Evaluation of Bubble Tea Drink on Metabolism *Streptococcus Mutans* ATCC 25175

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Abstract

Bubble tea drinks containing tea and tapioca pearl are currently very popular. *Streptococcus mutans* ATCC 25175 is known as caries-causing bacteria. Metabolism of *S. mutans* ATCC 25175 by producing glucosyltransferase (GTF). The GTF enzyme has the function of converting sucrose into fructose and glucan which plays a role in the process of biofilm formation. This study aims to evaluate of bubble tea drink on the metabolism *S. mutans* ATCC 25175.

The study was experimental method and the subjects was *S. mutans* ATCC 25175 from the Integrated Research Laboratory of Faculty of Dentistry Universitas Gadjah Mada. The test materials were divided into 4 groups, consisting of bubble tea drinks no sugar, no sugar and bubble, sugar no bubble, sugar and bubble. The study was carried out consist of the biomass index, biostability, changes in pH, the activity of the GTF enzyme and fructose release by *S. mutans* ATCC 25175. Microtiter Plate Assay was measured using an ELISA reader at wavelengths of 560 nm and 590 nm.

Statistical results using ANOVA and LSD or Dunnett T3 showed the bubble effect could reduce the biomass index, biostability and pH after interacting with *S. mutans* ATCC 25175 ($p<0.05$). The bubbles in the bubble tea drink were also able to activate the GTF activity of *S. mutans* ATCC 25175 after 24 hours of incubation and reduce the fructose enzyme by *S. mutans* ATCC 25175 after 72 hours of incubation ($p<0.05$). It was concluded that the metabolism of *S. mutans* ATCC 25175 was inhibited after interacting with bubble tea drinks.

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Introduction

Bubble tea drinks are currently very popular all over the world. This drink consists of tea mixed with a filling in the form of balls of tapioca pearls. The nutritional content in bubble tea drinks is 16 fl oz (472 ml), namely 317.5 calories; total fat as much as 10.6 (g); carbohydrates as much as 56 (g); sugar as much as 36 (g); and protein as much as 1.8 (g).[1] The main components in bubble tea are tea, black tapioca made from cassava or sweet potato starch, and brown sugar.[2]

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Dental caries is known as one of the most common oral diseases in the community. The caries prevalence rate in Indonesia reaches 45.3%.[3] *Streptococcus mutans* is known as a bacterium that has a major role in the occurrence of dental caries.[4] *Streptococcus mutans* have the ability to synthesize glucan extracellular polysaccharides α (1-3)-linkages which are insoluble in water; producing lactic acid through a homofermentation process; form colonies that adhere tightly to the tooth surface; and is acidogenic to other Streptococcus species.[5] *Streptococcus mutans* can coagulate and collectively irreversibly bind to the tooth surface in the presence of glucan (water insoluble glucan) synthesized from sucrose. Adhesive and water-insoluble dextran mediates the attachment of *S. mutans* and other bacteria to the tooth surface.[6]

There are 2 types of enzymes produced by *S. mutans* namely glucosyltransferase (GTF) and fructosyltransferase (FTF). This enzyme acts as

a substrate for sucrose for the synthesis of glucans and fructans. The GTF enzyme catalyzes the formation of soluble and insoluble glucans from sucrose and plays a role in the composition of the polysaccharide matrix in dental plaque. Glucan is known as an important virulence factor because it helps the attachment of bacteria to the dental pellicle, and also contributes to the structural integrity of the biofilm.[6] Metabolic processes can convert fructose from sucrose into glycolysis intermediates and rapidly converted to lactic acid. The presence of these acids will cause a decrease in pH which can cause demineralization of hydroxyapatite in tooth enamel.[7]

