Extraction of Hydroxyapatite by Alkaline Acid from Budu Waste and Synthesis Using Calcination Method

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Abstract. Hydroxyapatite powders were synthesised using anchovy fish bone waste collected from the Budu production process. Liquid Budu factory leaved much fish bones waste containing valuable calcium and phosphorous. It was extracted by two different acid solutions (HCl and HNO_3) and was subsequently precipitated by NH_4OH. Finally, as-prepared products were calcined at 800 ºC for 2 hours in a muffle furnace. The per cent yield of both obtaining products was 80%. The obtained samples were characterised by X-ray diffractometry (XRD), Fourier-transform infrared (FT-IR) spectroscopy and energy dispersive X-ray spectrometry (EDS). As a result, the XRD patterns of the products prepared from two different acids were identical; these acids have no significant influence on the structure of a product. The XRD patterns were identified as calcium magnesium sodium pentaphosphate [$Ca_9MgNa(PO_4)_7$] or merrillite (MER) crystallisation in the rhombohedral structure, and the crystal sizes of these products were estimated as nanometers. The FT-IR spectra showed the vibration modes of PO_4^{3-} at 562, 596, 983, and 1034 cm^{-1}, while the peak located at 369 cm^{-1} correspondings to the vibration of the metal-oxygen bonding in the merrillite structure. Moreover, the presence of Mg^{2+} in the product was confirmed by EDS technique. This paper showed the excellent potential for the phase transformation of merrillite from hydroxyapatite at low temperatures.

1. Introduction

Budu or fish sauce southern Thailand style is a favourite side dish of the Southern Border Provinces with various cooked forms such as kneading rice, mixed with vegetables, and pepper source, especially in Saiburi district, Pattani Province Industrial production. The bottle is sold in the form of OTOP products there are two brands, i.e., Budu Heng and Budu Yiseng. Budu is based on the digestion of enzymes and microorganisms from naturally occurring fish, which uses small fish such as *Stolephorus indicus, Clupeoides sp., Sardine sp., Pinialo pingalo* or *Decapterus russelli*. These fishes are taken without removing their tripe and by fermentation with sea salt in the ratio of 3:1 for 8-12 months. Fish and bone are fermented and produced a liquid called Budu of black colour or rather black. Liquid Budu will leave the residue containing fish bones, fins, and also contained fish meat. Therefore, Budu residue is one of the major reservoirs of both phosphorus and calcium in which can be used to prepare many species of calcium phosphate such as hydroxyapatite [1], whitlockite [2], and merrillite [3]. In addition, Budu residue has been synthesised for triphasic between hydroxyapatite: $Mg_2(P_2O_7)$: whitlockite in the weight percentage of 79: 11: 10 [4].

The most common method of synthesising merrillite powder is solid-state sintering, which involves heating relevant powdered calcium phosphate chemistry to >1025 °C. Another method of synthesising merrillite is through the heating of whitlockite to >1000 °C for at least 24 hours to drive off the "essential hydrogen" [5]. With the ever-growing need to develop pure, clean, non-toxic and environment-friendly techniques, this paper presents methods based on low temperature and short calcination time to generate large quantities of single-phase merrillite from Budu waste. The physical and chemical properties of samples are prepared by various characterisation techniques, such as X-ray diffraction (XRD), Fourier transform infrared (FTIR) and scanning electron microscopy (SEM).

2. Methodology

2.1 Materials

Anchovy fish bone as waste was obtained from the final step of the Budu production process at Budu Yiseng Factory, Amphor Saiburi, Pattani Province, Thailand. Ammonium hydroxide (J.T. BAKER), hydrochloric acid (Lab Scan), nitric acid (Lab Scan) were used in this study. All chemicals were reagent grade and used without further purification.

