EFFECT OF CALCINATION TEMPERATURE ON MORPHOLOGICAL, STRUCTURAL AND THERMAL PROPERTIES OF HYDROXYAPATITE DERIVED FROM GOAT BONE

Lin Lin Naing[1], Ni Ni Sein[2], ThidaWin[3]

Abstract

Hydroxyapatite (HAp) is included in the calcium phosphate compound family having the formula $Ca_{10}(PO_4)_6(OH)_2$.Calcination method was used to extract hydroxyapatite from waste goat bone, a useless resource, after deproteinization. This study focuses the study of HAp derived from waste goat bone subjected to different calcination temperatures (800ºC, 900ºC, 1000ºC and 1100ºC) with regard to their morphological, structural and thermal properties. Calcination treatment has eliminated the collagen and organic compounds from the HAp derived from goat bones. EDXRF spectrum showed the highest amount of calcium and phosphorus in HAp samples at all calcination temperatures. Only a single phase of hydroxyapatite was noted in the XRD pattern at each calcination temperature and the crystal system was indexed as hexagonal. Crystallinity percent increased from 56.77% at 800ºC to 62.73% at 1100ºC. With increase in calcination temperature crystallite size also increased but the increase was not pronounced at higher temperature. The absorption bands of 1548 and 1662 cm^{-1} originated by the collagen disappeared after calcination at 800 ºC. Thermal analysis revealed that the highest weight loss (32.215 %) accompanied by an exothermic peak was observed for uncalcined hydroxyapatitedue to the loss of organic constituents like collagen whereas the weight losses were negligible for the calcined samples.

Keywords: goat bone, hydroxyapatite, calcination method, crystallinity, hexagonal

Introduction

Hydroxyapatite (HAp), a main inorganic crystalline component in bones and teeth, is included in the calcium phosphate compound family having the formula $Ca_{10}(PO_4)_6(OH)_2$. Hydroxyapatite (HAp) crystallizes in a hexagonal system, though with some exceptions in a monoclinic system. The system possesses the hexagonal space group with hexagonal rotational symmetry and a reflection plane and cell

1. Assistant Lecturer , Department of Chemistry, Mandalar Degree College
2. Professor (Retired), Department of Chemistry, University of Yangon
3. Rector, University of Mandalay

parameters of a=b=9.418 Å and c=6.884 Å. HAp structure is formed by a tetrahedral arrangement of phosphate (PO_4^{3-}), which constitute the skeleton of the unit cell. The two oxygens are aligned with the c-axis and the other two are in a horizontal plane (Figure 1).

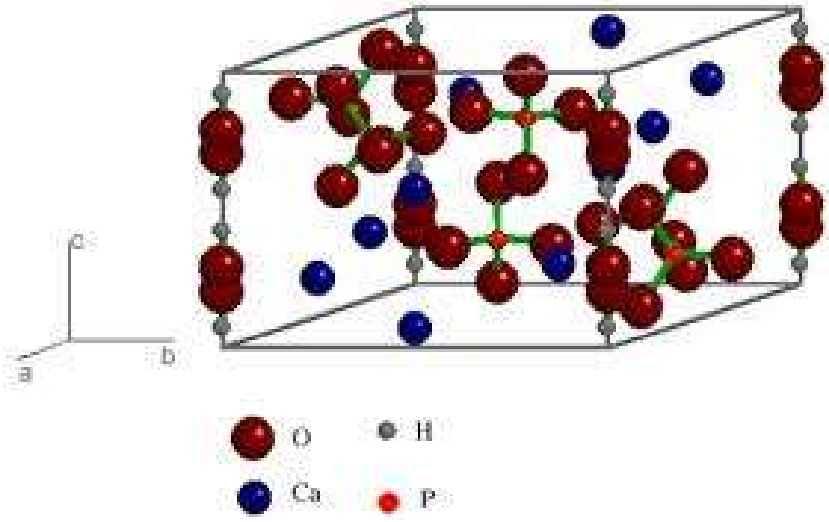


Figure 1: Crystal structure of hydroxyapatite

Hydroxyapatite is one of the most attractive materials for human hard tissue implants because it has close similarities with inorganic mineral component of bone and teeth (Nayak,2010). Naturally occurring HAp is hexagonal in structure with the chemical formula of one unit cell being $Ca_{10}(PO_4)_6(OH)_2$. HAp is also a potential implant material due to its excellent osteoconductive properties. HAp is not only a biocompatible, osteoconductive, non-toxic, non-inflammatory and non-immunogenic agent but also bioactive, *i.e.,* it has got the ability to form a direct chemical bond with living tissues (Fathi *et al*.,2008; Barakat *et al*., 2009).HAp can be derived either from a natural source or by a synthetic method. Both natural and synthetic HAp are widely used as bioceramic in forms of particle, block and coating for the hard tissue repair (Xiaoying *et al*., 2007). HAp having good biocompatibility with human tissues and similarities with the mineral fraction of natural bone is used as a bone graft material in medicine and dentistry. HAp can also be used not only as a bone replacement implants heart valves,

hip extension and other implants in the human body but also as a filler to replace amputated bone or as a coating of metallic prostheses. Besides, HAp are applied for the reconstruction of skull defects, tissue engineering, artificial bone synthesis, biosensor, removal of heavy metals, and as drug carrier (Venkatesan and Kim, 2010).

HAp material manufactured from animal bones can form strong chemical bonds with bone tissues and also it has the advantages of inheriting some properties of the raw materials *viz*. its chemical composition and structure. The composition of HAp from goat bone has similar properties to human bone mineral that it can be used for filling or replacement of bone and teeth. Biowaste such as goat bone is widely available in Myanmar and it can be used as a source of hydroxyapatite. Extraction of hydroxyapatite from goat bone is biologically safe and economic.

