

# Synthesis and characterisation of composite based biohydroxyapatite bovine bone mandible waste (BHAp) doped with 10 wt % amorphous SiO<sub>2</sub> from rice husk by solid state reaction

Dwi Asmi' , Ahmad Sulaiman' , Irene Lucky Oktavia' , Muhammad Badaruddin' , and Anne Zulfia'

Citation: **1725**, 020009 (2016); doi: 10.1063/1.4945463

View online: <http://dx.doi.org/10.1063/1.4945463>

View Table of Contents: <http://aip.scitation.org/toc/apc/1725/1>

Published by the [American Institute of Physics](#)

---

---

# Synthesis and Characterisation of Composite based Biohydroxyapatite Bovine Bone Mandible Waste (BHAp) Doped with 10 wt % Amorphous SiO<sub>2</sub> from Rice Husk by Solid State Reaction

Dwi Asmi<sup>1,a)</sup>, Ahmad Sulaiman<sup>1,b)</sup>, Irene Lucky Oktavia<sup>1,c)</sup>, Muhammad Badaruddin<sup>2,d)</sup>, and Anne Zulfia<sup>3,e)</sup>

<sup>1</sup>*Department of Physics, Faculty of Mathematics and Natural Sciences, University of Lampung Jl. Sumantri Brojonegoro No.1 Gedung Meneng Bandar Lampung 35145, Indonesia.*

<sup>2</sup>*Department of Mechanical Engineering, Faculty of Engineering, University of Lampung Jl. Sumantri Brojonegoro No.1 Gedung Meneng Bandar Lampung 35145, Indonesia.*

<sup>3</sup>*Department of Metallurgy and Materials Engineering, Faculty of Engineering, University of Indonesia, Kampus Baru-UI, Depok 16424, Indonesia.*

<sup>a)</sup>Corresponding author: dwiasmi82@yahoo.com; dwi.asmi@fmipa.unila.ac.id

<sup>b)</sup>ahmadsulaiman@yahoo.co.id

<sup>c)</sup>ireneluckyo@gmail.com

<sup>d)</sup>mbruddin@eng.unila.ac.id

<sup>e)</sup>anne@metal.ui.ac.id

**Abstract.** Effect of 10 wt% amorphous SiO<sub>2</sub> from rice husk addition on the microstructures of biohydroxyapatite (BHAp) obtained from bovine bone was synthesized by solid state reaction. In this study, biohydroxyapatite powder was obtained from bovine bone mandible waste heat treated at 800 °C for 5 h and amorphous SiO<sub>2</sub> powder was extracted from citric acid leaching of rice husk followed by combustion at 700°C for 5 h. The composite powder then mixed and sintered at 1200 °C for 3 h. X-ray diffraction (XRD), Fourier transformed infrared (FTIR) spectroscopy and Scanning electron microscopy (SEM) techniques are utilized to characterize the phase relations, functional group present and morphology of the sample. The study has revealed that the processing procedures played an important role in microstructural development of BHAp-10 wt% SiO<sub>2</sub> composite. The XRD study of the raw material revealed that the primary phase material in the heat treated of bovine bone mandible waste is hydroxyapatite and in the combustion of rice husk is amorphous SiO<sub>2</sub>. However, in the composite the hydroxyapatite, β-tricalcium phosphate, and calcium phosphate silicate were observed. The FTIR result show that the hydroxyl stretching band in the composite decrease compared with those of hydroxyapatite spectra and the evolution of morphology was occurred in the composite.

