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Original Article

In vitro Evaluation of the Erosive Effect of Probiotic Drink on Tooth Enamel

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Abstract

Purpose: The study aimed to evaluate the erosive activity of six probiotic drinks (PD) on tooth enamel. **Methods:** Forty-eight extracted human teeth premolar free of hypocalcification and carious were used in this study. The erosive activity was evaluated by measuring the pH, titratable acidity (TA), tooth weight loss, and the rate of calcium release after 5-min exposure daily over 7 days. **Results:** The pH of the PD was found to be in acidic range (3.08–4.10) with different TA values depending on the bacterial strain presence. The highest pH value showed minimum TA. The erosion was determined by the rate of calcium release and weight loss from the enamel surface on exposure to PD. Most of the samples showed consistent calcium reabsorption to the tooth enamel after 5 min of exposure daily over 7 days. However, all the samples showed persistent weight loss over 7 days' exposure. **Conclusion:** Although PD was found to be acidic, it exhibited low erosive activity, most probably due to the presence of high calcium content and certain bacterial strains in the drink. The number and type of bacterial strain in the drink did not significantly contribute to the erosion of the enamel, as no calcium loss was found except for certain drinks of repeated exposure in days 3 and 5. However, consistent weight loss was measured over a period of 7 days. In conclusion, PD does not cause any calcium loss on tooth enamel. Other than beneficial to the gut, it also promotes calcium reabsorption to the tooth enamel.

Keywords: Cultured drink, demineralization, probiotic drink, titratable acidity, tooth enamel

Introduction

Teeth are like a bone that contained phosphate-based mineral hydroxyapatite (HA) in the outermost layer of the tooth which is called enamel, the hardest tissue of the body; it can be distinguished from bone based on the anatomical position and arrangement.[1-4] At the early phase of its formation, enamel composed of inorganic and organic components. Later, a mature enamel will eventually have 97% of its matrix mineralized, with approximately 3% water and <1% organic matrix.[5] Having a crucial role in processing food, teeth have developed a high resistance to localized demineralization, mainly due to the enamel layer that covers the crown of the teeth.[6,7] Scaramucci *et al*.[8] stated that demineralization is caused by acidic attacks via two means, from dietary acids from food and drink, and by the microbial attack from bacteria contained in the mouth. Acidic attack involves the chemical dissolution of both organic and inorganic matrix components where there are acid diffusion and mineral loss from the tooth.[9-11]

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As an organic tissue, the integrity of the enamel is very much under the influence of pH. A study showed a fall in salivary pH after consuming soft drinks which might be attributed to the pH of the drink as well as its buffering capacity. Enamel dissolution occurs when it reaches the critical pH 5.5.[4] In the oral cavity, an acidic attack can be classified according to the pH of the acid.[12] Exposing teeth to the acidic pH <1 can cause surface etching even though for a short duration. While nanoscale surface softening occurs with short exposure at acidic pH 2–4, it does not affect the macroscale.[6] The acidic attack through weak acid (pH 4.5–6.9) is the most common acidic condition that will cause subsurface dissolution.[9]

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There are numerous aspects that contribute to dental erosion in an individual such as pH, salivary flow, buffering capacity, calcium content, and pellicle formation.[13] The routes of direct acidic attack have been well discussed and well-documented. Regardless of the type of acid in the drink, a lower pH dissolves HA in the enamel rapidly and causes more harm than it would at a higher pH. Chelation causes the destabilization of HA surface at low pH, weakening the phosphate coordination bonds. Acid concentration in molarity should also be taken into consideration. Titratable acidity (TA) is used to measure the concentration of hydrogen ion present in any drinks and the bound compounds, so it provides a broad overview of the acidity of a solution.[14,15] Alkali solution will be added to the drink until it reaches its natural value.[14] However, pH is not necessarily a crucial factor as pH merely measures the dissociated ions in a drink, which could be in the form of acids or alkalis.[16,17]

Many researchers agreed on the general health-promoting effects of the probiotic's products. Nevertheless, their recommendation for dental health purposes is not yet justified.[18,19] As minimal research has been conducted looking into the effects that probiotic drinks (PDs) have on dental health, this study specifically aims to look into the effects that PDs have on teeth mineralization.

Methods

Characterization of probiotic drink

The PD was labeled as sample A, sample B, sample C, sample D, sample E, and sample F and mineral water as a negative control and calcium chloride ($CaCl_2$) as a positive control.

