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RESEARCH ARTICLE OPEN ACCESS

Characterization of Hydroxyapatite from Bovine Bone by Mechanical Combination Method

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Abstract:

Hydroxyapatite is a chemical compound similar to human bone. For implant materials, the needs for hydroxyapatite is increasing. Therefore, sources for cheap hydroxyapatite with abundant availability are needed. Hydroxyapatite can be produced from bovine bone by a combination of mechanical processes. This was done by crushing, boiling and calcining the bovine bone. The calcination process was carried out at a temperature of 1000° C with a time variation of 3 and 6 hours. Characterization was done by X-Ray Diffraction (XRD), X-Ray Fluorescence, Scanning Electro Machine and EDX. The obtained bovine bone hydroxyapatite has a content similar to commercial hydroxyapatite. Produced hydroxyapatite has a ratio of Ca and P of 1.61, 1.74 and 1.68. The results of SEM-EDX show the porosity of hydroxyapatite. This result showed how this material can be applied as implant material.

Keywords — **Hydroxyapatite, bovine bones, mechanical treatment, characteristic, inplant**

I. INTRODUCTION

Implant materials from non-metallic suurces is continues to be developed, for several reasons mainly in term of cost and material availability. Because the use of hydroxyapatite as a biomaterial more rapidly. As the needs of cheap and biocompatible inplant materials.

Hydroxyapatite can be obtained by several methods including: sol-gel, hydrothermal, and precipitation[1-4]. Hydroxyapatite is formed from calcium and phosphate [5]. Source of hydroxyapatite can be obtained from nature, such as:eggshells, seashells, limestone, pig bones and bovine bone.

Bovine bone has a similar structure and chemical arrangement as human bones [6]. The process of obtaining hydroxyapatite from bovine bone can be done by extracting or by chemical reaction [7].

In comparison, the process of obtaining hydroxyapatite from bovine bone by hydroxyapatiteprocess yields a ratio of Ca and P of 1.69 and its size is 50 – 100 nm [8].

Calcination temperature has an effect on calcium phosphate compound. The process of bovine bone calcinations requires high temperatures. Hydroxyapatite from bovine bone was calcined at 1000°C [9].

The heating duration also affects the calcinations resul. The duration of the heating process will cleans hydroxyapatite from contaminants and cleaner hydroxyapatite will obtained.

Currently hydroxyapatite needs continue to increase, while the number is small and the price is expensive. People need materials and large quantities. To overcome this the hydroxyapatite material is produced from bovine bone waste.

Therefore, in this study in the productionof hydroxyapatite derived from bovine bone with an easy process that is by a combination of mechanical process with the parameters. So it can be obtained cheaper hydroxyapatite and abundant availability.

II.EXPERIMENT DETAILS

The raw material used was bovine bone. The process of obtaining hydroxyapatite from bovine bone was as follow: The bovine bone was cleaned with clean water. Clean bovine bones were cut into small pieces. Then, the bone was soaked in 1 % NaOH liquid. To remove the fat and dirt, the bovine bone was boiled in a presto pan. In the next process, the bones was dried and cruished with a cruiser machine to obtained bone powder. To found out the characteristic, hydroxyapatitewas tested by X-Ray diffraction X-Pert Powder PW 30/40, X-Ray Fluorescent (XRF) epsilon3 and Fourier Transform Infrared (FTIR) Perkin Elmer brands, while to observed the morphology and size of pore was done by scanning electron microscope (SEM) Hitachi brand,

III. RESULTS AND DISCUSSION

A. XRD

XRD was used to find out the crystal structure, phase change and degree of crystallinity. Then the phase was analyzed based on the result of the graph formed by adjusting each peak with the database Joint Committee on Powder Diffraction Standards (JCPDS) with the desired phase. The matching of XRD test results with this database was done by detecting 2θ angles and the both grid gap (d).

