



Synthesis and Characterization of Hydroxyapatite Gel - Nanosilver - Clove Flower Extract (*Syzygium Aromaticum* L.) as a Toothpaste Forming Gel

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ABSTRACT: Dental caries is a disease caused by the bacteria *Streptococcus mutans* and *Lactobacillus*, and affects everyone regardless of age. One way to overcome dental caries is to clean the teeth with a toothpaste made from hydroxyapatite-nano silver-clove extract. Hydroxyapatite is a biomaterial that has biocompatibility, bioactivity, and osteoconductive so that it can remineralize teeth. Nano silver is an antibacterial component that is able to kill microorganisms that cause dental caries. The addition of clove extract as an antimicrobial and aromatic compound can increase the attractiveness of the product. This study aims to determine the physical and chemical characteristics of the hydroxyapatite-nano silver-clove extract gel formulation with varying concentrations of hydroxyapatite 1%, 2%, and 3%, and nano silver concentrations of 1 ppm, 5 ppm, and 10 ppm. The results of the homogeneity test, pH level, dispersion, and adhesion showed that the gel was in accordance with the test standard. The results of organoleptic tests and statistical analysis showed that variations in the concentration of hydroxyapatite and nano silver affected the color and aroma ($P < 0.05$), but did not affect the texture ($P > 0.05$) of the gel. FTIR spectrophotometric analysis showed that the formulation already contained OH, CO_3^{2-} , PO_4^{3-} groups derived from hydroxyapatite, C=O derived from nano silver, and OCH, CH_3 , C-C, =C-H derived from clove extract.

KEYWORDS: Clove, Gel, Hydroxyapatite, Nano silver, Toothpaste

INTRODUCTION

Dental disease has become the 6th highest patient rate in Indonesia due to the lack of awareness in cleaning their oral and dental so that caries or cavities become a menace to its health. Dental health, eventually, affects the quality of human life. Dental abnormalities and disease could interfere with mastication function and cause infection. Moreover, the lack of awareness to do dental checkups has worsened the patient rate in Indonesia [1]. Another factor that causes tooth decay is the constant eating of sweet and sticky foods. This kind of food is the main source of life for bacteria that have the potential to damage our teeth. The higher the caries, the greater possibility of having a toothache. Everyone could experience toothache with pain symptoms in their oral area, therefore, they found it difficult to identify the cause of toothache [2]. The high number of tooth decay cases has increased the need of using biomaterial to form hard tissues on teeth. Biomaterial is a synthetic material that interacts with the biological system of living things. Biomaterial could be used to make an implant to substitute organs with certain processes and mechanisms. Biomaterial consists of several types, for instance, composites, polymer, ceramics, and metals [3].

Hydroxyapatite is a type of biomaterial that is widely applied in the medical field regarding both bones and teeth regeneration and implants. This, because hydroxyapatite has several properties that are in accordance to the nature of tissue: namely biocompatibility (the ability to adapt to the body), bioactivity (the interaction with the body), and good osteoconductive (able to increase hard tissue cells) [4]. In addition, biomaterials can be applied as autograft (replacement of body parts with other body parts in one individual), allograft (replacement of human bones with bones from other humans), and xenograft (replacement of human bones with bones from animals) where all three have the ability to chemically bonded to living human tissue [5]. Hydroxyapatite is a biomaterial that is included in the apatite mineral complex compound $[\text{M}_{10}(\text{ZO}_4)_6\text{X}_2]$ with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. This compound is a combination of tricalcium phosphate (Ca_3PO_4) and calcium hydroxide ($\text{Ca}(\text{OH})_2$). Hydroxyapatite synthesis can be carried out by mixing calcium precursors with phosphate precursors [6]. Hydroxyapatite can be synthesized by various methods, one of which is using the calcination method. This method uses the principle of removing the content of organic compounds and water in the material to produce a hydroxyapatite composite. The advantage of this method compared to other methods is that the material can decompose thermally and eliminate organic components, thereby increasing the level of safety of biological factors significantly in its use [7].



Tooth decay is not limited to solutions to replace damaged or lost tooth enamel. However, it is necessary to add other compounds as antibacterial to support hydroxyapatite to be of maximum benefit, such as silver nanoparticles (nano silver). Nano silver has antimicrobial properties so it is applied in the medical field as wound dressings, cotton fibers, and antiseptic mixtures to inhibit bacterial growth as well as an air sterilizer [8]. Synthesis of nano silver was carried out by various methods, namely physical, chemical, and biological methods. One of the common methods carried out by various studies is the chemical reduction method by carrying out a reduction reaction on silver salts such as silver nitrate by reducing agents to accelerate the formation of nanoparticles. This method uses sodium citrate as a reducing agent and produces nano silver compounds with a size of 30-40 nm [9].

