Engineering and Technology Journal e-ISSN: 2456-3358
Volume 09 Issue 11 November-2024, Page No.- 5482-5488
DOI: 10.47191/etj/v9i11.04, I.F. – 8.227

Innovative Use of Hydroxyapatite (HAp) from Pokea Clam Shells (*Batissa violacea var. celebensis*) for Toothpaste Manufacturing

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ABSTRACT: Innovation research on the use of hydroxyapatite from pokea clam shells (*Batissa violacea* var. celebensis) for toothpaste manufacturing has been successfully conducted. This study aims to determine how to synthesize and characterize hydroxyapatite (HAp) from pokea shells and the efficiency of its use in making toothpaste. The research process was conducted through hydroxyapatite synthesis and hydroxyapatite toothpaste formulation. The success of this synthesis was proven by the characterization of XRF spectrophotometer, FTIR spectrophotometer and digital optical microscope. The results of XRF analysis showed that the CaO content in the material was 88.21%. The results of FTIR analysis showed that in hydroxyapatite material there was absorption at wave numbers 926 cm^{-1} and 1024 cm^{-1} which showed asymmetric strain vibrations and bending vibrations of PO_4^{3-} groups. The results of XRD characterization of HAp show that the diffraction peak is at an angle of $2\theta = 20\text{-}57$ ° with a crystal size of 46.15 nm and a degree of crystallinity of 85.43%. Digital optical microscope test results at 500x and 1000x magnification, showed hydroxyapatite has a uniform and non-porous shape. The hydroxyapatite toothpaste produced is bone white in color with menthol aroma and slightly rough texture. The resulting toothpaste is homogeneous, the diameter of the distribution that meets the quality requirements of toothpaste is 6.833 cm. The pH measurement result on toothpaste is 5.82 which meets the toothpaste quality requirements. The result of viscosity measurement on toothpaste is 20,000 cpa.s which meets the requirements of toothpaste viscosity. The foam formed is 10 mm where the height of the foam formed has met the requirements. Hydroxyapatite toothpaste has good stability and strong antibacterial activity, where the clear zone formed is 18.7 mm

KEYWORDS: Pokea, hydroxyapatite, toothpaste and hydrothermal

I. INTRODUCTION

Oral and dental health cannot be separated from each other because the condition of both will have an impact on overall body health. Dental caries disease is still a major problem in oral health to date, the prevalence of which reaches more than 80% in Indonesia which continues to increase every year [1]. Caries is a disease of the hard tissues of the teeth caused by bacterial acids, resulting in erosion and tooth decay characterized by demineralization of the hard tissues of the teeth, namely enamel, dentin, and cementum. The formation of a tooth hole can take place after several demineralization events in the hard tissues of the tooth to show various colors, ranging from yellow to black [2].

Streptococcus mutans bacteria are one of the main pathogens that play an important role in the formation of dental plaque and the development of dental caries. This bacterium is a Gram positive acidogenic and aciduric that naturally occurs in the oral cavity [3]. Research shows that Streptococcus mutans can convert salivary sucrose into extracellular polysaccharides (PSE) through the process of glycoxidation and has a low pH, creating a favorable environment for the growth of other acidogenic and aciduric species [4].

Saliva has a protective effect against caries, but the protective effect and remineralization of caries naturally is not only a slow process but also clearly insufficient to protect individuals against caries [5]. Regular brushing with toothpaste is one of the common ways to promote remineralization and inhibit demineralization of enamel and dentin to prevent dental caries [6]. The most used toothpastes contain fluoride due to its wide availability and ease of use. Fluoride toothpastes also contain abrasives that help remove dental stains and provide adequate cleaning. However, this fluoride remineralization process is only effective on the outer surface while the inside is difficult to remineralizer. The efficacy of fluoride depends on the dose level used, the higher the fluoride, the better its role in demineralizing teeth. On the other hand, the addition of high doses of fluoride in toothpaste can result in the risk of fluorosis in children and toxicity in all ages. This limitation makes the use of fluoride less effective [5].