The explanation with JCPDS showed that CaO has been phase formation can occur because at 900ºC there was a transformation of calcium from calcium carbonate ($CaCO_3$) into calcium oxide (CaO) by releasing carbon dioxide (CO_2) (Gerlely, 2009). And it can also be seen that the hydroxyapatite phase has been fully formed after sintering at 1000 °C for 2 hours , where at that sintering temperatures calcium phosphate will undergo phase transformation to hydroxyapatite. Based on the suitability, it can be seen that the powder of CaO and phosphoric acid has been successfully reacted and resulted in a hydroxyapatite phase with the source of bovine bone (Gergely *et al.*, 2009).

From the XRD the existence of PO_4^{3-} and OH which states the existence of Hap was known. And to know the degree of crystallization of HAp through the peaks of intensity that emerged by using XRD. The result of XRD test was obtained by comparing the intensity of the peaks on the measured difractogram with JCPDS HAp. The XRD test results in Figure 4.2 in the sample show high intensity peaks, at 2θ angle is 1469 cts, 1477 cts, 2278 cts, 4362 cts, 5962 cts. This was indicated the presence of hydroxyapatite and how the result is similar to JCPDS HAp.

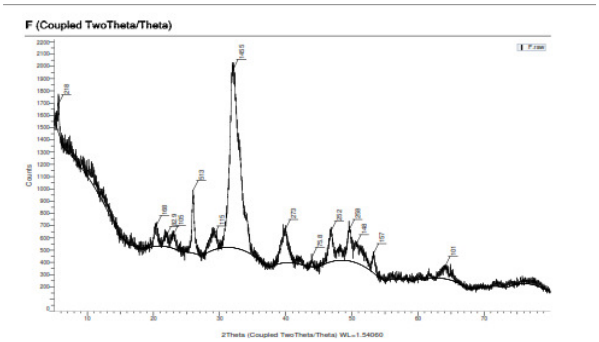

Figure 1. Raw material of bovine bone powder

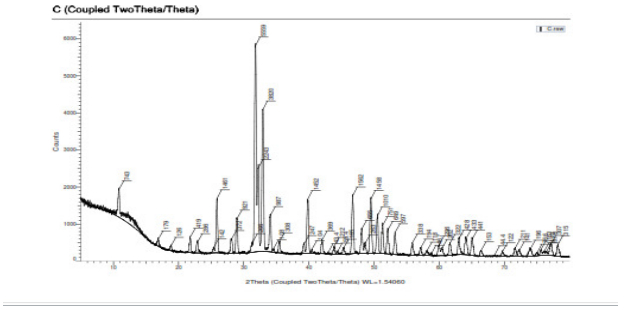

Figure 2. Once boiling HAP and 1000°C calcining for 3 hours

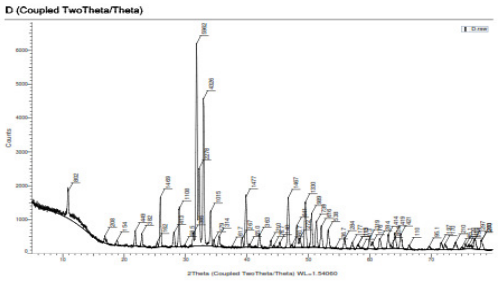

Figure 3. Once boiling HAP and 1000°C calcining for 6 hours

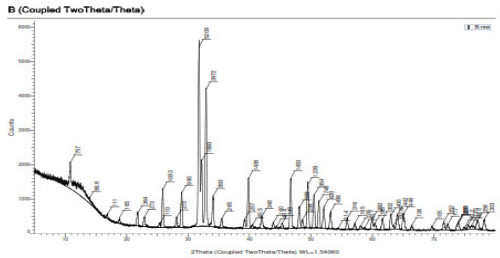

Figure 4. Twice boiling HAP and 1000°C calcining for 6 hours

The purity of HAp produced is not optimum because of the presence of $CaCO_3$ and Ca (OH)2 in the hydroxyapatite synthesis results. But to obtain a pure HAp can be done by improving the process.