2.2 Synthesis method

The dissolution of the fish bone waste into hydroxyapatite powder was performed according to a previously described procedure [6]. The fish bone was collected from Budu Yiseng residue and washed with water to remove the traces of meat and skin. After thorough washing the bones were dried at 60 ◦C and ground in a mortar pestle. The Budu waste (5 g) was first dissolved in 80 mL of deionised water supplemented with 20 mL of concentrated hydrochloric acid (37 %) or nitric acid (65 %) to obtain the solvated Ca^{2+} and hydrogenophosphate species. The remaining solution was neutralised using 100 mL of a concentrated NH_4OH solution (25%). The pH value of the precipitation reaction was maintained at pH 10. The dark white powder of amorphous hydroxyapatite was precipitated and recovered by filtration, washed several times with deionised water and dried in an oven at 100 °C. The precipitate sample is noted as A-HAP according to the amorphous phase of hydroxyapatite. The dark white powder indicates the powder still contains organic components and has not shown a high degree of purity. One (1) g of A-HAP was placed in a silica crucible and subjected to a temperature of then calcined in an electrical muffle furnace at 400, 600 and 800 °C for 2 hours to study the effect of calcination. The clear white powder of single-phase merrillite was obtained and noted as MER-Cl and MER-N according to the inorganic acid (HCl and HNO_3), respectively.

2.3 Sample characterisation

The XRD spectra were collected using a Philips PW 3710 powder diffractometer (PHILIPS X'Pert MPD, The Netherlands), Cu Kα (Ni filtered) radiation λ=1.5406 Å. Intensity data were collected by the step counting method in the 2θ range of = 10-90º. The average crystallite size was calculated from the broadening in the XRD pattern according to Scherrer's equation [7]:

$$D_{hkl} = \frac{K\lambda}{\beta 1/2 \cos \theta} \quad (1)$$

Where Dhkl is the average crystallite size, K is the broadening constant, λ is the wavelength of Cu Kα radiation (1.5406 Å), $\beta_{1/2}$ is the full-width at half-maximum of the main peak, and θ is the diffraction angle.

The FT-IR spectra were performed using a Spectrum JASCO 6800 spectrometer in the range of 400-4000 cm^{-1}. The morphological and microstructure analyses of all samples were carried out using the instrument Quanta 400 (SEM, Quanta 400, FEI). In addition, the elemental composition was analysed by an energy dispersive spectrometer (EDS) equipped with the SEM microscope system.

3. Results and Discussion

3.1 XRD results

The XRD pattern of the sample prepared without the calcination process contained low-intensity broad peaks, confirming the formation of mainly amorphous hydroxyapatite (A-HAP) as shown in Figure 1a. The XRD pattern of the as-synthesised A-HAP contained broad peaks at 26.03°, 32.28°, 46.82°, 49.68° and 53.49°, i.e., assigned to the (002), (112), (222), (213) and (141) respectively of hydroxyapatite (JCPDS card no 01-086-0740) [8]. When the calcination was operated at 800, the intensity and sharpness of all crystal planes increased considerably, confirming the increase in the degree of crystallinity. These diffraction peaks were observed at 2θ values of 13.73°, 17.13°, 26.02°, 28.06°, 31.33° and 34.68°, corresponding to (104), (110), (1010), (214), (217) and (220) Miller planes of $Ca_9MgNa(PO_4)_7$ or merrillite structure [3] (JCPDS card no 01-088-0797) (blue and red line). These results suggest that merrillite is fully formed after 800 °C. The average crystallite size is calculated using followed equation (1). The sample shows a nanosized crystal for A-HAP and MER-Cl as well as MER-N, respectively.