Measurement of pH

The pH was measured using a pH meter (Toddler Mettler).[15] Fifty milliliters of each sample were placed into a separate clean beaker. The pH electrode was calibrated using standard buffers of pH 4.0 and 7.0 and was rinsed thoroughly between uses to avoid contamination. Three measurements were taken from each sample drink to ensure the reproducibility of the results.

Determination of titratable acidity

The TA of the drinks was measured.[15] Fifty milliliters of the newly opened PD was placed in a clean beaker. The drink was titrated with 0.5 M of sodium hydroxide (NaOH) added in 20 µl increments until the pH reached 5.5, 7.0, and 10.0. The volume of NaOH required to raise the drink to pH 5.5, 7.0, and 10.0 was recorded. The volume of NaOH added was then plotted against pH. The determination of TA repeated three times on different days to ensure the reproducibility of readings. The same procedures were carried out on all other PDs. This was done for the measurement of the total TA. The TA of each drink was compared using the Kruskal–Wallis test.

Determination of calcium content

The titration method was used to determine the content of free calcium ions in the cultured drinks.[20] Two hundred microliter of each drink was added in the clean beaker that contained 1 ml of potassium hydroxide (1.25 N KOH) and 100 µl of calcon, a dye that used in the experiment for an indication of the endpoint of the titration. The content was titrated with ethylenediaminetetraacetic acid (EDTA) (1%) until the appearance of a blue coloration reached the endpoint. The volume of EDTA used until it reached the endpoint was recorded. Similar steps were carried out on a standard control (positive control) which contained a known amount of $CaCl_2$ (0.1 mg/ml). EDTA is chelating which is complexed with calcium ions. Depending on the volume of EDTA that complexes with a known content of calcium (standard control), the concentration of calcium present in the drinks then was calculated.

Assessment of calcium loss after expose to probiotic drink

The same cultured drinks were used and labeled as sample A, sample B, sample C, sample D, sample E, and sample F, and mineral water as a negative control and $CaCl_2$ as a positive control.

Preparation of tooth enamel specimen

According to Abdul Razak *et al.*,[15] the extracted human tooth premolar free of hypocalcification and carious was used in this study. Ethics approval was attained from the Universiti Teknologi Mara (UiTM) research ethics committee with ethics number REC/172/18. Forty-eight teeth were selected and sterilized in an autoclave. Then, the whole tooth was covered with nail varnish to expose enamel with an area of about 30 mm^2 to standardize the whole samples. The samples were then divided randomly into three groups:

a. Positive control group: The teeth specimen fully immersed in $CaCl_2$
b. Negative control group: The teeth specimen fully immersed in mineral water
c. Samples group: The teeth specimens fully immersed in PDs were labeled sample A, sample B, sample C, sample D, sample E, and sample F.

Determination of the rate of calcium release

The rate of calcium release was determined.[20] The teeth specimens were immersed in a clean beaker that contained 50 ml of each CD. Two hundred microliter of the drinks was pipetted out after 5 min and the concentration of calcium in the drinks was determined. The whole process was repeated everyday over a period of 7 days using a fresh sample of drinks. The amount of calcium that been released from the enamel surface was calculated as the amount of calcium determined following exposure to each drink minus the amount of calcium already present in the drinks.

Determination of weight loss

The method described by Abdul Razak *et al*.[15] was employed in this section with slight modification.[21] The initial weight (W_0) of the tooth specimens for the respective drink was recorded. The tooth then was immersed in 25 ml of the respective drinks and was constantly stirred. After 30 min, the

tooth was removed, washed with distilled water, and left to dry in an oven at 30°C for 24 h. After that, the tooth was reweighed and the weight loss due to the first exposure to the PD was calculated. The whole procedure was repeated everyday over a period of 7 days using fresh sample of drinks.

Statistical analysis

Data obtained were nonparametric and were descriptively analyzed using SPSS Statistical Program version 25. The differences of pH between cultured drink, TA, calcium content, and calcium released were compared and analyzed by Kruskal–Wallis followed by *post hoc* test by Mann–Whitney U-test with the Bonferroni correction. The level of significance was set at $P < 0.05$. The weight loss was analyzed by repeated-measures analysis of variance.

Results

Characterization of probiotic drink

pH of probiotic drink

The pH of PD and mineral water is shown in Figure 1. All cultured drinks were acidic; the pH values recorded were lowest in sample C, 3.08 ± 0.02 ($P < 0.001$), and highest in sample D, 4.10 ± 0.02 ($P < 0.001$). The pH of mineral water was recorded as neutral.

