

PHASES OF CATTLE BONES AT ELEVATED TEMPERATURES

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Abstract

The high temperature phases of cattle bones were studied with a view to the potential use of this material as prosthetic bone. Bone samples from the middle part of cow and buffalo legs were radially cut into pieces and heat-treated to temperatures ranging from 900° to 1400°C. The phases of air-quenched and slow-cooled samples were investigated using an x-ray diffractometer and an infrared spectrophotometer.

The results indicated that from 900° to 1300°C, the quenched samples from the middle part of the leg bones were composed entirely of calcium hydroxy-oxy apatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2(1-x)}\text{O}_x$, which dissociated into alpha-tricalcium phosphate, $\alpha\text{-Ca}_3(\text{PO}_4)_2$ and tetracalcium phosphate, $\text{Ca}_4\text{O}(\text{PO}_4)_2$ at 1400°C. In the case of slow cooling, the same behaviour was observed but the $\alpha\text{-Ca}_3(\text{PO}_4)_2$ was transformed to the beta-tricalcium phosphate, $\beta\text{-Ca}_3(\text{PO}_4)_2$.

Introduction

Calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), a mineral found in natural teeth and bone¹, has been recently commercialized for prosthetic implants². Hayek and Newesely³ successfully precipitated very fine and pure hydroxyapatite from aqueous media containing $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{HPO}_4$ and ammonia under controlled pH and temperature. Various kinds of starting material⁴⁻⁸ have been substituted based on the assumption that calcium hydroxyapatite obtained from the reaction between water-soluble salts of calcium and phosphate should be insoluble in aqueous solution.

Solid state reaction at high temperature can also be used for the preparation of hydroxyapatite. This must be carried out in an atmosphere of water vapor and nitrogen. Lehr *et al.*⁹ recommended heating a stoichiometric mixture of minus 200 mesh $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ and CaCO_3 for 3 h at 1200°C in an equal volume of water and nitrogen. Seuter¹⁰ showed that calcium hydroxyapatite could also be prepared by thermogravimetric experiments, by heating a 2:3 molar ratio of CaCO_3 and CaHPO_4 in the flow of dry

nitrogen and water vapour. The hydrothermal method is yet another technique for synthesizing apatite. For example, Mosebach¹¹ prepared hydroxyapatite single crystals hydrothermally using $\text{Ca}(\text{NO}_3)_2$, KH_2PO_4 and NaOH . Other attempts using different conditions have been reported¹²⁻¹⁷.

However, the preparation of hydroxyapatite powder as a starting material for a prosthetic ceramic implant is considered to be very expensive compared to that obtained from natural cattle bone. Cattle bone ash has been used in the whiteware industry and is the main raw material for "bone china". Degreased bones are calcined at 600°C , milled, dried and powdered. The white powder is composed of 67% to 85% calcium phosphate, 3% to 10% calcium carbonate, 2% to 3% magnesium phosphate and small amounts of caustic lime and calcium fluoride; it is given an approximate formula of $4 \text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaCO}_3$, with a molecular weight of 1340¹⁸.

Comparative studies of synthetic and natural bone hydroxyapatite have been reported by Osanov *et al.*¹⁹ The results indicated that their chemical compositions were similar, but bone contained a greater admixture of metal ions than the synthetic material. During heating to 400°C , the decrease in weight of both substances was nearly equal. In addition, the crystal structures of the two materials were identical. Other studies have been carried out by Scheicher²⁰, who calcined human teeth and bones at 900°C - 1100°C for 20-120 minutes after removing all organic matter and treating with an oxidizing agent. The ash was dried, ground, degreased with ethyl alcohol and treated with H_2O_2 . After heating the dried powder to 900°C - 1100°C the product was identified as hydroxyapatite and used as fillers for implant areas in contact with bone.

The fabrication of bone ash into ceramic must be done through a sintering process, where the powder is compacted and fired. Temperatures should be sufficiently high to join particles of bone ash together to form a dense body. The phases of bone at high temperatures are significant in controlling its biological properties² and also restrict the range of its potential applications.

