# Characterization of Toothpaste Made With Unfermented Cocoa Powder (Theobroma cacao L) Againts Bacteria Streptococcus mutans

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**Abstract.** Cocoa beans contain polyphenolic compounds/phenolic include catechin, epicatechin, anthocyanin, proanthocyanidin, phenolic acids, condensed tannins, alkaloids, teroid/terpenoids, flavonoids-other flavonoids, and some minor components. Polyphenol group compounds have antibacterial and anti-inflammatory effects. This research aims to determine the physico-chemical properties and effects of toothpaste containing active component of unfermented cocoa powder to the growth of *Streptococcus mutans* bacteria. This study uses a completely randomized design by varying concentration of unfermented cocoa powder used as active ingredient in toothpaste. Toothpaste was prepared with active ingredients of unfermented cocoa powder with concentration of 1.0; 2.5; 4, 0; 5.5% (w/w) and each repeated three times. The results indicated that the four concentrations of unfermented cocoa powder contained in toothpaste show an inhibitory effect against 20.936; and 21.039 mm. The fourth toothpastes have a viscosity above 400 dps, pH value above 7, therefore meet SNI 01-3524-1994 standard. Panelist scored this toothpaste with 3. **Keywords**: unfermented, cocoa powder, antibacteria, active compound, *Streptococcus mutans*, toothpaste

## 1 Introduction

The most common tooth and mouth disease found in societies is dental caries. Dental caries is a disease attacks hard dental tissue caused by the activity of microorganisms in the leaven of carbohydrates. Bacteria normally found in oral cavity are *S. mutans*, *S. viridians, Staphylococcus epidermidis, S. pneumoniae*, *and S. aureus* [1]. Among the bacteria, a type of bacteria *S. mutans* is often used in testing as an indicator of plaque on the teeth layer [2].

Advances in science and technology lead to a variety of toothpaste manufacturers to innovate by adding other substances, which have benefit to dental health. Addition of certain ingredients in toothpaste can reduce the amount of bacteria cause caries [3]. Certain ingredient commonly added to toothpaste is antibacterial agent, usually from herbal [4].

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Research on toothpaste made from natural or herbal active ingredient has been widely reported, such as tea leaves toothpaste, betel leaves toothpaste, gambier toothpaste, bay leaves toothpaste, ginger toothpaste, cocoa pods toothpaste and others. However, research toothpaste that uses active ingredients from unfermented cocoa powder as inhibiting the growth of bacteria S. mutans still not been done. Toothpaste in the market generally contains fluorine in the form of sodium fluoride, stannous fluoride, and sodium monofluorophosphate as antibacterial active component that works to inactivate enzymes in bacterial cells. Anti-bacterial ingredients that are commonly used to control plaque include phenol, hexetidine, fluoride and chlorhexidine [5]. However, the use of toothpaste with high fluoride concentrations is not recommended because it can cause side effects such as tooth enamel fluorosis [6].

One effort to reduce the negative impact of the use of fluoride in toothpaste is to use natural or herbal active ingredients. The return of attention to natural active ingredients known as back to nature regarded as beneficial. [7]. showed that the use of natural materials can reduce the side effects of chemicals on the body; therefore, the addition of natural ingredients in toothpaste can support the dental and oral health services

Cocoa beans contain polyphenolic compounds / phenolic include catechin, epicatechin, anthocyanin, proanthocyanidin, phenolic acids, condensed tannins, alkaloids, teroid / terpenoids, flavonoids-other flavonoids, and some minor components [8], [9]. Cocoa beans contain polyphenolic compounds 12-18% of the dry weight. Approximately 60% polyphenols is flavonoids prosianidin compounds, catechins 33-34%, 23-25% leukosianidin and has antibacterial and antioxidant activity [10]. In the fresh cocoa beans are found prosianidin containing 2 to 10, also called prosianidin oligomer, but it contains polyphenols such as quercetin and glycosides form [11]. [12], reports a compound from the class of polyphenols found in all plants have antibacterial and anti-inflammatory effects

Research in Japan found that the extract of cocoa beans have an antibacterial effect in the mouth and effectively prevent caries. Alleged active ingredient extracts of cocoa beans is possible to put into toothpaste as a supplement [13]. Based on the research conducted by [14], states that the test based on the qualitative component of the extract of cocoa bean turns cocoa beans contain tannins, polyphenol, flavonoids, alkaloids, and steroids which is one of the active components that can be utilized in oral health.