Besides, the gel product for toothpaste preparation requires additional aromatic compounds as an allure in its use. There are many aromatic compounds available in nature, e.g., eugenol. Clove (*Syzygium aromaticum* L.) is an example of a natural ingredient with eugenol content >90% and the rest is the total content of other components, such as caryophyllene and benzoic acid [10]. According to Sofihidayanti and Wardatun (2021), the clove plant has 3 main parts that can be used for its essential oil: leaves (1-4%), stalks (5-10%), and flowers (10-20%) with the main content of which is eugenol and other ingredients in the form of caryophyllene, ethyl nitrobenzene, and methyl ethyl benzoic acid. The essential oil of the clove plant has the characteristics of a yellowish color, thick, has a spicy taste, and has a distinctive aroma. Several methods to obtain essential oil from clove plants include extraction, water distillation, steam distillation, and soxhlation methods [11]. In Prasetyo (2018), the clove extraction method produces yields ranging from 61-87% with a variety of solvents. Essential oils are needed in various industries, such as raw materials for food flavors and fragrances, cosmetics, pharmaceuticals, and insecticides. This is because the eugenol compound in clove extract has biological activity as an antioxidant, antifungal, antibacterial, and antiseptic, and is even used as an anesthetic for fish. Cloves also have advantages where the price is cheaper and the extraction process is relatively easy. The properties and characteristics of eugenol that have been mentioned are in accordance with the needs of current research.

Based on the aforementioned basis, researchers will develop a gel as a toothpaste preparation with basic ingredients of hydroxyapatite derived from bovine bone (*Bos taurus*), nano silver, and clove extract (*Syzygium aromaticum* L.) with concentrate variation of hydroxyapatite and nano silver then afterwards will be carried out characterization using several tests, including homogeneity, pH level, spread ability, adhesion, organoleptic, and identification of functional groups using FTIR spectrophotometer.

MATERIALS AND METHOD

Tools and Materials

The tools used are analytical balance, stirring rod, beaker, measuring cup, watch glass, spatula, measuring flask, mortar pestle, dropper, spin bar, magnetic stirrer, hot plate, vacuum pump, evaporator, pH meter, filter paper, furnace, and FTIR spectrophotometer. The materials used are beef bone (*Bos taurus*), hydrogen peroxide (H_2O_2), phosphoric acid (H_3PO_4), silver nitrate ($AgNO_3$), sodium citrate ($Na_3C_6H_5O_7$), clove (*Syzygium aromaticum* L.), propylene glycol, carboxymethyl cellulose (CMC), and distilled water.

Synthesis and Preparation of Hydroxyapatite Solution

The synthesis of hydroxyapatite was carried out by the calcination method. Beef bones (*Bos taurus*) were pre-soaked using a 3% H_2O_2 solution for 2x24 hours with a change of solution every 24 hours. Then, the beef bones were heated at 900°C for 6 hours and mashed using a pestle mortar. Hydroxyapatite solutions with concentrations of 1%, 2%, and 3% were prepared by dissolving 1 gram, 2 grams, and 3 grams of beef bone in a solution of phosphoric acid. After that, it was stirred until homogeneous and transferred to a 100 mL volumetric flask for storage.

Synthesis and Preparation of Nano silver Solution

Synthesis of nano silver was carried out by chemical reduction method. A total of 5 mL of 1% $Na_3C_6H_5O_7$ solution was added little by little to 50 mL of 0.001 M $AgNO_3$ solution while stirring and heating. The heating and stirring process was stopped when the color changed to greenish yellow. Nano silver solutions with concentrations of 1 ppm, 5 ppm, and 10 ppm were prepared by dissolving 0.7 mL, 3.5 mL, and 7 mL of nano silver solution into 100 mL of distilled water. After that, it was stirred until homogeneous and transferred to a 100 mL volumetric flask for storage.

Clove (*Syzygium aromaticum* L.) Extraction

Clove extraction was done by maceration method. Cloves are dried and cut into small pieces. Then, 250 grams of clove were soaked in 1.5 L of distilled water for 3x24 hours. Afterwards, it was filtered and concentrated using an evaporator at 100°C for 8 hours until the extract was blackish brown in color and had a strong characteristic aroma.

Making Toothpaste Forming Gel

Heat 25 mL of distilled water at 80°C for 15 minutes. Then, add 8.5 mL of propylene glycol, 2 mL of hydroxyapatite, 2 mL of nano silver, and 0.5 mL of clove extract. Then, the mixture was stirred until completely dissolved and slowly added 0.65 grams of CMC until a gel began to form. Several gel formulations with varying concentrations of hydroxyapatite and nano silver are listed in the following table:

Table 1. Gel Formulation

Sample	Composition		
F1	HAp 1%	AgNPs 1 ppm	Clove
F2	HAp 1%	AgNPs 5 ppm	Clove
F3	HAp 1%	AgNPs 10 ppm	Clove
F4	HAp 2%	AgNPs 1 ppm	Clove
F5	HAp 2%	AgNPs 5 ppm	Clove
F6	HAp 2%	AgNPs 10 ppm	Clove
F7	HAp 3%	AgNPs 1 ppm	Clove
F8	HAp 3%	AgNPs 5 ppm	Clove
F9	HAp 3%	AgNPs 10 ppm	Clove

Homogeneity Test

Homogeneity test was carried out by smearing the sample on a flat transparent glass object. Then, it is observed and declared homogeneous if it does not show the presence of coarse grains [12].

pH Level Test

The pH level test was carried out using a calibrated pH meter by inserting it into the sample until the pH value displayed was constant.

Spread Ability Test

Spread ability test was carried out by placing 0.5 grams of the sample between 2 flat transparent glass objects. Then, the glass is pressed with a load of 50 grams and 100 grams for 5 minutes. After that, the diameter of the sample spread across and longitudinally was measured [13].

Adhesion Test

The adhesion test was carried out by placing 0.5 grams of the sample between 2 flat transparent glass objects. Then, the glass is pressed with a load of 1 kg for 5 minutes. After that, the slide is released with a load of 100 grams and the time is calculated until the two glasses are released [13].

