to the inclusion and exclusion criteria. The samples were divided into two groups, with 10 subjects in each treatment and negative control group. Saliva was collected before and after rinsing with 10 mL of 20% pineapple core extract solution in the treatment group and 10 mL of distilled water in the negative control group. Salivary flow rate was measured by weighing the collected saliva per unit of time; salivary pH was measured using a digital pH meter; and total protein was measured using the Pierce™ BCA Protein Assay Kit. The results were analyzed using a paired t-test. **Results:** Rinsing with 20% pineapple core extract solution produced significant differences in salivary flow rate ($p=0.019$) and salivary pH ($p=0.005$), whereas the difference in total protein was not significant ($p=0.059$). **Conclusion:** Salivary flow rate and salivary pH increased significantly, while salivary total protein decreased without statistical significance after rinsing with 20% pineapple core extract solution.

Keywords

Pineapple, bromelain, salivary flow rate, pH, and total protein.

Perbedaan efek berkumur menggunakan larutan ekstrak bonggol nanas 20% terhadap laju alir, pH dan total protein saliva: eksperimental studi

ABSTRAK

Pendahuluan: Saliva memiliki peran penting dalam menjaga kesehatan rongga mulut yaitu menjaga pH optimal rongga mulut. Penurunan pH saliva yang menyebabkan demineralisasi enamel. Tanaman nanas (*Ananas comosus*) memiliki khasiat bagi rongga mulut, yaitu dapat meningkatkan sekresi saliva dan memiliki enzim bromelain yang banyak didapatkan pada bonggol nanas yang mampu menghambat bakteri sehingga dapat mencegah terjadinya karies. Konsentrasi 20% dipilih berdasarkan penelitian sebelumnya dan lebih rendah dibandingkan konsentrasi 25%, 50%, dan 75% yang telah dilaporkan. Tujuan penelitian menganalisis perbedaan efek berkumur larutan ekstrak bonggol nanas 20% terhadap laju alir, pH dan total protein saliva. **Metode:** Penelitian eksperimental kuasi dengan rancangan penelitian pretest-posttest design with control group design. Populasi pada penelitian ini adalah mahasiswa FKG USU berusia 18-22 tahun. Pengambilan sampel menggunakan purposive sampling dengan kriteria inklusi dan eksklusi. Sampel terbagi menjadi 2 kelompok dan berjumlah 10 subjek pada setiap kelompok perlakuan dan kontrol negatif. Saliva dikumpulkan sebelum dan sesudah berkumur larutan ekstrak bonggol nanas 20% 10 mL dan kelompok kontrol negatif dengan berkumur aquades 10 mL. Laju alir saliva diukur dengan cara menimbang saliva yang terkumpul dibagi per waktu, pH saliva diukur dengan menggunakan pH meter digital, total protein diukur dengan menggunakan Pierce™ BCA Protein Assay Kit. Hasil penelitian kemudian dianalisis menggunakan uji T-berpasangan. **Hasil:** Penelitian menunjukkan berkumur larutan ekstrak bonggol nanas 20% memiliki perbedaan yang signifikan pada laju alir saliva ($p=0,019$) dan pH saliva ($p=0,005$), sedangkan total protein memiliki perbedaan yang tidak signifikan ($p=0,059$). **Simpulan:** Terdapat peningkatan laju alir saliva dan pH saliva secara signifikan dan terdapat penurunan total protein saliva yang tidak signifikan setelah berkumur larutan ekstrak bonggol nanas 20.

