

## Effect of cassava (*Manihot esculenta cranz*), rice (*Oryza sativa* L.), and potato (*Solanum tuberosum*) water extract to decrease pH phase fermentation of *Streptococcus mutans* ATCC 25175

Reza Afri Imelda<sup>1</sup>, Marry Siti Mariam<sup>1\*</sup>, Mieke Hemiawati Satari<sup>1</sup>

<sup>1</sup>Department of Oral Biology, Faculty of Dentistry Universitas Padjadjaran, Indonesia

### ABSTRACT

**Introduction:** Dental caries is the localised damage of the hard tooth tissue caused by acid production results of carbohydrate fermentation by acid-producing bacteria. *Streptococcus mutans* are one of the aetiological bacteria which fermented carbohydrate causes a decrease in the oral pH. Carbohydrate is generally consumed, included cassava, rice, and potato. The purpose of this study was to determine the mechanism of the water extract of rice, cassava, and potato in decreasing the pH of *Streptococcus mutans* culture to know their potential in causing caries and also determined decreasing differences between three water extracts. **Methods:** The experimental method was used in cultured *Streptococcus mutans* ATCC 25175 and tested against the treated rice, cassava, and potato water extract. Cultured bacteria were incubated for 48 hours in facultative anaerobes then measured the pH with a digital pH meter. The measurement result was statistically tested with the paired t-test and ANOVA. **Results:** The paired t-test ( $\alpha = 0.05$ ) showed that the p-value of rice, cassava, and potato water extract were 0.001, 0.001, and 0.018 respectively. ANOVA test with an  $\alpha$  value of 0.05 showed the p-value of 0.000. **Conclusion:** There was a decrease in pH of cultured *Streptococcus mutans* after administration of the water extract of cassava, rice, and potato, and there was a difference between the pH decrease of the *Streptococcus mutans* between administration of water extract of rice, cassava, and potato.

**Keywords:** *Streptococcus mutans*, *Manihot esculentacrantz*, *Oryza sativa* L., *Solanum tuberosum*, pH phase fermentation

P-ISSN 1979-0201, e-ISSN 2549-6212; Available from: <http://jurnal.unpad.ac.id/pjd/article/view/21156>

DOI: [10.24198/pjd.vol31no1.21156](https://doi.org/10.24198/pjd.vol31no1.21156)

Submission: Apr 12, 2018; Accepted: Feb 14, 2019; Published online: Mar 29, 2019

---

### INTRODUCTION

Dental caries is an infectious disease of global concern and infects all age groups.<sup>1</sup> Nowadays, caries is a common problem, but the frequency

of caries differs in every country. The prevalence of caries is lower in developed countries than in developing countries in the 20<sup>th</sup> century.<sup>2</sup>

The criteria of the DMF-T index of Indonesian society is still high; this means that the oral health

---

<sup>1</sup>Corresponding author: Marry Siti Mariam, Department of Oral Biology, Faculty of Dentistry Universitas Padjadjaran, Indonesia. Sekeloa Selatan I, Bandung, West Java, Indonesia, 40132. Phone: +6281905088875; Email: [marry.mariam@fkg.unpad.ac.id](mailto:marry.mariam@fkg.unpad.ac.id)

status of Indonesian people is still poor. Indonesia's DMF-T index is 4.6 with the value of: Decay = 1.6; Missing = 2.9; Filling = 0.08. The index describes caries damage, filled teeth, and teeth loss in all Indonesians by 460 teeth per 100 persons.<sup>3</sup>

Caries is tooth infectious disease which resulting in the solubility and damage of the hard tissues, and the process of occurrence on the tooth surface begins with plaque retention on the tooth surface for long periods.<sup>2</sup>

Dental caries process occurs due to the plaque bacteria from carbohydrate fermentation.<sup>4</sup> The bacteria found in dental plaque metabolise carbohydrates to produce acid and cause the plaque pH level decrease to 5 within 1-3 minutes.<sup>2</sup>

Acids will penetrate to the dental layer and dissolve various mineral salts such as calcium and phosphate.<sup>1</sup> The release of mineral salts on the dominant (demineralised) enamel and for some time resulted in the formation of cavities.<sup>5</sup>