Organoleptic Test

Organoleptic tests were carried out using the senses of sight, smell, and touch by observing the color, smelling the aroma, and feeling the texture of the sample [12].

Functional Group Characterization using FTIR Spectrophotometer

Functional group characterization was carried out by placing the sample in the FTIR spectrophotometer holder set in the wave number range of 4000-550 cm⁻¹. The functional group can be identified based on the absorption peak of a certain wave number through infrared radiation that is passed through the sample. The analysis can be done by determining the functional groups based on the peaks produced by IR spectra between wavenumber and transmission [14].

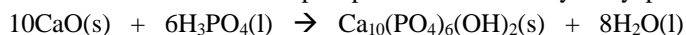


ANALYSIS AND DISCUSSION

Synthesis and Preparation of Hydroxyapatite Solution

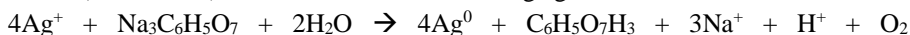
The synthesis of hydroxyapatite was carried out by the calcination method. This method has advantages compared to other methods because it is able to remove organic components and disease genomes in bones as to increase the safety of biological factors [7]. The beef bones are first soaked in a 3% H₂O₂ solution. Hydrogen peroxide is an irreversible acceptor capable of binding electrons and producing OH⁻ ions, resulting in the formation of hydroxyl radicals to oxidize organic compounds. The more hydroxyl formed; the more organic compounds are degraded so that the organic components will decrease [15]. Immersion using a 3% H₂O₂ solution is carried out for 2x24 hours with the replacement of the solution every 24 hours so that the effectiveness of this solution as an oxidizing agent, bleaching agent, and antiseptic can be maintained [16].

Then, the beef bones are heated using a furnace at a temperature of 900°C. This temperature is the optimum temperature because at that temperature calcium carbonate will be formed. If the temperature is below or above 900°C, then the phase formed is tricalcium phosphate (TCP) [17]. At this stage, full evaporation of water occurs when the temperature is 250°C, then the organic component content will be fully oxidized at a temperature of 450°C and there is a complete decomposition of CaCO₃ into CaO at a temperature of 750-1,000°C as evidenced by a change in the color of the bones to white [18]. Based on the research of Afifah and Cahyaningrum (2020), the use of high temperatures in the calcination process can produce purer hydroxyapatite composites and increase the value of the degree of crystallinity of the composite. Then, the bovine bone is reacted with a solution of phosphoric acid which serves as a source of phosphate and forms a hydroxyapatite composite with the reaction:



Synthesis and Preparation of Nano silver Solution

The synthesis of nano silver was carried out using a chemical reduction method by reacting silver nitrate (AgNO₃) and sodium citrate (Na₃C₆H₅O₇) which functioned as reducing agents with the reaction:



The method with sodium citrate or sodium borohydride as a nitrate salt reducing agent is the most frequently used method because it has a simple process step when compared to other methods, such as electrochemistry, ultrasonic irradiation, photochemistry, and sonochemistry [19]. The addition of Na₃C₆H₅O₇ solution into the AgNO₃ solution was carried out drop by drop constantly until the color changed to greenish yellow which is the typical color of nano silver (AgNPs) [20]. The color change is caused by a collective oscillating reaction of electrons in the conduction band which indicates the formation of nano silver compounds. The higher the AgNO₃ concentration, the faster the reaction will occur so that the resulting nano silver color will be darker [19].

Clove (*Syzygium aromaticum* L.) Extraction

Clove used must be dried first to reduce the water content so as to produce extracts in large quantities during the extraction process. Then, the cloves are cut to the smallest size possible so that the extract can be taken up completely while accelerating the rate of solvent evaporation when separated from the extract [21]. Then, the cloves were macerated in distilled water for 3x24 hours which was considered to have higher safety, compared to other solvents, such as ethanol, n-hexane, and benzene, especially if the extraction results were applied to living things. In addition, distilled water is also stable and volatile, thus accelerating the solvent evaporation process [22]. Then, solvent evaporation is carried out using an evaporator at a temperature of 100°C because the boiling point of distilled water is at 100°C, while the boiling point of clove extract in the form of oil is at 253°C so that it can make the water evaporate completely and leave a very thick clove extract, dark brown in color, and has a strong distinctive aroma [21].

Making Toothpaste Forming Gel

The distilled water is heated at 80°C to speed up the reaction that occurs between the components because the reaction will tend to run faster if it is carried out at high temperatures. Temperature is one of the causes of the increase in the rate of reaction, in addition to catalyst, volume, and concentration [23]. The temperature of 80°C was also chosen because distilled water has a boiling point of ±100°C. If the temperature is too high then solvent evaporation occurs and if the temperature is too low then the solution is not hot enough so that the reaction that occurs will run slowly. Then, the main ingredients in the form of hydroxyapatite, nano silver, and clove extract were added, as well as complementary materials in the form of CMC as a gelling agent to increase the viscosity because it has good stability in both acidic and alkaline conditions (pH 2-10) by forming a network structure capable of maintaining the gel system [24]. The added propylene glycol and glycerin function as humectants to maintain stability and moisture so that the

gel does not dry out easily because humectants will maintain water content by absorbing moisture and reducing the evaporation process [25].

