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REVIEW ARTICLE: UTILIZATION OF FISH BONES INTO HYDROXYAPATITE

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ABSTRACT

Hydroxyapatite (Ca5(PO4)3(OH)) is a naturally occurring inorganic element derived from bone that can be utilized for bone regeneration, repairing, filling, expanding and reconstructing bone tissue. Hydroxyapatite has perfect biocompatibility properties when implanted in the bones. In addition, hydroxyapatite can also be used as an adsorbent to overcome environmental pollution against heavy metals. This article aims to explain how to process waste from fish bones into hydroxyapatite. Based on study literature obtained information that the manufacture of hydrosiapapatite from fish bones there are 4 methods namely hydrothermal, electrodeposition, sol gel and precipitation.

Keywords: method, biocompatibility, adsorbent, waste

INTRODUCTION

The demand for biomaterial applications has increased in recent years, including due to an increase in the number of the world's population in old age, increasing numbers of accidents, and the presence of various diseases in Indonesia, where there are many cases of bone damage or trauma triggered by unhealthy dietary factors, cases of accidents and natural disasters, birth defects, and infections and tumors. Based on data from bone trauma sufferers who were treated at DR. Soetomo General Hospital Surabaya in 2001-2005, showed that bone fractures due to traffic accidents were about 64.38%, where cases in mandibles (lower jaw) and maxillary (upper jaw) ranked the most at 29.85%, zigoma fractures (facial skeleton) 27.64% and nasal (nose) fractures 12.66%. To help the healing process of bone fractures in large fracture cases, one strategy is to provide bone implant material that is temporary and often in the form of bone scaffold (bulk shape) or bone

graft (thin plate). Aside from synthetic materials such as hydroxyapatite, bone graft material can be taken from the patient's own body (autograft), from the body of another patient who has close family relationships (allograft), or from animals (xenograft).

Fish bones are one of the residual forms produced from the processing of fish that contain minerals that are quite high compared to other parts of the body because the main elements of fish bones are calcium, posfor and carbonate (Mutmainnah, Sitti Chadijah, 2017). Bones are composed mainly of calcium (Ca) and posfor (P) which biomineralize and form calcium phosphate compounds (Indrani & Adi, 2012). Fish bones contain 60-70% minerals with constituent components in the form of 30% collagen protein and most bioapatites, including hydroxyapatite, carbonated apatite or dahlite (Riyanto, Maddu & Rahman, 2013). Utilization of fish bone waste in a flour processing industry as a source of calcium (Ca) is one alternative in order to provide a food source rich in calcium (Ca) while reducing the adverse effects of environmental pollution due to the disposal of waste from the fish processing industry. The purpose of writing this paper is to explain how to process waste from fish bones into hydroxypiteite.

Product Manufacturing Process

Hydroxyapatite for industrial applications is generally obtained by synthetic methods. Some synthetic methods used to produce hydroxyapatite include hydrothermal, electrodeposition, gel soles and precipitation (Nayak & Kumar, 2010). Here is the synthesis method used to produce hydroxyapatite:

1. Methods of precipitation

It is the most commonly used method because it is considered the simplest for hydroxyapatite synthesis (Mohammad, Othman & Yee-Yeoh, 2014). The stability of hydroxyapatite synthesized through precipitation methods is easily influenced by pH, so it is necessary to add a solution that is alkaline until it reaches pH 10.

Working Procedure:

a. Sample Preparation

- Fish bones washed with tap water
- boiled for ±2 hours
- then cut into pieces and dried for 14 days with sunlight.
- After that the fish bones are cleaned from soft tissue and washed with aquades.
- Then dried with sunlight.

- After drying, tuna bones are soaked with acetone (CH3COCH3) for 3 x 24 hours (every 1 x 24 hours solvent replacement) is done)
- Steam at room temperature until the aroma of acetone is gone.
- After that, tuna bones are heated in the oven for 4 hours at a temperature of 105 °C and forged into smaller parts.

b. Manufacture of Calcium Oxide (CaO)

- Tuna bone weighed as much as 80.04 grams
- Oven for 2 hours.
- Tuna fish bones are calcined at 900 °C for 5 hours
- The weight of the sample and smooth it with a lumpang. Then iedakwith a sying 125 mesh. The resulting CaO powder is analyzed with Energy Dispersive XRay Flouresence (EDXRF).

c. Analysis with XRF

The tools and monitors are turned on so that on the monitor will appear display display for the operation steps of the XRF tool. Before the tool is optimized, the tool calibration is first carried out. When the XRF is operated the spinner of the holder sample will move towards the holder and stop automatically. The DX-95 digital display display will show the numbers. The measurement conditions are at a voltage of 14 kV and the current strength is 90 μA. Sample measurements will occur for up to 5 minutes.