Kata kunci

Nanas, bromelain, laju alir, pH dan total protein

INTRODUCTION

One indicator of poor healthcare in the community is dental caries. Dental caries remains a major public health concern, affecting 60%-90% of schoolchildren and nearly all adults worldwide. According to the Global Burden of Disease Study 2016, dental caries is an oral health problem experienced by a substantial portion of the global population (3.58 billion people). In Indonesia, 72.3% of the population experiences caries, placing oral diseases first, with a prevalence of 61% among the Indonesian population. Data from the 2018 Basic Health Research (Riset Kesehatan Dasar [RISKESDAS]) showed that the prevalence of caries in North Sumatra Province was 43.07%, whereas the prevalence in Medan City was 39.15%.^{1,2}

Caries is a disease of the hard tissues of the teeth, namely enamel, dentin, and cementum. It causes cavities in the teeth, characterized by enamel demineralization caused by acids produced from carbohydrates fermented by bacteria colonizing the tooth surface. The occurrence of caries is influenced by the host, substrate, time, and microorganisms. *Streptococcus mutans* is the predominant cariogenic microorganism in the oral cavity. In addition, *Lactobacillus* plays a role in the development of dental caries. *Streptococcus* plays a role in the early stage of caries by destroying the outer enamel, whereas *Lactobacillus* is involved in deeper caries.³⁻⁵

Saliva is one of the factors involved in preventing caries because it protects the oral cavity and is one of the body's defense mechanisms. It is secreted by three pairs of major salivary glands and contains 99.5% H₂O and 0.5% electrolytes and proteins. Saliva plays a role in lubrication, mastication, taste perception, prevention of oral infections, and prevention of dental caries. Saliva is a complex oral fluid resulting from the mixture of secretions from major and minor salivary glands in the oral cavity.^{6,7}

Saliva also plays a role in the caries process, depending on its viscosity, pH, and microbial composition. Saliva contains ions that regulate oral pH and act as a buffering system. The buffering system in human saliva consists of phosphate, bicarbonate, and protein buffer systems. Bicarbonate is an important buffer in saliva; its concentration is not constant but varies according to salivary flow rate. Unstimulated saliva contains little bicarbonate, whereas stimulated saliva contains more bicarbonate, depending on the salivary secretion rate.^{7,8}

An increased salivary flow rate increases the concentrations of bicarbonate, phosphate, and calcium, thereby increasing salivary pH. Saliva contains numerous proteins, such as lysozyme, lactoferrin, and salivary peroxidase, which are involved in soft-tissue repair and prevention of oral infections. These proteins act directly or indirectly through various mechanisms on plaque and bacteria, thereby modulating tooth susceptibility to dental caries. Salivary protein composition is influenced by time, food consumption, psychological conditions, smoking, diseases such as periodontal disease and diabetes, age, and sex.⁸⁻¹⁰

One chemical method for preventing caries is the use of mouthwash; however, continuous use may cause adverse effects, such as dehydration of the mucosal tissues. The use of traditional mouthwash as an alternative is considered safer than chemical agents. Therefore, a natural, inexpensive, efficient mouthrinse solution with minimal side effects is needed. Traditional plants that may prevent caries include pineapple and starfruit. Pineapple is a plant that grows abundantly in Indonesia and has potential for development as an alternative mouth rinse to maintain an optimal oral pH.^{11,12}

Pineapple is rich in fiber and water and has numerous health benefits due to its content of magnesium, calcium, potassium, zinc, iron, vitamin C, vitamins B1, B2, B3, and B6; vitamin E; tannins; the bromelain enzyme; and citric acid.^{13,14} The amount of bromelain enzyme differs among the stem, stalk, leaves, fruit, and peel; however, bromelain is abundant in the pineapple core. Bromelain consists of a mixture of thioendopeptidases and other compounds, including carbohydrate glycoprotein

phosphatases, glucosidases, peroxidases, cellulases, and several other protease inhibitors.^{13,14}

Scientifically, bromelain can break glutamine–alanine and arginine–alanine bonds. In addition, it can inhibit the growth of acid-producing anaerobic and aerobic bacteria. The citric acid content of pineapple can indirectly activate the autonomic nervous system through the central nervous system and stimulate the salivary glands to secrete saliva, producing a watery salivary secretion; this indicates cholinergic stimulation. A 1% citric acid concentration can stimulate parotid gland secretion up to fivefold.