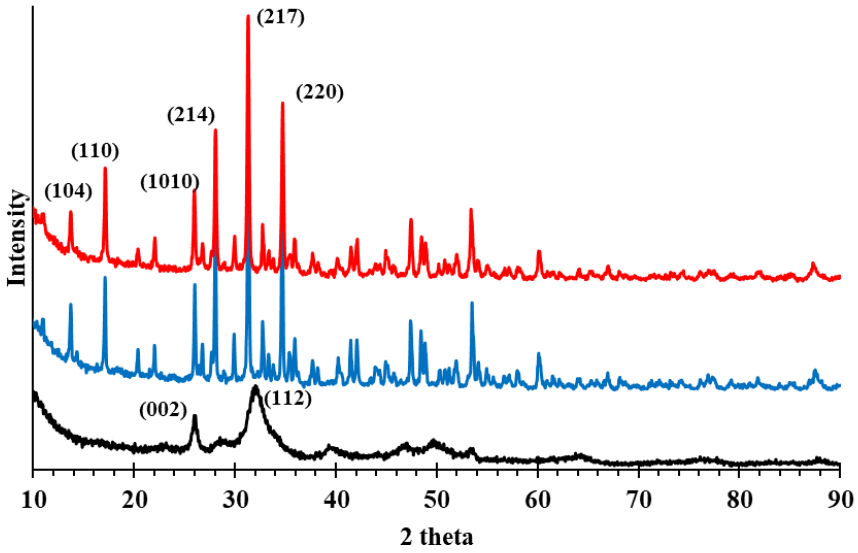

Figure 1. XRD pattern of A-HAP (black) MER-Cl (blue) and MER-N (red)

3.2 SEM-EDX results

The particles synthesised at low temperatures were more irregular and had less clear contours with the size of 100-300 nm, as shown in Figure 2a. Also, the particles showed a high tendency to agglomerate. For the sample prepared at higher temperatures, the particles were more regular in shape and had less aggregation with a uniform size of 80-100 nm (Figure 2c). The change from irregular to regular particle morphology with the increase in synthetic temperature was corresponding to the increase of the crystallinity of the merrillite nanocrystals; that is, a more regular shape of the particles was observed when the powders had higher crystallinity.

EDX analysis for the obtained samples was performed and the resulted are shown in Figure 2b and 2d. The figure shows that the Ca/P ratio for the A-HAP and MER-Cl were 2.32 and 1.97, respectively. These values lie within the unacceptable range for hydroxyapatite. Variation of these values than the standard hydroxyapatite value (Ca/P 1.67) might be due to the implication of the Mg ions (1.4-1.6%) obtained by those methods.