Titratable acidity

The amount of NaOH required for PD and mineral water to reach pH 5.5, 7.0, and 10.0 is shown in Figure 2. The amount of NaOH required for all samples to reach pH 5.5, 7.0, and 10.0 was within the range of 0.88–2.66 ml, 1.93–4.08 ml, and 2.81–5.39 ml, respectively. The volume of NaOH required was lowest in sample D and highest in sample C compared to other samples for pH 5.5, 7.0, and 10.0. Based on the rapid response of the drinks to the addition of NaOH, it can be inferred that sample D required the least amount of NaOH for buffering.

Calcium content

Calcium content in PD is shown in Figure 3. The calcium content was highest in sample E, 100.2 ± 0.00 µg/ml, and lowest in sample A, 47.5 ± 0.25 µg/ml.

Assessment of calcium loss after expose to probiotic drink

Rate of calcium release

No calcium ions were released from all PDs including the $CaCl_2$ (positive control) and mineral water after repeated immersions over 7 days' exposure except for sample D and sample E. The calcium released was recorded as 8.35 µg/mL and 6.27 µg/mL, respectively, in day 5 as shown in Table 1. For samples A, B, C, and F, the remineralization process occurred over a period of 7 days.

Weight loss

Figure 4 demonstrates changes in the weight of tooth specimens after repeated immersions in the respective PD over a period of 7 days. A persistent decrease in weight from day 0 to day 7 was observed in all tooth specimens following the repeated exposure, as shown in Figure 4. The weight loss was highest in sample B and lowest in sample C.

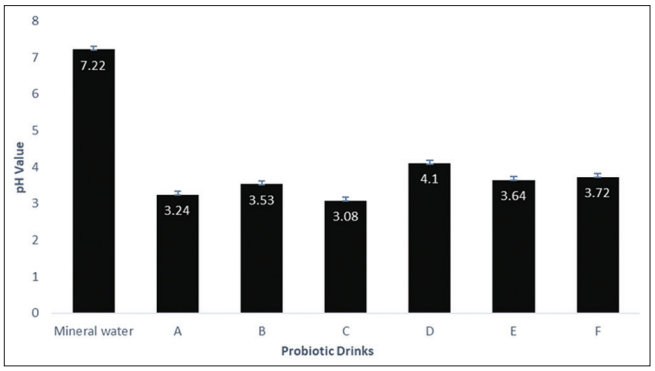

Figure 1: The pH of probiotic drinks recorded using pH meter

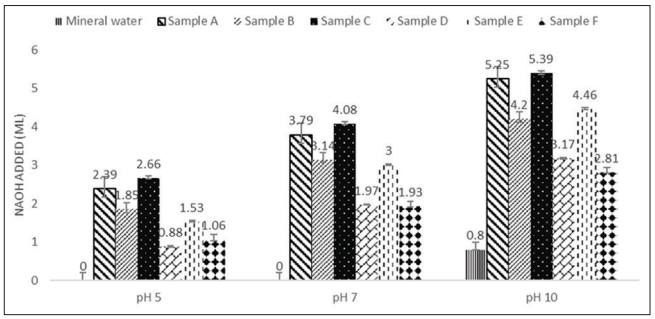

Figure 2: Titratable acidity of probiotic drink. The amount (ml) of sodium hydroxide (0.5 M) required to increase the pH of standard deviation to pH 5.5, pH 7.0, and pH 10. The readings were mean ± standard deviation from three determinations ($n = 21$)

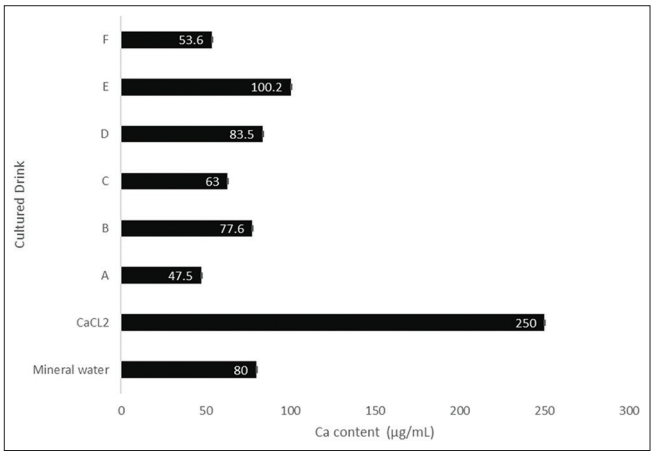

Figure 3: Calcium content of probiotic drink. The readings were the mean ± standard deviation from triplicate samples ($n = 21$)