The cholinergic antagonist atropine is completely unable to inhibit secretion, and no interruption occurs in the cholinergic neural pathway, the chorda tympani. This indicates that citric acid can reflexively activate the sympathetic neural pathway. An increased salivary flow rate may also increase salivary protein, whereas the tannin content of pineapple may affect salivary composition, resulting in astringency and decreased salivary protein.^{11,15}

Bromelain extract from pineapple has been widely used as a therapeutic agent because *in vitro* and *in vivo* studies have demonstrated various fibrinolytic, antiedematous, antithrombotic, and anti-inflammatory activities. Various clinical studies have shown that bromelain may aid in the treatment of several diseases, including cardiovascular disease, osteoarthritis, diarrhea, and cancer. It has also been widely used in burn wound debridement, coagulation, and immunogenicity.^{13,16}

In dentistry, bromelain has been widely used as an anti-inflammatory agent. A study by N. C. Praveen et al., entitled *In vitro Evaluation of Antibacterial Efficacy of Pineapple Extract (Bromelain) on Periodontal Pathogens*, demonstrated the preliminary antibacterial efficacy of bromelain.¹³ Bromelain is used as an antibacterial agent by reducing bacterial surface tension through hydrolysis of proteins in saliva. Pineapple has antibacterial effects, both by inhibiting bacterial growth (bacteriostatic effect) and by killing *S. mutans*, the bacteria responsible for caries.¹⁷

A previous study by Wanda Lewapadang et al., entitled *The Effect of Consuming Pineapple (Ananas comosus) on Salivary Flow Rate in Elderly Patients with Xerostomia*, found that consuming pineapple affected salivary flow rate in elderly patients with xerostomia.¹⁸ Another study by Minarni, entitled *The Effect of Rinsing with Pineapple Core Extract Solution on Oral Salivary pH*, found that rinsing with pineapple core extract solution increased salivary pH. Another study found that drinking tea could increase salivary flow rate, and an increased salivary flow rate could increase total salivary protein concentration.^{9,19}

Previous studies have not examined the effect of rinsing with pineapple core extract solution on salivary protein. Pineapple core extract is expected to increase salivary flow rate, maintain pH within physiological limits, and balance salivary protein levels. The hypothesis of this study is that rinsing with the extract solution will provide an effective natural alternative for preventing caries and serve as a basis for the development of phytopharmaceutical-based products in dentistry.

A concentration of 20% was selected based on previous studies showing that pineapple core extract has biological activity and may affect salivary parameters. This concentration was used as an initial test concentration to evaluate the effectiveness of pineapple core extract as a mouthrinse solution. The novelty of this study lies in the simultaneous evaluation of the effect of 20% pineapple core extract on salivary flow rate, pH, and total protein, because previous studies have primarily assessed salivary flow rate and pH, whereas its effect on total salivary protein remains limited. This study aimed to analyze the effect of rinsing with 20% pineapple core extract solution on salivary flow rate, pH, and total protein.

METHODS

This was a quasi-experimental study using a pretest–posttest control group design. All procedures, including the inclusion and exclusion criteria, intervention methods, and data-collection techniques, were carried out according to the original protocol without modification. The analyzed parameters included salivary flow rate, salivary pH, and total salivary protein. All parameters were specified as primary outcomes before the study began and were retained without modification through the final analysis.

The sample size was calculated using a one-population mean hypothesis test with 80% power. The required sample size was six participants per group; however, to account for potential bias, the sample size was increased to 10 participants per group.¹¹ The study was conducted among students of the Faculty of Dentistry, Universitas Sumatera Utara. The inclusion criteria were as follows: age 18–22 years, complete dentition, and no more than two teeth with caries. The exclusion criteria were as follows: allergy to pineapple, a history of systemic disease, use of medications affecting saliva, smoking, alcohol consumption, and engagement in strenuous physical activity.