Another approach to preventing caries is to focus on biomimetic agents that stimulate the remineralization process and inhibit demineralization of dental hard tissues. One of these biomimetic agents is hydroxyapatite (HAp) particles. Hydroxyapatite has been the subject of study in various fields of preventive oral health care [7]. Unlike fluoride, when hydroxyapatite is accidentally ingested through toothpaste, it does not pose systemic health risks such as fluorosis because hydroxyapatite is a major inorganic component of all human hard tissues, including teeth and bones [8]. The content of hydroxyapatite in toothpaste as an active ingredient has two roles, namely as an antibacterial agent and as an agent to remineralizer teeth [9]. Compared to fluoride, which is limited to surface remineralization, toothpaste containing hydroxyapatite has been shown to be able to remineralizer the deeper layers of enamel and can remineralizer the tooth surface, reduce caries and dentin hypersensitivity in long-term use [10].

Hydroxyapatite (HAp) is formed from calcium and phosphorus elements with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ which is the main calcium phosphate mineral in human mineralized tissues, namely teeth and bones. Bio metallic hydroxyapatite particles are opaque white in color that can repair enamel surface defects [11]. Hydroxyapatite can be synthesized using materials that can be extracted from calcium-containing sources found in the teeth and bones of living organisms [12]. Raw materials for making hydroxyapatite are widely found in nature, such as limestone, eggshells, coral and shells [13].

Pokea shells are one type of shellfish that can be used as raw material for hydroxyapatite. Pokea clams (*Batissa violacea var. celebensis*) belong to the *Corbicullidae* family which is distributed in several regions such as Sumatra, Java, West Papua, and Sulawesi [14]. Pokea clams have long been consumed by people in the Southeast Sulawesi region and even the public demand for Pokea meat is increasing. Agusriyaddin et al., [15] reported that in a year Pokea shells distributed in Pohara Market reached 152,879 kg wet weight. The shells caught by fishermen are only utilized while the shells become unused waste. Pokea shell waste can have a negative impact on the environment if not managed properly. Organic waste from decomposing Pokea shells can produce methane, a powerful greenhouse gas. Anaerobic decomposition (without oxygen) by bacteria produces methane, which contributes significantly to global warming and under aerobic conditions (with oxygen), the decomposition of organic matter can produce CO_2 as a major greenhouse gas.

Pokea shells also have great potential to be used in the manufacture of HAp which is a form of calcium phosphate that is widely used in biomedical applications. Pokea shells contain calcium carbonate ($CaCO_3$) which is high at 98% so that in this study hydroxyapatite will be made from pokea shells (Batissa violacea var. celebensis) which will be used for making toothpaste.

II. METHODS

Tools and Materials

The tools used in this study include Furnace, hydrothermal reactor, X-ray Fluorescence (XRF), Fourier Transform Infra-Red (FTIR) (Spectrum two system (L160000A) Perkin Elmer), X-Ray Diffraction (XRD) (D2 Phaser Merck Bruker) and digital optical microscope (DM-1000S).

The materials used in this study include Pokea shells taken from the Pohara river in Konawe Regency, ammonium hydrogen phosphate ($(NH_4)H_2PO_4$), Mueller Hinton agar (MHA), chloramphenicol, *Streptococcus mutans* bacteria, carboxymethyl cellulose sodium (Na-CMC), glycerin, sodium lauryl sulfate (SLS) and paper mint oil solution.

Pokea (*Batissa violacea var. celebensis*) Shell Preparation

The pokea shells were cleaned of impurities by washing with water and then drying in direct sunlight for two days until dry. Cleaning of the black-colored back of the pokea shell was done using sandpaper. The clean shells were then pulverized with a mortar and pestle and sieved with a 220 *mesh* sieve. The shell powder was then calcined using a furnace at 900°C for 4 hours to produce calcium oxide (CaO).