## INTRODUCTION

Conversion of biomaterial waste into value-added engineering materials may have an advantage increase in the availability of inexpensive resources and give contribution as part of the solution to the waste disposal issue. The use of biomaterials waste as a raw material in the manufacture of ceramics, especially bioceramics based hydroxyapatite to this day continues to be done. Hydroxyapatite with the chemical formula of Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub> is one of the emerging most bioceramics classes, which is widely used in orthopedics and dentistry<sup>1</sup>. There are a variety of raw materials sources for hydroxyapatite, including seashells<sup>2-3</sup>, corals<sup>4</sup>, eggshells<sup>5-6</sup>, oyster shell<sup>7</sup>, human teeth<sup>8</sup>, and bovine bone<sup>9-14</sup>. Among various raw material sources used in the preparation of hydroxyapatite, biomaterial waste derived bovine bone is the most popular and frequently available, however the use of mandible bovine bone waste as

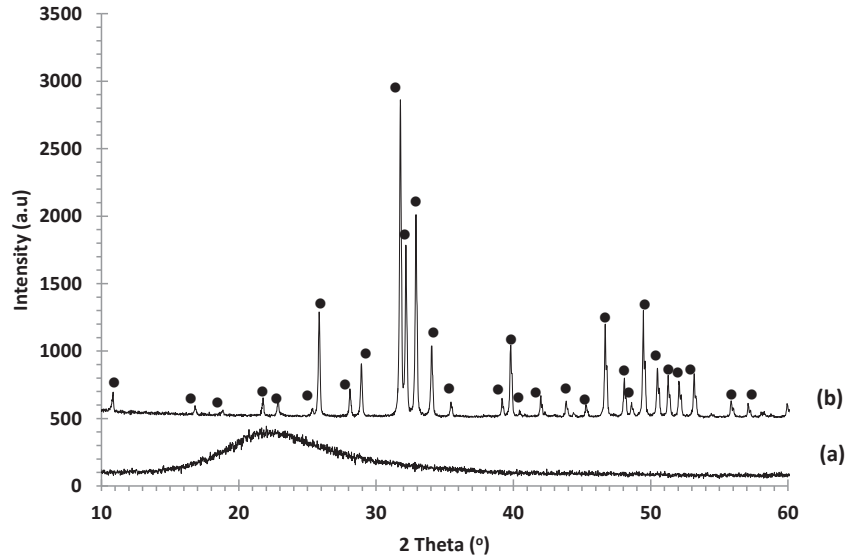
raw materials for the preparation of hydroxyapatite have not been conducted yet. Another biomaterial waste which has an amazing contribution in the solution to waste disposal issue is rice husk. The production of rice, one of the major food crops in the world, generates one of the major biomaterial wastes of the world, called, rice husk. The abundant and cheap, rice husks naturally have high contents of silica ( $\text{SiO}_2$ )<sup>15-16</sup>. Its production has been well reported.  $\text{SiO}_2$  has an important role in the biomineralisation and biological responses. Combine of both (hydroxyapatite and  $\text{SiO}_2$ ) biomaterial wastes into a new value-added composite materials is a challenge. In this study, an attempt has been taken to develop an alternative novel method to synthesize of bioceramic composites based biohydroxyapatite (BHAp) derived from bovine bone waste with addition of amorphous  $\text{SiO}_2$  rice husk and to evaluate the effect of silica substitution on the microstructure of the composites.