Homogeneity Test

Homogeneity test was carried out to prove that the components of the gel were well mixed. Based on the quality standard of SNI No. 12-3524-1995, the requirements for a good toothpaste are homogeneous, not coarse, there are no air bubbles, lumps, or separate particles [26].

Table 2. The Result of Homogeneity Test

Sample	Homogeneity Level
F1	Homogen
F2	Homogen
F3	Homogen
F4	Homogen
F5	Homogen
F6	Homogen
F7	Homogen
F8	Homogen
F9	Homogen

Based on the data in Table 2, the characteristics of each gel formulation are homogeneous. This indicates that the components that make up the gel have been mixed well and evenly so that it looks homogeneous, there are no air bubbles, and there are no coarse grains [27].

pH Level Test

The pH level test is a test to determine the degree of acidity of the sample to suit the conditions of the oral mucosa. The pH value is related to the stability of the active substance, effectiveness, and the state of the compound so that it can be classified as acidic, basic, or neutral [27]. Based on the quality standard of SNI No. 12-3524-1995, the pH value of toothpaste ranges from 4.5-10.5 [26] and the pH value of the oral mucosa ranges from 6.5-7.5 [28].

Table 3. The Result of pH Level Test

Sample	pH Level
F1	4,4
F2	4,3
F3	4,5
F4	4,3
F5	4,3
F6	4,5
F7	4,3
F8	4,5
F9	4,3

Based on the data in Table 3, the pH values of F3, F6, and F8 have met the quality standard, while the pH values of F1, F2, F4, F5, F7, and F9 have not met the quality standard. A low pH value can facilitate the growth of acidogenic bacteria that grow in an acidic environment (pH 4.5-5.5), such as *Streptococcus mutans* and *Lactobacillus*. A pH value below 4.5 is also able to increase the occurrence of demineralization and tooth enamel damage [26]. A high pH value has the opposite effect with a low pH which is able

to suppress the growth of *Streptococcus mutans* and *Lactobacillus* bacteria while strengthening the tooth enamel layer so that tooth remineralization occurs and prevents the potential for dental caries [29].

Spread Ability Test

The spread ability test was carried out to determine the ability of the gel to spread when used on the tooth surface. The gel has a semi-solid texture that is able to spread easily at the site of administration and will provide product user comfort. The easier the gel is to apply, the wider the contact surface area, thereby optimizing the absorption of the active substance. A good gel has a standard spread of 5-7 cm [25].

Table 4. The Result of Spread Ability Test

Sample	Massa (grams)	Without Load (cm)	Load 50 Grams (cm)	Load 100 Grams (cm)
F1	0,5100	5,2	5,45	5,6
F2	0,5062	5,15	5,25	5,4
F3	0,5049	5,15	5,25	5,3
F4	0,5024	5,1	5,75	6,0
F5	0,5020	5,6	6,05	6,3
F6	0,5063	5,1	5,45	5,65
F7	0,5041	5,3	5,75	6,1
F8	0,5090	5,15	5,35	5,55
F9	0,5020	5,15	5,9	6,15

Based on the data in Table 4, each formulation met a good gel dispersion scale. The spreading area increases which is directly proportional to the increase in the mass of the load and affects the object because the reaction occurs faster. The occurrence of increase and decrease in dispersion is caused by the consistency of the gel which is related to the viscosity value. If the viscosity is low, the dispersion will be wider and vice versa because the low viscosity makes the gel flow smoother and spreads well [13]. According to Rohmani and Kuncoro (2019), the dispersion is due to the stability of CMC as a gelling agent so that it affects the strength of the gel matrix. Therefore, the dominant factor that determines the dispersion response is the concentration and amount of CMC components involved. The temperature and storage packaging that are less impermeable also affect the viscosity of CMC because the gel will absorb water vapor and then release Na^+ ions which are replaced by H^+ ions to form HCMC compounds with a higher viscosity [30]. Other gel components, such as propylene glycol and glycerin with a liquid predominant phase, also reduce the viscosity of CMC which affects the spread ability of the gel [25].

Adhesion Test

The adhesion test was carried out to see the strength of the gel when it adhered to the tooth surface. A good gel has a standard of stickiness ranging from 1-6 seconds [30].

Table 5. The Result of Adhesion Test

Sample	Massa (grams)	Time
F1	0,5062	1,57 sec
F2	0,5044	1,85 sec
F3	0,5056	1,97 sec
F4	0,5011	1,64 sec
F5	0,5074	1,25 sec
F6	0,5029	1,83 sec
F7	0,5046	1,51 sec
F8	0,5005	1,71 sec
F9	0,5009	1,65 sec



Based on the data in Table 5, each gel formulation met the criteria for good adhesion. High adhesion indicates that the gel texture is denser, elastic, and easy to adhere, but has a poor spreadability. The low adhesion indicates that the gel texture is more liquid, less elastic, and does not adhere well, but has a very good spread ability [31]. Adhesion also affects the absorption of the active ingredients that make up the gel. The longer it sticks, the greater the active substance is absorbed [27].

Organoleptic Test

The organoleptic test aims to observe the gel visually if there is a phase separation or color change so that the product can be accepted by consumers [24]. This organoleptic test involved 10 respondents to assess the characteristics of the color, aroma, and texture of each gel. The increase in each characteristic is represented by adding a sign (+) at each increase. The results of the organoleptic tests are shown in the graph attached below.