Ghazi *et al.*²¹ have studied the phases of cattle bone char and bone char waste fired at different temperatures up to 1000°C . Distinct structural changes were detected on firing at 600°C , indicating the formation of $\alpha\text{-Ca}_3(\text{PO}_4)_2$ together with hydroxyapatite at temperatures as low as 600°C . Our present paper reports a study of the phases of cattle bones at temperatures ranging from 900°C to 1400°C which are the approximate sintering temperature ranges of synthetic calcium hydroxyapatite.^{5,8,22-25}

Materials and Methods

Sample preparation

Cow bones : bones from back-legs A-2, AO^x-2 were radially cut into pieces as shown in Fig. 1.

Buffalo bones : bones from a back-leg B-2 and a front-leg-B-1 were cut in the same way as AO^x-2 and A-2 respectively. No. 1 was used to characterize the starting bone, while No.2 was used to study high temperature phases.

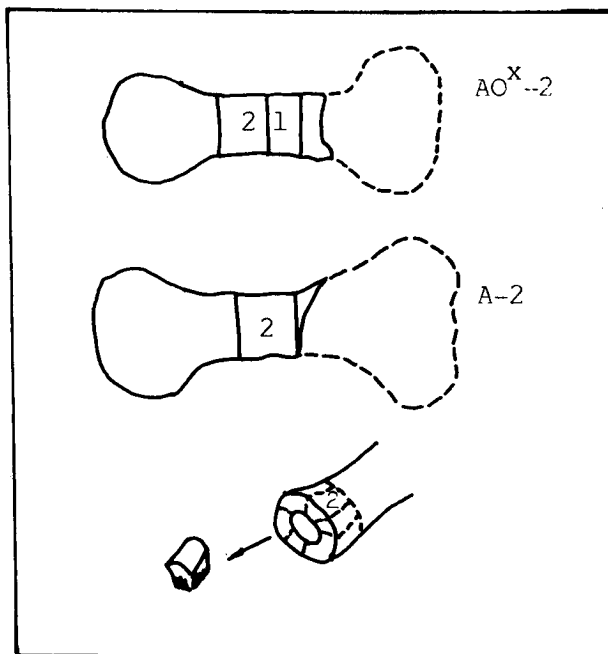


Figure 1. Schematic cutting of cattle bones

Heat treatment

Samples were fired in an electric furnace with a rate of heating of 10° per minute below 600°C and 7° per minute above 600°C. Fired samples were kept at the required temperature for 15 minutes. Two different techniques were used to cool the samples. Quenching was carried out by drawing fired samples from the furnace immediately after firing. Slow cooling was carried out by leaving the samples to cool to room temperature in the furnace.

Phase analyses

Nickel-filtered Cuk α radiation from a Philips X-ray diffractometer was used to identify the phases present after the sample was fired and powdered. Scanning was carried out in the range 10° - 60° of 2 θ at 2° per minute with a time-constant of 2.

An IR 440 Shimadzu spectrophotometer was used to study the infrared transmittance spectra 4000 to 300 cm^{-1} of the samples, which were prepared using the KBr pellet method. The OH absorption bands at 3572, 630 and 343 cm^{-1} , which have been assigned to the OH stretching mode, the OH vibrational mode and the translatory motions of OH-ions in the $2[\text{Ca}_3\text{-(OH)}]$ sublattice²⁶ were used to study any dehydroxylation that might occur²⁷. The Nujol mull technique was used when only the OH absorption band at 3572 cm^{-1} was to be studied since this band overlaps with the OH-bands of adsorbed water in the KBr pellet. A piece of polyethylene with a mineral oil on its surface was attached to a window of a specimen holder. The spectra of the compensated mineral oil and polyethylene were recorded as a blank, before adding the sample to the mineral oil and recording its spectrum at 4000-3000 cm^{-1} .