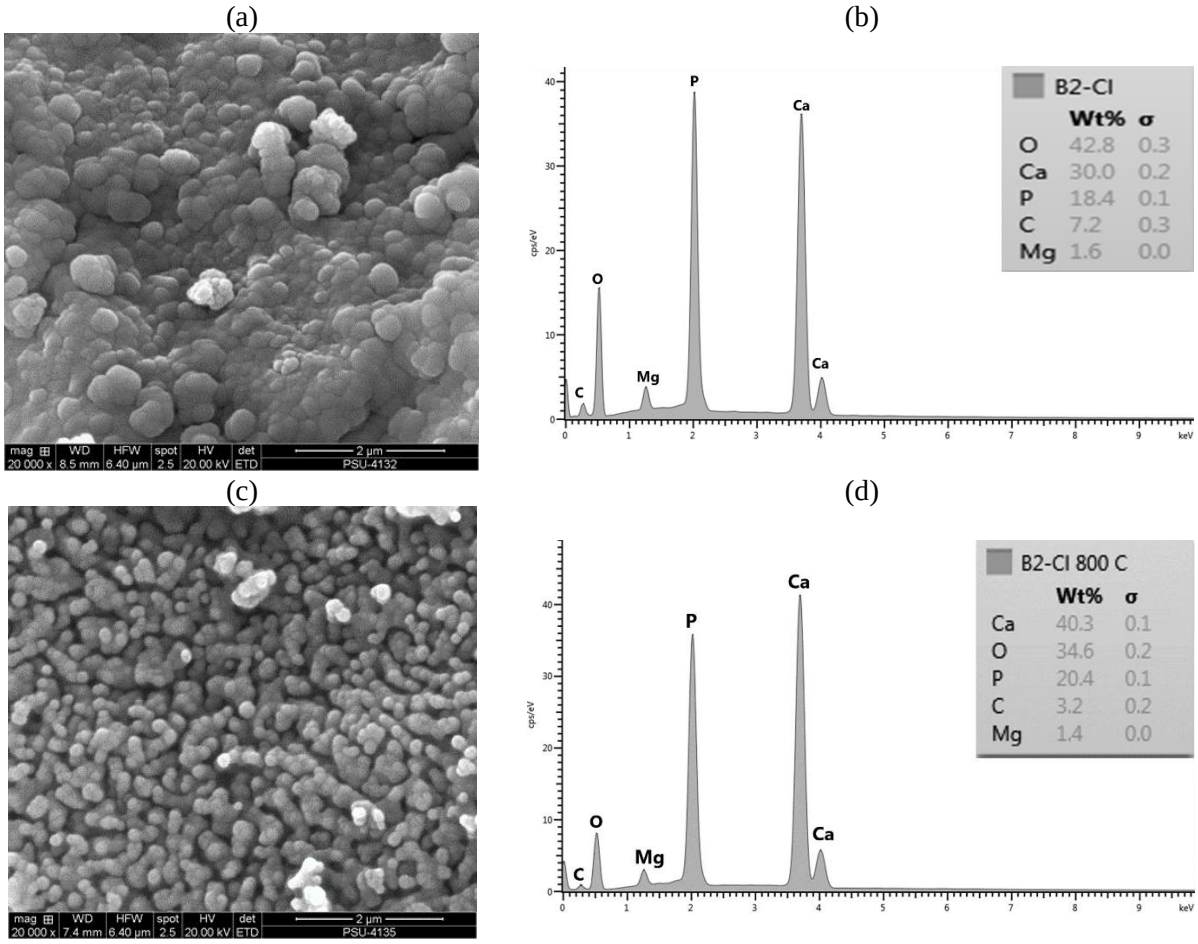

Figure 2. SEM-EDX image of A-HAP (a) – (b) and MER-Cl (c) – (d)

3.3 FTIR results

The FTIR spectra of the synthesised samples are shown in Figure 3. The bands at 1421 cm^{-1} correspond to CO_3^{2-} ions present in the A-HAP. The broad band located at 3500 cm^{-1} and 1644 cm^{-1} corresponds to H_2O adsorbed on the surface [9]. In the samples calcined to 800 °C this band is absent. Origin of the band at 982 cm^{-1} probably is from PO_4^{3-} ions presented in the non-apatite environment [10] may be attributed to the merrillite structure. The $\bar{\upsilon}_3$ carbonate bands at 892 cm^{-1} correspond to carbonated calcium substitution [11], i.e., PO_4^{3-}substituted by CO_3^{2-}. The absorption bands of significant intensity located at 1034-1123 cm^{-1} could be attributable to the factor group splitting of the $\bar{\upsilon}_3$ fundamental vibration mode of the PO_4^{3-} tetrahedral. The band observed at 945 cm^{-1} and doublet at 562-605 cm^{-1} correspond to $\bar{\upsilon}_1$ and $\bar{\upsilon}_4$ symmetric P-O stretching vibration of the PO_4^{3-} ions, respectively [12].