Shifting lifestyles and development of technological have resulted in one of the most popular bubble tea drinks today. Bubble tea drinks containing tea and tapioca pearls are also often added with milk and palm sugar. Tea is known to be rich in polyphenols (catechins). The results have proven that the polyphenol content of tea can inhibit the growth of bacteria that play a role in plaque formation, including *S. mutans*, *S. sobrinus*, and *Lactobacillus* which can cause formation dental plaque. The polyphenols in tea are also known to inhibit plaque formation.[8] On the other hand, it is known that the nutritional content of bubble tea drinks is carbohydrates. Samanarayake suggested that carbohydrates can be metabolized to acid by bacterial plaque.[6] The presence of acid will cause the pH to be low, allowing the growth of *S. mutans*. Biomass analysis was used to determine the activity of carbohydrate fermentation by *S. mutans*. Biostability analysis to determine the stability index after interaction with *S. mutans*. The biostability index was related to the activity of the GTF enzyme in releasing fructose as a source of nutrition for *S. mutans*. This study was important to evaluate the effect of bubble tea drink on the metabolism of *S. mutans* ATCC 25175. The evaluation includes the profile of biomass, biostability, pH against *S. mutans*, GTF enzyme activity and the release of fructose by *S. mutans* ATCC 25175.

Materials and methods

This study was an experimental laboratory and obtained ethical clearance from the Ethics Committee of the Faculty of Dentistry, Universitas Gadjah Mada (FKG UGM) No. 00698/KKEP/FKG-UGM/EC/2021 on June 15, 2021. The study was done at the Integrated Research Laboratory of FKG UGM according to letter No. 5114/UN1/FKG.1/Set.KG1/PT/2021 on June 18, 2021. The test materials were divided into 4 groups of bubble tea drinks, consisting of bubble tea drinks no sugar, no sugar and bubble, sugar no bubble, sugar and bubble. The study was carried out consist of the biomass index, biostability, and changes in pH, the activity of the GTF enzyme and fructose release by *S. mutans* ATCC 25175.

1. Identification of *S. mutans* ATCC 25175

Streptococcus mutans ATCC 25175 was re-cultured in Mitis Salivarius Bacitracin (MSB) selective media,[9,10] incubated in an anaerobic jar at 37°C for 2 x 24 hours. Colonies growing on solid media then cultured in 10 ml of BHI-Broth media, centrifuged at 3000 g for 15 minutes. The supernatant was stored for *S. mutans* develop solution.

2. Biomass Test

Bubble tea drink as much as 3 ml was weighed using an analytical balance. Incubation was carried out at 37°C for 24 hours, 48 hours, and 72 hours, then weighed again. This procedure was repeated on all materials by adding 100 µl *S. mutans*. Furthermore, *S. mutans* were cultured in MSB media to determine the relationship between biostability activity and colony growth.

3. Biostability Test

Biostability measurements according to de Olivera et al.[11] with modifications, it had been carried out in 2 ways, namely qualitative and quantitative. Qualitatively, the measurement was based on color absorption by giving 1% crystal violet and 1% safranin.

A total of 3 ml of each test material was added 100 µl *S. mutans* then incubated for 24 hours, 48 hours and 72 hours. In each tube, 150 µl of 1% crystal violet and 1% safranin were added to the test material. The change in the color of the solution was an indicator of the anti-*S. mutans* activity of the materials. If the dominant color was purple (negative) and between purple and red (stable), and red (positive).

Each test material was pipetted as much as 150 µl into a 96 well plate according time incubation then shook for 15 minutes at a speed of 210 g. The OD measured using an Elisa reader at a wavelength of 590 nm.

After incubation, the test material were

centrifuged at 3000 g for 15 minutes. The supernatant was produced as an anti-develop solution to determine the potential of the test material on the expression of GTF *S. mutans*.

4. pH test

Measurement of 4 group bubble tea drink was done using a digital pH meter. After checking the pH, the test and control materials (without *S. mutans*) based on 24 hours; 48 hours; and 72 hours were transferred much as 150 µl into a 96 well plate, and continued by measure the OD of the interaction between *S. mutans* and the four group test materials using an Elisa reader with a wavelength of 560 nm.