This study aims to determine the physicochemical properties and effects of toothpaste containing the active component of unfermented cocoa powder on the growth of Streptococcus mutans bacteria

## 2 Methodology

### 2.1 Material

Raw materials used in this study are unfermented dried cocoa beans obtained from Luwu, South Sulawesi, n-hexane (technical), Whatman filter paper No. 41, calcium carbonate ($CaCO_3$), gum arabic, texapon, glyserin, methyl paraben, sorbitol, NaCl, aquabidestilat, sodium benzoate, and saccharin.

### 2.2 Equipment

Equiment used are separating funnel, digital scales, petri dish, measuring cup 1500 ml, glass rod, universal conching, hydraulic press, sieve mesh 200, winnowing, blender, stainless steel pot, mixer, gas stove, capillary tube, pencil, cutter, ruler, FT-IR (Perkin

Elmer), GC (Agilent 6890)- MS (Agilent 5973 Inert MSD system), oven (Venticell 404 and 222 MMM medcenter GmbH D 82152 Germany), centrifuge (Hettich), vortex mixer (Barnstead International), ultrasonic (Elma), AAS Contra AA 700), hot plate stirer (Cimarec II), incubator (INB 500), vortex (Maximix II Thermo), pH meter (Brench top pH meter GLP 21 CRISON), waterbath (HH6 PRC), plastic, test tube (Pyrex), petridish (Pyrex), digital pH meter, scales capacity of 5 kg, analytical balance (Sartorius) and ose needle.

## 2.3 Research Methods

This study consists of four stages, they are: preparation of unfermented cocoa powder, preparation of toothpaste, methods of analysis and organoleptic test for resulted toothpaste

### 2.3.1 *Cocoa Powder*

Unfermented cocoa beans are separated from the podand pulp, and then sun-dried for 5 days with a temperature of ± 45ºC until it reaches the water content of 7%. Cocoa beans shells is being expelled using winnowing machine to obtain cocoa nib. Dried cocoa nibs are ground into cocoa liquor using universal counching tool. Cocoa liquor is separated from its fat using hydraulic pressing. Cocoa cake obtained is being crushed using blender, then immersed with technical n-hexane (1: 1.5) for 5 days. Filtration using Whatman paper No. 41 obtained filtrate and residue. Residues are the unfermented cocoa powder. Cocoa powder is aerated for three days, then sieved at 200 mesh [15]. Scheme of preparation of unfermented cocoa powder can be seen in Figure 1.

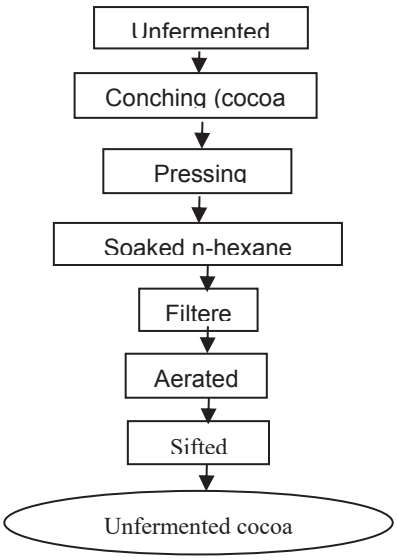


**Fig. 1**. Scheme of the process of making unfermented cocoa powder from unfermented cocoa nib

### 2.3.2 *Preparation of Toothpaste*

The basic formula used in the manufacture of toothpaste from unfermented cocoa powder is a modification of the formula toothpaste according to [12] ,[16]. Methyl paraben is

dissolved in hot water and added sorbitol, after all soluble salts are added (a). Texapon dampened little by little with glycerin is then stirred until homogeneous (b). In the container of texapon and glycerin (bb) solution of a mixture of methyl paraben (a) is added, stirred until it forms a gel mass (c). Into a gel mass (c) was added successively ingredient calcium carbonate, gum arabic, Na-saccharin, sodium benzoate, aquabidestilat and non-fermented cocoa powder dimixer until homogeneous to form a smooth paste base.