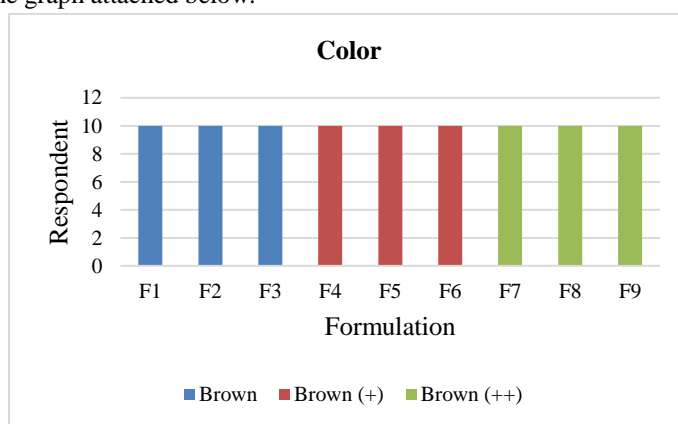


Figure 1. The Result of Color Organoleptic Test

Based on the data in Figure 1, each respondent gave an assessment of the visual characteristics of the visible color. At F1, F2, and F3, ten respondents stated that the gel was brown. At F4, F5, and F6, ten respondents stated that the gel was brown (+). At F7, F8, and F9, ten respondents stated that the gel was brown (++). These results indicate that increasing the concentration of hydroxyapatite and nano silver affects the color of the gel. The higher the concentration, the more the color characteristics appear.

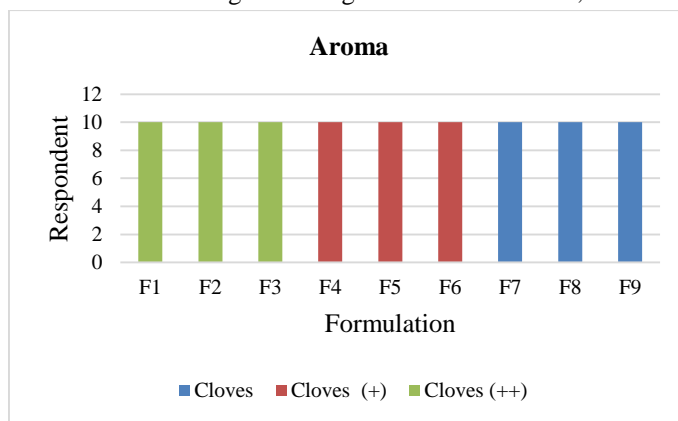


Figure 2. The Result of Aroma Organoleptic Test

Based on the data in Figure 2, each respondent gave an assessment of the visual characteristics of the aroma produced. In F1, F2, and F3, ten respondents stated that the gel had a distinctive clove scent (++). In F4, F5, and F6, ten respondents stated that the gel had a distinctive clove scent (+). At F7, F8, and F9, ten respondents stated that the gel had a distinctive clove scent. These results indicate that the increase in the concentration of hydroxyapatite and nano silver affects the aroma of the gel. The higher the concentration, the lower the characteristic aroma produced.

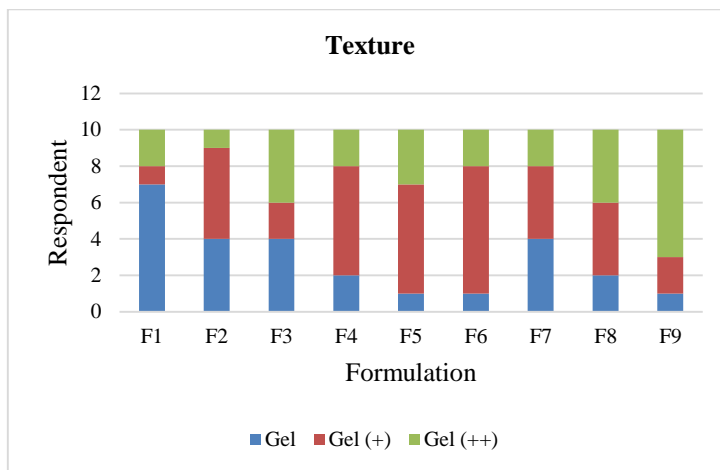


Figure 3. The Result of Texture Organoleptic Test

Based on the data in Figure 3, each respondent has given an assessment of the shape characteristics of the resulting texture. In F1, most of the respondents stated that the formulation was gel textured. In F2, F4, F5, and F6, most of the respondents stated that the formulation was gel textured (+). In F9, most of the respondents stated that the formulation was gel textured (++) . In F3, most of the respondents stated that the formulation was gel or gel textured (++) . In F7, most of the respondents stated that the formulation was gel (+) or gel textured. In F8, most of the respondents stated that the formulation was gel (+) or gel (++) . These results indicate that the difference in gel texture is influenced by the consistency of the CMC mass. The greater the mass of the gelling agent, the denser the texture of the gel because CMC affects the viscosity of the gel preparation.

Statistical tests were also carried out to determine the effect of variations in the concentration of hydroxyapatite and nano silver on the color, aroma, and texture of the gel preparation so that the hypothesis proposed was "there is an effect of the concentration of hydroxyapatite and nano silver on the color, aroma, and texture of the gel preparation". The data analysis was carried out by using the *Shapiro-Wilk* normality test first, if the data were not normally distributed, it was continued with non-parametric statistical tests. The *Shapiro-Wilk* test is a statistical test to determine whether the distribution of a data is normal or not. This test is generally applied in regression analysis examining random data of small size or amount ($N < 50$).