5. GTF enzyme purification

Extraction enzyme was done by taking 1 colony of *S. mutans* in MSB and incubated for 24 hours, 48 hours, 72 hours then cultured in BHI-Broth. Tubes of 4 group test material were centrifuged at 3000 g for 30 minutes, to obtain GTF, develop I and develop II. The supernatant of *S. mutans* as develop I (control) which was not interacted with the test material and the supernatant of *S. mutans* as develop II which interacted with the test material. Part of GTF in the precipitated material that has been prepared, added ethanol and centrifuged 3000 g for 30 minutes, put in the freezer for 1 hour, and added ammonium sulfate, centrifuged again at 2500 g for 1 hour, added with 8 M urea. Test material were shaken and left for 15 min, and centrifuged at 3000 g for 30 min.[12]

Develop I was produced from centrifugation with the addition of 50% ethanol in a 5:3 ratio, incubated at 0°C for 24 hours, continued to centrifuge again at 5500 g for 30 minutes, then the supernatant was discarded. The sediment was washed with 5 ml of PBS solution, then centrifuged again, and the supernatant was taken (develop II) which became an enzyme candidate.[13]

6. Preparation reagent solution of fructose and GTF.

The solution was containing 0.1 M sodium meleate (pH 6), 0.1 M fructose, 0.01% Merthiolate Iodine and *S. mutans* to 6 ml, which was incubated at 37°C for 15 hours then centrifuged at 1800 g for 20 minutes. The precipitate formed was water soluble glucan which was added with 70% ethanol, washed with 0.1 NaCl glucan and centrifuged 1500 g for 15 minutes. The supernatant was discarded and 0.1 NaCl was added, then dried. The results were analyzed by spectrophotometer in a 96 well plate as fructose release candidate by GTF *S. mutans*.

7. Measurement of enzymatic GTF *S. mutans*

The GTF enzyme that had prepared in the previous procedure was used to obtain value of *S. mutans* GTF expression. The test material and GTF *S. mutans* as a control were added 100 µl develop I GTF solution based on time into 96 well plates, incubated for 10 minutes. Plate was washed with 100 µl PBS, then incubated for 15 minutes and shook 100 g for 2 minutes. A total of 100 µl of *S. mutans* GTF was put into 96 well plates, incubated for 20 minutes, 100 µl of anti-sera was added, then incubated for 35 minutes, washed with 100 µl PBS, shook at 100 g for 2 minutes, then 100 µl of develop II GTF was added. Incubation was done for 20 minutes and added HCL 1 N 50 µl. The OD was read using an Elisa reader at a wavelength of 560 nm.

8. Measurement of fructose release by GTF *S. mutans* enzyme

The test material bubble tea drink and GTF *S. mutans* as a control were added 100 µl develop I fructose solution based on incubation time into 96 well plates, incubated for 10 minutes, washed with 100 µl PBS, then pipetted for each solution. Plate was shaken in a 100 g for 2 minutes, then pipetted *S. mutans* fructose into 96 well plates. Plate was incubated for 20 minutes, then added 100 µl of anti-sera, incubated again for 35 minutes, washed with 100 µl PBS and shook 100 g for 2 minutes. After that, 100 µl develop II fructose was added, incubated for 20 minutes, and 50 µl of HCL 1 N. Plate was read using Elisa reader at a wavelength of 560 nm.

Data were analyzed using IBM SPSS Statistics version 22 for Windows (IBM SPSS Inc., USA), and the significance level at $p < 0.05$. The Shapiro–Wilk and Levene tests were done to analyze normality and homogeneity of variance respectively. Data were calculated by using ANOVA and LSD or Dunnett T3 test.

Results

1. Identification of *S. mutans* ATCC 25175

The results of *S. mutans* ATCC 25175 on Mitis Salivarius Bacitracin (MSB) selective media after incubated in an anaerobic jar at 37°C for 2 x 24 hours are shown in Figure 1. The colonies growing spread out on MCB selective media.

The results of statistical calculations for normality data of biomass, biostability, pH, GTF

S. mutans and fructose release using the Shapiro-Wilk test showed p > 0.05 or normal data distribution. Homogeneity test using Levene's test showed biomass and biostability data p < 0.05 so the data was not homogeneous.