To know the hydroxyapatite characteristic of bovine bone, XRD test was done. The test result can be analyzed on graph showing the sharp peaks difraktogram with high intensity. The sharp peak at 5962 cts in the Post (°2Th) 31.792° is owned by the calcium (Ca) element and in the Post (°2Th) 32,925° with the peak height at 4326 cts is owned by the phosphorus (P) element. Calcium and phosphorus elements possessed by HA material from the $Ca_{10}(PO_4)_6(OH)_2$ functional group, so that the material is essentially hydroxyapatite.

Hydroxyapatite from the difraktogram test results with sharp peaks and high intensity illustrated that the sample is semi-crystalline and has high crystallinity (Pujianto *et al.*, 2005).

B. FTIR

FTIR test Result of bovine bone hydroxyapatite was strengthens the data from XRD test. From the FTIR results the presence of OH-group can be seen, it was showed by the presence of O-H bond at the highest wave peak of 2221.95 cm^{-1}, with intensity 98.20% T. Group clusters was showed by the presence of the P-O bond at the wave peak of 1019.69 cm^{-1}, with intensity 25.14% T and equal to the wave peak of 1087.03 cm^{-1}, with intensity 64.50% T. For the next sample wave 962.03 cm^{-1}, with intensity 58.39% T and wave 630.31 cm^{-1}, with intensity 76.09% T.

Then the FTIR results was indicated the presence of CO_2^{-3} which characterized by the presence of C-O bonds at the peak of wave 2026.99 cm-1, with an intensity of 98.47% T. The presence of this group was showed from the reaction of HAp with CO_2 which presented in the atmosphere at the time of synthesis and heat treatment. It was observed that the intensity of this peak decreases in the FTIR results and indicated by the wave peak of 2008.12 cm^{-1}, with an intensity of 98.10% T. Reduced peak intensity showed a decrease in CO_2^{-3} group concentration caused by an increase in heat treatment temperature (Bionatura, 2008).

FTIR is one of the infrared spectroscopy techniques that can identify complex cluster content in calcium phosphate compounds, but can not be used to determine the constituent elements. In infrared spectroscopy, infrared spectrum lies in wavelengths regions with ranging from 0.78 to 1000 µm or wave numbers from 12800 to 1 cm^{-1}. In terms of application and instrumentation, the infrared spectrum is divided into three types of radiation: near infrared (wave number 12800-4000 cm^{-1}), mid infrared (wave number 4000-200 cm^{-1}) and far infrared (200-10 cm^{-1}). FTIR is included in the category of mid-infrared radiation (wave number 4000-200 cm^{-1}).

The plot between transmitation percentages and wave numbers will produce infrared spectrum and each different type of bond has different frequencies located in slightly different environments, that is why there is no two different molecules of structure will have the precise infrared absorption or spectrum.

FTIR was utilized the vibrating energy of the hydroxyapatite constituent function group, which is the group of PO_4^{-3}, CO^{-2} group, and the (OH^-) group. The result of FTIR characteristic produced that IR spectrum such as picture 1, showed that there were many function clusters in bovines bone, there was clusters PO_4^{-3} in numeral phase 1023 cm^{-1} after the calcination, that has symmetry stretching vibration and 603,68 cm^{-1} as asymmetry vibration bending. OH^- bending cluster detected on 3283 cm^{-1}.

