Utilization of Environmental Wastes from Poultry Eggshell to Manufacture Hydroxyapatite for Dental Biomaterial

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Abstract

Eggshells are household waste that in large quantities can harm the environment. The very high content of Calcium Carbonate 90% is the main component of the composition of eggshells. This study aims to identify the manufacture of dental biomaterials; toothpaste. The eggshell was calcinated with the sintering temperatures of 350, 500, 600, and 900 ^{0}C for 10 minutes and reacted with Hydroxy o- phosphate. From the spectrum results obtained, the best hydroxyapatite was at a sintering temperature of 900 ^{0}C. The samples were compared to the addition of NaOH and the control variables. The additive fortification was carried out in preparations including glycerin, baking soda, and betel leaf extract for antimicrobials. The tested pH was the best in the range of 4.5-10.5. From the results obtained the best is sample 10 where the pH is neutral 7 organoleptic appearance was semi-solid.

Keywords
Environmental wastes, poultry eggshell, hydroxyapatite, dental biomaterial, sintering temperature

1. Introduction
Every day around the world, millions of tons of eggshells are disposed of as organic waste (Wu, Hsu, Hsu, Chang, & Ho, 2016). In Indonesia, according to data from the Central Statistics Agency (BPS), egg production in 2017 was 1,506,192 tons and in 2018 continued to increase to 1,644,460 tons. So far, eggshells have only been thrown away because it was considered to have no economic value, but instead polluted the environment (BPS, 2018). The eggshell contains $CaCO_3$ (calcium carbonate) which reaches 95%. The high calcium content can be utilized as the source of hydroxyapatite $(Ca_{10}(PO_4)_6OH)_2$ synthesis for synthetic bone, which can be described as a bone containing calcium ions that can be combined with orthophosphates, pyrophosphates, hydrogen, or hydroxides. Research on hydroxyapatite (HAp) is still being intensively refined. The results of several research findings found that HAp has the potential as a catalyst for various chemical reactions (Mori et al., 2002; Jun et al., 2004), as an adsorbent because of its high affinity for heavy metal ions (Lusvardi, Malavasi, Menabue, & Saladini, 2002); (Krestou, Xenidis, & Panias, 2004), for medical and dental applications because of its biocompatibility and bioactivity capabilities (Mansour, El-dek, Ahmed, Abd-Elwahab, & Ahmed, 2016); (Ravarian et al., 2010). HAp can be used as a base material for composite pastes, supported by an inorganic content of 95.1%, organic matter of 33%, and water at 1.6%. Most of the inorganic materials consist of calcium carbonate ($CaCO_3$) about 90.9% of forming bones and teeth. Toothpaste is a composite that can maintain the aesthetics and the health of teeth, in the form of a paste or gel. Most commercial toothpaste contains chemicals such as sodium lauryl sulfate which easily can cause irritation of oral cavity, ulceration, decreased salivary solubility, and changes in taste sensitivity. A good toothpaste should not cause tooth abrasion, changes color of the filling which will causes the imbalance of oral bacteria. The use of natural ingredients of toothpaste continues to be developed such as baking soda for tooth whitener, eggshell powder as a source of Ca^{2+}, clove oil as a sensitivity additive, glycerin as a preservative and moisturizer, lemon peel as a substitute for citric acid, orange peel, peel bananas that serve as a substitute for iron. The aim of this study is to identify the effectiveness of chicken eggshells as a source of calcium for toothpaste by using precipitation methods and thermal methods, and studying the effectiveness of hydroxy o-phosphate in extracting calcium from egg shells.

1.1 Objectives
Commercial toothpaste has been using synthetic active substances as the main ingredients, causing ulceration and other complaints if used for a certain period. In this research, we will study the production of toothpaste with active ingredient hydroxy apatite enriched with natural ingredient additives so that it meets the criteria as a toothpaste product.