### 2.4 Analytical Methods

This research uses experimental methods. In this study there were four treatments, namely the addition of unfermented cocoa powder with a concentration of 1%; 2.5%; 4.0%; and 5% and repeated three times. Combination treatment is 4x3 repetition so that acquired 12 units of the experiment. Data analysis using analysis of variance (ANOVA) and carried out further tests when $F_{counted} > F_{table}$ at the level of 95% and 99% use Tukey's Honest Significant Difference (HSD) Test. Non-fermented cocoa poder toothpaste is then analyzed its acidity using pH meter; viscosity using a viscometer, water content using Indonesian Pharmacopoeia IV method, and heavy metals content (Cu, Pb, Hg, and As) using the AAS method. Antibacterial activity of unfermented cocoa poder toothpaste is being tested t using Indonesian Pharmacopoeia IV.

#### 2.4.1. Experimental Toothpaste Organolaptic Test

Assessment of toothpaste containing active ingredient non-fat non-fermented cocoa powder conducted by 15 panelists. Assessment is written in the form of hedonic scale of 1-5 with increasing preference level, as increasing numbers of the scale (1 = strongly dislike; 2 = dislike; 3 = neutral; 4 = like; and 5 = very like). The data were analyzed statistically if there is a noticeable difference continued with HSD test [17].

## 3 Result and Discussion

### 3.1 Effect of Unfermented Cocoa Powder Toothpaste.

Results of unfermented cocoa powder's effect on *Streptococcus mutans* bacter are shown on Table 1.

Table 1. Average inhibition diameter of toothpaste made with unfermented cocoa powder

| No. | Concentration of Unfermented cocoa powder | Inhibition Diameter (mm) |
|---|---|---|
| 1. | 1,0 % | 18,326 |
| 2. | 2,5 % | 19,415 |
| 3. | 4,0 % | 20,936 |
| 4. | 5,5 % | 21,039 |

As shown on Table 1, the fourth toothpaste containing unfermented cocoa powder has antibacterial power which gives an inhibitory effect on the growth of bacteria *S. mutans*. The effects of unfermented cocoa powder as the active ingredient is due to phenol and derivatives that can change the nature of the bacterial cell protein. Toksit phenols are compounds that contain secondary metabolites that some tannins, catechins, flavonoids, steroids/ terpenoids, and alkoloid. The compound can lead to disrupted protein structure

without any damage to the structure of the resulting protein covalently framework changed properties.

According [5], the amino acid sequence of proteins remained intact after changing nature, but the biological activity to be damaged so that the protein can not perform its function. The results are consistent with research conducted by[14], [18], [19]. that the addition of the active ingredient in toothpaste to give an inhibitory effect against *S*. *mutans* and *S.viridans*. According to [19], that a new material can be said to have antimicrobial activity when the diameter of the barrier formed is 6 mm or 0.6 cm. The antibacterial properties of polyphenols derived from catechin and proanthocyanidin compounds contained in polyphenols. Catechins are naturally occurring polyphenol compounds are secondary metabolites and included in class compilers tannins, while proanthocyanidin is another name for condensed tannins [3]. In general, the antibacterial effect of tannins is reacted with the cell membrane, enzyme inactivation and destruction or inactivation of the function of the genetic material of bacteria [20].

Catechin and anto prosianidin addition, the antibacterial properties of polyphenols are also derived from the flavonoid compound. Flavonoids are the largest group of phenolic compounds and have the mechanism of action in inhibiting the growth of bacteria by inactivating the enzyme protein in the cell membrane, resulting in protein structure becomes damaged. Instability in the cell wall and the cytoplasmic membrane of bacteria will result in the function selective permeability, function active transport, controlling the composition of proteins from bacterial cells to be disrupted, so will cause the loss of macromolecules and ions out of the cell, so that the bacterial cells into a loss of form and will lyse [21].

Damage of cell membrane will affect microbial growth process because cell membrane is the site of some of the enzymatic reaction cell. While the mechanism of antimicrobial activity of alkaloids is to insert between the cell wall or DNA and thus prevent microbial DNA replication so that microbial growth will be interrupted [22]. The permeability of the disruption caused by the terpenoids can act as a solvent that is able to incorporate other secondary metabolites into the membrane [23].

