BIOACTIVITY (IN VITRO) OF HALAL HYDROXYAPATITE FROM BLACK TILAPIA FISH BONE FOR BIOMEDICAL APPLICATIONS

SITI KHADIJAH BINTI DERMAWAN

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Universiti Tun Hussein Onn Malaysia

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DEDICATION

This thesis is dedicated to my mother, late father, siblings, supervisor, co-supervisor, and friends, who leads me through the valley of darkness with hope and support, continual motivation, and everlasting encouragements toward the success of this research.

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ABSTRACT

Hydroxyapatite (HAp), $Ca_{10}(PO_4)_6(OH)_2$, is extensively utilized in biomedical field because it mimics inorganic part of human bone and teeth. Synthetic HAp is difficult to produce and costly. Large volumes of by-product waste from fishery factories have a negative impact on the environment. Hence, this research has extracted the halal HAp from waste black tilapia fish bones, characterized the properties of that HAp powder and determined their bioactivity. The bones were calcined at 600 °C to 1000 °C with a heating rate of 10 °C/min. Characterized by using Thermogravimetric Analysis (TGA) for thermal stability, Scanning Electron Microscopy (SEM) for morphology, X-ray Diffraction (XRD) for mineralogy, Fourier-transform Infrared Spectroscopy (FTIR) for chemical bonding and Energy Dispersive Spectroscopy (EDX) for elemental analysis. The extracted HAp was immersed in a simulated body fluid (SBF) solution for 3, 7, and 14 days to evaluate their bioactivity. The Kirby-Bauer test assessed the antimicrobial behaviour of the samples against Staphylococcus aureus and Escherichia coli bacteria. The cytotoxicity effect of the sample was tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte tetrazolium bromide (MTT) assay with human fetal osteoblast cells (hFOB 1.19). There are three stages of weight loss found by TGA which are dehydration, decomposition of organic compounds, and decarbonization. The derived HAp was compatible with standard HAp and the biphasic material was found at 900 and 1000 °C. Raw samples have denser and less porous microstructures than calcined samples, with grain size rising as temperature increases. Organic chemicals were gradually eliminated during the calcination process. The presence of magnesium and sodium showed as their trace elements and the Ca/P molar ratio is 1.63 (1000 °C). Then, apatite formed on the surface of pellet after the immersion in SBF. The extracted HAp does not possess any antimicrobial properties against both bacteria. The sample is non-toxic as the cell viability were 120.10 % and 162.62 % for Day 1 and Day 2 of incubation respectively. Thus, these findings have potential as biomaterial for biomedical applications.

ABSTRAK

Hidroksiapatit (HAp), $Ca_{10}(PO_4)_6(OH)_2$, digunakan secara meluas dalam bidang bioperubatan kerana ia menyerupai sifat bahagian bukan organik tulang dan gigi manusia. HAp sintetik sukar untuk dihasilkan dan mahal. Alam sekitar terjejas akibat sejumlah besar sisa sampingan produk daripada kilang perikanan. Oleh itu, kajian ini telah mengekstrak HAp halal daripada sisa tulang ikan tilapia hitam, mencirikan sifat serbuk HAp tersebut dan menentukan bioaktivitinya. Tulang telah dikalsin pada suhu 600 °C sehingga 1000 °C dengan kadar pemanasan 10 °C/min. Dicirikan dengan menggunakan Analisis Termogravimetri (TGA) untuk kestabilan terma, Mikroskop Elektron Pengimbas (SEM) untuk morfologi, Belauan Sinar-X (XRD) untuk mineralogi, Spektroskopi Inframerah Transformasi Fourier (FTIR) untuk ikatan kimia dan Spektroskopi Sinar-X Sebaran Tenaga (EDX) untuk analisis unsur. HAp yang diekstrak telah direndam di dalam larutan Simulasi Cecair Badan (SBF) selama 3, 7, dan 14 hari untuk menilai bioaktiviti sampel. Ujian *Kirby-Bauer* menilai tingkah laku antimikrob sampel terhadap bakteria *Staphylococcus aureus* dan *Escherichia coli*. Kesan sitotoksisiti sampel telah diuji menggunakan ujian *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte tetrazolium bromide* (MTT) dengan sel osteoblas janin manusia (hFOB 1.19). Terdapat tiga peringkat penurunan berat yang ditemui iaitu dehidrasi, penguraian sebatian organik, dan penyahkarbonan. HAp yang diperolehi adalah sepadan dengan HAp standard dan bahan dwifasa ditemui pada 900 dan 1000 °C. Sampel mentah mempunyai struktur mikro yang lebih tumpat dan kurang berliang daripada sampel dikalsin, dengan peningkatan saiz butiran apabila suhu meningkat. Bahan kimia organik dihapuskan secara beransur-ansur semasa proses pengkalsinan. Kehadiran magnesium dan natrium ditunjukkan sebagai unsur surihnya dan nisbah molar Ca/P ialah 1.63 (1000 °C). Kemudian, apatit terbentuk pada permukaan pelet selepas rendaman dalam SBF. HAp yang diekstrak tidak mempunyai sebarang sifat antimikrob terhadap kedua-dua bakteria. Sampel ini tidak toksik kerana daya maju sel masing-masing adalah 120.10 % dan 162.62 % untuk Hari 1 dan Hari 2 pengeraman. Oleh itu, penemuan ini berpotensi sebagai biomaterial untuk aplikasi bioperubatan.