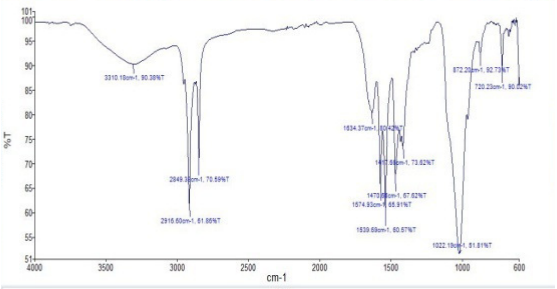

Figure 5. Raw material of bovine bone powder

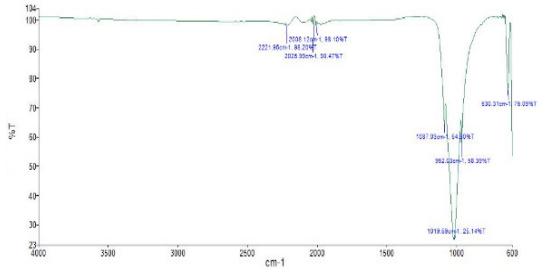

Figure 6. Once boiling HAP and calcining at 1000°C for 3 hours

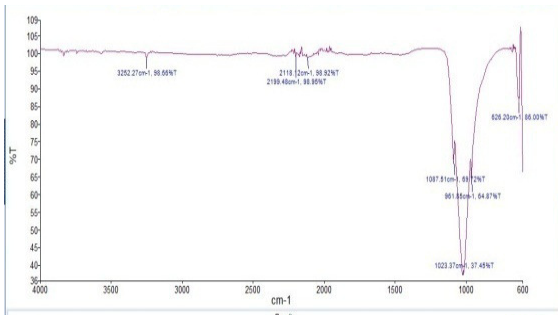

Figure 7. Once boiling HAP and calcining at 1000°C for 6 hours

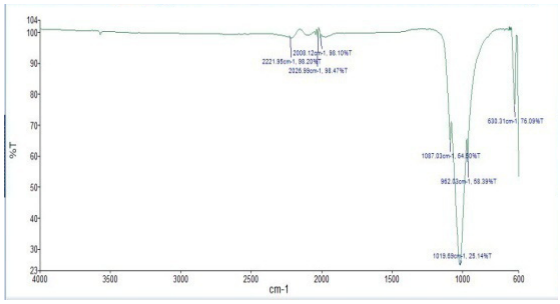

Figure 8. Twice boiling HAP and calcining at 1000°C for 6 hours

C. XRF

XRF is used to determine the percentage of elements contained in a chemical compound. For this hydroxyapatite compound can be seen composition ratio of Ca and Phosfor element.

Table. 1: Amount of components from hydroxyapatite bovines bone from XRF test

Components	Amount (%)			
	A.	B.	C	D
Al	0.983	0.587	0.62	1.049
Si	1.126	0.568	0.59	0.735
P	28.167	32.323	32.478	32.059
S	0.612	0.131	0.147	
Cl	0.102	0.094	0,.065	
Ca	67.082	64.211	62.111	62.599
Mn	0.064	0.073	0.082	0.047
Fe	0.3	0.613	0.562	0.153
Sr	0.104	0.088	0.08	0.095

A= . Bovines bone powder
B.= HA once boiling sintering 3 hours
C= HA omce boiling sintering 6 hours
D= HA twice boiling sintering 6 hours

According to table 1 there is a change in the content of the elements contained in hydroxyapatite, this is caused during boiling and sintering occurs the cleaning process so that the reduction of some impurities elements that exist. Comparison between the Ca and P element content of 1.6-1.9

Both of these clusters are supplements for XRD analysis results that showed that the synthesis result is HAp where PO_4^{3-} and OH- was a HAp builder with chemical formula $CO_{10}(PO_4)_6(OH)_2$.

D. SEM

SEM is needed to see the changes that exist in the microstructure, as shown in Fig. 9-12 .