2. Literature Review

A. Hydroxyapatite (HAp)

Hydroxyapatite (HAp) is a crystalline molecule composed of phosphorus, oxygen, hydrogen, and calcium with the molecular formula $Ca_{10}(PO4)_6(OH)_2$ which is a bioactive ceramic material with high affinity. The crystal structure of hydroxyapatite namely monoclinic and hexagonal. In general, hydroxyapatite crystals made by synthesis have a hexagonal crystal structure, while the composition of the constituent elements (% ideal weight) are Ca 39.9%, P 18.5%, OH 3.38%, and the ideal ratio of calcium-phosphate (Ca to P) of 1.67 (Jeong, Kim, Shim, Hwang, & Heo, 2019).

Synthetic hydroxyapatite is one of the materials that is often used in biomedical applications because this hydroxyapatite has an identical appearance to teeth and bones and has biocompatible and osteoconductive properties. Synthetic hydroxyapatite is still imported, with such an expensive price, which is USD 455 (IDR 6.6 million) per 5 grams under the brand name Sigma Aldrich, whereas hydroxyapatite can be synthesized by various methods namely: the dry method, the wet method commonly known as precipitation, and hydrothermal method (Wu et al., 2013). The wet method was common because is simple and can produce mostly amorphous hydroxyapatite powder. There are various hydroxyapatite (HAp) synthesis to be developed, such as sol-gel (Luo, Li, Wang, & Zou, 2011), water precipitation (Cox, Walton, & Mallick, 2015), hydrothermal technique (Method, Fatah, & Kareem, 2017); (Jinawath, Pongkao, & Yoshimura, 2002); Wu et.al, 2013), and solid reaction (Rhee, 2002); (Jokić et al., 2011); and (Wu et al., 2013). The calcium-containing natural materials such as fish bones, beef bones, coral, oyster shells, and eggshells, can be converted into useful HAp biomaterials. The transformation of cuttlefish bone by hydrothermal at 200 ^{0}C (Lemos et al., 2006). The porous HAp from bovine bone was obtained by heating treatment at a range of 400-1200 ^{0}C, the conversion of coral into single-phase HAp through a low-temperature hydrothermal process (Sivakumar & Manjubala, 2001). Recently, HAp could be synthesized through hydrothermal treatment of eggshells (Wu et al., 2013) and combined to waste materials; pomelo, grape, and potato peel extract. This would be a promising opportunity, considering that the raw material is wasted.

B.Calcium Carbonate

Calcium carbonate ($CaCO_3$) is the largest commercial source of calcium compounds. It is found as a major component of marine shells, shellfish, snails, coral reefs, pearls, and eggshells. Calcium carbonate is the active ingredient in limestone, performed is the most important ingredient in toothpaste as an abrasive removing food particles from the teeth. Generally, almost half of the total weight of toothpaste is calcium carbonate.

C. Toothpaste Biocomposite Additives

1. Glycerin

The uses of glycerin as a preservative for fruit in cans, as a base for lotions, as a freeze guard in hydraulic jacks, and as a raw material for printer ink, cakes, and candies. In the manufacture of composite pastes, glycerin functions as a moisturizer for the teeth as well as prevents drying and hardening of the paste. Glycerin can be used as a humectant, that will form a satisfactory gloss and semisolid consistency which does not irritate, its hygroscopic property can be mixed with almost many substances. Humectants in toothpaste according to the standard Harry's Cosmeticology formula range from 10% - to 30%. In toothpaste formulations, it is necessary to add glycerin as a humectant because it can maintain moisture and prevent dryness of toothpaste and prolong the contact of active substances on teeth.

2. Baking Soda

One of the chemicals used in the manufacture of composite paste is baking soda (sodium hydrogen carbonate or sodium bicarbonate) with the chemical formula $NaHCO_3$. Baking soda in the manufacture of composite pastes is an abrasive for removing plaque. The advantages of using baking soda in the field of dentistry were its buffering capacity to saliva, effectively neutralizing acids, and its water-soluble properties. Toothpaste containing baking soda with a high concentration of 65% is effective in removing intrinsic stains on teeth compared to those that do not contain baking soda.