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LIST OF SYMBOLS AND ABBREVIATIONS

λ	-	Wavelength
θ	-	Theta
%	-	Percentage
$\leq$	-	Less or equal
°	-	Degree
°C	-	Degree Celsius
°C/min	-	Degree Celsius per minute
μL	-	Microlitre
μm	-	Micrometre
$(NH_4)_2HPO_4$	-	Ammonium hydrogen phosphate
ACP	-	Amorphous calcium phosphate
Ag	-	Silver
ALP	-	Alkaline phosphatase
ASTM	-	American Society for Testing and Materials
at%	-	Atomic-percent
β-TCP	-	Beta-tricalcium phosphate
Ba^{2+}	-	Barium ion
Ca	-	Calcium
Ca/P	-	Calcium/Phosphorus
CaP	-	Calcium phosphate
$CaCl_2$	-	Calcium chloride
CaO	-	Calcium oxide
$CaCO_3$	-	Calcium carbonate
$Ca(OH)_2$	-	Calcium hydroxide
$Ca_5(PO_4)_3F$	-	Fluorapatite
$Ca_{10}(PO_4)_6(OH)_2$	-	Hydroxyapatite

Cd	-	Cadmium
Cl	-	Chlorine
Cl^-	-	Chloride ion
cm	-	Centimeter
CMC	-	Carboxymethyl cellulose
Co	-	Cobalt
CO_2	-	Carbon dioxide
CO_3^{2-}	-	Carbonate ion
CPC	-	Calcium phosphate crystal
CTAB	-	Cetyltrimethylammonium bromide
CV	-	Cell Viability
DMEM	-	Dulbecco's Modified Eagle's Medium
DMSO	-	Dimethyl sulfoxide
E. coli	-	Escherichia coli
E.g.	-	Example
EDTA	-	Ethylene diamine tetraacetic acid
EDX	-	Energy Dispersive Spectroscopy
EELS	-	Electron energy-loss spectroscopy
EPD	-	Electrophoretic deposition
F^-	-	Fluoride ion
FAO	-	Food and Agriculture Organization
FBS	-	Fetal Bovine Serum
FTIR	-	Fourier-transform Infrared Spectroscopy
g	-	Gram
g/mol	-	Gram/mole
GPa	-	Gigapascal
H	-	Hydrogen
HAp	-	Hydroxyapatite
HCl	-	Hydrochloric Acid
HCO_3^-	-	Bicarbonate
hFOB 1.19	-	Human fetal osteoblast cell line
HPO_4^{2-}	-	Hydrogen phosphate
ICDD	-	International Centre for Diffraction Data

JCPDS	-	The Joint Committee on Powder Diffraction Standards
K	-	Potassium
K	-	Kelvin
$K_2HPO_4.3H_2O$	-	Potassium Phosphate Dibasic Trihydrate
KBr	-	Potassium bromide
KCl	-	Potassium chloride
kV	-	Kilovolt
LB	-	Lysogeny broth
M	-	Molar
mA	-	Milliampere
Mg	-	Magnesium
$MgCl_2.6H_2O$	-	Magnesium chloride hexahydrate
mm	-	Millimetre
mL	-	Millilitre
mol	-	Mole
MPa		Megapascal
MRI	-	Magnetic Resonance Imaging
MTT	-	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte tetrazolium bromide
Na	-	Sodium
NaCl	-	Sodium Chloride
$NaHCO_3$	-	Sodium bicarbonate
Na_2SO_4	-	Sodium sulfate
NIR	-	Near-infrared
nm	-	Nanometer
O	-	Oxygen
OD	-	Optical density
OH^-	-	Hydroxyl ion
P		Phosphorus
PCC	-	Precipitated calcium carbonate
Pen-Strep	-	Penicillin Streptomycin
PBS	-	Phosphate Buffered Saline
PM	-	Particulate matter