The present study is therefore focussed on the preparation of ydroxyapatite from waste goat bone and to study the effect of calcination temperature on its morphological, structural and thermal properties.

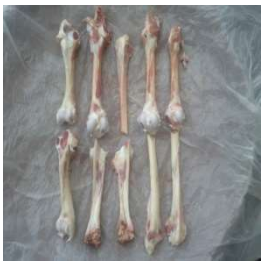
Materials and Methods

Sample Collection

The raw goat bone samples were directly collected from a local butcher shop in Mandalay Region.

Sample Preparation

The bones were firstly cleaned with water to remove dirty substances and then cooked in a steel pot on a hotplate for several hours to get rid of any remaining unwanted materials. The dried bones were crushed into small chips and transformed to fine powder (Figure 2).



(a) Raw goat bone

(b) Cleaned goat bone

(c) Raw goat bone powder

Figure 2: Preparation of raw goat bone powder

Preliminary Investigation of Raw Goat Bone

Moisture, protein and ash contents were determined by oven drying method, Kjeldahl digestion method and muffle furnace respectively. Phosphorus content was determined with Molybdivanadophosphoric acid method by using UV Vis Spectrophotometer PD-303 UV at Department of Agricultural Research, Ye Zin, Nay Pyi Taw. Calcium content was determined by wet digestion with HNO_3:$HClO_4$ (4:1v/v) followed by measuring with Atomic Absorption Spectrophotometer AA-6200, SHIMADZU at Department of Agricultural Research, Ye Zin, Nay Pyi Taw.

Preparation of Hydroxyapatite from Goat Bone

Hydroxyapatite was prepared according to the procedure of Mondal *et al*.,2012 with some modifications. The collected bones were initially washed with cleaned water to remove dirty substances and then cooked in a steel pot on a hot plate for several hours to get rid of any remaining unwanted materials. The bones were then deproteinized through immersion in 1M hydrochloric acid solution for 24 h at room temperature. Next, the deproteinized goat bones were thoroughly washed several times with distilled water. After that, the bones were immersed in 1M sodium hydroxide solution for 24 h to remove the remaining proteins. Then, the filtered goat bones were thoroughly washed with distilled water again and were dried at 60˚C in hot air oven for several hours. The dried bones were crushed into small chips and transformed to fine powder. Finally, a yellowish white hydroxyapatite powder was obtained. Hydroxyapatite powder was calcined at four different temperatures *viz*., 800ºC, 900ºC, 1000ºC and 1100ºC in a muffle furnace (LEF-103S, DAIHAN LABTECH Group, Korea) for 3 h. The calcined powder samples were cooled and examined the colour. The samples were stored for further studies.

Characterization Techniques

Relative abundances of elements in uncalcined and calcined hydroxyapatite samples derived from goat bone were qualitatively determined by EDXRF analysis using EDX-702 spectrometer (Shimadzu Co. Ltd., Japan) at Universities' Research Center, Yangon. The scanning electron microscope (SEM, JEOL-JSM-5610LV, Japan) was used for the morphological study of uncalcined and calcined hydroxyapatite samples. The phase purity was examined by using Rigaku X-ray diffractometer (Rigaku Co., Japan) with Cu K_α (λ=1.54056 Å) radiation over a range of 2θ angles from 10° to 70°. Various functional groups present in the prepared HA powder as well as the powders calcined at different temperatures were identified by FTIR (Perkin Elmer). Thermal analysis of hydroxyapaptite samples was investigated by TG-DTA employing Thermal Analyzer (DGH-60H), Shimadzu, Japan.

Results and Discussion

Composition of Raw Goat Bone Sample

The experimental results of composition of raw goat bone powder sample are shown in Table 1. The protein and ash contents of the sample were found to be 25.2 % and 2.0 % respectively. The moisture content of the sample was found to be 6.0 %.The calcium and phosphorus contents of the sample was found to be 11.7 % and 11.8 % respectively.

Table 1: Composition of Raw Goat Bone Sample

No	Component	Results (%)
1.	Protein	25.2
2.	Ash	2.0
3.	Moisture	6.0
4.	Calcium	11.7
5.	Phosphorus	11.8

Change of Colour during Calcination

Figure 3 shows the change of colour of hydroxyapaptite samples upon calcination. Before calcination, hydroxyapatite powder was pale yellowish white. After calcination at 800ºC the yellow colour diminished and at temperature 900ºC and above it changed to white colour.

(a)Uncalcined sample (b) 800˚C (c) 900˚C (d) 1000˚C (e) 1100˚C

Figure 3: Colour of hydroxyapatite derived from goat bone (a) uncalcined sample (b) at 800ºC (c) at 900ºC (d) at 1000ºC and (e) at 1100ºC

Relative Abundances of Elements in Hydroxyapatite Samples Derived from Goat Bone

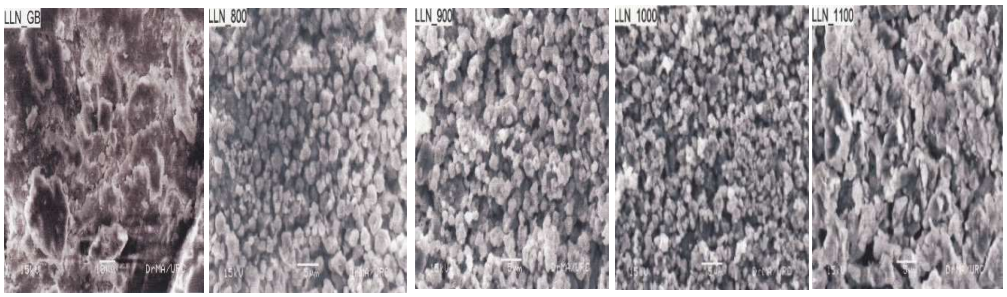
Table 2 shows the relative abundances of elements in uncalcined and calcined hydroxyapatite samples. In all of these samples calcium was found to have the highest percentage followed by phosphorus. Other elements such as iron and strontium were found in all hydroxyapatite samples. Potassium was observed in these calcined samples and zinc and zirconium were found in uncalcined sample and calcined sample at 1000ºC respectively.