## EXPERIMENTAL METHOD

Biohydroxyapatite (BHAp) powder used for preparation of composite was adopted from Asmi experiment<sup>17</sup>. Mandible bovine bones waste was collected from local slaughter around city of Bandar Lampung, Indonesia. The sample then were cut in a small piece with average dimension of  $2 \times 3 \text{ cm}^2$  and washed thoroughly in running tap water followed by boiling in pressure cooker for 10 h and dried in oven at  $100^\circ\text{C}$  for 24 h. The collected mandible bovine bones then immersed by using 1 N HCL (Merck, 35%) solution for 12 h and washed thoroughly several time with warm distilled water. The remaining proteins of bone waste were immersed again in 1 N NaOH solution for 12 h and washed with distilled water for several times until pH equal to 7 in the filtrate was reached, then dried in oven at  $100^\circ\text{C}$  for 5 h. The dried bovine bone then grinding with alumina mortar and pestle to obtained bone powder and sieving by using siever with grid size of 200 mesh followed heat treated in furnace at  $800^\circ\text{C}$  for 5 h to obtained biohydroxyapatite (BHAp) powder. The synthesis method to produce of amorphous  $\text{SiO}_2$  derived from rice husks waste was modified from Umeda and Kondoh experiment<sup>15</sup>. Rice husks waste were soaked in 5 wt% citric acid solutions at  $50^\circ\text{C}$  and stirring for 30 minutes, followed by distilled water rinse at  $25^\circ\text{C}$  for 30 minutes with stirring to remove the citric acid residue from rice husk. After drying at  $100^\circ\text{C}$  for 2h, the pretreated rice husks were heat treated at  $700^\circ\text{C}$  for 3h to produce amorphous  $\text{SiO}_2$ . The heat treated amorphous  $\text{SiO}_2$  then grinding with alumina mortar and pestle to obtained bone powder and sieving by using sieve with grid size of 200 mesh. To synthesize BHAp- $\text{SiO}_2$  composites, the processed BHAp powder was wet ball-milled in ethanol for 3 h with 10 wt % of processed amorphous  $\text{SiO}_2$  powder. The slurry was then dried in oven at  $60^\circ\text{C}$  for 36 h and ground with mortar and pestle. The obtained powder then screened through a  $35 \mu\text{m}$  sieved and heat treated at  $500^\circ\text{C}$  for 30 minutes, followed by  $1200^\circ\text{C}$  for 3h, and then furnace-cooled. Processed BHAp powder without addition of  $\text{SiO}_2$  also heat treated at  $500^\circ\text{C}$  for 30 minutes, followed by  $1200^\circ\text{C}$  for 3h, and then furnace-cooled and used as a control sample. The crystal structure analysis of all samples phase were monitored by using x-ray diffractometer (PW3040/60 X'pert Pro) using  $\text{CuK}\alpha$  radiation, 40 kV and 30 mA in 2 theta range of 5-80 degrees. The compositional of functional group in the samples were monitored by using FTIR spectrophotometer (Perkin Elmer Optima 100), and the surface morphology observed by Scanning Electron Microscopy (JEOL JSM-5610LV).

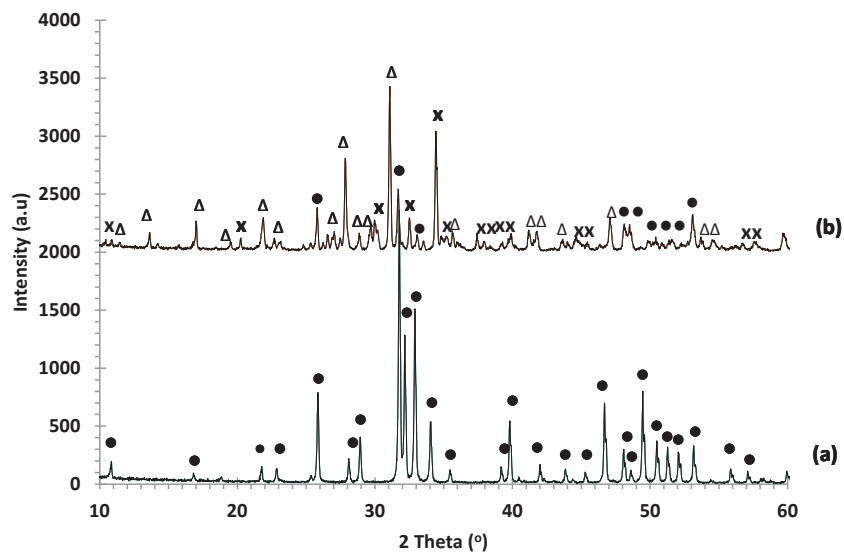
## RESULTS AND DISCUSSION

Figure 1 show XRD results of processed raw materials used in the synthesis of BHAp-10wt%  $\text{SiO}_2$  composite. The representative diffraction (XRD) pattern of the  $\text{SiO}_2$  rice husk heated at  $700^\circ\text{C}$  is shown in Fig.1a which indicates a single diffuse band centered at 2 theta around  $22^\circ$  (Fig. 1a). This indicates that the silica in this material was in an amorphous state<sup>15,18-19</sup>. The diffraction pattern of processed BHAp powder heated at  $800^\circ\text{C}$  is shown in Fig. 1b. The primary phase in this material is hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ - HAp related with JCPDS file # 09-0432 having hexagonal crystal system and 63/m space group. This result strongly declares the formation of crystalline pure hydroxyapatite<sup>17,20</sup>.