Most Indonesian population consume rice as its main food. In Indonesia, staple food other than rice is cassava and potatoes.<sup>6</sup> Generally, Indonesian people consume carbohydrates as much as three times a day as the staple food.<sup>7</sup> The frequency of high and continuous carbohydrate consumption in adjacent time intervals cause more acid to be produced, and the risk of caries may increase.<sup>1</sup> Rice, cassava, and potatoes contain starch which is a complex carbohydrate (polysaccharide) and simple carbohydrates (monosaccharide and disaccharides).<sup>6</sup> Monosaccharide is the most simple carbohydrates including glucose, fructose, galactose, and dextrose. Disaccharides are two monosaccharides that bind to include sucrose, lactose, and maltose. The simple carbohydrate content in cassava is 2-17% sucrose with a small amount of dextrose, fructose, glucose, and maltose.<sup>7,8</sup> Potatoes contain monosaccharide in the form of glucose and fructose and disaccharides in the form of sucrose. The total content of simple carbohydrates in potatoes is about 0.5 wt% wet.<sup>9</sup> White rice contains a simple carbohydrate of 0.3-0.5% with most of the sucrose and small fructose and glucose.<sup>10</sup>

Based on the explanation above, the three kinds of carbohydrate which are rice, cassava and potatoes, have the content of sucrose. Sucrose will be digested by *Streptococcus mutans* to produce fructan and glucan; then glucan helps

bacterial attachment to the biofilm to form larger colonies of plaque bacteria. *Streptococcus mutans* synthesises glucans into intracellular polysaccharides which then fermented to produce acid (acidogenic) and thrive in an acidic (aciduric) atmosphere continuously.<sup>2,11</sup> In a certain period, the acid causes the demineralisation process of the enamel resulting in the formation of tooth cavities.<sup>4</sup> The purpose of this study was to determine the mechanism of the water extract of rice, cassava, and potato in decreasing the pH of *Streptococcus mutans* culture to know their potential in causing caries and also determined decrease differences between three water extracts.

## METHODS

This research was experimental by measuring the pH of *Streptococcus mutans* ATCC 25175 fermented cultures on water extract of rice, cassava, and potatoes. The research material was carbohydrate from water extract of rice, cassava, and potatoes by using water solvent (aquadest) fabricated at Chemical Laboratory of Universitas Padjadjaran. This research was conducted in several stages which were the extract fabrication; bacterial culture; suspension of test bacteria fabrication; planting of test bacteria in extract medium; and pH measurement of *Streptococcus mutans* culture.

Fabrication of the water extract of rice, cassava, and potatoes was performed through extraction by maceration process using water solvent so that the extract was obtained with a numerical scale and milligram units. Each ingredient of rice, cassava, and potatoes was weighed as much as 1000 grams, then finely cut, added with 2000 ml of aquadest then blended for 30 seconds, let stand until the precipitation forms. After the decantation precipitate was formed (the liquid was separated from the solid) then filtered. The filtrate from the filtered product was added with 2000 ml of aquadest and let stand again for 3 days in the refrigerator, then decanted and filtered. The filtered filtrate was dried and weighed afterwards. The breeding medium used was the Mueller-Hinton bullion. The culture medium used was trypticase-yeast-cysteine-sucrose-bacitracin (TYCSB) medium. The test bacteria were pure cultures of *Streptococcus mutans* ATCC 25175. *Streptococcus mutans* ATCC 25175 was

cultured on a TYCSB medium with 20% sucrose, then incubated in an anaerobic environment for 48-72 hours at 37°C. On examination under a microscope, an image of a bacterial colony was formed in a cauliflower-like shape and small white crystals which were attached to the medium and developing a long chain formation.

Fabrication of *Streptococcus mutans* culture was inoculated into Mueller-Hinton bullion. The bacteria that have been compared to turbidity with Mc Farland solution 0.5 (0.5 ml, 175% solution  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  and 99.5 ml of 1%  $\text{H}_2\text{SO}_4$  solution), as a comparable standard solution equivalent to 108 CFU/ml were cultivated. The culture was stored in an anaerobic environment for 1-2 hours at 37°C. The culture pH is then measured. Then a medium contained Mueller Hinton (MH) bullion was made for each extract.

The rice medium contained 5 ml of Mueller Hinton bullion and 0.5 g of rice water extract. Cassava medium contained 5 ml of Mueller Hinton bullion and 0.5 g of cassava water extract. Potato medium contained 5 ml of Mueller Hinton bullion and 0.5 g of potato water extract. The test bacteria culture with turbidity by the Mc Farland standard was inoculated on each medium as much as 0.1 ml. The medium was incubated inside an anaerobic environment at 37°C for 48 hours, then each medium pH was measured – the pH measurement was performed using a digital pH meter. The pH meter was already calibrated with a neutral pH solution (pH = 7). Then the pH meter was cleansed with sterile aquadest and dried using a sterile tissue before being used to measure the pH of *Streptococcus mutans* culture in the test medium. The pH meter cleansing was performed in every measurement time to make the results more accurate. The measurement of culture pH was carried out before and after 48 hours.