3. Betel Leaf

Betel is a kind of vine that rests on the trunk of another tree. The single leaf is heart-shaped, pointed with a pointed edge, flat edge, curved leaf bone, leaf width 2.5-10 cm, leaf length 5-18 cm, grows alternately, stemmed, and emits a pleasant odor when crushed. Betel spread throughout Indonesia, often found in the yard. The preferred growing place is at an altitude of 200-1000 m above sea level which has rainfall of 2250-4750 mm per year. This plant grows in slightly humid forest areas with moist soil conditions, and shady areas and is protected from the wind (Das, Da, Sriram

Sandeep, Nayak, & Mohanty, 2016). Betel leaf has a distinctive aromatic odor with anti-inflammatory, antiseptic, and anti-bacterial. The plant parts that can be used are the leaves, roots, and seeds. The leaves are used to treat bad breath, sore eyes, vaginal discharge, inflammation of the respiratory tract, cough, canker sores, and nosebleeds (Patra, Das, Kumar Dey, & Das, 2016).

3. Methods

3.1 Material and Equipment

Wastes of chicken eggshells, NaOH, Hydrogen o-phosphate, Aquadest, Glycerin, Baking soda, Betel leaf extract. Collect chicken eggshell waste, then cleaned and washed using running water. Dried using an oven at a temperature of 110 ^{0}C for 2 hours. Mashed using a mortar and pestle until forming a fine powder then sieved. The equipment used was a Glass beaker, porcelain cup, stirring rod, Analytical balance, Oven, Furnaces, Volume pipette, Bulb, universal pH, Watch glass, Sieve, Funnel, Mortar, Fourier Transform Infrared (FTIR) Shimadzu. In this study, calcium carbonate was obtained from eggshells. Eggshells are used because they contain about 90% calcium carbonate as the main component of eggshells. The type of eggshell used is chicken eggshell because it is affordable and easy to obtain as well as the amount of production is abundant. To obtain a good shell powder, the eggshell must be cleaned and dried in the oven first so that it is not contaminated with unwanted substances. The results of the sieve for shell powder are 100 mesh size. Then it is synthesized by doing the calcination process first by making 4 samples for comparison. Add Hydrogen-o phosphate and aquadest with a composition ratio then dry. At a calcination temperature of 900 ^{0}C, 2 batches were carried out, with the second batch adding 5% NaOH.

The use of NaOH is intended to determine the percentage addition of the OH functional group to HAp by using XRD. Then separate the sample for testing and the sample to be used as a toothpaste product. Weighed the CaO powder obtained from the synthesis of hydroxyapatite and mixed with additives such as baking soda which is useful for removing plaque stains, but does not change the color of the teeth, glycerin which functions as a moisturizer to moisturize teeth as well as prevent drying and hardening of the paste, and leaf extract. Betel is efficacious as anti-inflammatory, anti-septic, and anti-bacterial. The selection of the formula in this study is based on the ratio. Some of the samples were used for the FTIR characterization test, which is a tool or instrument that can be used to detect functional groups, identify compounds and analyze mixtures from the analyzed samples without damaging the sample.

3.2 Research Procedure

1. Synthesis of Hydroxyapatite

Eggshell fine powder was calcined using a furnace at temperatures: 350 ^{0}C (for 10 minutes), 500 ^{0}C (for 10 minutes), 600 ^{0}C (for 10 minutes) and 900 ^{0}C (for 10 minutes) which resulted as product A, product B, product C, and product D. Then add Hydrogen-o phosphate (HoP) and aquadest, with the composition of (HoP); 65.g, 56. g, and 47 g and constant amount of 3 g aquadest which resulted as (result #1, #2, and #3) classified as the product from calcination with temperature 350 ^{0}C, (result #4, #5, and #6) classified as the product from calcination with temperature 500 ^{0}C, (result #7, #8, and #9) classified as the product from calcination with temperature 600 ^{0}C, and (result #10, #11, and #12) classified as the product from calcination with temperature 900 ^{0}C. Then dry with an oven at 180 ^{0}C for 20-24 hours or until dry. Among four thermal treatment, being optimized. The best intermediate product will be added to NaOH to be identify its FTIR spectrum and its organoleptic appearance. All the research stages was illustrated in the flow diagram.

2. Toothpaste Fabrication

Weighed 3 g of CaO powder obtained from the synthesis of hydroxyapatite at a temperature of 900 ^{0}C. Then mixed with baking soda in succession 3.0 g; 5.0 g and 7.0 g. After that, 2.52 g of glycerin were added sequentially; 3.78 g and 5.04 g. Then add betel leaf extract to the mixture as much as 2.76 g. Stir until it blended in homogeneous for 10 minutes (Figure 1).