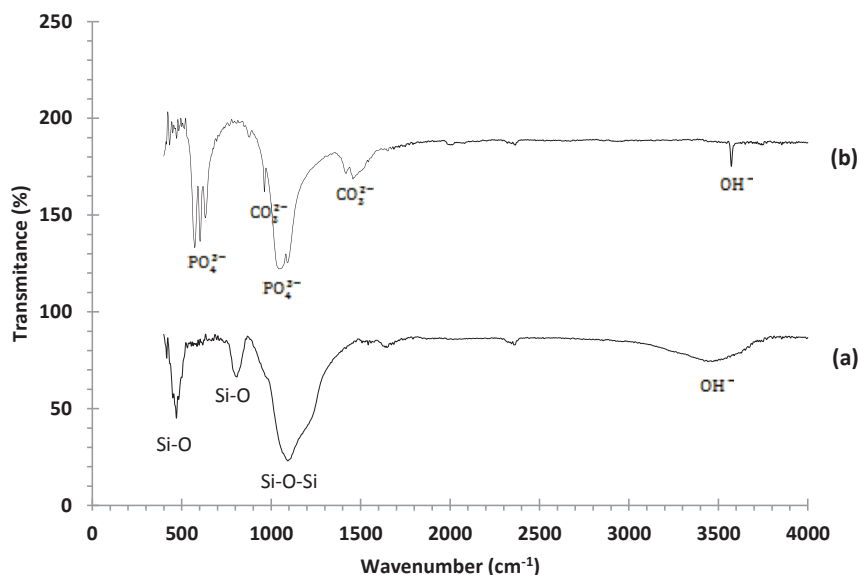
**FIGURE 1.** X-ray diffraction (XRD) patterns of SiO<sub>2</sub> rice husk heat treated at 700°C (a) and mandible bovine bone was (BHAp) heat treated at 800°C (b). ● = hydroxyapatite.

The diffraction pattern (XRD) of BHAp powder without SiO<sub>2</sub> used as a control sample and composite of processed BHAp with addition of 10 wt% SiO<sub>2</sub> heat treated at 1200 °C for 3h is shown in Fig. 2. The phase hydroxyapatite was the only phase observed for the heat treated BHAp without SiO<sub>2</sub> sample. In this sample no transformation phase was occurred. However, for the composite of BHAp with addition of 10 wt% SiO<sub>2</sub> the phase presents of hydroxyapatite (HAp or Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>), β-tricalcium phosphate (β-TCP or β-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>), and calcium phosphate silicate (CPS or Ca<sub>5</sub>(PO<sub>4</sub>)<sub>2</sub>SiO<sub>3</sub>) were observed. The appearance of peaks of β-TCP corresponds to the JCPDS file # 70-2065 belongs to Rhombohedra (R3c) symmetry, whereas the appearance of another peaks of CPS correspond to the JCPDS file # 40-0393<sup>21-22</sup>.