Hydroxyapatite (HAp) Synthesis from Pokea Shells

14 g CaO and 17.25 g $NH_4H_2PO_4$ were dissolved in 100 mL distilled water to obtain a CaO: $NH_4H_2PO_4$ substrate mole ratio of 1.67. The mixture was put into an autoclave to be heated in an oven at 230ºC for 48 hours. The results of heating in the form of solids were filtered with Whatman filter paper no. 42 and washed until the pH was neutral. The process of removing the remaining water in the solid was carried out by heating the solid at 105ºC for 24 hours and continued with the sintering process at 900ºC.

Formulation of Hydroxyapatite (HAp) into Toothpaste

A total of 0.75 g Na CMC was put into the mortar and dissolved with enough hot water, then stirred until homogeneous or until it got a gel-like texture. After that, 1 g of SLS and 12.5 mL of glycerin were added and stirred until homogeneous. The next step, 21 g of HAp powder was added little by little and stirred until homogeneous. The last step is the addition of 0.25 mL of paper mint oil solution and stir until homogeneous or the texture becomes toothpaste.

Hydroxyapatite Toothpaste (HAp) Test

Organoleptic Test

Organoleptic testing of toothpaste is done by observing visually and taste which will be carried out by 20 respondents on color, taste, aroma and texture.

Homogeneity Test

The homogeneity test was carried out by applying 1 g of toothpaste preparation on a transparent glass and then visually observed in an inverted position.

Spreadability Test

The spreadability test was carried out by applying 1 g of toothpaste on the glass and then covering it again with transparent glass and then given a load weighing 200 g, then measuring the diameter of the spread.

pH test

The pH test was carried out by dissolving the paste preparation (1 g) with 10 mL distilled water. The pH meter used was the OHAUSS brand pH meter.

Viscosity Test

Toothpaste was weighed as much as 0.5 g, then placed on the viscometer plate and installed a cone and plate type spindle 5/30 mm. Then set the Rheosys Micra software on the computer connected to the viscometer according to the test to be carried out and wait until the test results appear.

Foam Formation Test

0.1 g of toothpaste was put in a measuring cup and dissolved with 10 mL of water. Shake the measuring cup for 1 minute, then measure the height of the foam formed at minute 0 and minute 5 after shaking.

Stability Test

The toothpaste was placed at 4°C for 24 hours, then placed at 40°C for 24 hours (1 cycle). Six cycles of testing were conducted and physical changes in the preparation at the beginning and end of each cycle were observed, including organoleptic evaluation and phase separation.

Anti-bacterial Test

Antibacterial activity against *Streptococcus mutans* was performed using the pitting technique. *Streptococcus mutans* bacteria were used as test bacteria. Making MHA media in a Petri dish using the pour plate method and allowed to stand until it solidifies, then placed a shard on top of the solid media to form pits. The test bacteria were then poured into the liquid media and homogenized, then poured back into the petri dish. test samples were taken and inserted into the wells formed. The positive control used is chloramphenicol, while the negative control uses sterile distilled water. Then the bacteria were incubated for 1×24 hours at 37°C and then observed for inhibition, namely the clear zone formed and determined the results of the bacterial inhibition test.

III. RESULTS AND DISCUSSION

Hydroxyapatite synthesis begins with the preparation of Pokea clam shells. This process involves heating at high temperatures using a furnace with temperatures reaching 900°C. The weight of the pokea shell sample before calcination was 28.204 g and 18.647 g after calcination with a % yield of 66.11%.

The synthesis of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) was carried out by hydrothermal method using calcium oxide (CaO) and ammonium hydrogen phosphate ($(NH_4)\ H_2PO_4$)) precursors which can be represented by the following chemical equation:

$$10Ca\ (OH)_2 + 6(NH_4)\ HPO_4 \rightarrow Ca_{10}\ (PO_4)_6\ (OH)_2 + 6NH_3 + 18H_2O$$

In this reaction, calcium hydroxide ($Ca\ (OH)_2$) and ammonium hydrogen phosphate react to form hydroxyapatite, ammonia (NH_3), and water (H_2O). The resulting yield of hydroxyapatite synthesis is 67.54%.