Based on the analysis of variance showed that the diameter of the barriers to the growth of *S*. *mutans* was not significantly different between the treatment at the level of 95% (Fcounted <Ftable) thus differences in the concentration of unfermented cocoa powder is added to each experimental toothpaste does not exert significant influence. According [24]. The effect of antimicrobial agents depends on the concentration, generally a concentration greater than the minimum inhibitory concentration [24], the addition of organic compounds in antibacterial material can reduce the antibacterial activity.

### 3.2 Viscosity of Toothpaste Made With Unfermented Cocoa Powder

Viscosity test results of toothpaste unfermented cocoa powder are shown in Table 2.

**Table 2**. Viscosities of unfermented cocoa powder toothpaste

| Sample | Parameter | Result | Tools |
|---|---|---|---|
| Toothpaste (1,0%) | Viscosity | 455 dps | Visco-meter |
| Toothpaste (2,5%) | | 449 dps | |
| Toothpaste (4,0%) | | 445 dps | |
| Toothpaste (5,5%) | | 442 dps | |

The analysis showed that the viscosity decreases with increasing concentrations of unfermented cocoa powder. Viscosity grades to four toothpaste experiments ranged between 442 to 455 dps dps (Table 2). This indicates that the four toothpastes unfermented cocoa powder is more dilute. The low value of viscosity possessed toothpaste containing unfermented cocoa powder because one of the properties of unfermented cocoa powder is easy to absorb moisture from surrounding environment (higroscopis). Based on the analysis of variance showed that the toothpaste viscosity grades four experiments was not significantly different at the 95% confidence level (Fcounted <Ftable) therefore the concentration of unfermented cocoa powder does not exert significant influence on the difference in viscosity.

Viscosity is an important parameter in emulsion products, especially toothpaste because it related to the viscosity stability of an emulsion. The higher the value of the viscosity of a material, then the material will be more stable due to the movement of the particles tend to be difficult [25].

### 3.3 pH of Unfermented Cocoa Powder Toothpaste

Results of the degree of acidity (pH) toothpaste experiment with several concentrations of unfermented cocoa powder are shown in Table 3.

**Table 3**. Degree of acidity (pH) of experimental toothpaste and commercially available toothpaste

| Sample | Parameter | Result | Tools |
|---|---|---|---|
| Toothpaste (1,0%) | pH | 7,38 | pH meter |
| Toothpaste (2,5%) | | 7,45 | |
| Toothpaste (4,0%) | | 7,68 | |
| Toothpaste (5,5%) | | 7,50 | |

The values of pH for the fourth experimental toothpastes (Table 3) are on the range 7.38-7.68. The value meets SNI 01-3524-1994 requirements for toothpaste, therefore resulted toothpastes are safe to be used [26].

Analysis of varians shows that the impact of addition unfermented cocoa powder to toothpaste did not significantly change pH value of the entire toothpastes with 95% and 99% confidence level (Fcounted < Ftable). Therefore, toothpaste made with unfermented cocoa powder does not harmful human health.

According to microbiology test, toothpaste made with unfermented cocoa powder shows no microbial activity (Table 4) at pH range 7.38–7.68. Microbial activity does not occur at pH range of 7–7.7 or neutral condition. Microbial activity is expected to occur below pH 7. This assumption is in line with [27] study, which stated that microbe can grow under degree of acidity (pH) of 2.73 with optimum pH value of 6. [28], (2009) stated that *S. mutans* is aciduric because it can resist and breed in acidic nature until pH 4,5.

### 3.4 Microbiology Test of Unfermented Cocoa Powder Toothpaste

Result of microbiology test of unfermented cocoa powder toothpaste with concentration of 1,0; 2,5; 4,0; and 5,5% are shown in Table 4.

In Table 4, it shows that *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Candida albicans* were not detected for the four experimental toothpaste and control toothpaste. Absence of those type of bacteria were caused by unfermented cocoa powder containing

polyphenols and flavonoid class of compounds, which are able to inhibite the growth or development of three types of bacteria through destruction of the bacterial cell wall. Mechanism of action of polyphenols in the inhibition of bacteria is through protein denaturation and coagulation of bacterial cells or through the destruction of the bacterial cell wall. According to [19], antimicrobial mechanism of action of phenols is by means of protein denaturation and coagulation of bacterial cells, which interact with the phenol derivative bacterial cells through adsorption processes involving hydrogen bonds. At low levels, it can lead to lysis of cytoplasmic wall, formation of complex protein-phenol with weak bonds and immediate decomposition, followed by phenol penetration into cells and cause precipitation and denaturation of proteins.