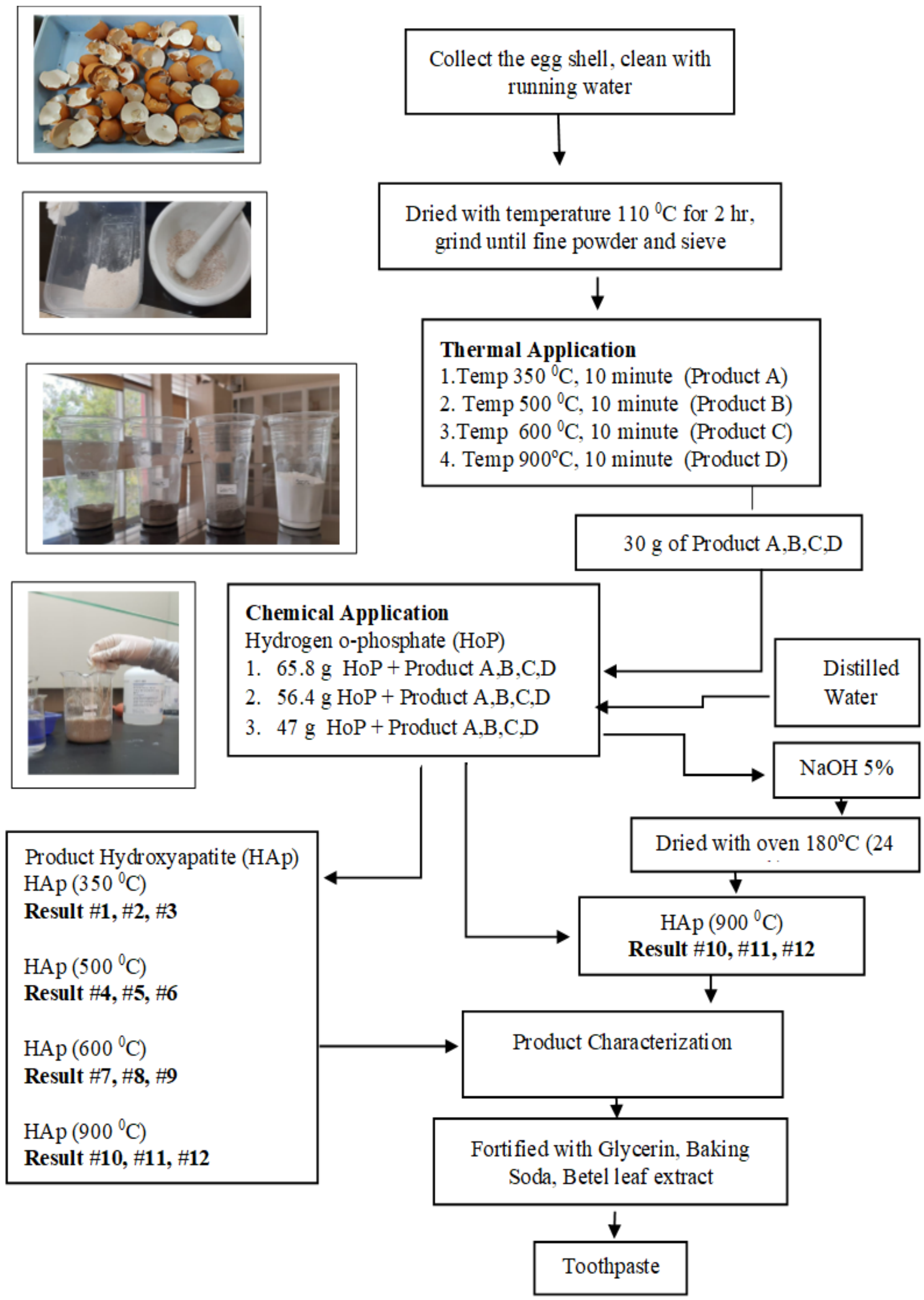


Figure 1. Flowchart of Hydroxyapatite Synthesis on Fabricate Toothpaste

4. Results and Discussion

The FTIR test in this study aims to detect functional groups, identify compounds and analyze mixtures in each product. In this test, there are four samples taken from different temperatures that have gone through the calcination process first.