X-Ray Fluorescence (XRF)

The results of compounds in Pokea shells are presented in Table 1.

Table 1. XRF analysis results on pokea shells

No.	Compound	Content (%)
1	CaO	88.214
2	SiO_2	1.059
3	MgO	0.721
4	Al_2O_3	0.211
5	SO_3	0.172
6	Fe_2O_3	0.135
7	K_2O	0.105
8	Na_2O	0.032
9	MnO	0.026
10	P_2O_5	0.016
11	SrO	0.011
12	LOI	9.26

Table 1 shows that the dominant compound contained in pokea shells is calcium oxide (CaO), which is 88.214% after the calcination process. In addition, there are other metal oxides such as MgO, Al_2O_3, SiO_2 , SrO, K_2O, Fe_2O_3, SO_3, MnO, P_2O_5 and Na_2O with low presentations.

Fourier Transform Infra Red (FTIR) Analysis

Analysis using an FTIR instrument was carried out to determine the functional groups contained in the hydroxyapatite constituent compounds. The results of the functional group analysis on the sample are shown in Table 2.

Table 2 Results of FTIR Analysis of HAp

Function Group	Waves numbers (cm^{-1})
PO_4^{-3} strain	561; 926
PO asymmetry$_4^{-3}$	1024
OH strain	3572

in Table 2 show that there are peaks of PO_4^{3-} vibrational at 561-926 cm^{-1} , PO_4^{3-} asymmetry strain at 1024 cm-^{1} , OH strain at 3572 cm^{-1}. Typical carbonate (CO_3^{2-}) absorption identified at 1407 cm^{-1} and 1518 cm-^{1} is not visible, this is thought to be due to the sintering temperature at a high temperature of 900ºC which allows the release of CO_3^{2-} groups from the hydroxyapatite structure.

X-Ray Diffraction (XRD) Analysis

XRD characterization was carried out on hydroxyapatite material with testing carried out at angles 2θ = 20° - 60° and **λ** Cu-ka = 0.154 nm. The results of XRD characterization are shown in Figure 1.

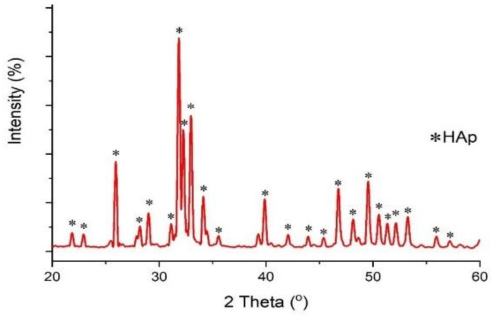

Figure 1 XRD pattern of hydroxyapatite material

Figure 1 shows that the hydroxyapatite material is indicated at 29 angular peaks of 2θ = 20-57° and the typical peak of HAp identified at 31,6°. This result was confirmed with Hutabarat research on HAp-La_2O composites[3] that the typical peak of hydroxyapatite was identified at of 2θ = 31.7°. Calculation of hydroxyapatite crystal size at angles 2θ = 20-57° obtained an average of 46.15 nm with a degree of crystallinity of 85.43%.

Digital Optical Microscope Characterization

Analysis using a digital optical microscope aims to identify the morphology of the hydroxyapatite composite. Morphological images were taken using a digital microscope with 1000x magnification. The *capture* results were then processed using the Image application to produce clearer images. The analysis results obtained are presented in Figure 2.

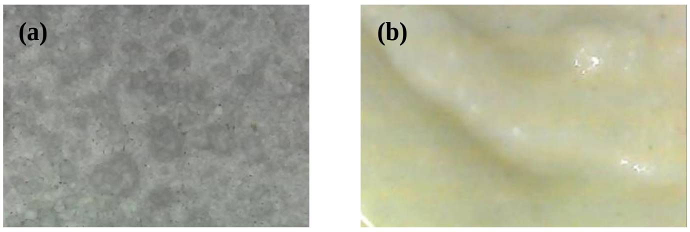

Figure 2 Optical microscope analysis results (a) HAp micrography (b) hydroxyapatite toothpaste micrography

Figure 2 shows that the distribution of hydroxyapatite particles is evenly distributed that indicating that the dispersion particle is homogeneous. The shape and morphology of the hydroxyapatite particles are quite uniform, indicating good homogeneity in the toothpaste.