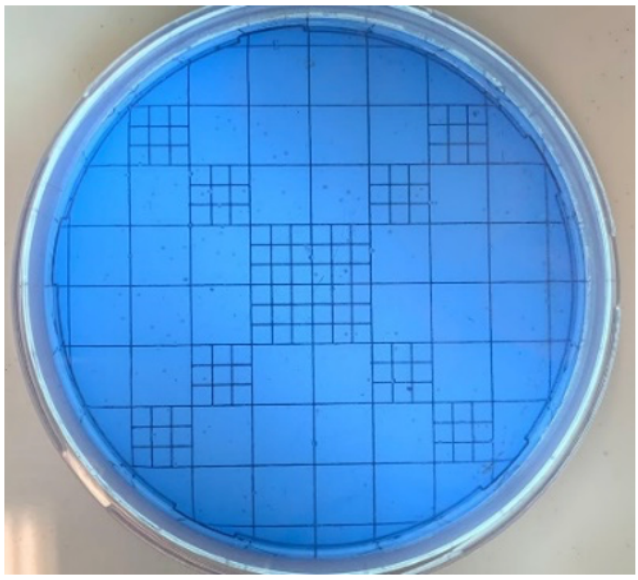


Figure 1. Colonies of *S. mutans* ATCC 25175 on Mitis Salivarius Bacitracin (MSB) selective media.

2. Biomass Test

The biomass index of bubble tea and tea without bubble tea, both containing sugar and without sugar, is shown in Table 2.

The overall yield of the biomass index is in the strong category or less than 0.5. The results of statistical testing using One-way ANOVA showed p = 0.000, these results indicated that *S. mutans* 25175 bacteria interacted with bubble tea drinks significantly changed biomass. The results of the comparison of each biomass according to the incubation time are shown in Table 1.

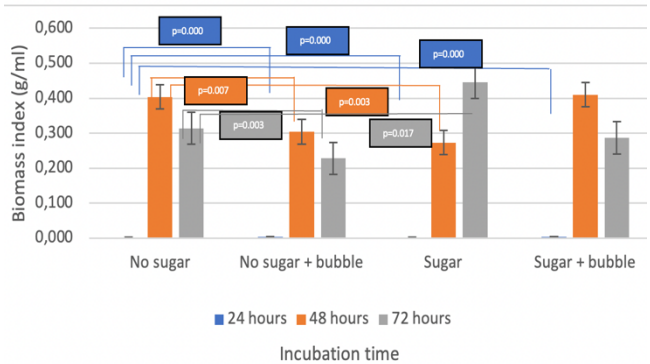


Table 1. Biomass index of bubble tea drinks and the comparison each biomass according to incubation time.

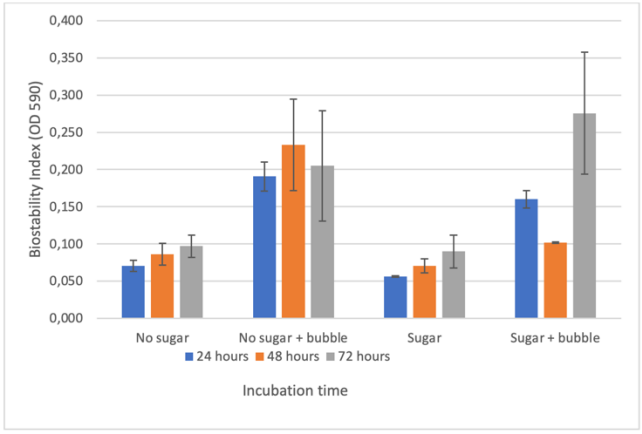


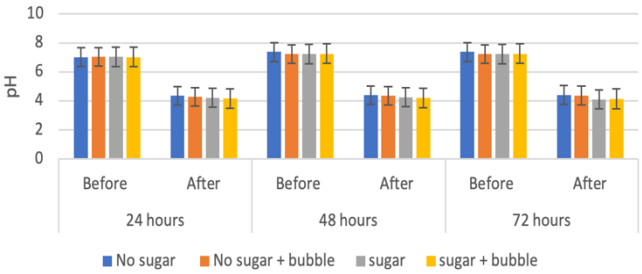
Table 2. Biostability index of bubble tea drinks according to incubation time.

3. Biostability Test

The results of qualitative biostability testing showed that the whole group no sugar, no sugar and bubbles, sugar, sugar and bubbles were incubated for 24 hours, 48 hours and 72 hours, which were purple in color. These results indicated that all bubble tea drinks didn't have anti-*S. mutans* activity. Quantitative measurements using a spectrophotometer with a wavelength of 590 nm are shown in Table 2.