Discussion

Acidic foods or beverages are considered as exogenous acids that have a wide implication on causing dental erosion.[21-23] The common effect of the frequent contact is demineralization of the enamel that softens the tooth surface and its underlying region.[24] Reducing intake and

Table 1: Calcium release in probiotic drink for 7 days

Group	Calcium (μg/mL)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Negative control							
Mineral water	−3.50	−3.50	−3.50	−3.50	−3.50	−3.50	−3.50
Positive control							
$CaCl_2$	0.00	0.00	0.00	0.00	0.0000	0.00	0.00
Probiotic drinks' sample							
A	−8.16	−10.75	−11.21	−13.59	−15.12	−15.12	−15.12
B	−5.95	−18.79	−22.65	−22.65	−16.38	−22.65	−22.65
C	−32.03	−34.10	−34.10	−34.10	−34.10	−34.10	−34.10
D	0.00	−4.92	4.40	0.00	8.53	0.00	0.00
E	0.00	0.00	0.00	0.00	9.66	0.00	0.00
F	−9.02	−10.63	−10.62	−9.02	−9.02	−9.02	−9.02

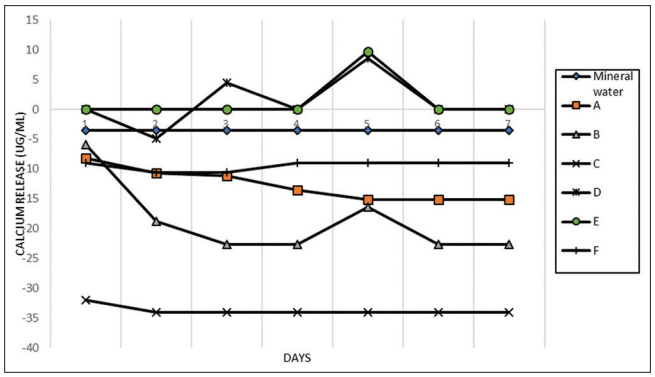

Figure 4: Measurement of calcium loss from tooth enamel after repeated immersion to probiotic drinks over 7-day period

modifying consumption habits can minimize the effect of acids on dental tissues.[25] Studies prove that acidic beverages modified with the addition of calcium, phosphate, or fluoride have the distinct capability to reduce the formation of erosive lesions in the enamel.[26,27]

Extensive research has proved the benefits of probiotic consumption to general health such as hypocholesterolemic effects, preventing and ameliorating bowel diseases by improving the immune system.[28-31] Studies have also showed the advantage of probiotics in oral health whereby probiotics when incorporated into dairy products are able to neutralize acidic conditions in the mouth, thus interfering with cariogenic bacteria activities.[32] Most probiotics are in dairy forms that contain calcium and have the possibility of reducing the demineralization effect on tooth enamel.[28] However, some studies have also claimed that PDs can cause dental erosion on enamel.[22,33]

Therefore, in this *in-vitro* study, the erosive effects of PD were assessed in the laboratory by measuring the pH, neutralizable acidity, and their ability to cause enamel erosion. Moreover, this study investigates mineral tooth loss by repeated immersions of PD over 7 days' exposure to determine the detrimental effect over the continuous consumption of PD. Calcium content of each PD was also determined due to calcium capability to buffer the erosive effect of the drinks.[27]

All six PDs used were proven to be acidic, in agreement with Lodi *et al*.[33] and Nadelman *et al*.,[34] as they had a pH range from 3.0 to 4.15. The pH value was lowest in sample C, 3.08 ± 0.02 ($P < 0.001$), and highest in sample D, 4.10 ± 0.02 ($P < 0.001$). The acidic nature of PD will contribute to the acidity of the oral environment caused by lactic acid produced by mouth bacteria and sugars from foods, soft drinks, and sweets. Acidic conditions can cause tooth decay and demineralization of the tooth enamel.[35] Acidic conditions over time can cause dental erosion whereby the enamel slowly dissolves creating tooth cavities.[36] However, pH only measures the hydrogen ion concentration in the drinks and does not provide a good indication of the presence of undissociated acid.[19]

TA is known to give a realistic measure to determine the buffering capacity of drinks. Buffering capacity is the measurement of alkali volume that is required to achieve pH 5.5, 7.0, and 10.0. The volume of NaOH required was lowest in sample D and highest in sample C compared to other samples. The lowest pH means the hydrogen concentration is higher, thus high TA , as it required a high amount of neutralizable acid. This shows the correlation between pH and TA values. The amount of available H+ ion in a drink will determine the total amount of neutralizable acids needed for the drink.

