

RESEARCH ARTICLE

Chewing gum supplemented with *Brassica oleracea var. capitata f. rubra* extract for pH detecting of artificial saliva

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ABSTRACT

The prevalence of oral diseases in Indonesia is 25,9% in 2018, the highest percentage is recorded being caused to dental caries. Oral condition which may be acidic due to foods is potential to cause dental caries, as oral bacteria activities such as *S. mutans* may transform foods into becoming acids. In current condition, it is difficult for people to detect their own acid and base oral condition independently. This study aimed to create an innovation of a chewing gum supplemented with red cabbage (*Brassica oleracea var. capitata f. rubra*) extract which has functions as oral pH detector. The chewing gum was made by mixing the maceration extract of red cabbage and gumbase. This study was performed by using a qualitative test of pH 1-13 buffer solution colour change and pH 5-9 artificial saliva which was added by the extract, and then tested with manufactured chewing gum. Anthocyanin level was tested by using a 520 and 700 nm UV-Vis spectrophotometer. The study resulted in colour change in colour qualitative test. On pH 5-9 artificial saliva, colour change happened in order of orange, orange-brown, brown, brown-green, and green, after it was mixed with chewing gum. Colour compatibility test on chewing gum and artificial saliva showed a compatibility percentage of 80. Chewing gum supplemented with red cabbage can be used as pH detector of pH 5-9 artificial saliva.

Keywords: artificial saliva; chewing gum; pH indicator; red cabbage

INTRODUCTION

Dental caries is a pathologic process depending on several etiologic factors, which cause the destruction of the dental tissues.¹ Generally, caries is caused by four factors in mouth, there are teeth (host), bacteria (agent), time, and substrate or diet. The main bacteria that cause caries is *Streptococcus mutans* which works by convert polysaccharides into glucan using glucosyl transferase enzyme, a virulent factor in dental caries pathogen.² The glucosyl transferase enzyme can catalyze the formation of soluble and insoluble glucan.³ Glucan plays an important role in facilitating bacteria adherence on the tooth surface which is lead to the increasing accumulation of plaque and initiate caries. *Streptococcus mutans* could stand at acidic condition in oral cavity,⁴

that means this bacteria can't live and growth in basic environment. Metabolism of *Streptococcus mutans* causes a decrease in pH of plaque within 1-3 minutes. A decrease in pH below 5.5 (critical pH) initiate demineralization⁴ and the pH of human saliva is considered normal limits when values are between 6.0 and 7.5.⁵

In dental office, the present detection methods for caries are based on visual diagnosis, such as inspection, palpation, and X-ray imaging. There are also several methods have been proposed to realize more precise and sensitive detection, such as a pH measurement, radiography, and fluorescence. The pH detection is one of the simplest choices which related to microorganism activity and state of caries.⁶ People often didn't know the pH condition in their mouth whether acidic

or basic. So it can increase caries prevalence due to acidic condition and this would be great if people could detect their own pH condition in their mouth. So, that people can be aware and try to prevent the acidic condition of their oral cavity from being related to the process of caries.

Chewing gum is one of candy which people like. Chewing gum can increase the salivary flow rate and the pH.⁵ The salivary flow rate would have been increased the buffering action of saliva caused to neutralize the salivary pH and the pH would have been increased.⁵

The chewing gum will be made of gumbase, softener and natural colorant from red cabbage (*Brassica oleracea*) extract. Red cabbage is chosen because it has purple pigment that was known as anthocyanin.⁷ Based on research from Yusuf, Indriati, and Attahmid, the total anthocyanin concentration is 1354.01 mg/l from 3 days maceration.⁸ Based on research from Wiczowski, Nowak, and Topolska which anthocyanins in red cabbage products were analysed using HPLC-DAD-MS/MS method,⁹ among twenty anthocyanins identified and quantified seven of them predominated and the seven main compounds covered almost 68% of the total anthocyanin content in fresh red cabbage.⁹ Anthocyanin is pH sensitive and stable enough to be pH indicator.⁷ Its color will change to more reddish color in acidic environment and to greenish color in basic environment.¹⁰

Anthocyanin also has many biological activities such as antioxidant, anti-inflammation, analgesic, antibacterial and antidiabetic.⁷ Anthocyanin is a natural pigment of red cabbage which contains antioxidants with the strength of 150 flavonoids.¹¹ Flavonoid and anthocyanin are active substances that have antibacterial properties that can inhibit the growth of *Streptococcus mutans*.¹² The purpose of this research is to create chewing gum that can be used as pH indicator of artificial saliva.