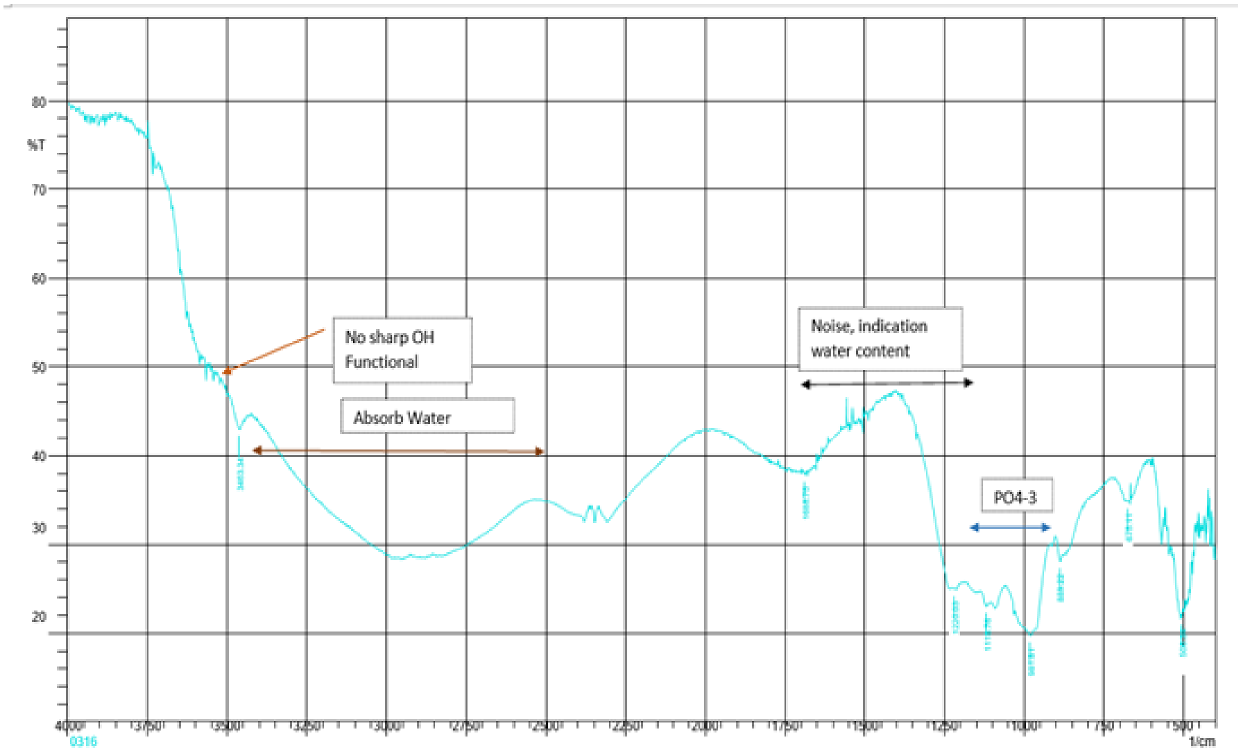


Figure 2. FTIR result 350 ^{0}C Calcination

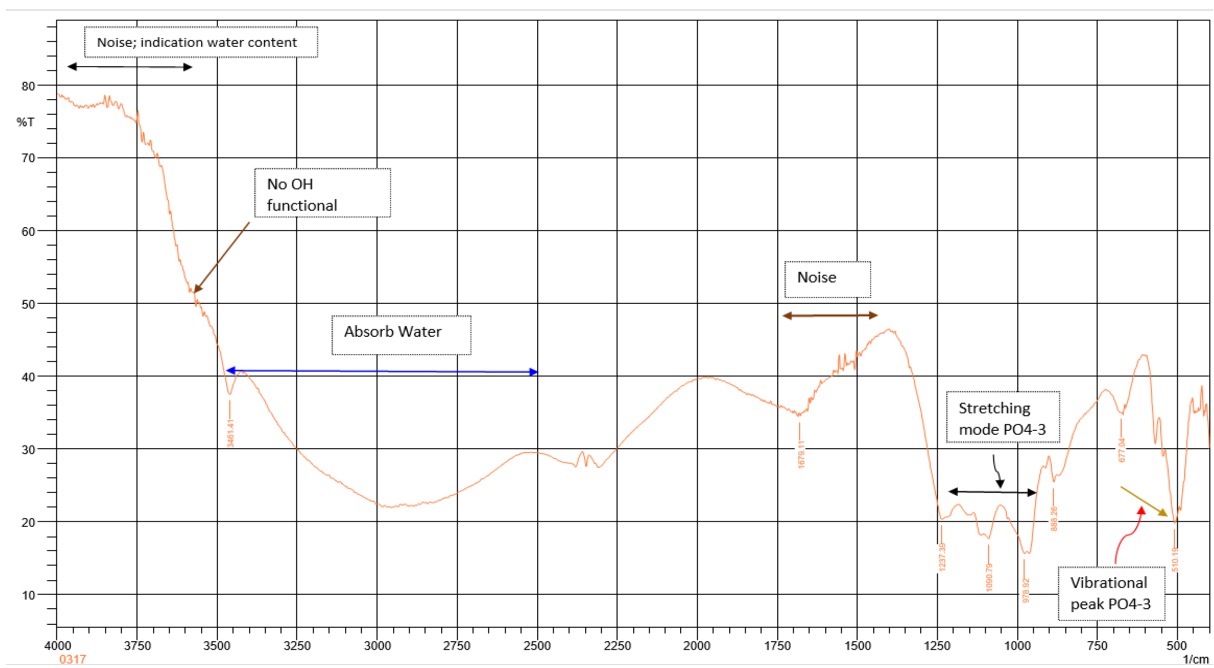


Figure 3. FTIR result 500 ^{0}C Calcination

At 350 ^{0}C there is no OH functional group. There is enough noise a lot in the wave range of 1350 – 1700 cm^{-1}. At the wave number 981.81 cm^{-1} – 1220.03 cm^{-1} which indicates the group (PO_4^{-3}) with a strong band that usually ranges in the wavenumber 1000-1150 cm^{-1}. Likewise, at a temperature of 500 ^{0}C, there is no OH functional group (Figure 2 and Figure 3). There is noise which was quite a lot in the wave range of 1450 – 1700 cm^{-1}. The wavenumber 978.92 cm^{-1} – 1237.39

cm^{-1} shows the (PO_4^{-3}) group which is marked by a strong and sharp band caused by the greater the amount of