The study involved 20 participants: 10 participants rinsed with 20% pineapple core extract solution, and the other 10 rinsed with distilled water as the negative control. The study was conducted between 10:00 AM and 12:00 PM.¹¹ If more than 20% of participants reported adverse effects during the study, such as oral irritation, allergy, or pain, the study was discontinued. An early analysis was also conducted to determine whether there was a trend toward increased salivary flow rate and salivary pH. The study was conducted by an independent dentist at the Faculty of Dentistry, Universitas Sumatera Utara, and the results were subsequently reviewed by an independent dentist before being provided to the researcher.

The pineapple cores used were from the Cayenne variety (*Ananas comosus*) obtained from Sipahutar District, North Sumatra, Indonesia. The pineapple core extract mouthrinse solution was prepared by blending pineapple core with 70% alcohol, followed by evaporation. After a concentrated extract was obtained, it was mixed with distilled water. Participants underwent caries and Simplified Oral Hygiene Index (OHI-S) examinations and were instructed not to eat or drink for 1 hour before the study. Participants were instructed to brush their teeth using the Bass method without toothpaste for 1 minute. They then consumed a carbohydrate source consisting of three wafers and waited for 10 minutes. After 10 minutes, participants were asked to collect the initial saliva sample using the draining method.

After the initial saliva sample was collected, participants were instructed to rinse for 1 minute with either pineapple core extract solution or distilled water (negative control). After rinsing, participants waited for 10 minutes. They were then asked to collect a second saliva sample using the draining method. Immediately after saliva collection, salivary flow rate was measured by weighing, salivary pH was measured using a digital pH meter, and total salivary protein was measured using a spectrophotometer with the Pierce™ BCA Protein Assay Kit.^{11,17}

The study data were tested for normality using the Shapiro–Wilk test. A paired *t*-test was used to compare the mean salivary flow rate and salivary pH between the treatment and control groups because the data were normally distributed. The Wilcoxon test was used for total salivary protein data because they were not normally distributed. All analyses were performed using SPSS version 25, with a significance level of $p < 0.05$.

RESULTS

All study participants met the inclusion and exclusion criteria. Participants were divided into two groups: the group that rinsed with 20% pineapple core extract solution

and the negative control group that rinsed with distilled water. The characteristics of the study participants are presented in Table 1.

Table 1. Characteristics of the study participants

Charateristics		Pineapple core extract solution 20% (n=10)	Aquades
Gender	Men	5	5
	Women	5	5
Age	18	2	3
	19	2	1
	20	2	2
	21	3	3
	22	1	1

The study participants consisted of 20 students from the Faculty of Dentistry, Universitas Sumatera Utara, aged 18–22 years, with good systemic health, complete dentition, and no more than two teeth with caries. All participants were non-smokers, did not consume alcohol, did not use medications affecting salivary secretion, and did not engage in strenuous physical activity before the study.

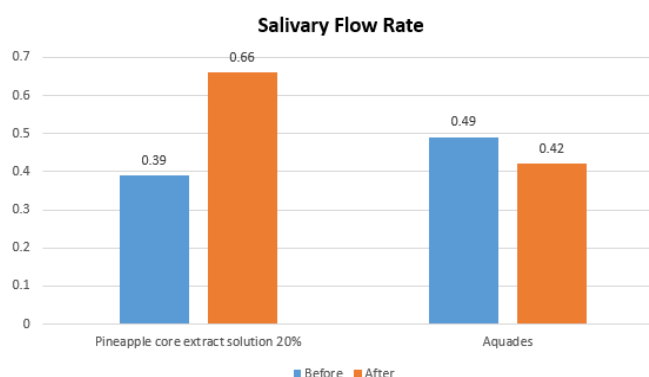


Figure 1. Salivary Flow Rate Results

As shown in Figure 1, the mean salivary flow rate before rinsing with 20% pineapple core extract solution was 0.39 ± 0.32 mL/min. The mean salivary flow rate after rinsing with 20% pineapple core extract solution was 0.66 ± 0.37 mL/min. The statistical test showed a value of $p = 0.019$, indicating a significant difference in salivary flow rate before and after rinsing with 20% pineapple core extract solution. The mean salivary flow rate before rinsing with distilled water was 0.49 ± 0.30 mL/min, whereas the mean salivary flow rate after rinsing with distilled water was 0.42 ± 0.19 mL/min. The statistical test showed $p = 0.187$, indicating no significant difference in salivary flow rate before and after rinsing with distilled water.