Table 2 Relative Abundance of Elements in Hydroxyapatite Samples Derived from Goat Bone at Different Calcination Temperatures

No	Temperature (˚C)	Relative abundance (%)						
		Ca	P	Fe	Sr	K	Zn	Zr
1	Room temperature (uncalcined sample)	77.853	20.332	0.592	1.107	-	0.116	-
2	800	78.813	19.386	0.714	0.426	0.661	-	-
3	900	77.153	21.861	0.560	0.425	-	-	-
4	1000	76.288	21.347	1.248	0.439	0.597	-	0.082
5	1100	76.469	21.638	0.841	0.371	0.682	-	-

Surface Morphology of Uncalcined and Calcined Hydroxyapatite Samples Derived from Goat Bone

The changes of morphology in SEM images of the hydroxyapatite samples derived from goat bone are shown in Figure 4. Densely compacted organic molecules including collagen fibrils along with inorganic mineral structure were observed in uncalcined hydroxyapatite. With increase in calcination temperature the organic polymers were removed and smaller size hydroxyapatite was observed in the SEM image obtained at 800 ºC. Porosity decreased as seen in hydroxyapatite derived from goat bone samples calcined at 800 ºC. At 900 ºC, the particles were found to coalesce and at 1000 ºC necking was observed. As the temperature increased porosity decreased and increased amount of neck formation was observed in the samples calcined at 1100 ºC. The porosity was found to decrease indicating low strength, but as the temperature increased discontinuous cavities were visible due to the formation of neck which increases the strength. This observation was also reported by Pattanayak *et al.,* 2005.



(a) (b) (c) (d) (e)

Figure 4: SEM images of hydroxyapatite samples derived from goat bone (a) uncalcined goat bone and calcined samples at (b) 800ºC (c) 900ºC (d)1000ºC and (e) 1100ºC

X-ray Diffraction Analysis of Hydroxyapaptite Samples Derived from Goat Bone

XRD analysis is a highly trustable technique utilized to investigate the crystalline compounds. Figure 5 shows the XRD spectra of the uncalcined and calcined hydoxyapatite samples derived from goat bone. The XRD spectrum of uncalcined hydroxyapatite sample shows less crystalline peaks and not all the standard hydroxyapatite peaks have been obtained. The intensity of raw goat bone was found to be dispersed by X-ray radiations with a lower intensity and wider peak. This may be due to the presence of extracellular matrix and fibrous collagen. When subjected to calcination at higher temperatures, the broad peak gradually became split into five distinct crystalline peaks, (211), (112), (300), (130) and (222) at 2θ values of 31.805º, 32.234 º,32.936 º, 39.830 º and 46.733 º, respectively, which are similar to the standard HAp (74-0565). Sharp and narrow peaks with high intensity of crystalline patterns were observed. The intensity count of XRD peaks increased due to the detection of more number of diffracted rays developing from the larger number of same group of planes. Therefore, it can be concluded that the calcination treatment has eliminated the collagen and organic compounds from the goat bones and does not affect the molecular skeleton of the hydroxyapatite. The present XRD results suggest that HAp stability has not been affected and no other peaks were observed apart from standard HAp.

Phase purity

Furthermore, X-ray diffraction was employed to evaluate the phase purity and the crystallographic structural properties of the goat bone after calcination at four selected temperatures (800ºC, 900ºC, 1000ºC and 1100ºC). Table 3 shows the phase purity of the hydroxyapatite derived from goat bone at 800ºC, 900ºC, 1000ºC and 1100ºC. It was noted that only single phase of hydroxyapatite with no other phase was found. The well-resolved XRD spectra could be easily indexed on the basis of hexagonal crystal system with equal axial length of 'a' and 'b' and shorter length 'c'.

Crystallinity and crystallite size

Crystallinity percent was calculated by the following equation.

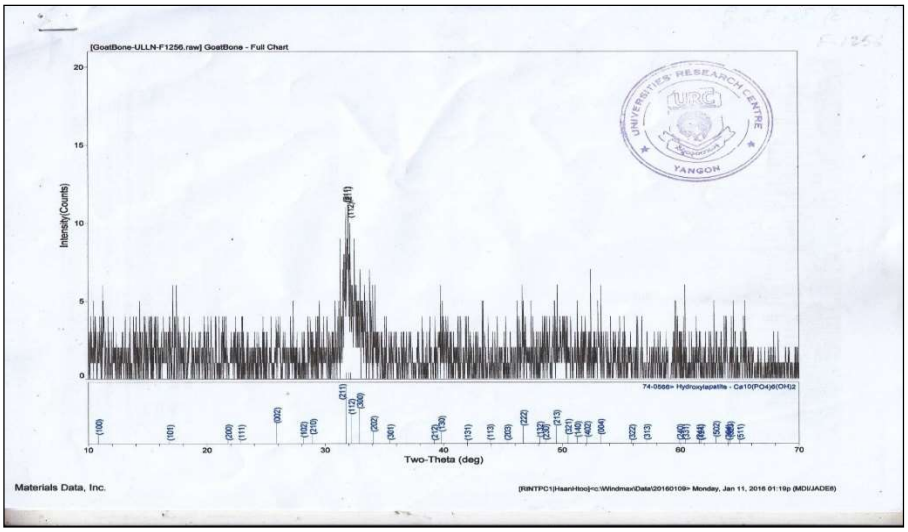
$$\text{Crystallinity \%} = \frac{\text{total crystalline peak area}}{\text{total area of all peaks}} \times 100$$