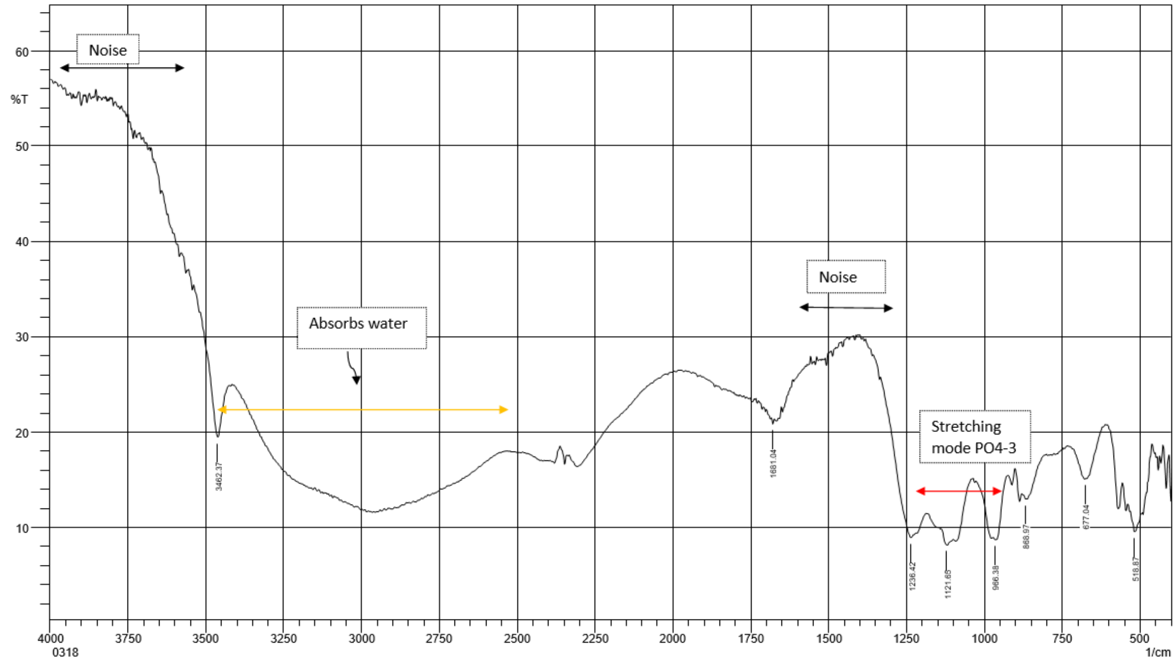


Figure 4. FTIR result 600 ^{0}C Calcination

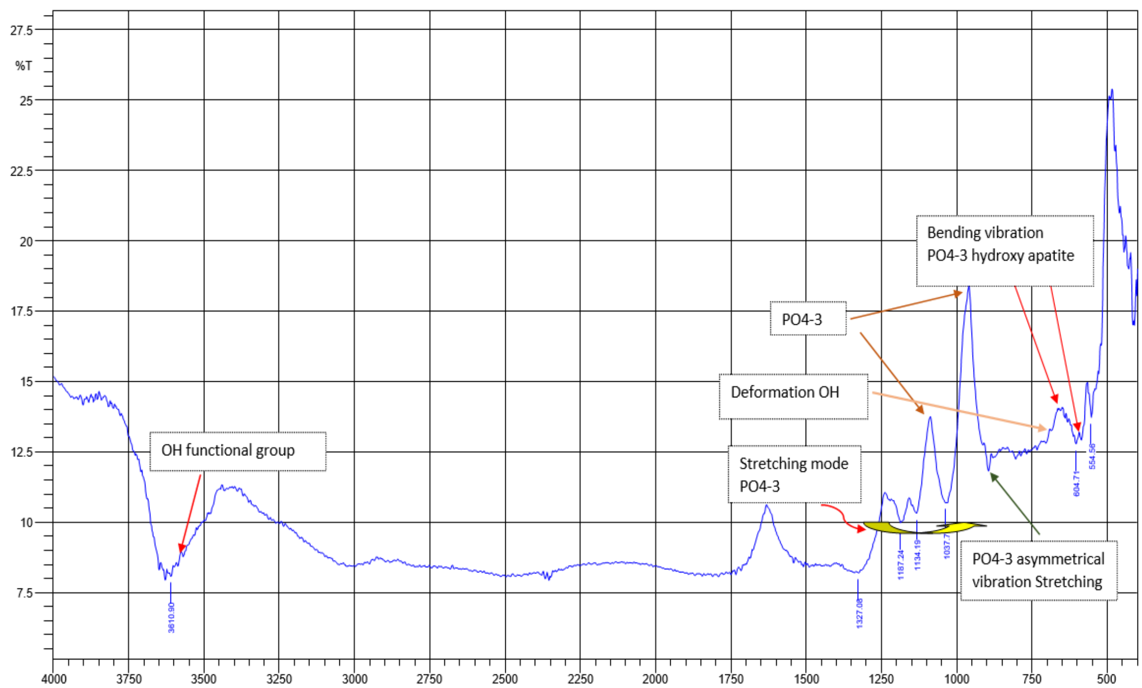


Figure 5. FTIR result 900 ^{0}C Calcination

phosphate reacted with calcium, the more phosphate groups are formed. formed which is found in the wavenumber 1000-1150 cm^{-1} (Pattanayak et al., 2005) (Figure 4 and figure 5). At a temperature of 600 ^{0}C, there is still no OH functional group. The noise is less in the wave range of 1350 – 1450 cm^{-1}. The wavenumber 996.38 cm^{-1} – 1267.42 cm^{-1} shows the group (PO_4^{-3}) which is marked by a strong and sharp band. At a temperature of 900 ^{0}C, there is an OH functional group in the wave range of 3500 - 3600 cm^{-1}. The wave range of 950 cm^{-1} – 1100 cm^{-1} shows a group (PO_4^{-3}) that was characterized by a strong and very sharp band. Calcium carbonate from eggshells is used as a base for making composite pastes because of its ability to bind dirt and strengthen teeth (Table 1).