Organoleptic Test

The organoleptic test observation results are presented in Table 3.

Table 3 Organoleptic test results of hydroxyapatite toothpaste

Organoleptic test		Respondents (%)
Color	Bone white	100
Aroma	Menthol-scented	100
Texture	Smooth	20
	Somewhat rough	80
Taste	Slightly sweet	45
	There is no sense of	55

Based on the organoleptic test results presented in Table 3, it can be concluded that 100% of respondents said that the hydroxyapatite toothpaste preparation was bone white with a menthol aroma. In addition, as many as 20% of respondents said the toothpaste produced was smooth 80% of other respondents said the texture of the toothpaste was slightly rough when palpated. The slightly rough texture comes from hydroxyapatite powder. The hydroxyapatite particles will settle in demineralized areas and help restore the strength and hardness of tooth enamel. The presence of hydroxyapatite powder in toothpaste also indicates that the toothpaste has strong abrasive properties so that it can remove stains and plaque on the teeth optimally [16]. As many as 45% of respondents said that the taste of toothpaste was slightly sweet coming from the paper mint oil solution, but 55% indicated that there was no taste in the toothpaste.

Spread ability Test

The results of the hydroxyapatite toothpaste spread ability test can be seen in Figure 3

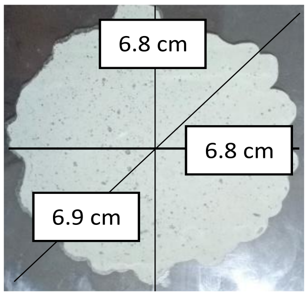

Figure 3 Spread ability results of toothpaste

Based on the test results that can be seen in figure, which is in the range of diameter distribution of 6.8 - 6.9 cm with an average diameter distribution of 6.833 cm. These results are still categorized as safe because the spread ability obtained is in accordance with the requirements for good spread ability for toothpaste preparations, namely 5-7 cm, which shows a semi-solid consistency that is very comfortable in use.

pH test

The pH measurement test results on hydroxyapatite toothpaste are 5.82 as shown in Figure 4.9 which meets the quality requirements of SNI 12-3524 toothpaste, 1995, with a pH range of 4.5-10.5 [17] so that the toothpaste is safe to use and will not cause irritation in the oral cavity.

Viscosity Test

Based on the results of the hydroxyapatite toothpaste viscosity test which can be seen in Figure 4.10, it shows that the viscosity value of hydroxyapatite toothpaste is 2000 dpa.s or 20,000 cpa.s which meets the toothpaste viscosity requirements because according to SNI standards (12-3524-1995) the viscosity value of toothpaste ranges from 20,000-50,000 cpa.s [18].

Foam Formation Test

The results of the foam formation test on hydroxyapatite toothpaste can be seen in Table.

Table 4 Foam height of hydroxyapatite toothpaste

Observation after shaking (minutes)	Foam height (mm)
0	10
1	9
2	6
3	3
4	1
5	1

The results of the toothpaste foam height test which can be seen in Table 4 are in the range of 10 mm in the 0th minute observation after shaking. Based on the test results, the hydroxyapatite toothpaste preparation formula has met the requirements. The maximum foam height requirement for toothpaste is 15 mm. Hydroxyapatite toothpaste can be a good choice for those with sensitive mouths or who experience irritation from detergents contained in toothpaste.

Stability Test

Accelerated stability tests were conducted to illustrate the conditions of storage over a long period of time. The preparation was placed in a climatic chamber at 4° C for 24 hours and 40° C for 24 hours (1 cycle). The treatment was repeated for 5 cycles. The results of the stability test can be seen in Figure 4.