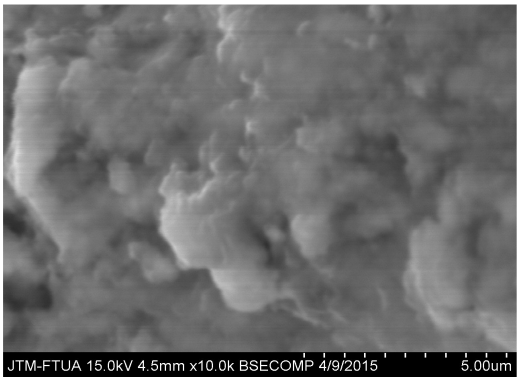

Figure 9. Raw material of bovine bone powder

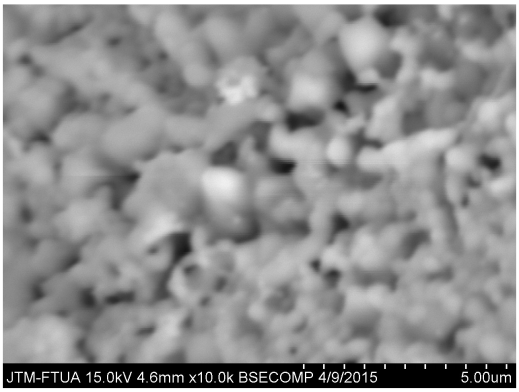

Figure 10. Once boiling HAP and calcining at 1000°C for 3 hours

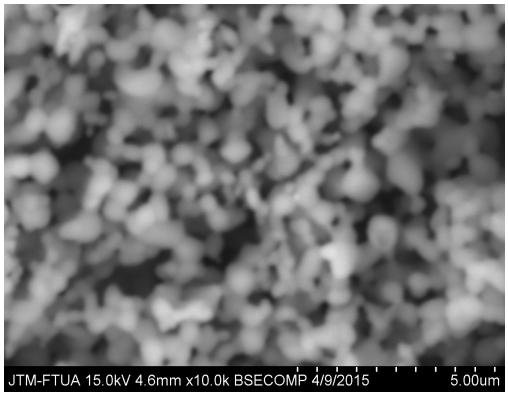

Figure 11. Once boiling HAP and calcining at 1000°C for 6 hours

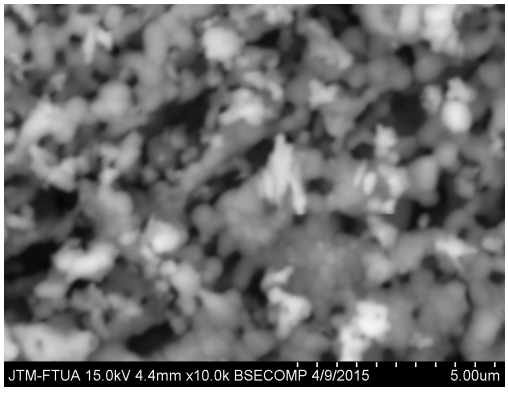

Figure 12. Twice boiling HAP and calcining at 1000°C for 6 hours

From the observation it can be seen that the cow bone powder with no treatment is very different from those that have experienced the treatment either boiling or sintering

As the result from observation with SEM, there was bovines bone powder with treatment level that gave micro structure morphology and relative pore like in picture 2. There was closed pore on picture A that was caused by protein and fat. While on picture 10, 11 dan 12, the pore was opened, because of boil and calcination treatment's influence.

Hydroxyapatit production with thermal decomposition method (8), by cutting a part of the bone, gave not good enough result, so after boiling process resulted in fat substance, there was still useless substance on the bone such as protein.

IV. CONCLUSION

Production of hydroxyapatite from bovine bone by a combination of mechanical processes was gave good significant result. This was done by crushing, boiling and calcination the bovine bone, with time allocation of 3 and 6 hours. Good hydroxyapatite is produced by the process of boiling and repeated destruction.

As a result from XRD, hydroxyapatite is approached from commercial hydroxyapatite, it also from XRF and FTIR characteristic that gave appropriate result with literature. From this research produced hydroxyapatite had good composition, with average Ca:P comparison 1.68 and hydroxyapatite morphology with visible pores that is enable for synthetic bone application.

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