ISSN 1392–1320 MATERIALS SCIENCE (MEDŽIAGOTYRA). Vol. 26, No. 1. 2020

Effect of $Ca(OH)_2$ and Heat Treatment on The Physico-Chemical Properties of Bovine Bone Powder; a Material Useful for Medical, Catalytic, and Environmental Applications

Naseer A. KHAN [1]*, Mukhtiar AHMED [1], Naveed ul Hasan SYED [1], Matthew DREWERY [2]

[1] *Department of Chemical Engineering, University of Engineering and Technology (UET) Peshawar, Khyber Pakhtunkhwa (KPK) 25120, Pakistan*
[2] *Faculty of Engineering and Built Environment, The University of Newcastle, Callaghan, New South Wales (NSW) 2308, Australia*

crossref http://dx.doi.org/10.5755/j01.ms.26.1.21219

Received 13 July 2018; accepted 10 December 2018

In this study the authors investigated the effect of alkali ($Ca(OH)_2$) and heat treatment on the physico-chemical properties of bovine bone powder. For this purpose, raw and alkali treated samples were heated separately at temperatures of 400 °C, 600 °C, 800 °C, and 1000 °C. A combination of characterization techniques, such as TGA, XRD, N_2-adsorbtion isotherms, and EDX were used. It was found that the boiling of cleaned solid pieces of bones in 2 molar $Ca(OH)_2$ solution results in a mass loss of about 10 % (mainly discards oily liquid). TGA analysis affirms that the hydrocarbons of bone matrix are partially extractable (~ 10 %) in the boiling alkaline solution. The color of raw and treated bone samples remained similar, that is changing from yellowish white to grayish black before turning into white over temperatures ranging from 30 °C (room temperature), 400 – 600 °C, and 800 – 1000 °C, respectively. Moreover, XRD signatures were also comparable at unified temperature ranges, however, it was noted that carbonization due to heating engenders a significant change in the intensities of the x-ray reflections. Despite of having similarities, surface area of raw and treated bones at 400 °C, 600 °C and 800 °C were found to be different, indicative of a chemical interactions of calcium ions with bone. Quite interestingly, TGA, XRD, and N_2-adsorbtion isotherms support the argument that a limited amount of calcium ions diffuses into the vacancies or interstitial sites of bone lattice. Furthermore, EDX analysis of the samples calcined at 1000 °C confirms that the $Ca(OH)_2$ treatment increases the total calcium content of hydroxylapatite (inorganic part of bone matrix).
Keywords: $Ca(OH)_2$ treatment, calcium diffusions, and hydroxyapatite (HAp).

1. INTRODUCTION

Modern agriculture has improved the quantity and quality of comestibles with consistent productions. Currently, for most countries agriculture products comprises a major percentage of their total economy [1, 2], however the high level of consumption is generating huge quantities of bio-wastes, with improper disposal raising serious environmental concerns [3]. Furthermore, waste generated from food handling in developed countries is about 40 % of the total mass [4]. For example, meat processing industries are producing enormous volumes of skin, bones, skull, feet, and entrails on a daily basis [5]. Unfortunately, the traditional methods of bio-waste disposals are no longer acceptable, for example previously tons of crushed bones were consumed as cattle feed, but this practice is now outlawed due to an increased risk of bovine spongiform encephalopathy disease [6]. In short, environmental researchers are looking for strategies to dispose wastes properly and if possible to convert them into useful products [7 – 9].

In this particular work we studied the physico-chemical properties of the waste bovine bones, and hopefully the attained knowledge will be used for several economic, social, and environmental benefits. Briefly, bones are the hard tissues of animal species, a factory of blood cells, and provides the essential structure for holding muscles [10, 11]. This solid matrix is a chemical combination of highly complex organic and inorganic compounds, whereas the inorganic part is between 60 – 70 %, and the remaining components are organic species and water [12]. Inorganic components within the structure consists of calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), with presence of trace amounts of calcium chlorapatite ($Ca_{10}(PO_4)_6(Cl)_2$), and calcium fluorapatite ($Ca_{10}(PO_4)_6(F)_2$) [11, 13]. Furthermore, 3 – 8 % of carbonate ions (CO_3^{2-}) are also part of the calcium apatite as a hydroxyl (OH^{-1}) substitution, and have a vital role in several biological reactions [14]. On the other hand, collagen (fibrous protein) is the dominant organic component (95 % of total hydrocarbon) along with minute quantities of chondroitin sulfate (structural sugar forming cartilage), keratin sulfate (hydrated carbohydrates of bone joints), and lipids (phospholipids, triglycerides, fatty acids, cholesterol, etc.) [15]. These hydrocarbon molecules can be converted into several valuable products, such as gelatin, porous carbon, soaps, detergent, and glues, etc. [16, 17].