Crystallinity of the hydroxyapatite derived from goat bone was found to increase with increasing temperature (Table 4). In raw goat bone the crystallinity percent was 26.28 %. The broad peaks in uncalcined hydroxyapatite reflect a poor crystallinity. Increasing the temperature results more intense and sharp peaks corresponding to an increase in the mineral crystallinity. Further increase of the calcinations temperature to 900ºC, 1000ºC and 1100ºC the crystallinity percents were comparable, i.e., about 60%. The increase in crystallinity indicated that organic portions were completely removed from hydroxyapatite.

Crystallite size τ (tau) of the hydroxyapatite powder was evaluated from the peak broadening of XRD patterns based on Scherrer's equation (Landi *et al*., 2000) as follows: $\tau = \frac{0.9\lambda}{\beta \cos\theta}$

in which τ is the crystallite size (nm), λ is the diffraction wavelength (0.154059 nm for Cu K_α radiation) , θ is the diffraction angle (degree) and 'B' is the full width at half maximum (FWHM) for the diffraction peak (radian).

Table 4 shows the changes of crystallite size of hydroxyapatite derived from goat bone before and after calcination. Crystal size increased with increasing temperature and the sizes were not much different at higher calcination temperatures.



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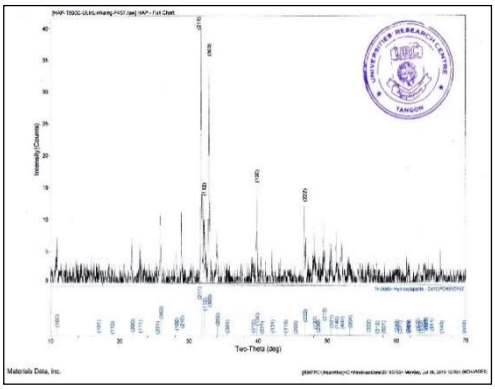

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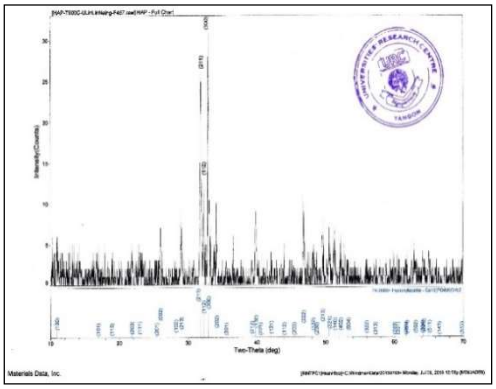

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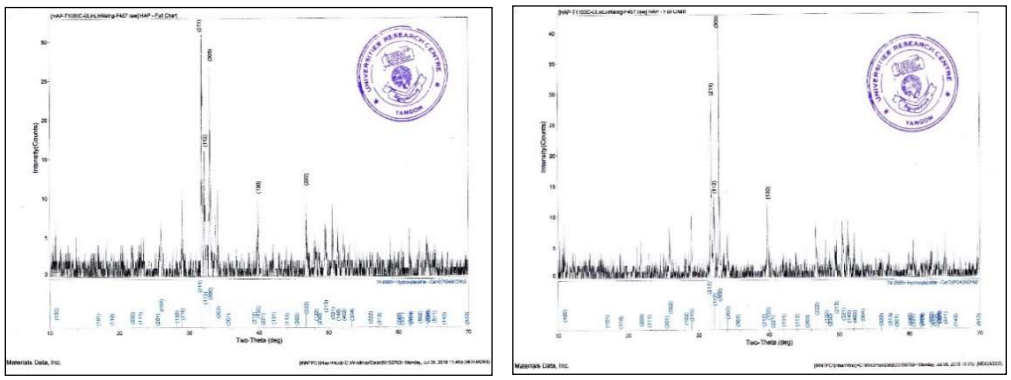


(d) (e)

Figure 5: X-ray diffraction patterns of uncalcined and calcined hydroxyapatite samples derived from goat bone (a) uncalcined sample and samples calcined at (b) 800˚C(c)900˚C(d) 1000˚C (e) 1100˚C

Table 3: Phase Purity and the Crystallographic Structural Properties of Uncalcined and Calcined HAp Samples Derived from Goat Bone

Samples	**Phase**	**Lattice constants (Å)**			**Crystal structure**
		a	**b**	**c**	
UncalcinedHAp	Hydroxyapatite	9.3826	9.3826	6.9600	Hexagonal
HAp at 800^{o}C	Hydroxyapatite	9.4167	9.4167	6.8929	Hexagonal
HAp at 900^{o}C	Hydroxyapatite	9.3893	9.3893	6.8688	Hexagonal
HAp at 1000^{o}C	Hydroxyapatite	9.4062	9.4062	6.8862	Hexagonal
HAp at 1100^{o}C	Hydroxyapatite	9.4168	9.4168	6.8766	Hexagonal