MATERIALS AND METHODS

The research was conducted as experimental laboratories that has acknowledged by Dentistry

Faculty Research Ethical Committee of Universitas Gadjah Mada (001476/KKEP/FGK-UGM/EC/2018). Red cabbage was washed and cut into small pieces. About 1.5 kg of fresh red cabbage was extracted by 7.5 L of ethanol 96% and citric acid which ratio 98:2 for 3 days. Then, it was filtered and the solvent was evaporated. The thick extract was kept in light-isolated container at 4 °C.¹³

Anthocyanin content determined by using pH differential method referring to Giusti and Wrolstad. The extract was diluted using pH 1 (0.025 M KCl) and pH 4.5 (0.4 M CH₃COONa) buffer with dilution factor (DF) of 100. The solution was left alone in dark room for 15 minutes. Its absorbance was read at 520 nm and 700 nm wavelengths using UV-Vis spectrophotometer. 520 nm was the maximum wavelength of cyanidin 3-glucoside which is the most common anthocyanin pigment found in nature and 700nm was correction factor¹⁴. Total anthocyanin was calculated based on dry and fresh material with milligram cyanidin 3-glucoside (Cy3G) per 100 gram of sample using the following equation.

$$\text{Total Anthocyanin (mg/L)} = \left[(A_{520} - A_{700}) \text{ Ph1} \right] - \left[(A_{520} - A_{700}) \text{ ph 4,5} \right] \times \text{DF} \times 1000 \times \text{Mr} / (\epsilon \times \text{P})$$

Where DF is dilution factor, Mr is molecular weight (449.2 g/mol for Cy3G), ϵ is molar absorptivity coefficient (26900 cm⁻¹ mg⁻¹ for Cy3G) and P is cuvette length.¹⁴ Red cabbage extract with concentration of 40%, 60%, and 80% in aquadest and buffer solution for pH ranged 1-13 was prepared. The use of different concentration is to compare the color intensity of buffer solution and artificial saliva color change. The color intensity will increase along with increased concentration¹⁵. The color of red cabbage extract is purple-red and the buffer solution is transparent like water. Two drops pipette red cabbage extract was mixed with 2 ml buffer solution and shaken. Then, the color change was observed visually.

Chewing gum was made from chewing gum kit 6.5 oz product of Glee Gum. Ingredients of the

package are confectioner's sugar, corn syrup, chewing gum base, and natural flavors. One package of chewing gum kit can be produced in about 50 chewing gum.

In this study only use chewing gum base and corn syrup from chewing gum kit, red cabbage extract concentration of 100%, and xylitol. Red cabbage extract concentration of 100% were used as coloring agent because of its consistency. It was thick like a sweet chocolate jam, so it can be mixed with gumbase and softener which also have a thick consistency and a little stickiness. Red cabbage extract diluted with aquades can not be mixed with gumbase and softener because of its consistency were not same, so it can not color the batter.

Twenty grams of gumbase was heated in a bowl on boiling water until it melted. Then, 1 g of softener and 5 grams of red cabbage extract concentration of 100% were added. The batter was stirred evenly. Homogeneous batter was moved to container that filled with xylitol powder. After the batter was cooled down, it was molded into about 10 chewing gum¹⁶. Fifty chewing gum were made to be a sample.

Artificial saliva is made with 36 gram NaCl, 1,6 gram KCL, 0,96 gram CaCl₂, 0,8 gram NaHCO₃, and 400 cc water.¹⁷ Artificial saliva was placed in 5 ml containers. A drop using a dropper pipette of red cabbage extract was dropped to the container with certain pH and the color change of artificial saliva observed visually. This treatment was done with 5 variation of pH (5-9).

In vitro study of chewing gum also done with artificial saliva. Artificial saliva was placed in 5 ml containers and the chewing gum was immerse in the container and treated with mechanical intervention that is pressing chewing gum from outside the container with both thumbs for about 3 minutes. Every color change of artificial saliva in container in photos was observed visually. This treatment also was done with 5 variation of pH (5-9) with 10 samples for each variation of pH artificial saliva. Artificial saliva's color change analysis is qualitative analysis was done by counting match

percentage of artificial saliva and chewing gum color change with gold standars.