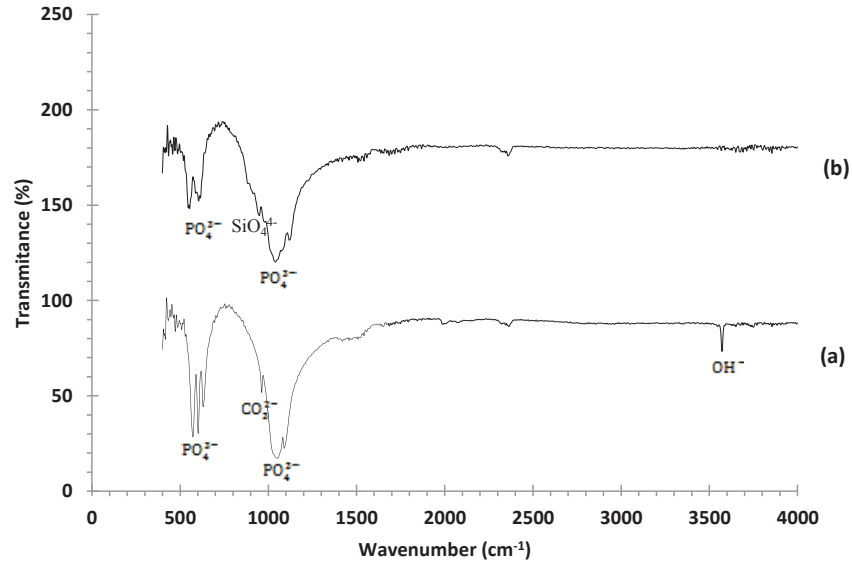
**FIGURE 2.** X-ray diffraction (XRD) patterns of (a) BHAp without SiO<sub>2</sub> sample heat treated at 1200°C and (b) BHAp-10 wt% SiO<sub>2</sub> heat treated at 1200°C. ● = hydroxyapatite (HAp), Δ=β-tricalcium phosphate(β-TCP), and x = calcium phosphate silicate(CPS).

Figure 3 shows the FTIR spectra of amorphous SiO<sub>2</sub> rice husk powder heat treated at 700°C 5h (a) and BHAp powder heat treated at 800°C 5h (b) in the range of 4000-400 cm<sup>-1</sup>. The FTIR spectra of both samples show a series of bands in the mid-infrared region. A large broad band around 3444 cm<sup>-1</sup> and a band centered at 1640 cm<sup>-1</sup> in Fig. 3a is due to the stretching and bending vibration of the O-H bond from silanol groups (Si-OH) and is attributed to the adsorbed water molecule on the silica surface<sup>23</sup>. The sharp peak band at 1099 cm<sup>-1</sup> is due to the Si-O-Si asymmetric stretching vibration, whereas the band at 803 cm<sup>-1</sup> has been assigned to the network Si-O-Si symmetric bending vibration<sup>23</sup>. The band peak at 480 cm<sup>-1</sup> is associated with bending frequency of O-Si-O modes<sup>23</sup>. The stretching band at 3588 cm<sup>-1</sup> and libration band at 631 cm<sup>-1</sup> showed in Fig. 3b is attributed to adsorbed water originate from OH<sup>-</sup> groups<sup>24</sup>. The characteristic bands for PO<sub>4</sub><sup>3-</sup> appear at 571 cm<sup>-1</sup>, 601 cm<sup>-1</sup>, 1050 cm<sup>-1</sup>, and 1089 cm<sup>-1</sup>. The observation of the asymmetric P-O stretching of the PO<sub>4</sub><sup>3-</sup> bands at 1050 cm<sup>-1</sup> as a distinguishable peak, together with sharp peaks at 571 cm<sup>-1</sup> and 601 cm<sup>-1</sup> correspond to the triply bending vibrations of PO<sub>4</sub><sup>3-</sup> in hydroxyapatite<sup>17,24-25</sup>. The characteristic bands of CO<sub>3</sub><sup>2-</sup> identified at 926 cm<sup>-1</sup> correspond to the C-O bending, and the bands at 1419, 1481 cm<sup>-1</sup> correspond to ν<sub>3</sub> mode of CO<sub>3</sub><sup>2-</sup><sup>2-17,24-25</sup>.