Results and Discussion

The main chemical compositions (Table 1) of natural bone expressed in terms of calcium oxide and phosphorous pentoxide are 32.1%, 28.3% for cow bone and 33.0%, 27.9% for respectively buffalo bone. Chemical analyses indicate that Ca:P ratios are 1.44 for cow bone and 1.52 for buffalo bone. These values are somewhat less than the 1.67 theoretically expected for calcium hydroxyapatite, indicating that cattle bones are calcium-deficient apatite.

TABLE 1. CHEMICAL ANALYSIS OF CATTLE BONES*

Type of Bone	NO.	LOI**	CaO	MgO	P ₂ O ₅	SiO ₂	Fe ₂ O ₃
Cow	AO ^x -2	33.4	32.1	1.4	28.3	4.0	0.4
Buffalo	B-2	32.0	33.0	2.8	27.9	2.4	0.4

* Conducted by the Department of Science & Services, Bangkok 10400, Thailand

** LOI = loss on ignition.

The phases found in natural and fired bones are summarized in Table 2. The X-ray diffractograms shown in Fig. 2 indicate that natural cattle bones gave broad peaks similar to that of calcium hydroxyapatite. This pattern was the same as that obtained from

TABLE 2. PHASE IDENTIFICATION OF BONES USING X – RAY DIFFRACTOMETER

Type of bone	no.	Heat-Treatment °C/min.	Phases present			
			Natural	Quenched sample	Slow-cooled sample	
Cow	AO ^x -2	-	HA ¹			
		900/15		HA	HA	
		1000/15		HA	HA	
		1300/15		HA	HA	
		1400/15		HA + α-Ca ₃ (PO ₄) ₂ + Ca ₄ O(PO ₄) ₂	HA + βCa ₃ (PO ₄) ₂ + Ca ₄ O(PO ₄) ₂	
	A-2	900/15			HA	
		1000/15			HA	
		1300/15			HA	
		1400/15			HA + βCa ₃ (PO ₄) ₂ + Ca ₄ O(PO ₄) ₂	
	Buffalo	B-2	-	HA ¹		
			900/15		HA	HA
			1000/15		HA	HA
			1200/15		HA	HA
			1300/15		HA	HA
1400/15				HA + α-Ca ₃ (PO ₄) ₂ + Ca ₄ O(PO ₄) ₂	HA + βCa ₃ (PO ₄) ₂ + Ca ₄ O(PO ₄) ₂	
B-2		900/15			HA	
		1000/15			HA	
		1200/15			HA	
		1300/15			HA	
		1400/15			HA + βCa ₃ (PO ₄) ₂ + Ca ₄ O(PO ₄) ₂	

HA¹ = broad peaks similar to that of calcium hydroxyapatite

HA = Ca₁₀(PO₄)₆(OH)₂ = Calcium hydroxyapatite

Ca₃(PO₄)₂ = Tricalcium phosphate

Ca₄O(PO₄)₂ = tetracalcium phosphate

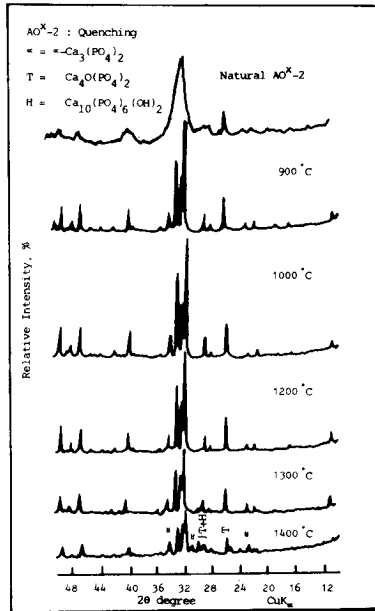


Figure 2. X-ray diffractograms of cow bone AO^X-2 heat-treated from 900° to 1400°C and quenched in air.