d. Hydroxyapatite Synthesis

- CaO weighed 7.4094 grams.
- put in a 300 mL chemical glass and add 100 mL aquades so that a suspension will form.
- After that, stirring is done at a speed of 700 rpm for 1 hour at a temperature of 90 ° C
- Add with a solution of posfat acid 0.6 M as much as 100 mL slowly at a speed of 1 mL / minute using burette.
- After the solution of posfat acid is exhausted, stirring continues the speed of 700 rpm for 1 hour at a temperature of 90 ° C. the pH of the solution is

regulated using a solution of sodium hydroxide (NaOH) 1 M until it reaches pH 10.

- let the solution be left for, 12, 24, 36, 48 and 60 hours so that the deposit will form.
- The obtained deposits are filtered using a buchner funnel ±4 hours and washed with aquades as much as 3 times washing.
- Heat the sediment for 2 hours at 105 °C and heated at 900 °C for 5 hours.
- The resulting weight of the powder is then weighed. After that, the rendmen of hydroxyapatite obtained are calculated.

e. Analysis with FTIR

- Samples in powder form are eroded to meet particle sizes of less than 2 μm.
- Put it in the pellet press evenly. The pellet press is connected to the hydraulic company pump as well as the vacuum pump for 15 minutes. Pellets formed in the brush have a thickness of 0.3 mm (transparent). Next the pellet is carefully opened and moved into a sle holder using a spatula. After that, the device is set at normal speed and transmission expansion of 100 x. after that, the function group analysis is carried out.

f. Hydroxyapatite Analysis with XRD

- use the tool calibrated first and XG control (in the form of current), water flow, shutter and door open set. While waiting for the calibration of the tool, as much as 2 mg of the sample
- placed inside the holder on the difraktometer. The voltage used is '40 Kv and the current generator is 30 mA with a wavelength source of 1.5406 Å. The results obtained in the form of a difractogram are identified based on intensity and angle 2θ. The phase determination appears in reference to the Joint Committee on Powder.

2. Sol-Gel Method

This method has the advantage of being widely developed as a technique in producing a powder with purity, crystallineity, high reactivity, and the process using low temperatures, this method can also increase the mixing of molecules from

calcium and phosphorus (Nayak 2010). So here researchers are interested in making hydroxyapatite from mackerel bones as a source of calcium.

Working Procedure:

a. Sample preparation

- Boil fish bones as much as 2 kg at a temperature of 80oC for 30 minutes. Do cleaning of meat that is still attached and washing with water and washed again with aquadest
- Soak with acetone solution for 3x24 hours with solvent change Every 1x24 hours.
- Dried tuna bones are then calcined using a furnace at a temperature of 900oC for 5 hours, The sample is eroded after which the sample is syak with a syak of 100 mesh before the dioven sample with a temperature of 105oC for 30 minutes.

b. Manufacture of 80% H3PO4 Solution

- Memipet posfat acid as much as 94.11 mL
- put in a 100 mL measuring pumpkin then dissolved with aquades after which it is crushed to the limit mark

c. Analysis with X-Ray Flourescence (XRF)

Turn on the device and monitor, when turned on appears display display on the monitor for the operation step of the XRF tool on the counter view on the screen shows the number 0 to 10 cps, calibration is done first. Next when XRF operated spinner sample holder with holder (sample place) measuring 3 cm which amounts to 10 holes in one The disc will move towards holder one position and stop automatically. The DX-95 digital dispaly display will show the same number.

d. Synthesis of hydroxyapatite by adding post acid (H3PO4)

- Weighing calcium oxide powder (CaO) as much as 5.0013 grams later dissolved with 96% ethanol as much as 25 ml,
- 80% H3PO4 solution of 25 mL that has been made is inserted into the burette. Samples mixed with Ethanol 96% dripped with posse acid

(H3PO4) while stirred with using magnetic stirr at a speed of 300 rpm at a temperature of 37^{o}C for 2 hour

- The sample is heated using a water heater at a temperature of 60^{o}C for 1 hour then the sample is silenced for 1x24 hours then the sample is stirred back using a magnetic stirer until the sample is gel-shaped.
- The gel that has been formed is heated by putting it in the oven at a temperature of 105^{o}C for 12 hours after which the sample is stretched to a temperature of 400^{o}C, 600 ^{o}C, 900^{o}C for 5 hours then samples tested using FTIR, XRD and XRF. After synthesis, the results obtained can be calculated by using the formula:

Yield = Final Weight of Sample / Initial Weight of Sample x 100%

e. Analysis with Fourier Transform Infra Red (FTIR)

- Prepare the sample then mixed with KBr with a ratio of 1:10 (sample: KBr)
- After mixing the sample is solidified by using pressure by using a hydraulic compression pump with a power of 100 tons (kg newton) and vacuum pump for 15 minutes. Which aims to Make pellets. The pellets formed have a thickness of 0.3 mm (transparent)
- Then opened pellet carefully and transferred into the holder cell using a spatula after the sample is inserted into the FTIR tool.
- Then peak-peak read monitor then determine and analyze the function group.

f. Identification of Hydroxyapatite using X-Ray Difraction (XRD)

- calibrate the tool and set the XG control in the form of current, water flow shutter and door open.
- Then while waiting for the calibration of the tool, as much as 2 mg The sample is placed in a holder measuring (2x2) cm2 on the difraktometer. The voltage used is 40 kV and the current of the generator is 30 mA with a CuKα source (λ = 1.5405 Å) resulting in a graph that Identified based on the intensity and angle of 2 theta formed.
- The determination of the phase that appears refers to the Joint Commite on Powder Difraction Standard (JCPDS).

3. Hydrothermal methods

In this method in the process a single crystalline solid, pure particle or nanoparticle is formed. The advantages of hydrothermal methods are that they can accelerate the interaction between solid and liquid matter, can form pure phases and homogeneous materials, high diffusion, low viscosity, and increased soluble power (Yoshimura 2008)

Working Procedure:

a. Sample Preparation

- The waste of fish bones is cut into smaller pieces and then boiled in boiling water for 30 minutes.
- Fish bones that have been boiled are washed thoroughly using running water then continued with aquades and then dried in direct sunlight.
- Then the fish bones are soaked with acetone solution for 3 X 24 hours with a different acetone solution every day
- dry fish bones at room temperature until acetone completely evaporates (Juraida, et al 2011)
- fish bones then in the oven for 30 minutes using a temperature of 115^{o}C
- Then calcined into a furnace at 1000oC for 5 hours after calcination of fish bones then eroded using mortal and lumpang until it becomes a smaller parikel and to uniformize particle size then in the ayak by using ayakan measuring 125 mesh.

b. Hydroxyapatite Synthesis

- CaO powder is weighed by 5,117 grams and then dissolved with 100 mL aquades in Erlenmeyer 250 mL. The mixture is then added a solution of ammonium dihydrogen phosphate (NH4H2PO4) 100 mL
- The mixture solution is then homogenized using a magnetic stirrer at a speed of 300 rpm with a temperature of 90oC for 1 hour.
- After magnetized stirrer solution then sterilized using autoclave for 2 hours with a temperature of 121OC and pressure of 1 atm, this process is a hydrothermal process.

- After going through the process of sterilization the solution is then filtered with

  Using whatman filter paper No. 42 then the deposits contained in the filter paper are washed by using aquades as much as 3 times to remove the remains of ammonium.

- The acquired deposits are then put into the crutch cup that has been

  Known empty weight. The precipitate is heated at 105^{o}C for 30 minutes to remove the rest of the water

- After heating continued for the calcination process using a furnace at a temperature of 900oC for 5 hours.

- Calculate the yield obtained after the synthesis process using the formula next

  Yield = Final Weight of Sample / Initial Weight of Sample x 100%

c. Analysis with X-Ray Flourescence (XRF)

Turn on the tool and monitor, when turned on appears display display on the monitor for the operation step of the XRF tool, calibrate first. Furthermore, when the XRF is operated the spinner of the sample holder with a holder (sample place) measuring 3 cm which amounts to 10 holes on one disc will move towards the position of holder one and stop automatically on the DX-95 digital dispaly display will show the same number. Measurement conditions at a voltage of 14 kV and a current strength of 90 μA each measurement take 5 minutes.

d. Analysis with Fourier Transform Infra Red (FTIR)

- Sample in the form of crystal / powder hydroxyapatite, pounded to meet the particle size less than 2 μm, then

- Put it in the press pellets evenly. The pellet press is connected to a hydraulic company pump with a force of 100 tons (kg newton) and a vacuum pump for 15 minutes. The pellets formed have a thickness of 0.3 mm (transparent),

- Then opened the pellet carefully and transferred into the holder cell using a spatula.

- After that set the Infra Red Spectrophotometer device with paper speed at a "normal" position and transmission expansion "100 x". Checked the scale of the paper through the making of the spectrum of filmpolystiren. When the

paper scale is precise, in the same way the Infrared spectrum is made from the sample that has been prepared.