The measurement of the biostability index uses a scale of 1> (strong); 0.91-0.99 (medium); <0.9 (weak). The average OD results from Table 2 showed that the entire group of bubble tea drinks is in the weak category. The results of ANOVA showed p < 0.05 or it could be interpreted that *S. mutans* 25175 interacted with bubble tea drinks significantly changed the biostability.

4. Measurement pH



Tabel 3. Measurement pH bubble tea drink before and after incubation.

Measurements of pH before and after incubation for 24 hours, 48 hours, and 78 hours were shown in Table 3. Table 3 showed that the overall pH decreased after incubation for 24 hours, 48 hours and 72 hours. The overall pH

after incubation was in the positive category, pH > 4 or could suppress the expression of *S. mutans* GTF. Changes in pH before and after interaction with *S. mutans* were shown in Table 4. Table 4 described the lowest pH changes in tea drinks without sugar or bubbles. The highest change in pH was in bubble tea drinks containing sugar. The pH index value was determined using a scale base of 1> (strong); 0.81-0.99 (medium); <0.8 (weak). The overall change in pH or pH index showed a weak category or it could be interpreted that *S. mutans* was able to ferment carbohydrates contained in bubble tea drinks.

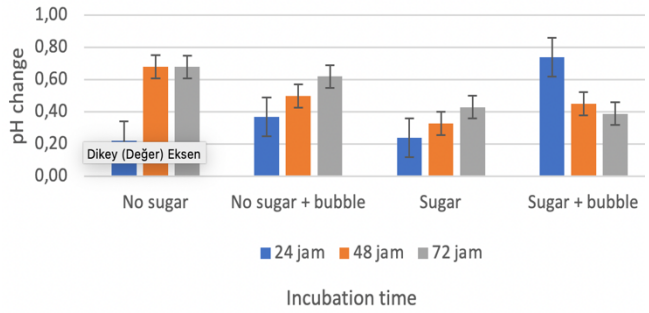


Table 4. Changes in pH after interaction with *S. Mutans*.

ANOVA result showed changes in pH before compared to after incubation according to each time obtained p = 0.000. These results indicated the interaction of *S. mutans* with bubble tea drinks significantly decreased the pH.

5. Measurement enzymatic of GTF *S. Mutans*

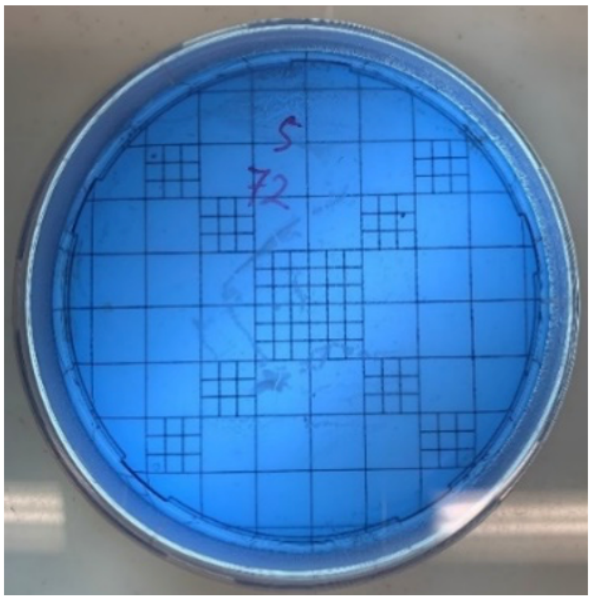


Figure 2. Colonies of *S. mutans* ATCC 25175 from the sugar group of bubble tea drink did not grow on Mitis Salivarius Bacitracin (MSB) media after 72 hours incubation.