Calcium hydroxyapatite (HAp), a solid bone mineral, has a unique chemical composition and can be used to produce materials having different medical, catalytic, and environmental applications [18, 19]. Calcination or

* Corresponding author. Tel.: +92919222261; fax: + 9291 9222213.
E-mail address: *naseerahmedkhan@uetpeshawar.edu.pk* (N.A. Khan)

pyrolysis, treating raw bones in alkaline solution, and bone hydrolysis (at higher temperature and pressure) were the most common methods employed for the extraction of HAp [14, 20 – 23]. Leaching parameters, such as temperature, pH, and treatment duration determines the final structure and chemistry of the crystalline HAp [13, 24 – 26]. In general, heat treatment above 600 °C eliminates the organic portion of the bone matrix forming pure HAp crystals [20, 27]. Whereas, at lower temperatures (< 600 °C), the material is commonly called bone char, consisting of different masses (depending on the treatment temperature) of degraded organic compounds [12, 28, 29]. Finally, severe heating for longer durations, for example ≥ 1000 °C may form secondary stable phases, such as tricalcium phosphate and metals carbonates [30 – 33].

Bone char is an excellent adsorbent (can adsorb heavy metals, fluoride, bacteria) and catalyst support (for loading zinc, cobalt, potassium) for pollution control and fuel production reactions, respectively [34 – 36]. Organic and pathogen freed HAp is biocompatible and nontoxic material which can be used in bone grafting, artificial implants coating, and is considered a suitable carrier for targeted drug delivery to the affected organs of human body [36 – 39]. More importantly, bone calcium (the amount of calcium may be increased by $Ca(OH)_2$ treatment) can react with CO_2 forming added amounts of structural CO_3^{-2} (carbonation reaction) ions and probably this modified material will be more compatible for medical applications [39]. In this study a systematic comparison was carried out to investigate the pyrolysis of pure and $Ca(OH)_2$ treated bovine bone by using TGA, XRD, N_2-adsorption isotherms, and EDX characterization techniques.

2. EXPERIMENTAL DETAILS

Approximately, 15 kilograms of waste bones were collected from a local butcher shop of district Peshawar, Khyber Pakhtunkhwa, Pakistan. Initially, these bones contained small lumps of meat which were carefully removed with a sharp knife. After that, the whole mass of the bones was dipped in heated distilled water (50 °C) for 4 hours to remove remaining dirt and other attached coarse particles. For drying, these washed bones were kept under direct sunlight for more than 6 hours and then were sliced into small pieces of about 2 inches' length.

For experiments, these sized bones were separated into two main classes. The first portion was crushed into fine particles, whereas the second was treated with a research grade alkaline solution (2 molar $Ca(OH)_2$) and then crushed into powder. Once again raw and treated samples were cleaned separately in distilled water (50 °C) and then dried at 110 °C for 3 hours. For every experimental study, a sample of 20 grams of powder bones was calcined with a ramp rate of 10 °C/ min to temperatures of 400 °C, 600 °C, 800 °C, and 1000 °C and held for a total of 2 hours. All calcined samples were gradually cooled to a room temperature for analysis.

All powdered samples were studied via several analytical instruments. Mass loss due to the gradual heating was carried out under inert nitrogen environment by using standard Thermogravimetric analyzer, Pyris diamond series (Perkin Elmer, USA). For this experiment, powdered raw and alkali treated samples were separately heated from a room temperature to a 1000 °C with a ramp rate of 10 °C/min.

X-Ray diffractometer, JDX-3532 (JEOL, Japan**)**, was used to investigate the phase variations (crystallinity) of the bone composites at different calcination temperatures (400 °C, 600 °C, 800 °C, and 1000 °C). Samples were examined at room temperature by Cu (Kα) radiation within a 2θ range of 20° to 55°. These sample were homogeneously grounded prior to XRD analysis.

The specific surface area of powdered samples (m^2/g) was determined by physical adsorption of a nitrogen gas at 77 K (– 196.15 °C). The graphs of N_2-adsorption isotherms were attained by physical adherence of saturated nitrogen with cleaned bone surface. BET (Brunauer, Emmett and Teller) equation was solved for the calculation of specific surface area by using NOVA2200e (USA). Prior to N_2 adsorption test all samples were degassed under vacuum at a temperature of 105 °C. The Energy Dispersive X-ray spectroscopy (EDX) of the targeted spot sites was investigated by using JSM5910 (JEOL, Japan).

3. RESULTS AND DISCUSSION

The variations in the physico-chemical properties of raw and treated bone samples were systematically compared in this study. As shown in Fig. 1, major mass loss (expected to be organic matter which dissolves in alkali solution [40, 41]) of bone matrix occurs within the first two alkali treatments. Therefore, further alkali treatments could not extracted the remaining structural hydrocarbon species (as known, bones contains 30 – 40 % bonded hydrocarbons) [26]. The measured mass loss was about 10 %, however this number was low when compared to the NaOH and KOH solution treatments (the measured mass loss for NaOH and KOH treatment was about 20 %) [42]. Most probably, as HAp is chemically made up of Ca^{2+} cations, thus there is insufficient driving energy for $Ca(OH)_2$ solution to break the interconnected polymeric structures as since Ca^{2+} cations are smaller in size as compared to K^+ and Na^+ ions, thus they have a coordination strength too low to cleave the phosphate (PO_4^{3-}) ions from the bone matrix.