RESULTS

Data of absorbance which is used to calculate the total value of anthocyanins using the total anthocyanin formula can be shown in Table 1. The result showed that anthocyanin concentration of red cabbage is 2252.267 mg/100 g or 2252.267 mg/L.

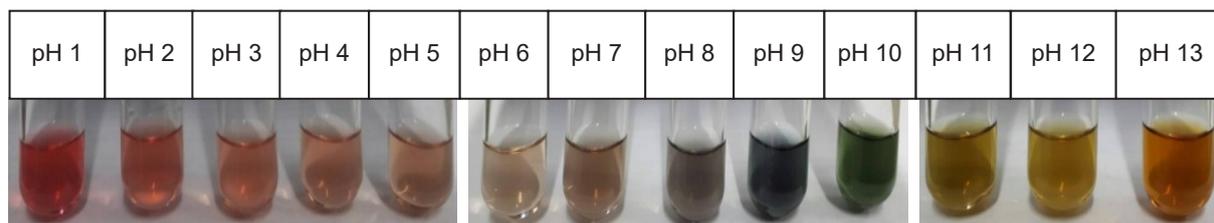
Table 2 showed the color change of buffer solution on red cabbage extract. In pH 1, 2, 3 buffer solution changed its color from trasparant color to red. In pH 4,5,6 pH buffer solution changed to pale red – orange, pH 7 changed to blue-purplish, pH 8,9 changed to bluish-green, pH 10-11 changed to green, pH 12 changed to green-yellow, and pH 13 buffer solution changed to yellow. Red cabbage extract which contain anthocyanin was tested the change of the color in buffer solution pH 5 until 9 (Figure 2 A) and artificial saliva which has range pH from 5 until 9 (Figure 2 B).

Chewing gum was tested in artificial saliva at pH 5 until 9. Then color change of each pH was matching between buffer-extract mixture, artificial saliva-extract mixture, and artificial saliva-chewing gum mixture. The result showed that color change of artificial saliva on chewing gum is appropriate at each pH value and the color change result data is in the Table 3 below.

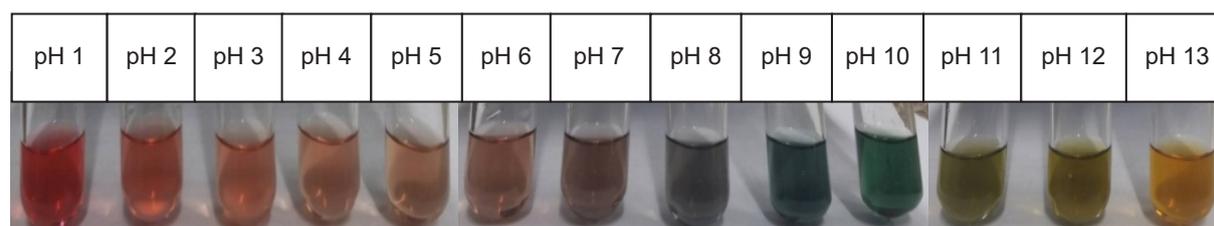
Figure 1 shows the result of color qualitative test of red cabbage extract at various pH buffer solution. The buffer solution changed color from red at acidic to yellow at basic. At the neutral pH color changes to purple then changes to blue in increasing pH. The color of red cabbage extract in buffer solution at pH 1 until 13 can be shown in Table 2 below.

Table 1. Absorbance data of red cabbage extract at 520 dan 700 nm using UV-Vis spectrophotometer.

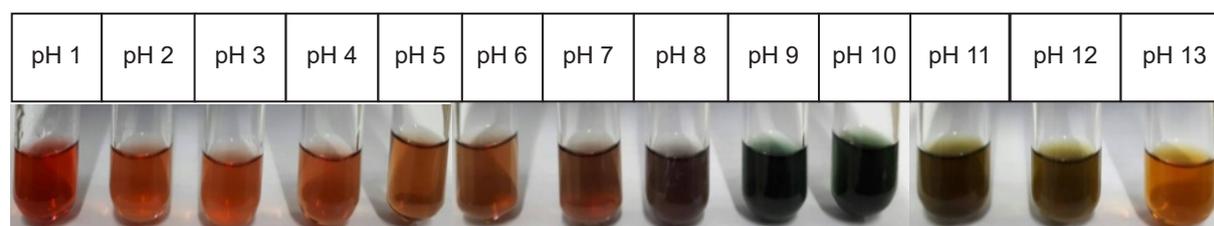
Wave Length (λ)	Absorbance	
	pH 1	pH 4.5
λ 520 nm	1.770	0.424
λ 700 nm	0.019	0.022



(A)



(B)



(C)

Figure 1. Color quality test of red cabbage extract with 40% (A) , 60% (B) , 80% (C) concentration.