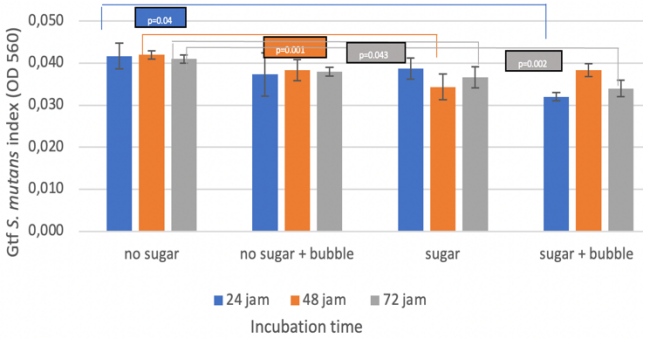


Table 5. *S. mutans* GTF enzyme activity after interaction with bubble tea drinks.

Testing of GTF enzyme *S. mutans* to measure the ability of sugar fermentation is shown in Table 5. The results of ANOVA test on the activity of the *S. mutans* GTF enzyme after interacting with bubble tea showed p = 0.001. These results indicated the interaction of *S. mutans* with bubble tea drinks was significant on the expression of *S. mutans* GTF.

The LSD results showed that the bubble effect of bubble tea drinks could significantly reduce the activity of the enzyme GTF *S. mutans* 25175 after incubation for 24 hours. At 72 hours of incubation, there was a significant difference, possibly because the *S. mutans* bacteria were no longer growing. This is shown from the observations on Mitis Salivarius Bacitracin media did not show the growth of *S. mutans* (Figure 2).

6. Measurement of fructose release by GTF *S. mutans* enzyme

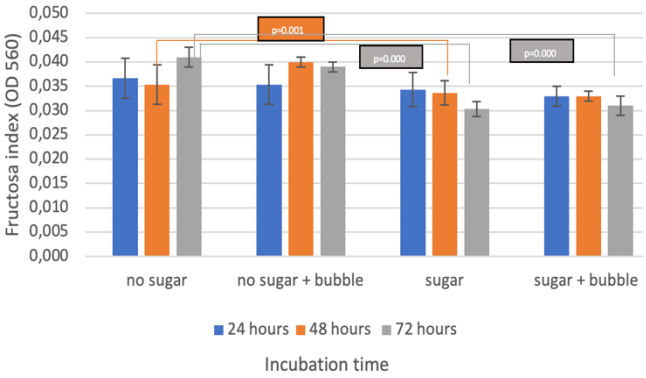


Table 6. Results for the release of fructosa by GTF *S. mutans* ATCC 25175 enzyme after interaction with bubble tea drinks

The results of the fructose release measurement using a spectrophotometer at a

wavelength of 560 nm are shown in Table 6.

The results of statistical tests using One-way ANOVA showed p = 0.001 or it could be interpreted that the interaction of bubble tea drinks significantly affected the release of fructose according to the incubation time. The LSD results show that the bubble effect of bubble tea drinks could significantly reduce the release of fructose by the GTF *S. mutans* 25175 enzyme after 72 hours of incubation.

Discussion

Measuring the metabolism of *S. mutans* ATCC 25175 after interacting with bubble tea drinks was done through biomass analysis. This parameter was used to measure the activity of carbohydrate fermentation in bubble tea drinks by *S. mutans*. The interaction activity could as indicator of bubble tea drinks to inhibit the development or fermentation activity of *S. mutans* or vice versa, namely the ability of *S. mutans* to metabolize to grow and develop in bubble tea drinks.

The results of the ANOVA and Post hoc Dunnett T3 statistical tests showed that bubble tea drinks could significantly change the biomass index produced by *S. mutans* ATCC 25175. The biomass index category produced was in the strong category or less than 0.5. The biomass index increased with increasing incubation time and the highest yield was at 72 hours incubation in the group interacted with sugar group. The decrease in the biomass index in the bubble tea drink group with an incubation time of 72 hours. These results indicated that *S. mutans* ATCC 25175 could increase the activity of fermenting sugars contained in bubble tea drinks while the bubble content is thought to be able to inhibit its fermentation activity.

Measurement of biostability aimed to determine the stability index when bubble tea drinks are interacted with *S. mutans* 25175. The results of Table 2 showed that *S. mutans* 25175 significantly changed the biostability ($p<0.05$) even though it was in the weak category. The results also showed that there was a significant difference in the effect of bubbles on biostability ($p<0.05$) compared to drinks without bubbles. These results indicated that bubble can reduce changes in biostability of bubble tea drinks.