Many strategies have been proposed to decrease the erosive effect of PDs with low acidity. The addition of various different ions or complexes to erosive drinks showed promising results, as the ions were found to supersaturate the drinks respect to the tooth mineral.[19,24] The free calcium content was determined in cultured drink using the titration method, and all PDs showed that the presence of calcium includes mineral water. All the PDs used showed the presence of calcium in the range of 47.5–100.2 μg/ml [Figure 3] in consensus with Lodi *et al*.[33] that measured calcium content in fermented beverages. The calcium content in PD is higher compared to sports drink. High saturation of calcium ions in PD explained the slow release of these ions from enamel after exposure to drinks.[19] No calcium ions released were

found from all PDs after the repeated immersions over 7 days' exposure contrary to significant calcium released in sport drinks reported by Abdul Razak *et al*.[19] Moreover, the reduction of calcium content in the drink was found after the exposure which indicates the calcium reabsorption by the tooth on exposure. However, minimum calcium released were recorded from sample D (8.35 µg/mL) and sample E (6.27 µg/mL) in day 5 as shown in Table 1.

Weight loss of tooth due to enamel dissolution was measured according to the method of Abdul Razak *et al*.[19] Sample B showed consistent and highest weight loss in day 7, as shown in Figure 5. A persistent decrease in weight from day 0 to day 7 was observed in all tooth specimens following repeated exposure, as shown in Table 1, an indication of demineralization process, but it is not due to the calcium release. As dental enamel also consists of other minerals such as Na, Cl, Zn, and P, further investigation should be carried out which mineral is most affected by the exposure to PD.

The cariostatic effect of milk and milk products has been attributed to their high content of Ca and P ions, the buffering capacity, and the presence of casein phosphopeptides (CPPs).[37] The CPPs are tryptic digestion products of casein, a bovine milk protein,[38] and produced partly by the proteolytic activity of the lactic acid bacteria present in the probiotics.[39] The CPPs have a remarkable ability to stabilize calcium and phosphate, preserving them in a soluble form as amorphous calcium phosphate (ACP).[40] The ACPs being highly soluble are readily converted to HA making them a suitable remineralizing agent. The CPP-ACP complex binds to the surface of the teeth as well as to dental plaque and deposits a high concentration of ACP in close proximity to the tooth surface. This localized CPP-ACP buffers the free calcium and phosphate ions under acidic conditions, substantially increasing the level of calcium phosphate in plaque and thereby maintaining a state of supersaturation that inhibits enamel demineralization and enhances remineralization.[41-43]

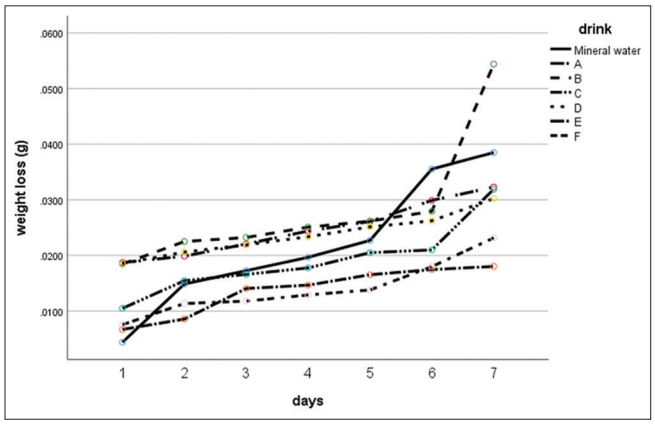

Figure 5: Measurement of weight loss of probiotic drinks following periodic exposures over a 7-day period

Although the detrimental effects of various drinks are compared, it is not possible to define the degree to which any drink will damage teeth, especially in the oral cavity, where the buffering action of saliva is very effective in neutralizing the acidic pH to neutral. However, buffering potential of saliva between individuals varies as there are many factors that can contribute to dental erosion.

Conclusion

PDs show characteristics that may cause demineralization of dental enamel, such as low pH and high buffering capacity. However, PDs also contain a high calcium level which may reduce the dissolution of the dental structure produced by acidic substances. In this *in vitro* study, PDs do not promote erosion of the dental enamel but rather only a superficial mineral loss which requires further investigation and confirmation. Further *in situ* studies are recommended to evaluate the erosive potential of PDs.

Ethics of study

This study was accepted by the UiTM research ethics committee. The ethic reference code is REC/172/18.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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