Table 4: Crystallinity Percent and Average Crystallite Size of Uncalcined and Calcined HAp Samples Derived from Goat Bone

No	Temperature (˚C)	Total area of crystalline peaks (nm^2)	Total area of all peaks (nm^2)	Crystallinity (%)	Average crystallite size (nm)
1	Room temperature (Uncalcined sample)	22.6	86.0	26.28	25.45
2	800	43.2	76.1	56.77	74.53
3	900	45.7	77.0	59.35	81.13
4	1000	45.3	75.7	59.84	83.10
5	1100	46.8	74.6	62.73	83.53

FT IR Analysis

The FT IR spectra of the uncalcined and calcined hydroxyapatite samples derived from goat bone are shown in Figures 6, 7,8, 9 and 10. The corresponding spectral data are shown in Table 5. The FT IR spectrum of uncalcined sample shows the characteristic peaks of hydroxyapatite at 563 cm^{-1}, 603 cm^{-1}, 960 cm^{-1} (shoulder) and 1030 cm^{-1}due to phosphate vibrations (Figueiredo *et al*., 2010). The presence of collagen in goat bone was indicated by C=O stretching vibration at 1662 cm^{-1} and N-H in plane bending at 1548 cm^{-1} . The double band at 1415 cm^{-1} and 1454 $cm^{-1}$and also a peak at 871 $cm^{-1}$are attributed to the vibration of carbonate group. Moreover, O-H stretching vibration of hydroxyl group was observed at 3435 cm^{-1}.

After calcination most of the bands due to the phosphate vibrations of hydroxyl apatite have largely increased in intensity. Within 500-700 cm^{-1} region of the spectra of calcined samples, three bands at 632, 601, 570 cm^{-1} were observed whereas in the spectrum of uncalcined sample only two bands were observed. The bands at 630 cm^{-1}appeared with low intensity in the spectra of calcined hydroxyapatite but it is not observed in the uncalcined sample. It is clearly resolved in the spectra of calcined samples. The absorption bands of 1548 and 1662 cm^{-1}due to the presence of collagen disappeared after calcinations at 800 ºC and above. Typical bands of carbonate at 871, 1410 and 1445 cm^{-1} attributed to lattice carbonate vibration

(Landi *et al*., 2003) show small intensity in the spectra of all calcined samples indicating the removal of carbonate.