## RESULTS

The result of pH measurement was done on the culture of *Streptococcus mutans* medium bullion Mueller-Hinton turbidity Mc Farland 0.5 as control and on the culture of *Streptococcus mutans* which was treated with rice water extract, cassava water extract, and potato water extract.

The results of the culture pH measurement of *Streptococcus mutans* on the Mueller-Hinton

bulge medium of Mc Farland's turbidity 0.5 after incubation for 2-3 hours in anaerobic facultative atmosphere showed a mean pH of 6.49 (Table 1).

Table 1. pH value of *Streptococcus mutans* on Mueller-Hinton bullion medium with McFarland's turbidity of 0.5

Repetition	pH
1	6.58
2	6.34
3	6.54
mean	6.49

The results of the measurement of the culture pH of *Streptococcus mutans* which was treated with rice water extract, cassava water extract, and potato water extract on Mueller-Hinton bullion medium after 48 hours incubation in an anaerobic facultative atmosphere can be seen from Table 2. The measurements showed a mean pH of rice water extract 3.773, cassava water extract 3.913 and potato water extract 5.98 (Table 2).

Table 2. pH value of *Streptococcus mutans* treated with water extract

Repetition	Rice	Cassava	Potato
1	3.72	3.92	5.95
2	3.82	3.91	5.95
3	3.78	3.91	6.04
mean	3.773	3.913	5.98

The result of paired t-test on cultures applied by rice extract can be with a degrees ( $\alpha$ ) 0.05% obtained p-value of cassava as 0.001. The test results showed significant changes in pH before and after (Table 3).

The result of paired t-test on cultures applied by cassava extract with a degree ( $\alpha$ ) 0,05% obtained p-value of cassava equal to 0.001. The test results showed significant changes in pH before and after (Table 4). The result of paired t-test on cultures applied by potato extract with degree ( $\alpha$ ) 0.05% obtained a p-value of cassava equal to 0.018. The test results showed significant changes in pH before and after (Table 5).

Analysis using an ANOVA test method to find out whether there is a difference of pH decrease between three rice water extract, cassava, and potato. The result of an ANOVA test with the degree ( $\alpha$ ) 0.05% shows p-value equal to 0.000.

The test results showed that the three groups have a different mean of each other. The three extracts showed a different pH decrease in each group (Table 6).

Table 3. Paired t-test result of the pH value of *Streptococcus mutans* culture treated with rice water extract

Repetition	pH before	pH after	p-value
1	6.58	3.72	0.001*
2	6.34	3.82	
3	6.54	3.78	
Mean	6.4867	3.7733	
$\alpha$	0.05		

Notes: \* = significant

Table 4. Paired t-test result of the pH value of *Streptococcus mutans* culture treated with cassava water extract

Repetition	pH before	pH after	p-value
1	6.58	3.92	0.001*
2	6.34	3.91	
3	6.54	3.91	
Mean	6.4867	3.9133	
$\alpha$	0.05		

Notes: \* = significant

Table 5. Paired t-test result of the pH value of *Streptococcus mutans* culture treated with potato water extract

Repetition	pH before	pH after	p-value
1	6.58	5.95	0.018*
2	6.34	5.95	
3	6.54	6.04	
Mean	6.4867	5.9800	
$\alpha$	0.05		

Notes: \* = significant

Table 6. ANOVA test result of the pH value of *Streptococcus mutans* culture treated with rice, cassava, and potato water extract

Repetition	Rice	Cassava	Potato	p-value
1	3.72	3.92	5.95	0.000*
2	3.82	3.91	5.95	
3	3.78	3.91	6.04	
Mean	3.773	3.913	5.98	
$\alpha$	0.05			

Notes: \* = significant

An advanced ANOVA test was performed to sort the pH decrease of the extract group respectively. The results of the test showed the extract of rice water has the greatest pH decrease, then cassava water extract had the second largest pH decrease and potato water extract is in third (Figure 1).