Table 6. The Result of Normality Test

Formulation	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Formulation 1	.422	10	.000	.628	10	.000
Formulation 2	.272	10	.035	.802	10	.015
Formulation 3	.300	10	.011	.815	10	.022
Formulation 4	.300	10	.011	.815	10	.022
Formulation 5	.324	10	.004	.794	10	.012
Formulation 6	.370	10	.000	.752	10	.004
Formulation 7	.245	10	.091	.820	10	.025
Formulation 8	.248	10	.082	.805	10	.017
Formulation 9	.416	10	.000	.650	10	.000

a. Lilliefors Significance Correction

Based on the data in Table 6, there are data that have a significance value of < 0.05 . These results indicate that the data are not normally distributed so that further tests are needed in the form of non-parametric statistical tests to prove the hypotheses that have been made previously. The next statistical test is the *Kruskal-Wallis* test (H-test) to show significant differences between the independent and dependent variable groups in the sample. The data from the *Kruskal-Wallis* test can be seen from the probability significance value (P-Value) because the P-Value value is related to the proposed hypothesis. Value > 0.05 then the proposed hypothesis can be rejected.

Table 7. The Result of *Kruskal-Wallis* Test

Test Statistics ^{a,b}			
	Color	Aroma	Texture
Kruskal-Wallis H	89.000	89.000	14.240
df	8	8	8
Asymp. Sig.	.000	.000	.076

a. *Kruskal-Wallis* Test

b. Grouping Variable: Formulation

Based on the data in Table 7, the color and aroma factors have a P-Value value < 0.05 while the texture has a P-Value value > 0.05 which means that variations in the concentration of hydroxyapatite and nano silver affect the color and aroma but have no effect on the texture of the gel preparation.

Characterization of Functional Groups using FTIR Instruments

FTIR (Fourier Transform Infrared) spectrophotometer is a chemical characterization method to analyze functional groups in samples qualitatively based on the absorbance value of infrared light. This method is characterized by a shift in wavelength in the wavenumber area of $4000-550\text{ cm}^{-1}$. Functional group analysis is known based on increasing or decreasing intensity, as well as the appearance and disappearance of spectra [7].

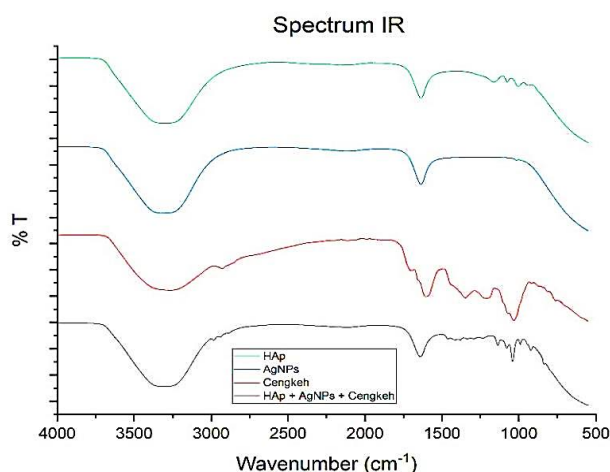


Figure 4. The Result of FTIR Gel Preparation Test

Typical functional groups of hydroxyapatites are the OH group in the $3400-2700\text{ cm}^{-1}$ area, the CO_3^{2-} group in the $1700-1400\text{ cm}^{-1}$ area, and the phosphate group (PO_4^{3-}) in the $1150-560\text{ cm}^{-1}$ area [32]. The spectrum of the hydroxyapatite gel shows the OH group at a wave number of 3307.32 cm^{-1} due to the vibration of the H-O-H molecule, the CO_3^{2-} group at a wave number of 1634.53 cm^{-1} which is produced during the calcination process where the carbonate group binds to calcium to form calcium carbonate, and the PO_4^{3-} group which consists of stretching vibrations at wave numbers 1161.42 cm^{-1} , 1075.67 cm^{-1} , 1003.73 cm^{-1} and medium vibrations at wave numbers 939.59 cm^{-1} [33]. The nano silver gel spectrum showed OH groups at 3307.72 cm^{-1} and a carbonyl group



(C=O) at 1634.70 cm^{-1} . A slight decrease in the intensity of the hydroxyl group and the appearance of a carbonyl group were due to the reduction of silver ions by other groups during the gelling process [34]. The clove extract gel spectrum showed OH group at wave number 3267.95 cm^{-1} , OCH group at wave number 2926.56 cm^{-1} , C-C aryl group (aromatic compound) at wave number 1603.25 cm^{-1} , methyl group (CH_3) at a wave number of 1695.74 cm^{-1} , and the functional group =C-H (typical of eugenol) in the fingerprint area with a cis position at a wave number of 759.63 cm^{-1} , while the trans position at a wave number of 1032.71 cm^{-1} and 917.63 cm^{-1} [35].