PO_4^{3-}	-	Phosphate ion
rpm	-	Rotation per minute
S. aureus	-	Staphylococcus aureus
SA	-	Sodium alginate
SBF	-	Simulated Body Fluid
SEM	-	Scanning Electron Microscopy
Si^{2+}	-	Silicon (II)
SO_4^{2-}	-	Sulfate
Sr	-	Strontium
TCP	-	Tricalcium phosphate
TGA	-	Thermogravimetric analysis
UVA	-	Ultraviolet A
UVB	-	Ultraviolet B
wt%	-	Weight-percent
XRD	-	X-ray Diffraction
Zn	-	Zinc

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

According to The State of World Fisheries and Aquaculture 2020 from the Food and Agriculture Organization (FAO), 179 million tons of global fish production is estimated in 2018. In most regions of the world, total fish loss and waste lie between 30 % and 35 %. The waste generates an undesirable environmental impact and over-exploitation. Therefore, high added-value products should be produced from the waste of fish by-products to minimize those impacts. Tilapia fish (*Oreochromis*) is a major species produced in world aquaculture (FAO, 2020). Typically, it will be commercialized after extracting the bones as frozen-at-sea-fillets. The bones were considered impracticable, worthless, and dismissed as a waste. However, the bones can be used as a cheap source of calcium phosphate (Boutinguiza *et al.*, 2012).

Hydroxyapatite (HAp) is a naturally taken form of calcium phosphate, the largest amount of inorganic components in human bones and teethes (Sadat-Shojai *et al.*, 2013). It has been widely used in orthopedic and dental applications due to its close similarity in composition with natural bone and teeth, Ca/P molar ratio = 1.67 (Sunil & Jagannatham, 2016). In addition, HAp is biocompatible, non-toxic, bioactive, non-immunogenic and non-inflammatory (Prabakaran & Rajeswari, 2006). Due to those properties, HAp has attracted interest for use in biomedical applications. But there are some differences between synthetic HAp and natural HAp: metabolic activity and dynamic response of natural HAp are better than synthetic HAp (Boutinguiza *et al.*, 2012). In general, HAp can be prepared either by chemical synthesis or extractionof HAp from natural sources. There are some methods used to synthesize HAp chemically, such as microwave irradiation (Indira & Malathi, 2021), chemical precipitation (Grande *et al.*, 2009), sol-gel (Jang *et al.*, 2021), hydrothermal processing (Xu *et al.*, 2011; Lin *et al.*, 2013), and solid-state reaction (Rhee, 2002).

Natural HAp bioceramics are usually extracted from natural sources likebovine bones (Luthfiyah *et al.*, 2022; Herliansyah *et al.*, 2009), porcine bones (Yunoki*et al.*, 2006), fish bones and scales (Zainol *et al.*, 2019; Sunil & Jagannatham, 2016), corals (Nandi *et al.*, 2015), cuttlefish shells (Venkatesan *et al.*, 2018; Cozza *et al.*, 2018), eggshells (Pu *et al.*, 2019), natural gypsum (Sassoni, 2018), and natural calcite (Herliansyah *et al.*, 2007). Chemical synthesis might be a complicated, time-consuming and expensive method. But the extraction of HAp from bio-wastes is considered a safe method because no foreign chemical is required and economically desirable due to the high global demand for HAp (Muthu *et al.*, 2020; Barakat *et al.*, 2008). Furthermore, natural sources are easy to obtain, low cost, simple to process, and available in unlimited supply, especially from waste by-products. The method for extraction of natural HAp commonly used are by calcination process, alkaline hydrolysis, hydrothermal or a combination of methods (Pu *et al.*, 2019). The calcination process (heat treatment) was done in a furnace in an atmosphere environment without any chemical solution, which was classified as a safe method.