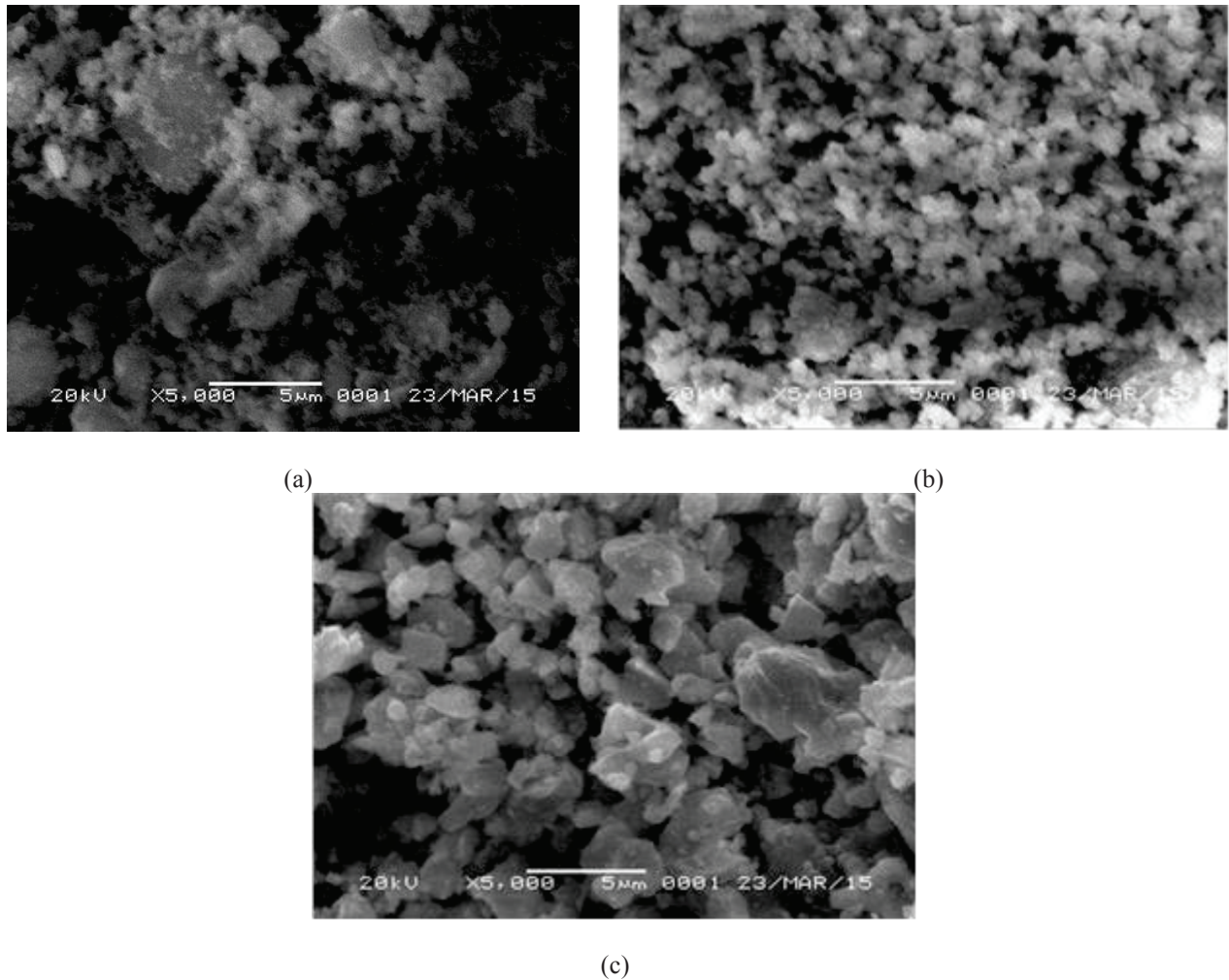
**FIGURE 3.** FTIR spectra of SiO<sub>2</sub> rice husk powder heat treated at 700 °C 5h (a) and BHAp powder heat treated at 800 °C 5h (b).