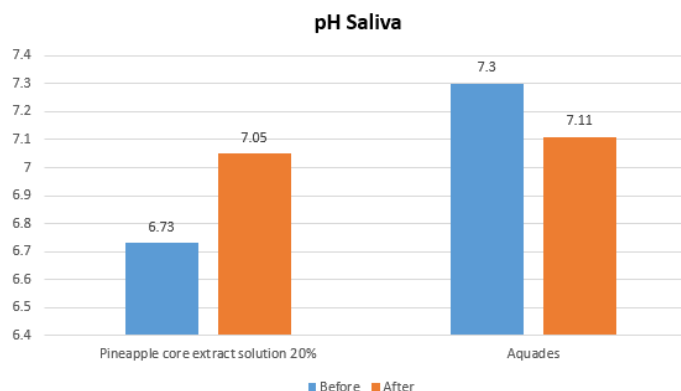


Figure 2. Salivary pH Results

Based on Figure 2, the mean salivary pH before rinsing with 20% pineapple core extract solution was 6.73 ± 0.33 . The mean salivary pH after rinsing with 20% pineapple core extract solution was 7.05 ± 0.28 . The statistical test showed $p = 0.005$, indicating a significant difference in salivary pH before and after rinsing with 20% pineapple core extract solution. The mean salivary pH before rinsing with distilled water was 7.30 ± 0.38 , whereas the mean salivary pH after rinsing with distilled water was 7.11 ± 0.46 . The statistical test showed $p = 0.147$, indicating no significant difference in salivary pH before and after rinsing with distilled water.

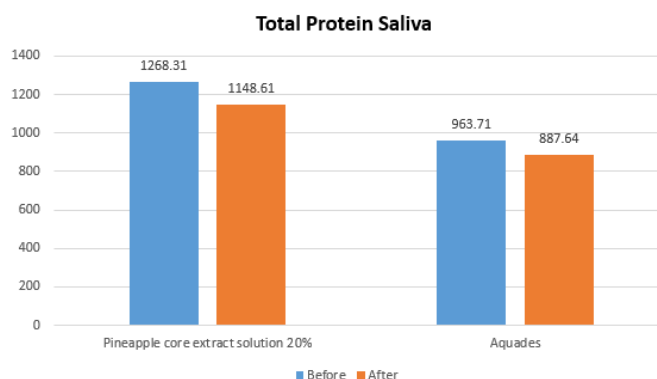


Figure 3. Total Salivary Protein Results

Figure 3 shows that the mean total salivary protein level before rinsing with 20% pineapple core extract solution was $1268.3121 \pm 288.20 \mu\text{mL}$. The mean total salivary protein level after rinsing with 20% pineapple core extract solution was $1148.6052 \pm 308.17 \mu\text{mL}$. The statistical test showed $p = 0.059$, indicating no significant difference in total salivary protein before and after rinsing with 20% pineapple core extract solution. The mean total salivary protein level before rinsing with distilled water was $963.7055 \pm 476.58 \mu\text{mL}$, whereas the mean total salivary protein level after rinsing with distilled water was $887.6366 \pm 251.95 \mu\text{mL}$. The statistical test showed $p=0.959$, indicating no significant difference in total salivary protein before and after rinsing with distilled water.

All 10 participants (100%) in the treatment group showed increased salivary flow rate and salivary pH to neutral values, whereas in the negative control group, 1 of 10 participants (10%) showed an increase in salivary flow rate and salivary pH. The absolute effect of the intervention was 90%, and the relative effect was nine times higher in the group that rinsed with 20% pineapple core extract solution than in the control group.¹⁸

The trial was conducted according to the protocol; therefore, no problems or events requiring early discontinuation occurred during the study. No adverse effects or undesirable events were observed in participants during or after the intervention in either the treatment or control group. All participants completed the procedure without clinical complaints.