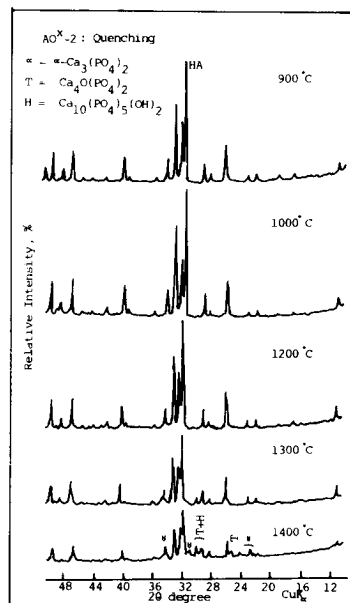


Figure 3. X-ray diffractograms of natural cow bone, AO^X-2 heat-treated from 900° to 1400°C and slow-cooled in a furnace.

synthetic materials²⁷, which was considered to be the result of very fine grain size. The x-ray results for cattle bones fired at selected temperatures up to 1300°C and air-quenched or slow-cooled were identified as calcium hydroxyapatite as shown in Figs. 2 and 3. Dehydroxylation from the apatite structure was not detectable. After firing at 1400°C and quenching, cattle bones showed phase dissociation to tetracalcium phosphate, $\text{Ca}_4\text{O}(\text{PO}_4)_2$ with d-spacing of 3.00 ($2\theta = 29.7$), 3.05 ($2\theta = 29.2$) and 2.76 ($2\theta = 32.4$) respectively, and alpha-tricalcium phosphate, $\alpha\text{-Ca}_3(\text{PO}_4)_2$ with d-spacing of 2.91 ($2\theta = 30.8$), 2.62 ($2\theta = 34.2$), and 3.91 ($2\theta = 22.7$). However, when cattle bones were slow-cooled after firing at 1400°C, the $\alpha\text{-Ca}_3(\text{PO}_4)_2$ transformed to beta-tricalcium phosphate $\beta\text{-Ca}_3(\text{PO}_4)_2$ which was noticeable from the d-spacing of 2.88 ($2\theta = 31.0$), 2.61 ($2\theta = 34.3$), and 3.21 ($2\theta = 27.7$). The transformation temperature has been reported as 1180°C²⁸. The tetracalcium phosphate phase was also found coexisting with $\beta\text{-Ca}_3(\text{PO}_4)_2$.

Buffalo bone B-2 obtained after quenching and slow-cooling exhibited identical patterns to those of AO^x-2. Other samples of cow bone A-2 and buffalo bone B-1 from slow-cooling also gave the same patterns as the slow-cooled sample AO^x-2.

The IR spectra from 4000-300 cm^{-1} of the KBr pellet of cow bone AO^x-2 after firing and quenching are shown in Fig. 4. The expected bands attributed to the PO_4 -ions and OH-ions in calcium hydroxyapatite were observed as described²⁶. However, there was a noticeable decrease in the intensity of the OH absorption bands at 3572, 630 and 343 cm^{-1} . Some carbonate bands were observed in the wavenumber range 1400 to 1500 cm^{-1} ²⁹ when heat-treatment was carried out at 900°C, but these disappeared when firing was carried out above 900°C. The absorption bands at about 3450-3300 cm^{-1} have been assigned to adsorbed water³⁰.

The IR spectra of the same sample from 4000 to 3000 cm^{-1} using the Nujol mull technique are shown in Fig. 5. The OH stretching mode of this peak was diminished in intensity from 1000°C up to 1200°C and disappeared at 1300°C.

The results of this investigation provide information on the high temperature phase changes of natural cattle bones and indicate that dehydroxylation gradually occurs eventually yielding oxyapatite before dissociating to tricalcium phosphate and tetracalcium phosphate. The observation is the same as that found with synthetic calcium hydroxyapatite²⁷.

The buffalo bone B-2 yielded similar IR spectra to AO^x-2, as shown in Fig. 6. Dehydroxylation was also noticed as the intensity of the OH absorption bands at 3572, 630 and 343 cm^{-1} decreased in agreement to the results with cow bone AO^x-2. The IR spectra of the slow-cooled AO^x-2, A-2, B-2, and B-1 displayed the same results.