Table 2. Data of buffer solution color change on red cabbage extract

pH	Color
1,2,3	Red
4,5,6	Pale Red – orange
7	Blue-Purplish
8-9	Bluish – Green
10-11	Green
12	Green-Yellow
13	Yellow

Table 3. Artificial saliva color change on chewing gum

pH value	Color
5	Orange
6	Brownish Orange
7	Brown
8	Greenish Brown
9	Green

Table 4. Data of in vitro study of artificial saliva color change on chewing gum

pH value	Color	Compatible	Not Compatible
5	Orange	9	1
6	Brownish Orange	8	2
7	Brown	8	2
8	Greenish Brown	6	4
9	Green	9	1

Table 3 shows the color change of artificial saliva on chewing gum. In pH 5 artificial saliva changed to orange, pH 6 changed to brownish-orange, pH 7 changed to brown, pH 8 changed to greenish brown, and pH 9 of artificial saliva changed to green.



Figure 2. Buffer solution (pH=5-9) color change after extract addition (A) and Artificial saliva (pH=5-9) color change after extract addition (B)

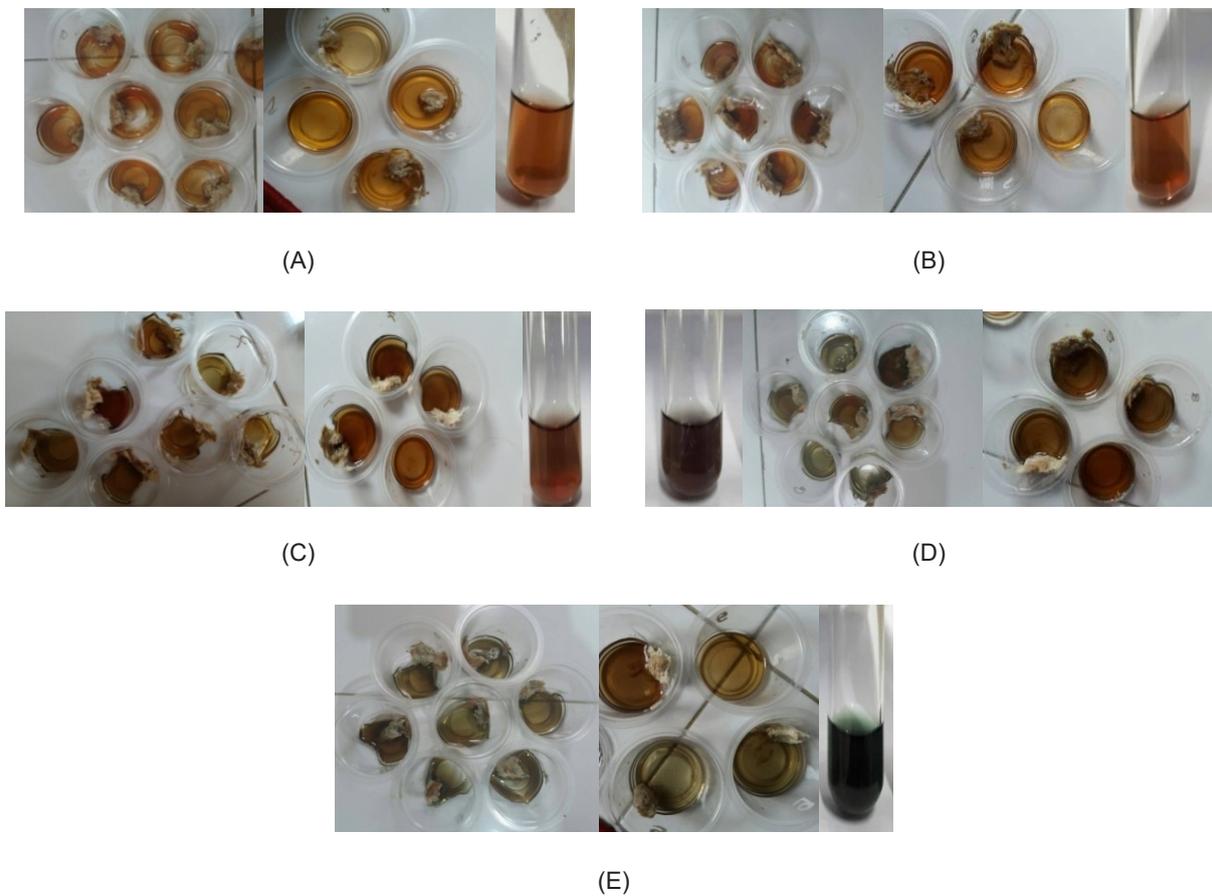


Figure 3. The color change of artificial saliva with pH 5 (A), pH 6 (B), pH 7 (C), pH 8 (D), pH 9 (E) after chewing gum addition

Buffer-extract mixture and artificial saliva-extract mixture color change used as gold standard to compare color change of artificial saliva-chewing gum mixture. The both color change was matching, and the result can be seen in Table 4.

In Table 4 also show the compatibility of ten samples of artificial saliva-chewing gum mixture

that was matched with the gold standard. If the color change same to the gold standar, it means compatible.

The compatibility data of in vitro study of chewing gum. Indicated that color change of artificial saliva at various pH mostly compatible with color change of buffer solutions. Among 50 chewing gum samples, only 10 samples that show

incompatibility. So that retrieved 80% compatible and 20% incompatible chewing gum.

DISCUSSION

Addition of red cabbage extract to pH buffer solution yield fresh red color, this color change is caused by anthocyanin structure change due to the effect of H⁺ and OH⁻ ions. In addition of H⁺ ion make pH lowered down so that the environment becomes acidic.¹⁸ At the acidic condition, the anthocyanin will established as flaviumcation where the solution has red color.¹⁹ When the extract was added to 4, 5, and 6 buffer solution, the color of solutions change to pale red. Anthocyanin color change at pH level 5 tend to make colorless solution, this was caused by anthocyanin at pseudo basic condition started to lost its color at pH scale 4-6.¹³ At pH 7 buffer solution, yield blue-purplish color.