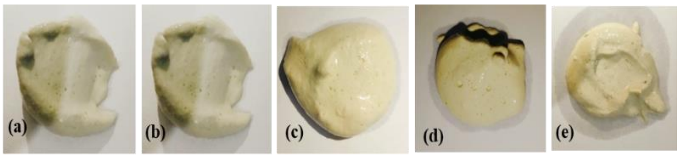

Figure 4 Hydroxyapatite toothpaste stability test results (a) 1st cycle (b) 2nd cycle (c) 3rd cycle (d) 4th cycle (e) 5th cycle

The results show that in the 1st cycle, 2nd cycle, 3rd cycle, 4th cycle and 5th cycle there were no significant changes in color, aroma, shape, and taste in the hydroxyapatite toothpaste preparation. This indicates that the hydroxyapatite toothpaste produced has excellent stability.

Anti-bacterial Test

The bacteria used were Streptococcus mutans, chloramphenicol as positive control and sterile distilled water as negative control. The presence of antibacterial activity is characterized by the appearance of a clear zone formed around the wells after incubation for 24 hours. The larger the diameter of the clear zone formed, the better the antibacterial activity of a substance. The results of the antibacterial test activity can be seen in Figure 5

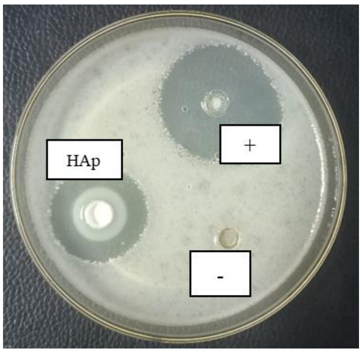

Figure 5 Antibacterial test of hydroxyapatite toothpaste

Table 5 Inhibition zone

Sample	Diameter (mm)			Inhibition zone	Inhibition Strength
	Dv	Dh	Dd		
Distilled water (-)	0	0	0	0	None
Chloramphenicol (+)	35	35	36	29,3	Very strong
Hydroxyapatite	22	26	26	18,7	Strong

Dv = vertical diameter, Dh = horizontal diameter, Dd = diagonal diameter

Antibacterial activity was carried out using a positive control of chloramphenicol which served as a control of the test substance, by comparing the diameter of the inhibition area formed. While the negative control in the form of distilled water serves to determine whether or not the solvent affects the growth of test bacteria. Based on Table 5 shows that the diameter of the inhibition zone of hydroxyapatite against *Streptococcus mutans* bacteria has a strong inhibition zone, this indicates that hydroxyapatite biomaterials have an effect on strong antibacterial activity [19].

CONCLUSIONS

Hydroxyapatite (HAp) synthesis from pokea shell waste (*Batissa violacea var. celebensis*) has been successfully conducted using hydrothermal method. The results of FTIR analysis showed that the hydroxyapatite material had absorption at 598 cm^{-1} and 1024 cm^{-1} which showed asymmetric strain vibrations and bending vibrations of the PO_4^{3-} group. The results of XRD characterization of HAp showed that the diffraction peak was at an angle of 2θ = 20-57° with a crystal size of 46.15 nm and a degree of crystallinity of 85.43%. HAp has been successfully used to make toothpaste. Organoleptic test results of hydroxyapatite toothpaste are bone white with menthol aroma and slightly rough texture which is hydroxyapatite nanoparticle powder. The resulting toothpaste is homogeneous, the results of a good distribution diameter of 6.833 cm. The pH measurement results on toothpaste are 5.82 which meets the quality requirements of SNI toothpaste (12-

3524, 1995). The results of viscosity measurements on toothpaste are 20,000 cpa.s which meets the requirements for toothpaste viscosity according to SNI standards (12-3524-1995). The foam formed from hydroxyapatite toothpaste is 10 mm where the height of the foam formed has met the requirements. Hydroxyapatite toothpaste has good stability and strong antibacterial activity, where the inhibition zone formed is 18.7 mm.

ACKNOWLEDGMENT

We would like to thank Department of Chemistry Laboratory and Faculty of Medicine Laboratory, Halu Oleo University, Indonesia for its support in conducting this study.

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