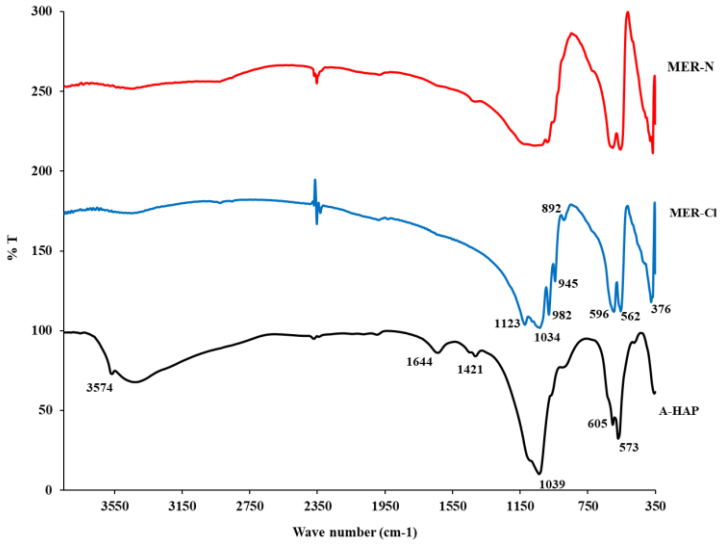

Figure 3. FTIR spectra of A-HAP (black) MER-Cl (blue) and MER-N (red)

4. Conclusion

Merrillite powder obtained by precipitated from Budu waste has been characterised. The results of the XRD analysis showed that the main component is calcium hydroxyapatite. However, as received A-HAP sample mostly consisted of an amorphous phase along the minor amount of crystalline phase. A highly crystalline single phase of merrillite was obtained from the initial A-HAP powder by heat treatment at the temperature of 800 °C.

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References

[1] Mohd Pu'ad N, Koshy P, Abdullah H Z, Idris M I and Lee T C 2019 Syntheses of hydroxyapatite from natural sources. *Heliyon* **5(5)** e01588
[2] Batool S, Liaqat U, Babar B and Hussain Z 2021 Bone whitlockite: synthesis, applications, and future prospects. *Journal of the Korean Ceramic Society*
[3] Xie X, Yang H, Gu X and Downs R T 2015 Chemical composition and crystal structure of merrillite from the Suizhou meteorite *American Mineralogist* **100** 2753-12756
[4] Daupor H, Kuwae P, Wijaya A R, Chelong I and Haji Samoh A 2018 Proceeding of the Pure and Applied Chemistry International Conference Thailand February 7-8
[5] Adcock C T, Hausrath E M, Forster P M, Tschauner O and Sefein K 2014 Synthesis and characterisation of the Mars-relevant phosphate minerals Fe- and Mg-whitlockite and merrillite and a possible mechanism that maintains charge balance during whitlockite to merrillite transformation *American Mineralogist* **99** 1221–1232
[6] El Asri S, Laghzizil A, Saoiabi A, El Abassi K, M'hamdi R and Coradin T 2009 A novel process for the fabrication of nanoporous apatites from Moroccan phosphate rock *Colloid and Surface A: Physicochemical and Engineering Aspects* **350** 73-78
[7] Rahavi S, Monshi A, Emadi R, Doostmohammadi A and Akbarian H 2012 Determination of crystallite size in synthetic and natural hydroxyapatite: A comparison between XRD and TEM results *Advanced Materials Research* **620** 28–34
[8] Wijesinghe W P S L, Mantilaka M M M G P G, Karunarathne T S E F and Rajapakse R M G 2019 Synthesis of a hydroxyapatite/poly(methyl methacrylate) nanocomposite using dolomite *Nanoscale Advances* **1** 86-88

[9] Anindya P, Sudeep P, Amit R C, Vamsi K B, Mitun D and Arijit S 2017 Synthesis of hydroxyapatite from Lates calcarifer fish bone for biomedical applications *Materials Letters* **203** 89-92
[10] Tomoyo G and Keiko S 2014 Effects of trace elements in fish bones on crystal characteristics of hydroxyapatite obtained by calcination *Ceramics International* **40** 10777-10785
[11] Trinkunaite-Felsen J, Stankeviciute, Z, Yana J C, Yang T C K, Beganskiene A and Kareiva A 2014 Calcium hydroxyapatite/whitlockite obtained from dairy products: Simple, environmentally benign and green preparation technology *Ceramics International* **40** 12717-12722
[12] Landi E, Logroscino G and Proietti L 2008 Biomimetic Mg-substituted hydroxyapatite: from synthesis to in vivo behaviour *Journal of Materials Science. Materials in Medicine* **19** 239