The pH test results showed the lowest change in pH was in drinks without sugar or bubbles, while the highest was in bubble tea drinks containing sugar. The overall change in pH or pH index showed a weak category or it could be interpreted that *S. mutans* is able to ferment carbohydrates contained in bubble tea drinks. These results supported the study of Hedberg et al. that carbohydrate fermentation occurred at pH < 5.2.[14]

Measurement of enzyme activity of GTF *S. mutans* after interacting with bubble tea drinks showed significant results, which indicated that bubble tea drinks had a significant effect on the expression of GTF *S. mutans*. The bubble effect on bubble tea drinks could significantly reduce the activity of the enzyme GTF *S. mutans* 25175 after incubation for 24 hours, while at 72 hours incubation also showed a significant difference, possibly because *S. mutans* is no longer growing. This is shown from the observations on the media Mitis Salivarius Bacitracin did not show the growth of *S. mutans*. The results of this change in pH are in line with the results of biostability testing which showed that the bubble effect can maintain biostability so that it can reduce the activity of the GTF *S. mutans* 25175 enzyme.

The metabolism of *S. mutans* 25175 is strongly influenced by carbohydrates as a source of energy and survival including for its pathogenicity. Carbohydrates that are the main source of the metabolism of *S. mutans* bacteria are glucose, fructose, or sucrose. Fructose alone is known to affect the expression of certain genes in relation to GTF. In the research of Zeng and Burne,[15] it has not been revealed why fructose in particular plays a role in gene expression in the association of GTF which is more than sucrose or glucose. The results of this study also added that the inhibition of the release of fructose by the GTF *S. mutans* enzyme as an effort to prevent dental caries. The results of this study indicated that the bubble effect of bubble tea drinks can significantly reduce the release of fructose by the GTF *S. mutans* 25175 enzyme after incubation for 72 hours.

The results of this study also supported the research conducted by Hasibul et.al.[16] that D-tagatose inhibits the activity of the GTF enzyme which will result in the release of D-fructose from sucrose. D-Fructose (and sucrose) are known to enhance gtfB expression. In addition, it is known that D-fructose induces higher gtfB expression than D-glucose in the early exponential phase. The mechanism of inhibition of *S. mutans* GTF

enzyme activity and the release of fructose after interaction with bubble tea is also thought to be due to the tea polyphenol content (EGCG) which can inhibit the formation of *S. mutans* biofilm by suppressing the Gtf gene.[17] This mechanism supported previous study that inhibition of biofilm by decreasing mRNA expression in gtfB, gtfD, and gbpB *S. mutans* by papain. The inhibition formation of biofilms by papain has the same effect to chlorhexidine as the golden standard mouthwash.[18] From the results of this study, it is possible that bubbles in bubble tea drinks without sugar could be used to prevent dental caries through a mechanism to reduce the release of fructose. This result also supported previous study that the extract of the white rice bran contains polyphenols and mouthwash that containing bran extract which is known to effectively inhibit the growth of *Streptococcus mutans* and *Porphyromonas gingivalis*.[19]

The results of this study also supported previous studies that bubble tea drinks could improve the quality of saliva by reducing salivary C-reactive protein (CRP) and increasing calcium levels. Chewing tapioca pearls (bubble) could stimulate salivary secretion mechanically. In addition, the effect of bubble in bubble tea drinks can increase levels of salivary alpha amylase and phosphate, also may improve saliva quality through a saliva buffer mechanism.[20,21]

Conclusions

The result of this study can be concluded that the bubble effect could decrease the biomass index, biostability and pH after interacting with *S. mutans* ATCC 25175. Bubble effect in bubble tea drinks was able to significantly inhibit the activity of the enzyme GTF *S. mutans* ATCC 25175 after 24 hours of incubation and significantly reduce the release of fructose by *S. mutans* ATCC 25175 after 72 hours of incubation. Metabolism of *S. mutans* ATCC 25175 after 24 hours of incubation is inhibited after interacting with bubble tea drinks.

Acknowledgments

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Declaration of Interest

The authors have no conflict of interest to declare.

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