**Table 4.** Microbiology test result of toothpaste made with unfermented cocoa powder

| No. | Sample | Parameter | Result |
|---|---|---|---|
| 1. | Toothpaste (1%) | *Streptococcus mutans* | Negative |
| | | *Pseudomonas aeruginosa* | Negative |
| | | Candida albicans | Negative |
| 2. | Toothpaste (2,5%) | *Streptococcus mutans* | Negative |
| | | *Pseudomonas aeruginosa* | Negative |
| | | *Candida albicans* | Negative |
| 3. | Toothpaste (4%) | *Streptococcus mutans* | Negative |
| | | *Pseudomonas aeruginosa* | Negative |
| | | *Candida albicans* | Negative |
| 4.. | Toothpaste (5,5%) | *Streptococcus mutans* | Negative |
| | | *Pseudomonas aeruginosa* | Negative |
| | | *Candida albicans* | Negative |

## 3.5 Flavor, Aroma and Color Test of Toothpaste Made With Unfermented Cocoa Powder

Evaluation results of flavor, aroma and color test of toothpaste made with unfermented cocoa powder with a fat-free concentration of 1%; 2.5%; 4%; and 5.5% conducted by 15 semi-trained panelists. The test results for flavor and color of toothpaste made with unfermented cocoa powder can be seen in Table 5.

Table 5. Result of average hedonic test of flavor, aroma and color of toothpaste made with unfermented cocoa powder

| Test Criteria | 1% | 2,5 % | 4% | 5% |
|---|---|---|---|---|
| Flavor | 3,2 | 3,4 | 3,7 | 3,5 |
| Aroma | 3,5 | 3,2 | 3,4 | 3,5 |
| Color | 3,6 | 3,5 | 3,6 | 3,7 |

Table 5. shows that the value of the hedonic test average of 15 panelists attribute to the highest sense given in toothpaste experiment with the active component concentration of 0.5% with a value of 4.2.

The higher the concentration of the active components given in toothpaste made with unfermented cocoa powder, the more declining ratings given by panelists attributed to the flavor of toothpaste. Panelist's assessment attributes to flavor is decreasing with the increasing of concentrations active components concentration. It is suspected that the use of unfermented cocoa powder resulted in unpleasant bitter taste. Results of analysis of variance analyst shows that preference level of panelists attribute to flavor is 95% of confidence level, which indicates that the difference in the concentration of defatted unfermented cocoa powder gives a significant influence (Fcounted> Ftable) against the attributes of flavor to toothpaste experiment. Results of further Honestly Significant Difference (HSD) tests is at error rate of 5%, indicates that the treatment which gives best impact to flavor in experiment toothpaste is 0.5% methanol extract concentration of defatted unfermented cocoa powder. Referring to Pasiga (2004), the active compound concentration commonly added to toothpaste is at range of 0.1-0.5%. Meanwhile, [29] stated that addition of active compound can be at range of 0.2-0.3%. It is assumed that the addition active compound to toothpaste above those range will result in bitter taste.

Analysis of variance attributed to aroma and color for experiment toothpaste (Table 6) shows that there is no significance difference at 95% of confidence level (FCounted < FTable). It means variation of concentrations added to experiment toothpaste do not give significant difference to aroma and color at the confidence level of 95%.

Overall, the results of the analysis of toothpaste products made from non-fermented cocoa powder in terms of the results of inhibition, viscosity, pH, flavor aroma and microbiology showed that the results met the SNI quality standards for toothpaste and the product could be recommended and utilized for small and medium industries.

## 4 Conclusion

Results of concentration variations treatment of unfermented cocoa powder in toothpaste do not have a significant influence on the diameter of inhibition of bacteria *S. mutans*. The diameter inhibition of fourth toothpastes made with unfermented cocoa powder as inhibiting growth of bacteria *S. mutans* are 18.326; 19.415; 20.936; and 21.039 mm respectively. The fourth toothpastes have pH above 7, which is in accordance to SNI 01-3524-1994, and panelists mark "like" with a score above 3.

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