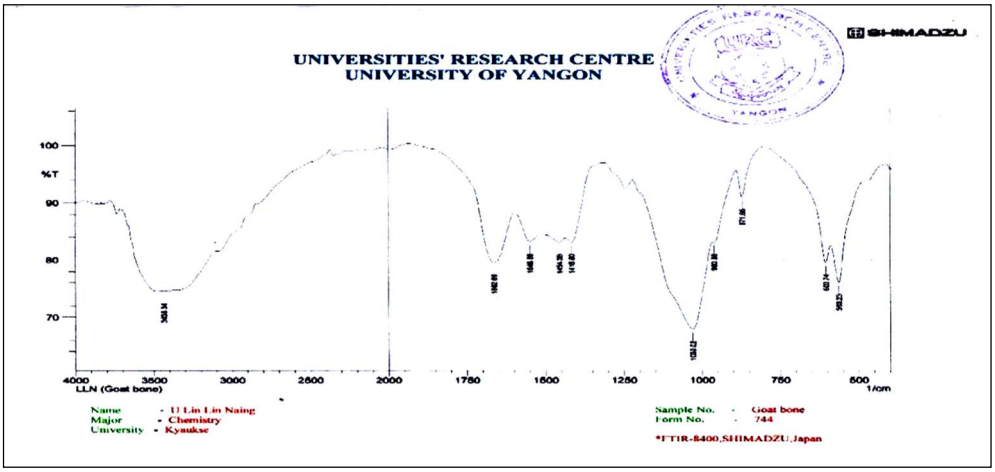


Figure 6: FT IR spectrum of uncalcined HAp sample derived from goat bone

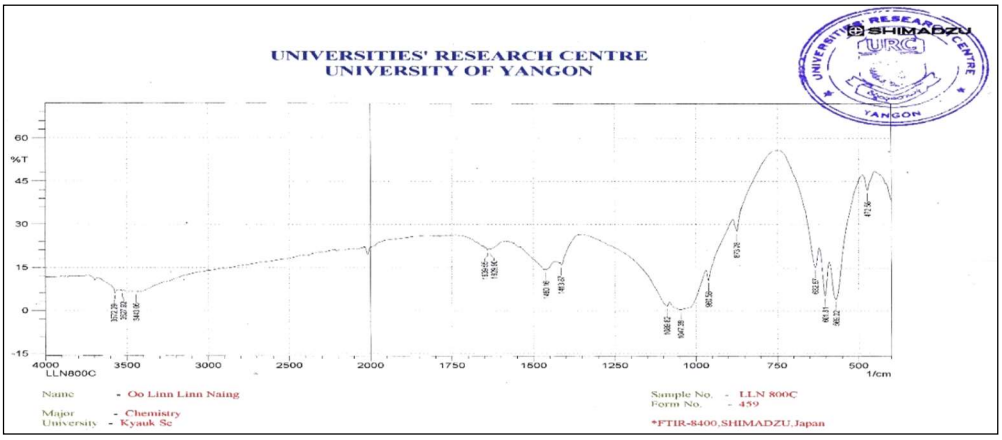


Figure 7: FT IR spectrum of HAp derived from goat bone calcined at 800˚C

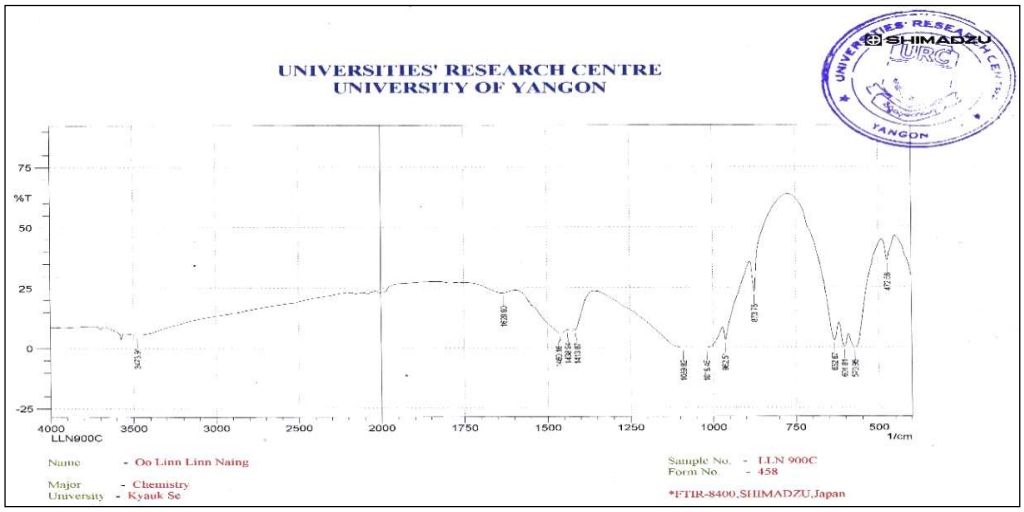


Figure 8: FT IR spectrum of HAp derived from goat bone calcined at 900˚C

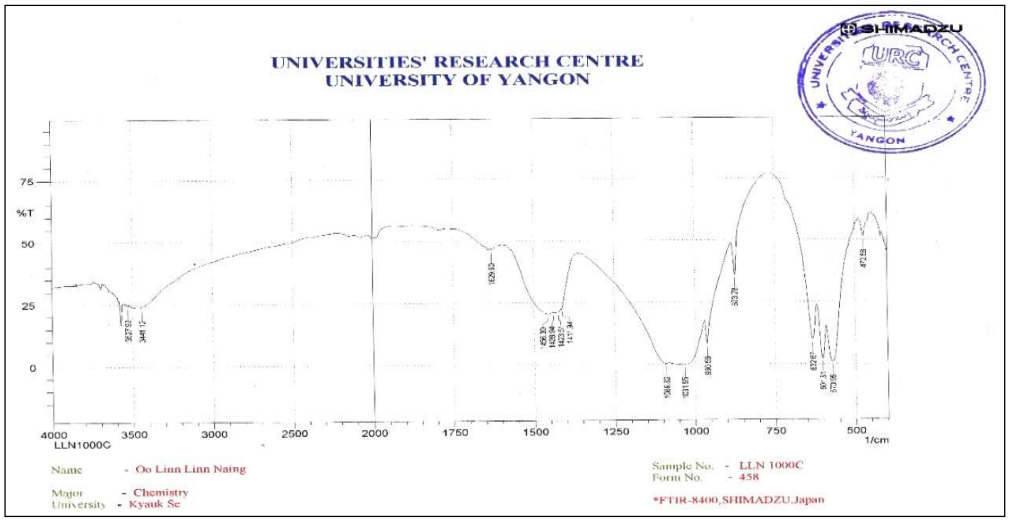


Figure 9: FT IR spectrum of HAp derived from goat bone calcined at 1000˚C

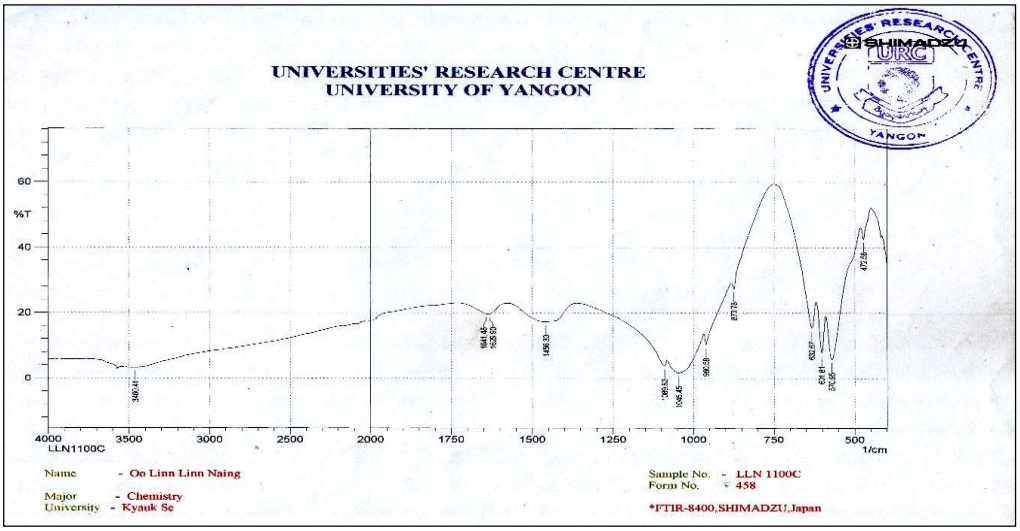


Figure 10: FT IR spectrum of HAp derived from goat bone calcined at 1100˚C

Table 5: FT IR Spectral Data of Uncalcined and Calcined Hydroxyapatite Samples Derived from Goat Bone