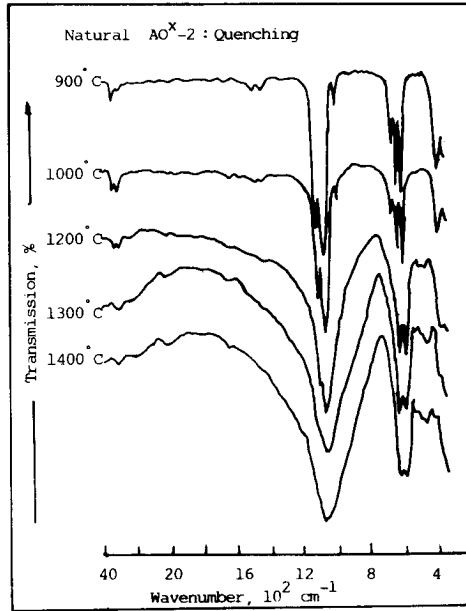


Figure 4. IR spectra of fired cow bone AO^x-2 quenched at selected temperatures using KBr method.

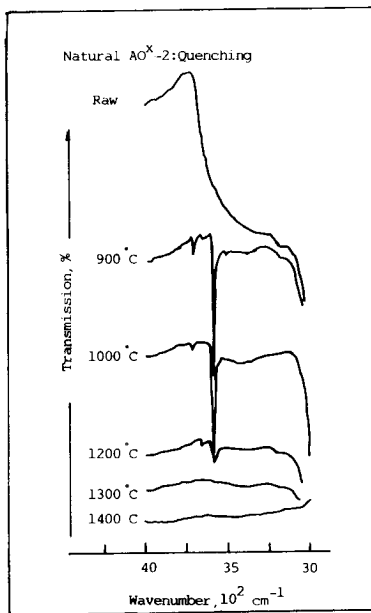


Figure 5. IR spectra of cow bone AO^x-2 quenched at selected temperatures using Nujol mull method.

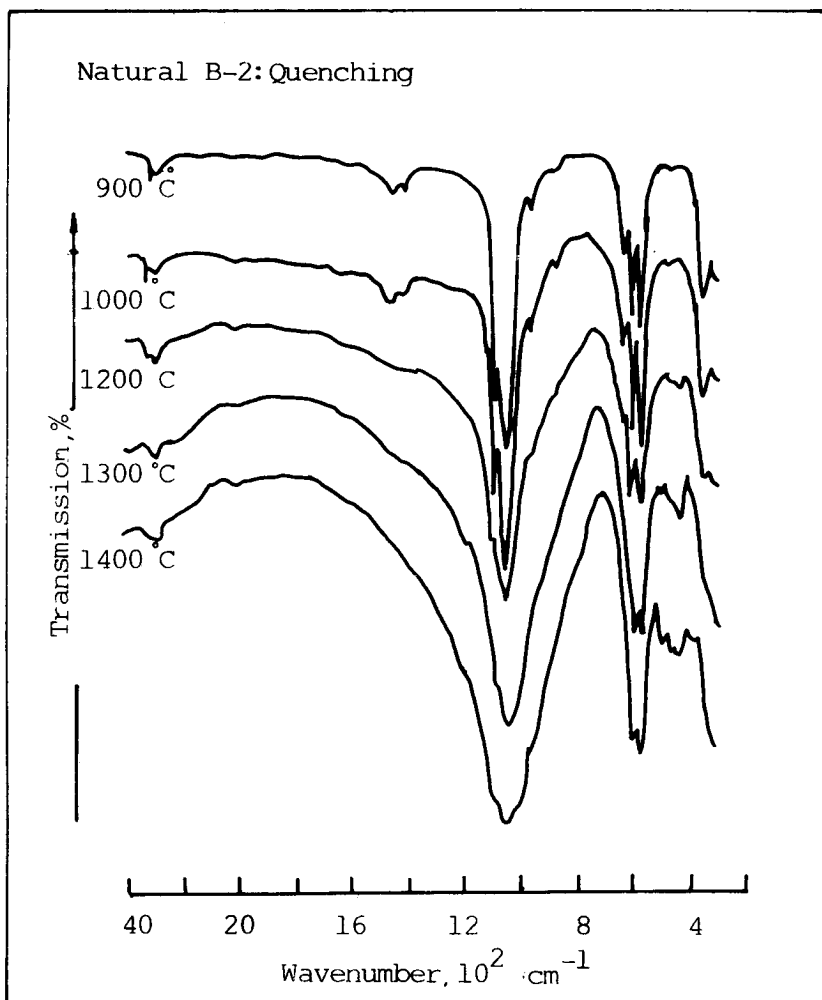
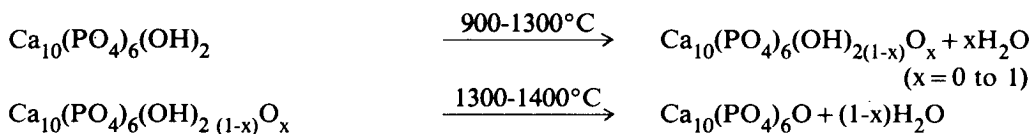
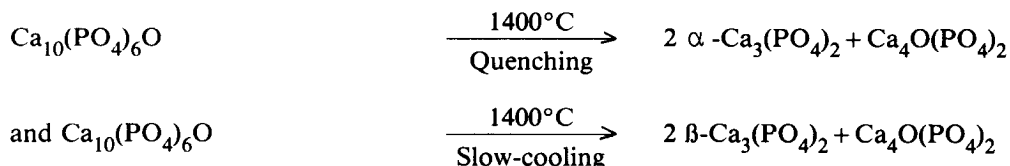