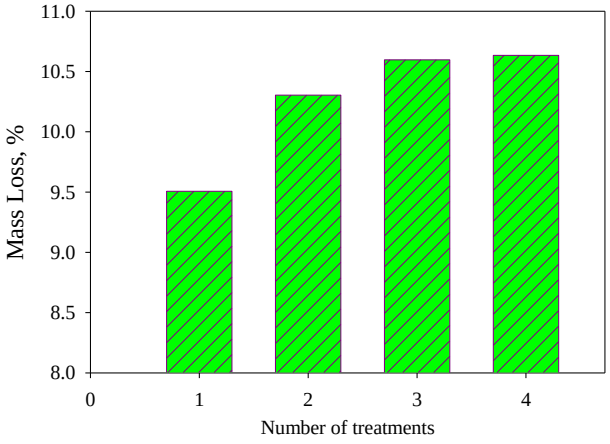

Fig. 1. Cumulative mass loss of bone verses number of $Ca(OH)_2$ treatments

In this study, the extracted hydrocarbon compounds

were not chemically analyzed, however from the physical appearance, it is expected to contain mainly lipids (oily appearance). Thermogravimetric analysis of the treated and raw bone samples were also compared in order to analyze the differences in the mass loss curves under heating as shown in Fig. 2. The deviation in the mass loss curves of the treated and raw bone begins at about 150 °C, however at temperatures > 250 °C, the trend is mostly same with raw samples having a consistent mass loss of approximately 10 % lower than treated samples. The difference in slope of curves above 150 °C and prior to 200 °C suggests that the $Ca(OH)_2$ treatment extracts water (mass loss below 200 °C is due to surface and bonded water) and hydrocarbons [14]. Furthermore, mass loss curves for both treated and raw samples above 250 °C is due to the decomposition of the polymeric organic compounds and their trend is very much similar up to a temperature of 800 °C [43 – 46]. Interestingly, the slope variation around carbonate decomposition range (700 °C – 1000 °C) suggests that the total number of bonds between calcium and carbonate (CO_3^{2-}) ions (bone surface consists considerable concentration of carbonates [47]) have increased due to $Ca(OH)_2$ treatment, with a considerable amount of calcium entering into the bone frame work forming primary bonds [13, 48, 49].

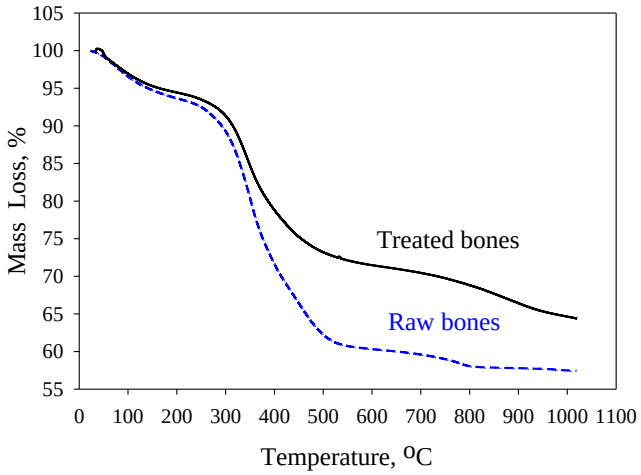

Fig. 2. Thermogravimetric analysis, TGA plot of treated and raw bones

The color change of prepared samples when heated to different temperatures were also examined and is presented in Table 1. As discussed earlier, the alkali treatment extracts a limited amount of hydrocarbons, therefore the overall chemical composition of both samples is quite similar and thus have identical color appearance at same temperatures. For example, initially the color of crushed samples is yellowish white at room temperatures. Whereas, when it is heated with a ramp rate of 10 °C/min to a temperature of 400 °C, the color changes to mostly grayish with presence of few blackish areas. Further heating to a temperature of 600 °C increases the total amount of blackish portion due to an increase in solid carbon. As a whole, prior and at a temperature of 600 °C, the carbonization reaction and volatilization of hydrocarbons (collagen, fats, etc.) are responsible for the grayish black appearance [50]. Above 600 °C, the degraded carbon sitting over HAp mineral cannot sustain its bonding and oxidizes to CO_2 and H_2O, therefore the powder samples calcined at 800 °C or 1000 °C are white in color [6].