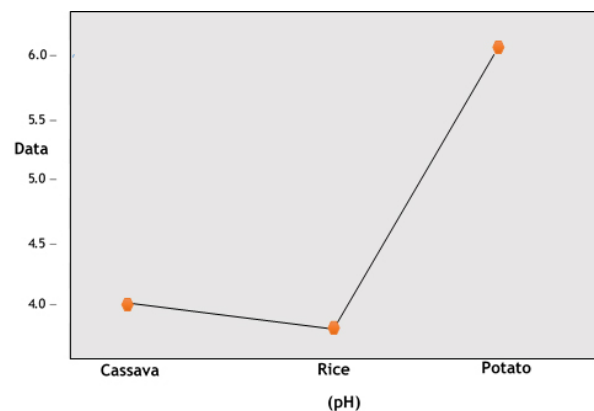


Figure 1. A. CD68<sup>+</sup> as brown cell membrane of macrophage on pulp polyp (40x10); B. CD68<sup>+</sup> as brown cell membrane of macrophage on gingiva polyp (40x10); C. CD68<sup>+</sup> as brown cell membrane of macrophage on fibroma (40x10)

## DISCUSSION

The results showed a decrease in culture pH which was treated with water extract of rice, cassava and potatoes. Decreased pH occurs because the process of carbohydrate metabolism contained in the three extracts by *Streptococcus mutans* to produce acid.

*Streptococcus mutans* is an acidogenic bacteria. It is called acidogenic because the bacteria can produce acids. The resulting acid is the final product of carbohydrate metabolism by *Streptococcus mutans*.<sup>8</sup> The process of carbohydrate metabolism of *Streptococcus mutans* occurs anaerobically known as fermentation. The product of the metabolism of *Streptococcus mutans* is lactic acid, the product other than lactic acid is acetic acid, formic acid, and ethanol. Carbohydrates prior to metabolism by cells should be transported into cells and broken down with specific enzymes against the type of carbohydrate to be metabolised. Carbohydrates transported into the cell will then be metabolized in the pathway of glycolysis.

Glycolysis pathway is a process of catabolism producing energy and end products. The final

product of metabolism in the form of lactic acid when high carbohydrate reserves, on the contrary, will produce ethanol, formic acid, and acetic acid when low carbohydrate reserves. Generally, the resulting acid will be released into the external environment causing a decrease in the external pH.<sup>11</sup> The theory has explained why pH decrease occurs in culture after the third treatment of extracts known to have carbohydrate content.

The results of the study also showed that there was a difference in the pH decrease in cultures that each treatment extracted. Study of Leme explaining combined sucrose and starch showed an increased pH reduction and cariogenic potential.<sup>12</sup> The differences in pH decrease in each extract are due to differences in the number of fermentable carbohydrates such as sucrose, glucose, and fructose in cassava, rice, and potatoes, in addition, complex carbohydrates (starch) are also known to be fermented with a greater degree of pH decrease.<sup>12</sup> These types of carbohydrates are present in rice, cassava, and potatoes with different amounts of content. The content of sucrose, fructose, and glucose in rice amounted to 0.3 - 0.5%, on cassava of 2 - 17% and in potatoes by 0.5%.<sup>9,13,14</sup>

Differences in pH decrease in each extract may also be due to differences in the number of complex carbohydrates that are starch contained in rice cassava and potatoes. The US Department of Agriculture stated that starch content in rice is 85%, 38% in cassava and 16% in potatoes. Rice has a higher starch content than cassava and potatoes. This explains why rice has the greatest decrease in pH. According to Bagge et al.<sup>15</sup>

Sucrose is the most cariogenic carbohydrate since it can be fermented to produce acid, sucrose can be synthesized by *Streptococcus mutans* into intracellular polysaccharides (IPS) as well as extracellular polysaccharides (EPS) in an example, glucan and fructan. EPS has an important role such as increasing the ability of bacterial attachment to form the accumulation of microorganisms, forming a thick and thick biofilm structure, and increasing the acidification of plaque biofilm.<sup>12</sup>

Glucans can be synthesized into IPS as energy reserves IPS will be stored in bacterial cells as energy reserves, when the reduced carbohydrate intake of IPS will be metabolised to produce energy and acid. IPS metabolism will

affect the external pH. This is because the IPS metabolism by *Streptococcus mutans* produces acids and then the acid is released into the external environment to maintain intracellular pH stability. Carbohydrates other than sucrose that can be fermented acidogenic bacteria are glucose and fructose with a lower degree of cariogenic than sucrose because the pH produced sucrose is lower than glucose.<sup>15</sup>

Consumption of fermentable carbohydrates leads to a rapid decline in pH from neutral values to 5 or less of 5. According to Heymann<sup>16</sup>, increasing the number of acidogenic bacteria will cause a constant pH in the acid state. High-frequency carbohydrate consumption can also increase the growth of acidogenic bacteria. Consumption of carbohydrates for long and continuous periods leads to an increase in the number of acidogenic bacteria such as *Streptococcus mutans*. The weakness of this research is not to test the carbohydrate content in each extract used.