No	Wavenumber (cm^{-1})						Remarks
	Uncalcined sample	800˚C	900˚C	1000˚C	1100˚C	Reported values*	
1	3435	-	-	-	-	3100-3500	O-H and N-H stretching
2	-	3443	3473	3441	3460	3100-3500	Stretching vibration of O-H
3	1662	-	-	-	-	1634	Stretching vibration of C=O (Collagen)
4	1548	-	-	-	-	1548	N-H in plane bending (Collagen)
5	1454	1460	1460	1456	1456	1400-1629	Carbonate groups
6	1415	1413	1413	1411	-	1400-1629	Carbonate groups
7	-	1089	1089	1089	1089	900-1200	Stretching P-O
8	1030	1047	1018	1031	1045	900-1200	Stretching P-O
9	960	960	962	960	960	900-1200	Stretching P-O
10	871	873	873	873	873	871	Carbonate group
11	-	632	632	632	632	500-700	Bending P-O
12	603	601	601	601	601	500 - 700	Bending P-O
13	563	569	570	570	570	500-700	Bending P-O

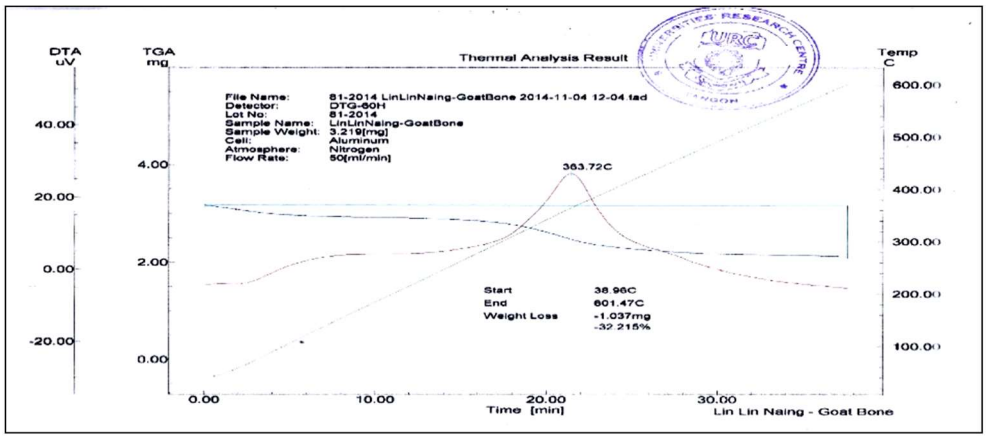
*Figueiredo *et al*., 2010

TG-DTA Analysis

TG-DTA thermogram of hydroxyapatite derived from goat bone is shown in Figure 11(a).

Within the temperature range of 38.96ºC to 90ºC small initial weight loss was observed as 8.045% due to the dehydration of goat bone (surface and bound water). Exothermic peak was observed at 363.72ºC in the temperature range of 90ºC to 445ºC. Appearance of the exothermic peak in DTA curve was due to the combustion of organic component of bone (mainly collagen). So weight loss of 24.324 % was noted in this temperature range. As the

temperature was increased from 445ºC to 601.47ºC unnoticeable weight loss was observed and the TG curve was found to be thermally stable. For calcined hydroxyapatite samples TG-DTA thermograms are depicted in Figures 11 (b,c,d and e).No inflection point was noted in each TG profile of calcined hydroxyapatite sample indicating the stability of the composition of hydroxyapatite. A very small endothermic peak occurred at around 400ºC may be due to the removal of residual organic moieties. The weight losses of the uncalcined and calcined samples subjected to TG analysis are shown in Table 6. The highest weight loss was observed for uncalcined hydroxyapatite (32.215%) due to the loss of organic constituents like collagen. On the other hand, negligible amount was noted for calcined samples. This finding confirmed the decomposition of organic moieties during calcination.



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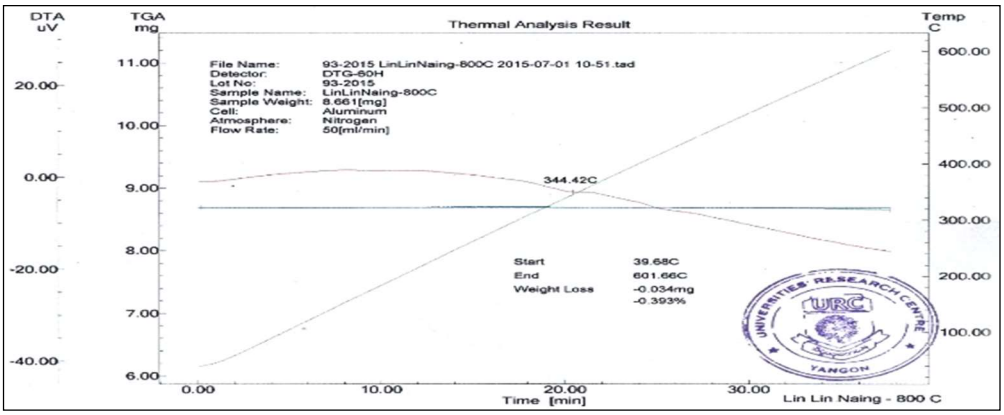