Table 1. Effect of calcination temperature on the color of treated/ raw bones samples

Temperature °C	Color
25 °C – 30 °C	Yellowish White
400 °C – 600 °C	Grayish black to dark black
800 °C – 1000 °C	White

The phase variations due to chemical changes at varied temperatures (30 °C, 600 °C, and 1000 °C) is shown in Fig. 3. In general, the XRD signature of all studied samples is in agreement with the positions of reference JCPDS (joint committee on powder diffraction standards) card number 9-432, confirming the presence of HAp as a bone mineral [51]. The phase composition of organic-hydroxyapatite varies greatly with temperature as marked by the variations in width, intensity, and primary peak positions (30° – 34°). As a whole, the crystallinity increases with temperature as previously analyzed by many researchers and can be verified by the formation of sharp diffraction peaks at a 2θ angle of 31°, 32°, and 33° [52, 53]. Both samples have similar crystallographic structure at uniform ranges of temperature, which essentially means that the extracted hydrocarbons (mostly lipids) are not chemically or structurally bonded with the HAp mineral. The overlapping of the leading diffraction peaks (30° – 34°) of uncalcined samples (room temperature) is because of amorphous hydrocarbons and therefore have a low crystallinity and crystal size. More interestingly, the alkali treated samples calcined at 1000 °C have comparatively higher order of crystallinity (sharp peaks) when compared to untreated samples. This result also suggests that during alkali treatment, calcium cations diffuse to the vacant or defective sites of HAp mineral and thus lowers the total number of lattice deformations.

As previously discussed in the methodology, both raw and treated samples were calcined under similar conditions, yet they have different sets of textual properties as shown in Table 2.

Table 2. Comparison of the textual properties of the raw and treated bones samples calcined at varies temperatures.

Temperature, °C	Crystallite size[a] τ, nm		Surface area[b], m^2/g		Pore radius[c], A°	
	Treated	Raw	Treated	Raw	Treated	Raw
30 °C	2.4	2.2	12.1	8.09	15.67	15.07
400 °C	-	-	125.5	115.8	18.63	19.3
600 °C	4.2	4.1	69.01	59.3	19.58	19.5
800 °C			25.6	21.2	16.81	15.09
1000 °C	34.3	26.8	14.9	14.6	14.99	14.9

[a] Scherrer equation [c] BJH method
[b] Multiple point BET equation

Most probably, hydrocarbon extraction and calcium deposition or diffusion (≥ 800 °C) into the bone structure contributes towards these variations. As a whole, the crystallinity improves with alkali treatment and calcination temperature as shown in Fig. 3 and Table 2. Briefly, the gradual heating discards the bonded hydrocarbons and improves the alignments of the Ca and PO_4 ions [54]. Furthermore, the determined pore diameter was approximately the same as temperatures increases, whereas calcium deposition (due to alkali treatment) on bone framework forms extraterrestrial faces and thus have a higher BET surface area as shown in Table 2 [55 – 57]. However, at a temperature of 1000 °C, both raw and treated samples have a constant surface area of 14 m^2/g. This constant surface area when compared to the XRD pattern of the samples calcined at 1000 °C suggests a limited diffusion of calcium into the HAp mineral. Similarly, for alkali treated sample and when calcined at 1000 °C, the chemical composition determined by the energy dispersive X- ray spectroscopy (EDX) affirms that the mineral mainly consists of calcium and phosphorous elements and have a higher Ca/P ratio (1.8) when compared to the literature [20, 29].

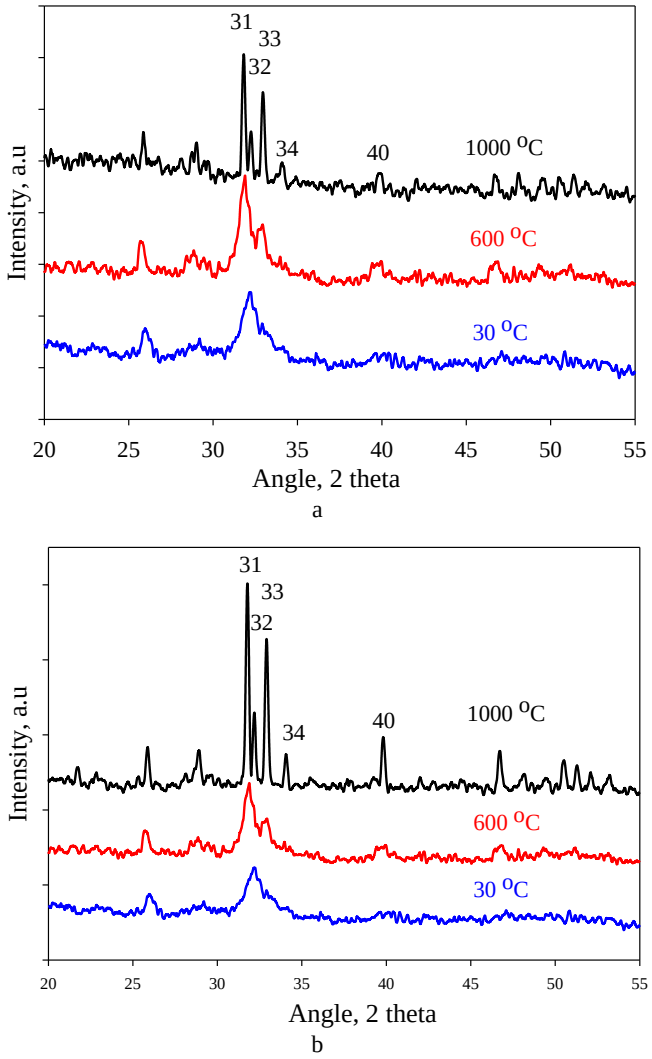

Fig. 3. Diffractograms: a – raw; b – treated bovine bone samples calcined at 30 °C, 600 °C, and 1000 °C