Figure 4 shows the FTIR spectra of BHAp without SiO<sub>2</sub> (a) and BHAp with 10 wt% SiO<sub>2</sub> (b) heat treated at 1200 °C 3h. The band centered at 3572 cm<sup>-1</sup> and 631 cm<sup>-1</sup> showed in Fig. 4a is attributed to the adsorbed water<sup>24</sup>. The characteristic bands for PO<sub>4</sub><sup>3-</sup> appear at 546 cm<sup>-1</sup>, 602 cm<sup>-1</sup>, 1050 cm<sup>-1</sup>, and 1090 cm<sup>-1</sup><sup>117,24-25</sup>. The characteristic bands of CO<sub>3</sub><sup>2-</sup> identified at 962 cm<sup>-1</sup> correspond to the C-O bending<sup>24-25</sup>. Comparing the FTIR spectra of BHAP without SiO<sub>2</sub> (Fig. 4a) and with SiO<sub>2</sub> (Fig. 4b), the most notable effect of SiO<sub>2</sub> addition on FTIR spectra of BHAP is the change in the hydroxyl stretching bands at 3572 cm<sup>-1</sup> and 631 cm<sup>-1</sup>. Addition of SiO<sub>2</sub> into biohydroxyapatite (BHAP) reduces the amount of hydroxyl group to counterbalance for the extra negative charge of the silicate group. No stretching band from OH<sup>-</sup> groups was observed in the BHAP spectra with SiO<sub>2</sub> addition as shown in Fig. 3b, however a new band appears at 945 cm<sup>-1</sup>. This band can be attributed to Si-O vibration of SiO<sub>4</sub><sup>4-</sup> and it is proved that the PO<sub>4</sub><sup>3-</sup> is replaced by SiO<sub>4</sub><sup>4-</sup> in the hydroxyapatite structure<sup>21</sup>.



**FIGURE 4.** FTIR spectra of BHAp without SiO<sub>2</sub> (a) and BHAp with 10 wt% SiO<sub>2</sub> (b) heat treated at 1200 °C 3h.

Figure 5 shows scanning electron microscopy (SEM) micrographs of amorphous SiO<sub>2</sub> rice husk powder heat treated at 700 °C 5h (a), BHAp powder heat treated at 800 °C 5h (b), and composite BHAp with addition of 10 wt% amorphous SiO<sub>2</sub> rice husk heat treated at 1200 °C 3h. In the amorphous SiO<sub>2</sub> rice husk powder heat treated at 700 °C (Fig. 5a), the microstructure composed of various size grains, most of them were large and irregularly shaped grains of a diameter approximately 4 μm, but in the BHAp sample heat treated at 800 °C (Fig. 5b), there were identified regular shaped grains resembling a sphere with a diameter of approximately ≤ 1 μm. The morphology of BHAp- 10 wt% amorphous SiO<sub>2</sub> composite (Fig. 5c) showed that there are many small spherical particles with a diameter of < 1 μm and large irregular shape particle with diameter of ≥ 5 μm distributed on the surface of the BHAp composite. Small spherical particle which indicate that the silica particles were melted and deformed from irregular to spherical shape, while large particle were still in irregular shape which can be attributed to unmelted silica particles<sup>26</sup>.



**FIGURE 5.** SEM of amorphous  $\text{SiO}_2$  rice husk powder heat treated at  $700^\circ\text{C}$  5h (a), BHAp powder heat treated at  $800^\circ\text{C}$  5h (b), and composite of BHAp with addition of 10 wt% amorphous  $\text{SiO}_2$  rice husk heat treated at  $1200^\circ\text{C}$  3h.

## CONCLUSION

Composite of BHAp- $\text{SiO}_2$  has been successfully synthesized by using the powders of hydroxyapatite obtained from mandible bovine bone waste and 10 wt% amorphous  $\text{SiO}_2$  rice husk and heat treated at  $1200^\circ\text{C}$  for 3h. The analysis of FTIR, XRD and results shows that the substitution of the silicate groups on the phosphate groups causes some  $\text{OH}^-$  loss to maintain the charge balance of hydroxyapatite, calcium phosphate silicate phase formed, and evolution of the composite microstructure.

## ACKNOWLEDGMENTS

Dwi Asmi is grateful to The Ministry of Research, Technology and Higher Education (RISTEKDIKTI) of Indonesia for research funding under Hibah Kompetensi research grant program with the contract No: 162/UN26/8/LPPM/2015.