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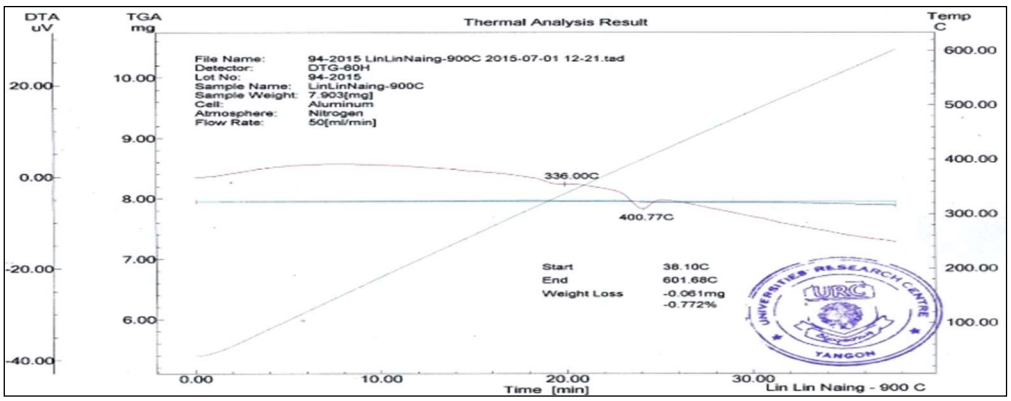

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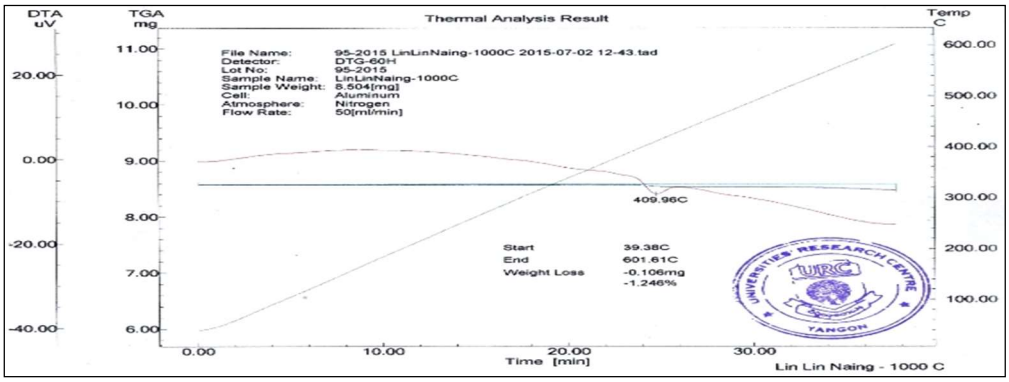

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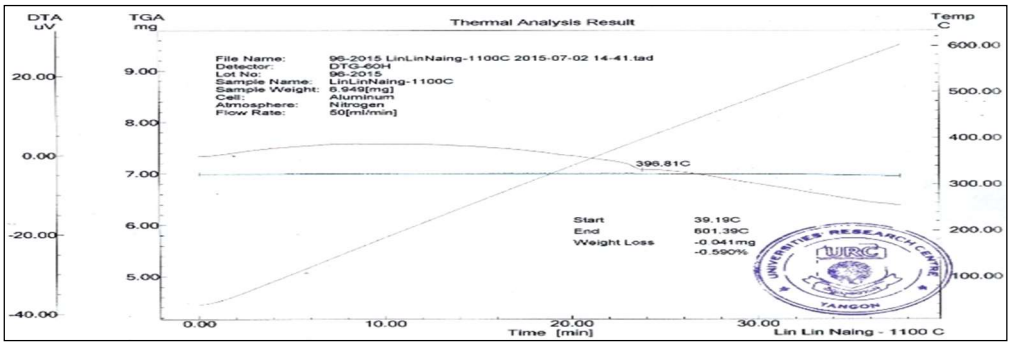

(e)

Figure 11: TG DTA thermograms of uncalcined and calcined hydroxyapatite samples derived from goat bone

Table 6: Weight Loss Percents of Uncalcined and Calcined Hydroxyapatite Samples Derived from Goat Bone after TG-DTA Analysis

No	Samples	Initial weight (g)	Final weight (g)	Weight loss (g)	Weight loss (%)
1	Room temperture	3.219	2.182	1.037	32.215
2	Calcined at 800°C	8.661	8.627	0.034	0.393
3	Calcined at 900°C	7.903	7.842	0.061	0.772
4	Calcined at1000°C	8.504	8.398	0.106	1.246
5	Calcined at 1100°C	6.949	6.908	0.041	0.590

Conclusion

Goat bone hydroxyapatite was prepared from waste goat bone by deproteinization followed by calcination. EDXRF analysis revealed that calcium and phosphorous were present in higher amount both in uncalcined and calcined samples. SEM analysis showed compact and dense structure of both organic and inorganic molecules before calcination of hydroxyapatite 1100 °C densification was observed due to the neck formation between particles.By XRD analysis sharp and narrow peaks with high intensity of crystalline patterns were observed in calcined samples compared to uncalcined sample indicating the elimination of the collagen and organic compounds from the goat bones. Crystallinity percent and crystallite size increased from 800 °C to at 1100 °C but the increases were not much different at high calcination temperatures.The absorption bands due to the presence of collagen (1548 and 1662 cm^{-1}) diminished after calcination. In TG-DTA thermogram of uncalcined sample showed high weight loss of 32.215% which is accompanied by an exothermic peak at 363.72°C. However, samples calcined at 800°C, 900 °C, 1000 °C and 1100 °C showed negligible weight losses indicating the stability of the hydroxyapatite. Among the calcination temperatures of 800 °C, 900 °C, 1000 °C and 1100 °C,morphological, structural and thermal properties were not much different.

This study revealed that calcination is an easy and affordable way to extract hydroxyapatite from waste goat bone resources.

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