The test was performed to determine the percentage of carbohydrate content such as sucrose, fructose, glucose, and starch contained in each extract. It is suggested in subsequent research to test carbohydrate content beforehand so it is known for certain comparison of the type of sucrose, fructose, glucose and starch contained in each extract of rice, cassava and potato.

## CONCLUSION

There was a decrease in pH of cultured *Streptococcus mutans* after administration of the water extract of cassava, rice, and potato, and there was a difference between the pH decrease of the *Streptococcus mutans* between administration of water extract of rice, cassava, and potato.

## REFERENCES

1. Bird DL, Robinson DS. Student Workbook for Modern Dental Assisting. 12<sup>th</sup> ed. Philadelphia: Saunders; 2017. p. 248.
2. Fejerskov O, Kidd E. Dental caries: The disease and its clinical management. 2<sup>nd</sup> ed. Hoboken: John Wiley & Sons, Inc; 2009. p. 4-27.
3. National Institute of Health Research and Development (NIHRD). Indonesia Basic Health Research (RISKESDAS) 2012-2013. Jakarta:

- Ministry of Health of the Republic of Indonesia; 2013. p. 118.
4. Miller CH, Infection Control and Management of Hazardous Materials for the Dental Team: Infection Control and Management of Hazardous Materials for the Dental Team. 5<sup>th</sup> ed. St. Louis: Mosby; 2013. p. 35-9.
  5. Fisher J, Glick M. A new model for caries classification and management. J Am Dent Assoc. 2012; 143(6): 546-51. DOI: 10.14219/jada.archive.2012.0216
  6. Sumardjo D. Pengantar Kimia: Buku Panduan Kuliah Mahasiswa Kedokteran dan Program Strata 1 Fakultas Bioeksakta. Jakarta: EGC; 2008. p. 205-25.
  7. Salvador EM, Steenkamp V, McCrindle C. Production, consumption, and Nutritional value of cassava (*Manihot esculenta* Crantz) in Mozambique: An overview. 2014. J Agricult Biotech Sustain Dev. 6(3): 29-38. DOI: 10.5897/JABSD2014.0224
  8. Afoakwa EO, Budu AS, Asiedu C, Chiwona-Karlton L, Nyirenda DB. Application of multivariate techniques for characterizing composition of starches and sugars in six high yielding CMD resistant cassava (*Manihot esculenta* crantz) varieties. J Nutr Food Sci. 2011; 1(4): 111. DOI: 10.4172/2155-9600.1000111
  9. Lister CE, Munro J. Nutrition and health qualities of potatoes - a future focus. New Zealand Institute for Crop & Food Research. Crop & Food Research Confidential Report number: 143, 2000. pp. 5-6.
  10. Houston DF, Houston DF, Kohler GO. Nutritional properties of rice. Washington D.C.: National Academies; 1970. p. 8.
  11. Marsh PD, Martin MV, Lewis MAO, Williams DW. Oral microbiology. 5<sup>th</sup> ed. London: Elsevier Health Science; 2009. p. 41-75.
  12. Leme, A.P.; Koo, H.; Bellato, C.M.; Bedi, G. and Cury, J.A. 2006. The role of sucrose in cariogenic dental biofilm formation—new insight. Journal of dental research, 85(10), 878-887pp.
  13. Lister, C.E. and Munro, J. 2000. Nutrition and health qualities of potatoes-a future focus. New Zealand: New Zealand Institute for Crop and Food Research Limited. 5-6pp.
  14. Salvador, E. M.; Steenkamp, V.; and McCrindle, C. M. E. 2014. Production, consumption and nutritional value of cassava (*Manihot esculenta* Crantz) in Mozambique: An overview 6(3):29-38.
  15. Bagg, J.; MacFarlane, T.W.; Poxton, I.R. and Smith, A.J. 2006. Essentials of microbiology for dental students 2<sup>nd</sup> ed. Oxford university press. 250-254pp.
  16. Heymann, H.O.; Swift Jr, E.J.; and Ritter; A.V. 2014. Sturdevant's art & science of operative dentistry. Elsevier Health Sciences. 41-88pp.