²⁰ The solution has bluish color at pH 8-9. Blue color was yielded because anthocyanin molecule formed quinoid.²⁰ While addition of extract at 10-13 (basic) buffer solution, color change happened from green till yellow. Anthocyanin was depended on pH, If the extract is added to basic solution, it's color will changes into green and often ends with yellow color.¹⁸ Color of anthocyanin was depended on pH, it will change because of acid and basic solution factor.

The chewing gum supplemented with red cabbage extract in 100% concentration was mixed with five milliliters of artificial saliva and then treated with mechanical intervention that is pressing chewing gum from outside the container for about 3 minutes. Mechanical intervention makes red cabbage extract release from chewing gum to artificial saliva. Anthocyanin which contained in red cabbage extract react with acid and basic solution in artificial saliva that makes color change of artificial saliva. Test was done on pH 5-9 of artificial saliva with chewing gum matched with artificial saliva color on saliva-extract mixture and buffer solution color on buffer-extract mixture for each pH variation. The result showed that artificial saliva color at pH 5, 6, 7, 8, 9 after gum addition are orange, brownish orange, brown, greenish brown,

and green. There are some studies of pH solution color change with anthocyanin from red cabbage extract but no studies that have seen color change of artificial saliva combined with anthocyanin from red cabbage extract. The color change of acid and base artificial saliva which mixed with chewing gum supplemented with red cabbage extract shows that chewing gum can be used as a pH indicator in artificial saliva. In acid artificial saliva (pH 5 & 6), artificial saliva will change color to orange and brownish orange. While in artificial saliva with neutral pH (pH 7), the color change of artificial saliva is brown, and in basic artificial saliva (pH 8 & 9) will greenish brown and green.

This study only used artificial saliva which is made from various chemicals not original human saliva. Red cabbage extract also have an unpleasant odor which create human discomfort if that chewing gum applied in human. So, it would be better if the odor can be removed. This research also would be better if the research use original human saliva, so it will show the original result on human saliva. The color change also better to be observed with machine like spectrophotometer or chromameter.

CONCLUSION

Chewing gum supplemented with red cabbage extract can be used as pH indicator for artificial saliva.

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