- Then determine and anlisa its function groups

e. Identification of Hydroxyapatite Using X-Ray Difraction (XRD)

- Calibrate the tool and set the XG control in the form of current, water flow, shutter, and door open.
- Then while waiting for the calibration of the tool, as much as 2 mg of the sample is placed in a holder measuring (2x2) cm2 on the difraktometer. The voltage The used is 40 kV and the generator current is 30 mA with a CuKα source (λ = 1.5405 Å).
- The result is an identified phase graph based on the intensity and angle of the 2 theta formed. The phase determination that arises refers to the Joint Committee on Powder Diffraction Standards (JCPDS).

4. Electrodeposition method

The principle of the electrodeposition method is the formation of metal deposits in the cathode using the help of electrical energy through an electrolyte. The results of electrodeposition are influenced by several things, among others, the selection of electrode materials, electrolytes, current tightness, overpotential, and the addition of complex agents.

Working procedure:

a. Preparation of HA from the Bones

- Heating bones using a muffle furnace for 1 hour with a temperature of 800^{o}C
- Grow roughly using mortar until slightly smoothly eroded using a powder grinding machine for 1.5 hours
- Ha powder produced is done by testing particle size analysis (PSA) to find out the size of the grain

b. Manufacture of ha and chitosan mixed solution

- Manufacture of HA and chitosan solution by dissolving in ethanol-water solvent solution
- Ha and chitosan mixture solution is divided into 3 variations in the composition of chitosan namely 0%, 10%, and 20%.

- Before mixing with HA solution, chitosan is first dissolved using magnetic stirrer for 30, followed by stirring using ultrasonic stirrer for 1 hour

c. Electrodeposition Process

- Done using a current meeting of 1.8 A / cm3, 3 variations in time, namely 1 hour, 3 hours and 5 hours and variations in the composition of HA + Kitosan
- 3 samples for each time variation with ethanol solution containing HA+chitosan 0%, HA+chiosan 10% and HA+Kitosan 20%.

d. Sample Characterization Process

Performed by X-Ray Diffraction (XRD) testing, Fuild Body Scanning Testing (SBF) and calculation of precipitate weight efficiency. For SEM-EDS testing uses 5000x and 2000x magnification for each sample. For SBF testing using a lactate ringer solution.

The quality of fish bone waste becomes hydroxyapatite

Based on ambar Teguh Sulistiyani et al research in 2016 on Community Empowerment of Utilization of Fish Bone Waste for Hydroxyapatite (HA) Study at Lekor Kuala Terengganu-Malaysia Cracker Processing Plant that the Introduction of hydroxyapatite benefits to the community will empower micro and macro conditions. Micro conditions are related to the development of the populist economy that can be created by the development of fish bone waste treatment. In addition, the provision of healthy and affordable organic calcium will greatly help public health. Macro-wise, this community empowerment can (a) maintain cleanliness and environmental health and ensure habitat survival so that the balance will remain sustainable, (b) build a more perfect industrial base from upstream to downstream, and (c) recommend policies on the utilization of fish bones for the food and health industries. . In addition, the benefits of hydroxyapatite as a healthy and cheap source of calcium can be used as a material for the disposal of heavy metals to overcome pollution that has very valuable value for maintaining sustainable health quality. The sustainability value of the utilization of fish bone waste treatment technology will be greater in capacity in the future considering the increasing need for fish consumption so that fish bone waste will also increase significantly.

Product Usage

Utilization of fish bone waste in a flour processing industry as a source of calcium (Ca) is one alternative in order to provide a food source rich in calcium (Ca) while reducing the negative

impact of environmental pollution resulting from the disposal of fish processing industry waste. Fish bone product in the form of hydroxyapatite (Ca5(PO4)3(OH)) is a natural inorganic element derived from bone that can be used for bone regeneration, repair, filling, expansion and reconstruction of bone tissue. This is because hydroxyapatite has perfect biocompatibility properties when implanted in bone. In addition, hydroxyapatite can also be used as an adsorbent to overcome environmental pollution of heavy metals (Aisyah, et. al., 2012). In addition, it is also used for making kepuruk lekor in Malaysia. The fish used in this waste treatment include tuna, yellowfin tuna. This fish has a high economic value in the form of dicroxyapatite (Ca5(PO4)(OH).

CONCLUSION

Based on the literature study, information was obtained that there are 4 methods of making hydrospatite from fish bones, namely hydrothermal, electrodeposition, sol gel and precipitation.

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