4. CONCLUSIONS

The increase in the total calcium content of bone matrix is not studied yet and this treatment process may improve its suitability for several medical, catalytic, and environmental applications. For this reason, bone pieces were treated with a 2 molar $Ca(OH)_2$ solution to investigate the possibility of calcium fusion in hard bone tissues. This alkali treatment procedure also extracts a limited portion of hydrocarbons (~ 10%), whereas the strongly bonded hydrocarbon can be removed via heating. More importantly, the total calcium content of the sample increases with alkali treatment. Furthermore, the variation in bone chemistry (because of heating) is also visible through a color change. The crystallinity of the bone (hydroxyapatite) mineral increases with alkali treatment as observed by a sharp intense XRD signature.

Acknowledgments

The authors greatly acknowledge the funds and facilities provided by the Department of Chemical Engineering, University of Engineering & Technology Peshawar, Pakistan.

REFERENCES

1. **Watson, D.D.** The Political Economy of Food Price Policy during the Global Food Price Crisis of 2006-2008 *Food Security* 9 (3) 2017: pp. 497 – 509. https://doi.org/10.1007/s12571-017-0685-z
2. **Yue, Q., Xu, X., Hillier, J., Cheng, K., Pan, G.** Mitigating Greenhouse Gas Emissions in Agriculture: From Farm Production to Food Consumption *Journal of Cleaner Production* 149 2017: pp. 1011 – 1019. https://doi.org/10.1016/j.jclepro.2017.02.172
3. **Mirabella, N., Castellani, V., Sala, S.** Current Options for the Valorization of Food Manufacturing Waste: A Review *Journal of Cleaner Production* 65 2014: pp. 28 – 41. https://doi.org/10.1016/j.jclepro.2013.10.051
4. **Mahro, B., Timm, M.** Potential of Biowaste from the Food Industry as a Biomass Resource *Engineering in Life Sciences* **7** (5) 2017: pp. 457 – 468. https://doi.org/10.1002/elsc.200620206
5. **Bhaskar, N., Modi, V.K., Radha, C., Govindaraju, K., Lalitha, R.G.** Utilization of Meat Industry by Products: Protein Hydrolysate from Sheep Visceral Mass *Bioresource Technology* 98 (2) 2007: pp. 388 – 394. https://doi.org/10.1016/j.biortech.2005.12.017
6. **Iriarte-Velasco, U., Sierra, I., Zudaire, L., Ayastuy, J.L.** Preparation of a Porous Biochar from the Acid Activation of Pork Bones *Food and Bioproducts Processing* 98 2016: pp. 341 – 353. https://doi.org/10.1016/j.fbp.2016.03.003
7. **Salminen, E., Rintala, J.** Anaerobic Digestion of Organic Solid Poultry Slaughterhouse Waste – a Review *Bioresource Technology* 83 (1) 2002: pp. 13 – 26. https://doi.org/10.1016/S0960-8524(01)00199-7
8. **Toldrá, F., Aristoy, M.C., Mora, L., Reig, M.** Innovations in Value-addition of Edible Meat by-Products *Meat Science* 92 (3) 2012: pp. 290 – 296. https://doi.org/10.1016/j.meatsci.2012.04.004
9. **Franke-Whittle, I.H., Insam, H.** Treatment Alternatives of Slaughterhouse Wastes, and their Effect on the Inactivation of

different Pathogens: A Review *Critical Reviews in Microbiology* 39 (2) 2013: pp. 139 – 151. https://doi.org/10.3109/1040841X.2012.694410