Figure 6. IR spectra of fired buffalo bone B-2 quenched at selected temperatures using KBr method.

The high temperature phases of cow and buffalo bones deduced from the present study can be summarized by the following equations:





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บทคัดย่อ

การศึกษาโครงสร้างของกระดูกสัตว์ที่อุณหภูมิสูง เพื่อใช้ทำกระดูกเทียม ทำโดยตัดกระดูกในแนวรัศมีของส่วนกลางของกระดูกขาหัวและควาย ผ่านความร้อนในช่วง 900° ถึง 1400°ซ และตรวจเฟสของตัวอย่างที่ได้จากการปล่อยให้กระดูกเย็นตัวจากอุณหภูมิดังกล่าวอย่างรวดเร็วและอย่างช้า ๆ โดยใช้เอกซเรย์ดิฟแฟรคโตมิเตอร์ และอินฟราเรดสเปกโตรโฟโตมิเตอร์

ผลการทดลองแสดงว่าในช่วงอุณหภูมิ 900° ถึง 1300°ซ ตัวอย่างที่ปล่อยให้เย็นตัวอย่างรวดเร็วของกระดูกบางส่วนกลางประกอบด้วย แคลเซียม ไฮดรอกซ์-ออกซีแอนาไฮต์ $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2(1-x)}\text{O}_x]$ ทั้งหมด ซึ่งจะแตกตัวให้เป็น แอลฟา-ไตรแคลเซียมฟอสเฟต $[\alpha\text{-Ca}_3(\text{PO}_4)_2]$ และเตตระแคลเซียมฟอสเฟต $[\text{Ca}_4\text{O}(\text{PO}_4)_2]$ ที่ 1400°ซ. ตัวอย่างที่ปล่อยให้เย็นตัวอย่างช้า ๆ ให้พฤติกรรมในทำนองเดียวกันแต่เฟส $[\alpha\text{-Ca}_3(\text{PO}_4)_2]$ นี้เปลี่ยนไปเป็น เบตา-ไตรแคลเซียมฟอสเฟต $[\beta\text{-Ca}_3(\text{PO}_4)_2]$