The spectrum of the hydroxyapatite-nano silver-clove extract gel showed an OH group at a wave number of 3307.22 cm^{-1} , an alkane group at a wave number of 2981.89 cm^{-1} , a methyl group (CH_3) at a wave number of 1642.37 cm^{-1} , a CO_3^{2-} group at wave numbers 1459.31 cm^{-1} and 1417.58 cm^{-1} , the PO_4^{3-} group with stretching vibrations at wave numbers 1136.56 cm^{-1} and 1078.84 cm^{-1} , and the functional group =C-H (typical of eugenol) in the fingerprint area with the cis position at wave number 835.95 cm^{-1} , while the trans position at wave number 1041.44 cm^{-1} and 990.89 cm^{-1} . There is a shift in wavelength, increase and decrease in intensity, as well as the appearance and disappearance of spectra caused by the reduction and oxidation reactions between groups during the gelling process [7].

CONCLUSION

The results showed that the gel formulations of toothpaste preparations with varying concentrations of hydroxyapatite and nano silver had met the test eligibility standards, such as homogeneity, pH level, spread ability, and adhesion. In the organoleptic test, variations in the concentration of hydroxyapatite and nano silver have an effect on color and aroma, but have no effect on texture which can be seen from the results of normality and *Kruskal-Wallis* tests. FTIR spectrophotometer analysis showed that the gel sample contained OH, CO_3^{2-} , and PO_4^{3-} groups from hydroxyapatite, carbonyl groups from nano silver, as well as methyl and eugenol groups (=C-H) from clove extract.

REFERENCES

1. B. Puspitasari, "The Relationship between Oral Health and Quality of Life for the Elderly at Pajang Health Center Surakarta," University of Muhammadiyah Surakarta, Surakarta, 2017.
2. Yuliyana and A. S. R. M. Sinaga, "Expert System for Diagnosing Dental Disease using the Naive Bayes Method," Fountain of Informatics Journal, vol. 4, no. 1, pp. 19-23, 2019.
3. H. Hermawan, Introduction to Biomaterials, Canada: Laval University, 2019.
4. Y. A. Hariyanto and A. Taufiq, "Synthesis, Characterization of Structure and Optical Properties of Hydroxyapatite/Magnetic Nanoparticles," Journal of Physical Science and Engineering, vol. 3, no. 1, pp. 16-24, 2018.
5. Syamsuddin, "Synthesis and Characterization of Bone Bioceramics as Implant Materials by Sintering Method," Journal INTEK, vol. 4, no. 2, pp. 84-86, 2017.
6. L. Anggresani, S. Nursing and I. J. Rahayu, "Waste of Mackerel (*Scomberomorus guttatus*) bone as a source of calcium in the manufacture of hydroxyapatite," Journal of Catalysts, vol. 4, no. 2, pp. 133-140, 2019.
7. F. Afifah and S. E. Cahyaningrum, "Synthesis and Characterization of Hydroxyapatite from Cattle Bone (*Bos taurus*) using Calcination Technique," UNESA Journal of Chemistry, vol. 9, no. 3, pp. 189-196, 2020.
8. V. A. Fabiani, M. A. Putri, M. E. Saputra and D. P. Indriyani, "Synthesis of Nanosilver using Bioreductant of Pelawan Leaf Extract (*Tristanopsis merguensis*) and Antibacterial Activity Test," Journal of Chemistry and Chemistry Education, vol. 4, no. 3, pp. 172-178, 2019.
9. D. Maharani, L. Mahmudin and Iqbal, "Effect of Trisodium Citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) Reducing Substance Concentration on Optical Properties of Silver Nanoparticles," Gravity, vol. 17, no. 2, pp. 34-42, 2018.
10. F. Y. Prasetyo, "Essential Oil Extraction from Clove Flowers Using Ultrasonic Methods," University of Muhammadiyah Surakarta, Surakarta, 2018.
11. T. Sofihidayanti and S. Wardatun, "Study of Effect of Clove Flower Stem Extraction Method (*Syzygium aromaticum* L. Merr) on Eugenol Levels," Phytopharmaca: Scientific Journal of Pharmacy, vol. 11, no. 2, pp. 155-164, 2021.
12. D. P. Astuti, P. Husni and K. Hartono, "Formulation and Physical Stability Test for Hand Antiseptic Gel Preparations Lavender Flower Essential Oil (*Lavandula angustifolia* Miller)," Farmaka, vol. 15, no. 1, pp. 176-184, 2017.