10. **Bajpai, A.K., Singh, R.** Study of Biomineralization of Poly(vinyl alcohol)-based Scaffolds using an Alternate Soaking Approach *Polymer International* 56 (4) 2007: pp. 557 – 568. https://doi.org/10.1002/pi.2171
11. **Janković, B., Smičiklas, I.** The Non-isothermal Combustion Process of Hydrogen Peroxide Treated Animal Bones Kinetic Analysis *Thermochimica Acta* 521 (1) 2011: pp. 130 – 138. https://doi.org/10.1016/j.tca.2011.02.044
12. **Narasaraju, T.S.B., Phebe, D.E.** Some Physico-Chemical Aspects of Hydroxylapatite *Journal of Materials Science* 31 (1) 1996: pp. 1 – 21. https://doi.org/10.1007/BF00355120
13. **Piccirillo, C., Silva, M.F., Pullar, R.C., Braga da Cruz, I., Jorge, R., Pintado, M.M.E., Castro, P.M.L.** Extraction and Characterisation of Apatite- and Tricalcium Phosphate-based Materials from Cod Fish Bones *Materials Science and Engineering: C* 33 (1) 2013: pp. 103 – 110. https://doi.org/10.1016/j.msec.2012.08.014
14. **Figueiredo, M., Fernando, A., Martins, G., Freitas, J., Judas, F., Figueiredo, H.** Effect of the Calcination Temperature on the Composition and Microstructure of Hydroxyapatite Derived from Human and Animal Bone *Ceramics International* 36 (8) 2010: pp. 2383 – 2393. https://doi.org/10.1016/j.ceramint.2010.07.016
15. **Florencio-Silva, R., Sasso, G.R.S., Sasso-Cerri, E., Sasso-Cerri, E., Simões, M.J., Cerri, P.S.** Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells *BioMed Research International* 2015: pp. 1 – 17. http://dx.doi.org/10.1155/2015/421746
16. **Ulfa, M.** Analysis of Microporosity of Porous Carbon from Gelatin (KPG) using N_2-adsorbtion by Dubilin-Radushkevich Model *ALCHEMY Jurnal Penelitian Kimia* 13 (1) 2017: pp. 103 – 118. https://doi.org/10.20961/alchemy.v13i1.4070
17. **Shreve, R.N., Austin, G.T.** Shreve's Chemical Process Industries. 1984: McGraw-Hill.
18. **Corro, G., Sánchez, N., Pal, U., Bañuelos, F.** Biodiesel Production from Waste Frying Oil Using Waste Animal Bone and Solar Heat *Waste Management* 47 (Part A) 2016: pp. 105 – 113. https://doi.org/10.1016/j.wasman.2015.02.001
19. **Patel, S., Han, J., Qiu, W., Gao, W.** Synthesis and Characterisation of Mesoporous Bone Char Obtained by Pyrolysis of Animal Bones for Environmental Application *Journal of Environmental Chemical Engineering* 3 (4, Part A) 2015: pp. 2368 – 2377. https://doi.org/10.1016/j.jece.2015.07.031
20. **Ooi, C.Y., Hamdi, M., Ramesh, S.** Properties of Hydroxyapatite Produced by Annealing of Bovine Bone *Ceramics International* 33 (7) 2007: pp. 1171 – 1177. https://doi.org/10.1016/j.ceramint.2006.04.001
21. **Sobczak-Kupiec, A., Wzorek, Z.** The Influence of Calcination Parameters on Free Calcium Oxide Content in Natural Hydroxyapatite *Ceramics International* 38 (1) 2012: pp. 641 – 647. https://doi.org/10.1016/j.ceramint.2011.06.065
22. **Barakat, N.A.M., Khalil, K.A., Sheikh, F.A., Omran, A.M., Gaihre, B., Khil, S.M., Kim, H.Y.** Physiochemical Characterizations of Hydroxyapatite Extracted from Bovine Bones by Three Different Methods: Extraction of Biologically Desirable HAp *Materials Science and Engineering: C* 28 (8) 2008: pp. 1381 – 1387. https://doi.org/10.1016/j.msec.2008.03.003