DISCUSSION

This study was a clinical trial that analyzed the effect of rinsing with 20% pineapple core extract solution and distilled water on salivary flow rate, pH, and total protein. The dependent *t*-test results showed significant increases in salivary flow rate and pH in the group that rinsed with 20% pineapple core extract solution, whereas the group that rinsed with distilled water showed no significant results. This finding is consistent with a study conducted in Jambi, which reported an increase in salivary pH after consumption of pineapple and starfruit.²³

The increased salivary flow rate observed in this study is supported by a study by Zulfan, which showed that consuming pineapple can increase salivary flow rate. Citric acid is the strongest chemical stimulus. Higher concentrations of citric acid result in greater increases in salivary flow rate than lower concentrations.

In this study, all participants rinsed with pineapple core extract solution without added sweeteners. Among all gustatory stimuli, sour taste is the strongest stimulus compared with other tastes. Chemical stimulation of the tongue can indirectly activate the autonomic nervous system through the central nervous system, thereby stimulating the salivary glands to secrete saliva. This stimulation primarily produces watery salivary secretion, indicating cholinergic stimulation. Secretion cannot be completely inhibited by the cholinergic antagonist atropine, and no interruption occurs in the cholinergic neural pathway, the chorda tympani. This study is consistent with research conducted in Palembang, which reported increases in salivary flow rate (from 0.96 mL/min to 1.11 mL/min) and salivary pH (from 6.84 to 6.95) after chewing cucumber.²⁴

This indicates that noncholinergic neural pathways are also involved in salivary secretion induced by citric acid. Citric acid has been shown to reflexively activate sympathetic neural pathways.^{11,19} A study conducted in Banda Aceh reported a greater increase in salivary flow rate after consuming pineapple (from 0.3520 mL/min to 0.7980 mL/min) than after consuming papaya (from 0.2840 mL/min to 0.7000 mL/min), because pineapple contains more citric acid than papaya.¹⁴

The dependent *t*-test results showed a significant increase in salivary pH after rinsing with 20% pineapple core extract solution, whereas no significant result was observed in participants who rinsed with distilled water. This finding is consistent with a study conducted in Padang investigating the effect of rinsing with pineapple core extract solution on oral salivary pH, which found that rinsing with pineapple core extract solution significantly increased salivary pH.

The results showed that rinsing with pineapple core extract solution significantly increased salivary pH because bromelain in pineapple at concentrations of 0.1%–50% has antibacterial effects against aerobic and anaerobic bacteria. These findings are consistent with a study by Minarni, which found that rinsing with macerated pineapple core extract at concentrations of 25%, 50%, and 75% significantly increased salivary pH.¹¹ These findings may be due to the anti-adhesive properties of bromelain, which prevent bacteria from binding to specific glycoprotein receptors, thereby inhibiting bacterial attachment and exerting antibacterial effects.¹¹

Sour taste stimulation after rinsing with pineapple core extract solution may affect salivary secretion, thereby increasing salivary volume and pH. The increase in pH resulting from increased salivary secretion is due to an increased amount of bicarbonate ions, which is directly proportional to the salivary secretion rate, particularly from the

parotid glands. An increased salivary secretion rate increases salivary pH. Conversely, a decreased salivary secretion rate decreases salivary pH because the quantitative and qualitative composition of electrolytes determines pH and buffering capacity.¹⁹

The Wilcoxon test results showed no significant effect on the reduction in total salivary protein in either the group that rinsed with 20% pineapple core extract solution or the group that rinsed with distilled water. This finding is consistent with the study by Francis Canon et al., which reported that food intake can cause changes in salivary secretion and composition. Astringency may result from interactions between astringent compounds and saliva. It is a trigeminal sensation generally described as dryness, roughness, and a puckering sensation in the mouth.