Table 1. Summarize of FTIR Spectrum

Sample	Thermal Application	Wave Number (cm^{-1})	Indication
1	350 ^{0}C	510	Vibration PO_4^{-3}
		900 – 1250	Vibration PO_4^{-3}
		3571	No OH functional group
2	500 ^{0}C	900 – 1250	Vibration PO_4^{-3}
		3571	No OH functional group
3	600 ^{0}C	900 – 1250	Stretching Mode PO_4^{-3}
		3571	No OH functional group
4	900 ^{0}C	560,610	Bending Vibration PO_4^{-3}
		740	Deformation of OH
		880 and 1300	Stretching mode PO_4^{-3}
		900	Asymmetrical vibration PO_4^{-3}
		910 – 1100	Existence of PO_4^{-3}
		3500 - 3600	OH functional group

As well as the addition of betel leaf extract which functions as an anti-bacterial. While the addition of 5% NaOH as a comparison. From the results of making toothpaste above, a pH test has been carried out before being allowed to stand for 2 weeks, the pH obtained is pH 7 of the three variations. Then the pH test was carried out again after being left for 2 weeks, the pH obtained was between 7-11. After the pH test, the shape of the three formulas that formed a paste was observed (Table 2).

Table 2. Observation Results of Toothpaste Samples Without NaOH sample 10-12

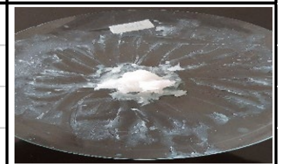
Eggshell fine Powder	Baking Soda	Glycerin	Betle Leaf Extract	pH	Organoleptic Appearance	Images
3 g	3 g	2.52 g	2.76 g	7	Semi Solid	
3 g	3 g	2.52 g	2.76 g	11	Dense Liquid	
3 g	3 g	2.52 g	2.76 g	10	Dense Liquid	

Table 3. Observation Results of Toothpaste Samples with NaOH sample 10-12

Eggshell fine Powder	Baking Soda	Glycerin	Betle Leaf Extract	pH	Organoleptic Appearance	Images
3 g	3 g	2.52 g	2.76 g	7	Liquid	
3 g	3 g	2.52 g	2.76 g	11	Liquid	
3 g	3 g	2.52 g	2.76 g	10	Liquid	

Based on previous research 3 parts have been obtained (He et al., 2015), namely the liquid part, wherein the manufacture of composite paste if it is in liquid form it will come out from the tube too quickly so it was not included in the requirements (Table 3). If the shape is semi-solid, it will be able to prevent the release of composite paste from the tube and will be able to hold on to the toothbrush so this was included in the requirements for the composite paste. Meanwhile, if it is solid, it will be hard to expel from the tube. From the results of the research that we have done, we get two parts, namely liquid and semi-solid. Where that meets the requirements in the manufacture of toothpaste is the result of samples 1 and 4 with a semi-solid form (Figure 6 and figure 7).

Figure 6. Result of sample 1 to sample 9 After Dried in the oven

Figure 7. Result sample 10 – 12 after dried in the oven

6. Conclusion

From the results of the research that we have done, we get two parts, namely liquid and liquid and semi-solid. Where that meets the requirements in making toothpaste is the result of sample 1 without NaOH and sample 4 with NaOH in semi-solid form. In the FTIR Spectrometry Test, there is a range of wavenumbers from 900 to 1250 cm-1 which is formed in all samples showing the PO_4^{-3} group. Only sample 10 formed the OH group in the wave range of 3500 – 3600 cm-1. In samples 1 and 4 there is more noise or disturbance due to the presence of water than in sample 7 which tends to be less. Because the presence of water causes the thermal process to be not optimal in samples 1, 4, and 7. Thus, it is an indication that the thermal treatment up to 600 ^{0}C has not yet formed Hydroxyapatite (HAp). To ensure this, it is necessary to do a further XRD Test.

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