23. **Lombardi, M., Palmero, P., Haberko, K., Pyda, W., Montanaro, L.** Processing of a Natural Hydroxyapatite Powder: From Powder Optimization to Porous Bodies Development *Journal of the European Ceramic Society* 31 (14) 2011: pp. 2513 – 2518. https://doi.org/10.1016/j.jeurceramsoc.2011.02.017
24. **Xu, J.L., Khor, K.A., Dong, Z.L., Gu, Y.W., Kumar, R., Cheang, P.** Preparation and Characterization of Nano-sized Hydroxyapatite Powders Produced in a Radio Frequency (rf) Thermal Plasma *Materials Science and Engineering: A* 374 (1) 2004: pp. 101 – 108. https://doi.org/10.1016/j.msea.2003.12.040
25. **Yan, L., Li, Y., Deng, Z.X., Zhuang, J., Sun, X.** Surfactant-assisted Hydrothermal Synthesis of Hydroxyapatite Nanorods *International Journal of Inorganic Materials* 3 (7) 2001: pp. 633 – 637. https://doi.org/10.1016/S1466-6049(01)00164-7
26. **Ozawa, M., Suzuki, S.** Microstructural Development of Natural Hydroxyapatite Originated from Fish-Bone Waste through Heat Treatment *Journal of the American Ceramic Society* 85 (5) 2002: pp. 1315 – 1317. https://doi.org/10.1111/j.1151-2916.2002.tb00268.x
27. **Lin, F.-H., Liao, C.J., Chen, K.S., Sun, J.S.** Preparation of a Biphasic Porous Bioceramic by Heating Bovine Cancellous Bone with $Na_4P_2O_7{\cdot}10H_2O$ Addition *Biomaterials* 20 (5) 1999: pp. 475 – 484. https://doi.org/10.1016/S0142-9612(98)00193-8
28. **Dimović, S., Smičiklas, I., Plećaš, I., Antonović, D., Mitrić, M.** Comparative Study of Differently Treated Animal Bones for Co^{2+} Removal *Journal of Hazardous Materials* 164 (1) 2009: pp. 279 – 287. https://doi.org/10.1016/j.jhazmat.2008.08.013
29. **Medellin-Castillo, N.A., Leyva-Ramos, R., Ocampo-Perez, R., Garcia de la Cruz, R.F., Aragon-Piña, A., Martinez-Rosales, J.M., Guerrero-Coronado, R.M., Fuentes-Rubio, L.** Adsorption of Fluoride from Water Solution on Bone Char *Industrial & Engineering Chemistry Research* 46 (26) 2007: pp. 9205 – 9212. https://doi.org/10.1021/ie070023n
30. **Chen, Y., Miao, X.** Thermal and Chemical Stability of Fluorohydroxyapatite Ceramics with Different Fluorine Contents *Biomaterials* 26 (11) 2005: pp. 1205 – 1210. https://doi.org/10.1016/j.biomaterials.2004.04.027
31. **Göller, G., Oktar, F.N.** Sintering Effects on Mechanical Properties of Biologically Derived Dentine Hydroxyapatit *Materials Letters* 56 (3) 2002: pp. 142 – 147. https://doi.org/10.1016/S0167-577X(02)00430-5
32. **Muralithran, G., Ramesh, S.** The Effects of Sintering Temperature on the Properties of Hydroxyapatite *Ceramics International* 26 (2) 2000: pp. 221 – 230. https://doi.org/10.1016/S0272-8842(99)00046-2
33. **Cheung, C.W., Chan, C.K., Porter, J.F., McKay, G.,** Combined Diffusion Model for the Sorption of Cadmium, Copper, and Zinc Ions onto Bone Char *Environmental Science & Technology* 35 (7) 2001: pp. 1511 – 1522. https://doi.org/10.1021/es0012725
34. **Delgadillo-Velasco, L., Hernández-Montoya, V., Cervantes, F.J., Montes-Morán, M.A., Lira-Berlanga, D.** Bone Char with Antibacterial Properties for Fluoride Removal: Preparation, Characterization and Water Treatment *Journal of Environmental Management* 201 (Supplement C) 2017: pp. 277 – 285.