Consumption of plant-derived foods, such as red grapes, pineapple, and several other fruits, may produce this sensation. Tannins are the principal molecules responsible for this sensation and belong to the polyphenol group. Tannins are known for their ability to bind and precipitate proteins. However, the molecular mechanism by which tannins produce astringency remains unclear and may involve activation of trigeminal chemoreceptors or mechanoreceptors.^{9,20}

A study conducted in Portugal found differences in total salivary protein concentration after yogurt consumption. Total protein concentration decreased immediately after yogurt intake and continued to decrease from 5 to 30 minutes after consumption. This decrease was associated with astringency occurring because tannins precipitate proteins, resulting in a reduced salivary protein concentration.²¹

Physiologically, this finding may be explained by an increase in salivary flow rate due to gustatory stimulation from the sweet-sour taste of pineapple extract. Increased flow rate, particularly from the parotid glands that predominantly produce serous saliva with lower protein concentrations, contributes to a dilution effect. Thus, protein concentration per unit volume may decrease even when total protein secretion per minute remains stable. This condition is consistent with findings from studies of saliva after food consumption, in which increased flow rate is often accompanied by small or nonsignificant changes in total protein levels due to high interindividual variability.^{25,28}

Total salivary protein is also an aggregate parameter that is not sensitive to shifts among protein fractions. Proteomic studies have shown that after oral stimulation, some proteins, such as immunoglobulins, may decrease, whereas others, such as cystatins or proline-rich proteins, may increase. Consequently, these changes among fractions may offset one another and result in only small variations in total protein values. Another factor to consider is the proteolytic nature of bromelain, which may break down large glycoproteins into smaller peptides, whereas saliva is rich in protease inhibitors, such as cystatins, that neutralize this activity. The combination of these opposing mechanisms may result in a tendency toward reduced total protein, although not sufficiently large to reach statistical significance.^{21,27,30}

Based on its proteolytic properties, bromelain has the ability to reduce salivary protein levels through direct degradation of soluble salivary proteins, such as mucins, amylase, and immunoglobulins, as well as through disruption of the protein-containing pellicle structure. This activity is supported by bromelain's ability to cleave peptide bonds in biological proteins; therefore, its use as a mouthrinse solution has the potential to significantly reduce total salivary protein concentration.^{22,26}

Further research is needed to evaluate the effectiveness of pineapple core extract for caries prevention, using a larger sample size and controlling stress levels, dietary patterns, and sleep duration to obtain better results. Future studies should include a larger sample size and control potential confounding factors, such as diet and stress. In addition, further clinical trials using other biochemical parameters are needed to explore the proteolytic mechanism of bromelain in greater depth.

Long-term studies may also evaluate the effectiveness of pineapple core extract solution as a natural caries-prevention agent. The limitations of this study are the limited

number of tests conducted to evaluate the effect of pineapple core extract on caries prevention and the small sample size, which may introduce bias.

CONCLUSION

There were significant increases in salivary flow rate and salivary pH before and after rinsing with 20% pineapple core extract solution. No significant difference in salivary flow rate was observed before and after rinsing with distilled water. No significant difference in total salivary protein was observed in either the group that rinsed with 20% pineapple core extract solution or the group that rinsed with distilled water. The findings of this study may contribute to the use of 20% pineapple core extract solution as an alternative mouthrinse to increase salivary flow rate and salivary pH as a potential agent for tooth remineralization.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and was approved by the Health Research Ethics Committee, Universitas Sumatera Utara (No. 1219/KEPK/USU/2022; December 16, 2022).

Informed Consent Statement: All participants voluntarily agreed to participate as study participants and stated that they would not raise any objections or claims at a later date.

Data Availability Statement: Research data will be made available, subject to the approval of all researchers, through the corresponding author's email address and in accordance with research ethics.

Conflicts of Interest: The authors declare no conflicts of interest.

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