## REFERENCES

1. L. L. Hench, *J. Am. Ceram. Society* **81(7)**, 1705-1728 (1988).
2. K. S. Vecchio, X. Zhang, J.B. Massie, M. Wang, and C.W. Kim, *Act. Biomaterial* **3(6)**, 910-918 (2007).
3. S. Santhosh and S.B. Prabu, *Mat. Letters* **97**, 121–124 (2013).
4. M. Sivakumar and K.P. Rao, *J. Biomed. Mat. Research Part A* **65A(2)**, 222–228 (2003).
5. S. Li, J. Wang, X. Jing, Q. Liu, J. Saba, T. Mann, M. Zhang, H. Wei, R. Chen, and L. Liu, *J. Am. Ceram. Society* **95(11)**, 3377-3379 (2012).
6. B. Chaudhuri, B. Mondal, D.K. Modak, K. Pramanik, and B.K. Chaudhuri, *Mat. Letters* **97**, 148-150 (2013).
7. S.-C. Wu, H.-C. Hsu, Y.-N. Wu, and W.-F. Ho, *Mat. Characterization* **62(12)**, 1180-1187 (2011).
8. D. S. Seo and J.K. Lee, *Ann. Biomed. Engineering* **36(1)**, 132-140 (2008).
9. N. A.M. Barakat, M.S. Khil, A.M. Omran, F.A. Sheikh, and H.Y. Kim, *J. Mat. Proc. Technology* **209(7)**, 3408-3415 (2009).
10. L. Duta, F.N. Oktar, G.E. Stan, G. Popescu-Pelin, N. Serban, C. Luculescu and I.N. Mihailescu, *App. Surf. Science* **265**, 41–49 (2013).
11. Y. G. Kim, D.S. Seo, and J.K. Lee, *App. Surf. Science* **255(2)**, 589-592 (2003).
12. J.S. Cho, H.-S. Kim, S.-H. Um, and S.-H. Rhee, *J. Biomed. Mat. Res. Part B: App. Biomaterials* **101B(5)**, 855-869 (2013).
13. S. Jinawath, D. Pongkao, and M. Yoshimura, *J. Mat. Sci. Mat. Medicine* **13(5)**, 491-494 (2002).
14. S. Joschek, B. Nies, R. Krotz, and A. Göpferich, *Biomaterials* **21(16)**, 1645-1658 (2000).
15. J. Umeda and K. Kondoh, *J. Mat. Science* **43(22)**, 7084-7090 (2008).
16. L. Sun and K. Gong K, *Ind. Eng. Chem. Research* **40**, 5861-5877 (2001).
17. D. Asmi, *Adv. Mat. Research* **112**, 155-159 (2015).
18. U. Kalapathy, A. Proctor, J. Schultz, *Bioresource Technology* **73**, 257-262 (2000).
19. U. Kalapathy, A. Proctor, J. Schultz, *Bioresource Technology* **85**, 285-289 (2002).
20. S. Pramanik, A. Agarwal, and K. N. Rai, *Trends Biomat. Arti. Organs* **19(1)**, 46-51(2005).
21. S.R. Kim, J.H. Lee, Y.T. Kim, D.H. Riu, S.J. Jung, Y.J. Lee, S.C. Chung, Y.H. Kim, *Biomaterials* **24**, 1389-1398 (2003).
22. A. El Yacoubi, A. Massit, M. Fathi, B. Chafik El Idrissi, K. Yamni, *J. App. Chemistry* **7(11)**, 24-29 (2014).
23. S. H. Javed, F.H. Shah, and M. Manasha, *JFET* **18**, 39-46 (2011).
24. M. Markovic, B.O. Fowler and M.S. Tung, *J. Res. Natl. Inst. Stand. Technology* **109**, 553-568 (2004).
25. K. Haberko, M.M. Bucko, J. Brzezinska-Miecznik, M. Haberko, W. Mozgawa, T. Panz, A. Pyda and J. Zarebski, *J. Europ. Ceram. Society* **26**, 537-542 (2006).
26. M. F. Morks and A. Kobayashi. *Trans. JWRI* **35(2)**, 11-16 (2006).