13. S. Rohmani and M. A. A. Kuncoro, "Stability and Activity Test of Basil Hand Sanitizer Gel Extract," *Journal of Pharmaceutical Science and Clinical Research*, vol. 1, pp. 16-28, 2019.
14. M. B. Satriawan and I. Illing, "FTIR Test of Bioplastics from Sago Dregs Waste with the Addition of Variations in Gelatin Concentrations," *Journal of Dynamics*, vol. 8, no. 2, pp. 1-13, 2017.
15. N. D. Perdana, S. Wardhani and M. M. Khunur, "The Effect of Addition of Hydrogen Peroxide (H₂O₂) on the Degradation of Methylene Blue Using ZnO - Zeolite Photocatalyst," *Chemistry Student Journal*, vol. 2, no. 2, pp. 576-582, 2014.
16. M. Adriati, A. Suseno and Taslimah, "Modification of Natural Zeolite Using Iron (Fe) and Cobalt (Co) for Phenol Degradation Catalyst," *Journal of Chemical Science and Applications*, vol. 16, no. 1, pp. 1-5, 2013.
17. C. Y. Ooi, M. Hamdi and S. Ramesh, "Properties of Hydroxyapatite Produced by Anneling of Bovine Bone," *Ceramic International*, vol. 33, pp. 1171-1177, 2017.
18. A. Haris, A. Fadli and S. R. Yenti, "Synthesis of Hydroxyapatite from Cow Bone Waste Using the Precipitation Method with Variations in Ca/P Ratio and H₃PO₄ Concentration," *JOM FTeknik*, vol. 3, no. 2, pp. 1-10, 2016.
19. S. Yanti, M. S. Arif and B. Yusuf, "Synthesis and Stability of Silver Nanoparticles (AgNPs) Using Trisodium Sulfate," in *Proceedings of the UNMUL National Chemistry Seminar, Samarinda, 2021*.
20. K. M. A. El Nour, E. T. A. Salam, H. M. Soliman and A. S. Orabi, "Gold Nanoparticles as a Direct and Rapid Sensor for Sensitive Analytical Detection of Biogenic Amines," *Nanoscale Research Letters*, vol. 10, p. 1186, 2017.
21. S. Hadi, "Clove Oil Extraction using n-Hexane and Benzene Solvents," *Journal of Renewable Natural Materials*, vol. 1, no. 2, pp. 25-30, 2017.
22. A. Mu'nisa, T. Wresdiyati, N. Kusumorini and W. Manalu, "Antioxidant Activity of Clove Leaf Extract," *Veterinary Journal*, vol. 3, pp. 272-277, 2012.
23. W. Irwanda, A. H. Alimuddin and Rudiyanayah, "Synthesis of Oxalic Acid from Stem Sap of Sri Rejeki Plant (*Dieffenbachia seguine* (Jacq.) Schott) Using Phosphoric Acid Hydrolysis Method," *Journal of Equatorial Chemistry*, vol. 6, no. 1, pp. 30-36, 2017.
24. F. R. H. Ayorbaba, "Optimization of CMC-Na and Propylene Glycol in Gel Preparations of Gotu kola (*Centella asiatica* (L.) Urban) Extract with the Simplex Lattice Design Method," *Sanata Dharma University, Yogyakarta, 2020*.
25. N. A. Sayuti, "Formulation and Physical Stability Test Preparation of Chinese Ketepeng Leaf Extract (*Cassia alata* L.)," *Indonesian Pharmaceutical Journal*, vol. 5, no. 2, pp. 74-82, 2015.
26. B. Gratia, P. V. Y. Yamlean and K. L. R. Mansauda, "Toothpaste Formulation of Nutmeg (*Myristica fragrans* Houtt.) Ethanol Extract Toothpaste," *Pharmacon*, vol. 10, no. 3, pp. 968-974, 2021.
27. Purgiyanti and R. I. Pratiwi, "Making Pain Relief Gel from Clove Flower (*Syzygium aromaticum* (L.) Merr. & Perry) Essential Oil," *Journal of the Thinkers*, vol. 8, no. 1, pp. 72-75, 2019.
28. N. Hamrun, F. Fitriani and R. Amalia, "Overview of Gingival Status, Oral Hygiene, pH, and Saliva Volume on Hormonal Contraceptive Users in Mappakasunggu District, Takalar Regency," *PDGI Journal*, vol. 65, no. 3, pp. 78-82, 2016.
29. Y. Khamisli, E. Dewi and B. E. M. Intan, "The Correlation of Caries Severity Index with Saliva pH in Mentally Impaired Persons at the Bina Grahita (SB) Harapan Social Institution, Padang City in 2018," *Journal of Dentistry, Baiturrohmah University*, vol. 6, no. 1, pp. 71-75, 2019.
30. T. Mappa, H. J. Edy and N. Kojong, "Sasaladahan (*Peperomia pellucida* (L.) H. B. K.) Leaf Extract Gel Formulation and Test of Its Effectiveness against Burns in Rabbits (*Oryctolagus cuniculus*)," *Scientific Journal of Pharmacy*, vol. 2, no. 2, pp. 49-55, 2013.
31. K. I. Doko, "Antibiofilm Activity Test against *Streptococcus mutans* and Optimization of CMC Na and Sorbitol in Toothpaste Formulation of Moringa Leaf Extract Gel (*Moringa oleifera* Lmk.)," *Faculty of Pharmacy USD, Yogyakarta, 2018*.
32. J. Sedyono and A. E. Tontowi, "The Process of Synthesis and Characterization of Hydroxyapatite FTIR from Natural Gypsum Kulon Progo," *Journal of Mechanical Engineering*, vol. 9, no. 1, pp. 6-12, 2018.
33. M. Khoiruddin, Yelmida and Zultiniar, "Synthesis and Characterization of Hydroxyapatite (HAp) from Blood Shells (*Anadara granosa*) by Hydrothermal Process," *JOM FTeknik*, vol. 2, no. 2, pp. 1-8, 2015.



34. S. Sutoyo, Tukiran, S. Khotijah and D. P. Sari, "Synthesis and Characterization of Silver Nanoparticles (AgNPs) Using a Bioreductor Methanol Extract of *Nephrolepis radicans*," in Proceedings of the National Chemistry Seminar (SNK), Surabaya, 2020.
35. E. Mulyono, A. Suryani, D. Mangunwidjaja and D. Setyaningsih, "Optimization of Eugenol Isomerization Conditions from Clove Leaf Oil Using Response Surface Methods," *Postharvest Journal*, vol. 7, no. 2, pp. 86-93, 2010.

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