https://doi.org/10.1016/j.jenvman.2017.06.038

35. **Rezaee, A., Rangkooy, H., Khavanin, A., Jafari, A.J.** High Photocatalytic Decomposition of the Air Pollutant Formaldehyde Using Nano-ZnO on Bone Char *Environmental Chemistry Letters* 12 (2) 2014: pp. 353 – 357. https://doi.org/10.1007/s10311-014-0453-7
36. **Iriarte-Velasco, U., Ayastuy, J.L., Zudaire, L., Sierra, I.** An Insight into the Reactions Occurring During the Chemical Activation of Bone Char *Chemical Engineering Journal* 251 (Supplement C) 2014: pp. 217 – 227. https://doi.org/10.1016/j.cej.2014.04.048
37. **Tadic, D., Epple, M.** A thorough Physicochemical Characterisation of 14 Calcium Phosphate-based Bone Substitution Materials in Comparison to Natural Bone *Biomaterials* 25 (6) 2004: pp. 987 – 994. https://doi.org/10.1016/S0142-9612(03)00621-5
38. **Figueiredo, M., Henriques, J., Martins, G., Guerra, F., Judas, F., Figueiredo, H.** Physicochemical Characterization of Biomaterials Commonly used in Dentistry as Bone substitutes—Comparison with Human Bone *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 92B (2) 2010: pp. 409 – 419. https://doi.org/10.1002/jbm.b.31529
39. **Szcześ, A., Hołysz, L., Chibowski, E.** Synthesis of Hydroxyapatite for Biomedical Applications *Advances in Colloid and Interface Science* 249 2017: pp 321 – 330. https://doi.org/10.1016/j.cis.2017.04.007
40. **White, E., Shors, E.C.** Biomaterial Aspects of Interpore-200 Porous Hydroxyapatite *Dental clinics of North America* 30 (1) 1986: pp. 49 – 67.
41. **Barakat, N.A.M., Khil, M.S., Omran, A.M., Sheikh, F.A., Kim, H.Y.** Extraction of Pure Natural Hydroxyapatite from the Bovine Bones Bio Waste by three Different Methods *Journal of Materials Processing Technology* 209 (7) 2009: pp. 3408 – 3415. https://doi.org/10.1016/j.jmatprotec.2008.07.040
42. **Brzezińska-Miecznik, J., Haberko, K., Sitarz, M., Bućko, M.M., Macherzyńska, B.** Hydroxyapatite from Animal Bones – Extraction and Properties *Ceramics International* 41 (3, Part B) 2015: pp. 4841 – 4846. https://doi.org/10.1016/j.ceramint.2014.12.041
43. **Herring, G.M., Geoffey, H.** Chapter 5 – The Organic Matrix of Bone A2 – Bourne, The Biology and Physiology of Bone (Second Edition) Academic Press 1972: pp. 127 – 189. https://doi.org/10.1016/B978-0-12-119201-3.50012-3
44. **Haberko, K., Bućko, M.M., Brzezińska-Miecznik, J., Haberko, M., Mozgawa, W., Panz, T., Pyda, A., Zarębski, J.** Natural Hydroxyapatite – its Behaviour during Heat Treatment *Journal of the European Ceramic Society* 26 (4) 2006: pp. 537 – 542. https://doi.org/10.1016/j.jeurceramsoc.2005.07.033
45. **Etok, S.E., Valsami-Jones, E., Wess, T.J., Hiller, J.C., Maxwell, C.A., Rogers, K.D., Manning, D.A.C., White, M.L., Lopez-Capel, E., Collins, M.J., Buckley, M., Penkman, K.E.H., Woodgate, S.L.** Structural and Chemical Changes of Thermally Treated Bone Apatite *Journal of Materials Science* 42 (23) 2007: pp. 9807 – 9816. https://doi.org/10.1007/s10853-007-1993-z
46. **Peters, F., Schwarz, K., Epple, M.** The Structure of Bone Studied with Synchrotron X-ray diffraction, X-ray Absorption Spectroscopy and Thermal Analysis *Thermochimica Acta* 361 (1) 2000: pp. 131 – 138. https://doi.org/10.1016/S0040-6031(00)00554-2
47. **Rajendran, J., Gialanella, S., Aswath, P.B.** Xanes Analysis of Dried and Calcined Bones *Materials Science and Engineering* 33 (7) 2013: pp. 3968 – 3979. https://doi.org/10.1016/j.msec.2013.05.038
48. **Landi, E., Celotti, G., Logroscino, G., Tampieri, A.** Carbonated Hydroxyapatite as Bone Substitute *Journal of the European Ceramic Society* 23 (15) 2003: pp. 2931 – 2937. https://doi.org/10.1016/S0955-2219(03)00304-2
49. **Lozano, L.F., Peña-Rico, M.A., Heredia, A., Ocotlán-Flores, J., Gómez-Cortés, A., Velázquez, R., Belío, I.A., Bucio, L.** Thermal Analysis Study of Human Bone *Journal of Materials Science* 38 (23) 2003: pp. 4777 – 4782. https://doi.org/10.1023/a:1027483220584
50. **Abdel-Fattah, W.I., Ghazy, T.M., Selim, M.M.** The Effect of Thermal Treatment on Some Characteristics of Animal Carbon *Thermochimica Acta* 51 (2) 1981: pp. 287 – 296. https://doi.org/10.1016/0040-6031(81)85166-0
51. **Markovic, M., Fowler, B.O., Tung, M.S.** Preparation and Comprehensive Characterization of a Calcium Hydroxyapatite Reference Material *Journal of research of the National Institute of Standards and Technology* 109 (6) 2004: pp. 553 – 568. https://doi.org/10.6028/jres.109.042
52. **Rogers, K.D., Daniels, P.** An X-ray Diffraction Study of the Effects of Heat Treatment on Bone Mineral Microstructure *Biomaterials* 23 (12) 2002: pp. 2577 – 2585. https://doi.org/10.1016/S0142-9612(01)00395-7
53. **Hiller, J.C., Thompson, T.J.U., Evison, M.P., Chamberlain, A.T., Wess, T.J.** Bone Mineral Change during Experimental Heating: an X-ray Scattering Investigation *Biomaterials* 24 (28) 2003: pp. 5091 – 5097. https://doi.org/10.1016/S0142-9612(03)00427-7
54. **Ruksudjarit, A., Pengpat, K., Rujijanagul, G., Tunkasiri, T.** Synthesis and Characterization of Nano-crystalline Hydroxyapatite from Natural Bovine Bone *Current Applied Physics* 8 (3) 2008: pp. 270 – 272. https://doi.org/10.1016/j.cap.2007.10.076
55. **Leofanti, G., Padovan, M., Tozzola, G., Venturelli, B.** Surface Area and Pore Texture of Catalysts *Catalysis Today* 41 (1) 1998: pp. 207 – 219. https://doi.org/10.1016/S0920-5861(98)00050-9
56. **Sepulveda, P., Ortega, F.S., Innocentini, M.D.M., Pandolfelli, V.C.** Properties of Highly Porous Hydroxyapatite Obtained by the Gelcasting of Foams *Journal of the American Ceramic Society* 83 (12) 2000: pp. 3021 – 3024. https://doi.org/10.1111/j.1151-2916.2000.tb01677.x
57. **Liu, H.S., Chin, T.S., Lai, L.S., Chiu, S.Y., Chung, K.H., Chang, C.S., Lui, M.T.** Hydroxyapatite Synthesized by a Simplified Hydrothermal Method *Ceramics International* 23 (1) 1997: pp. 19 – 25. https://doi.org/10.1016/0272-8842(95)00135-2