1.2 Problem Statement

HAp is a significant material for normal bone and teeth in biomedical applications. After implantation, HAp with good affinity and high biocompatibility will promote osteoconduction and slowly replace the host bone. Due to its excellent bioactive properties, various investigations have been carried out to produce this material by conducting chemical processes or extracting the HAp from natural sources. However, the chemical synthesis process of HAp might be complicated, time-consuming, and require high costs (Bas *et al.*, 2020). Most chemical synthesis methods can produce stoichiometric HAp but without other minerals. For example, Na, Mg, K, and Cl accelerate bone generation (Arokiasamy *et al.*, 2022).

Moreover, it is challenging to enhance the mechanical strength and stability of HAp structures, requiring additional chemical treatment (Boutinguiza *et al.*, 2012). Nevertheless, the natural-biological method requires low-cost materials, available in unlimited supply and needs a simple process to transform the raw materials into HAp

REFERENCES

Abdulgawad, I. A. I. (2016). Fermentation of Nile Tilapia (Oreochromis niloticus) Wastes using Lactobacillus plantarum for the Production of Lactic Acid and Fertilizer. *International Journal of Waste Resources, 06*(04).

Achar, T. K., Bose, A., & Mal, P. (2017). Mechanochemical synthesis of small organic molecules. *Beilstein Journal of Organic Chemistry*, *13*, 1907–1931.

Afshar, A., Ghorbani, M., Ehsani, N., Saeri, M. R., & Sorrell, C. C. (2003). Some important factors in the wet precipitation process of hydroxyapatite. *Materials and Design*, *24*(3), 197–202.

Ahmad Fara, A. N. K., & Abdullah, H. Z. (2015). Characterization of derived natural hydroxyapatite (HAp) obtained from different types of tilapia fish bones and scales. *AIP Conference Proceedings, 1669*.

Ahmad Fara, A. N. K., Yahya, M. A., & Abdullah, H. Z. (2015). Preparation and Characterization of Biological Hydroxyapatite (HAp) Obtained from Tilapia Fish Bone. *Advanced Materials Research*, *1087*, 152–156.

Ahmad Fara, A. N. K., & Abdullah, H. Z. (2016). Influence of calcination temperature on the microstructure and crystallographic properties of hydroxyapatite from black tilapia fish scale. *Materials Science Forum*, *840*, 151–155.

Arokiasamy, P., Al Bakri Abdullah, M. M., Abd Rahim, S. Z., Luhar, S., Sandu, A. V., Jamil, N. H., & Nabiałek, M. (2022). Synthesis methods of hydroxyapatite from natural sources: A review. *Ceramics International,* 48(11), 14959–14979.

Aydin, G., Terzioğlu, P., Öğüt, H., & Kalemtas, A. (2020). Production, characterization, and cytotoxicity of calcium phosphate ceramics derived from the bone of meagre fish, Argyrosomus regius. *Journal of the Australian Ceramic Society*.

Azis, Y., Jamarun, N., Zultiniar, Arief, S., & Nur, H. (2015). Synthesis of hydroxyapatite by hydrothermal method from cockle shell (Anadara granosa). *Journal of Chemical and Pharmaceutical Research*, *7*(5), 798–804.

Balonis-Sant, M., Ma, X., & Kakoulli, I. (2013). Preliminary results on biomimetic methods based on soluble ammonium phosphate precursors for the consolidation of archaeological wall paintings. *ACS Symposium Series*, *1147*, 419–447.

Balu, S., Sundaradoss, M. V., Andra, S., & Jeevanandam, J. (2020). Facile biogenic fabrication of hydroxyapatite nanorods using cuttlefish bone and their bactericidal and biocompatibility study. *Beilstein Journal of Nanotechnology*, *11*, 285–295.

Bano, N., Adzila, S., Jikan, S. S., Basri, H., & Kanasan, N. (2019). Extraction of biological apatite from cow bone at different calcination temperatures: A comparative study. *Key Engineering Materials*, 796, 46–52.

Barakat, N. A. M., Khalil, K. A., Sheikh, F. A., Omran, A. M., Gaihre, B., Khil, S. M., & Yong, H. (2008). Physiochemical characterizations of hydroxyapatite extracted from bovine bones by three different methods : Extraction of biologically desirable HAp. *Materials Science & Engineering C*, *28*(8), 1381–1387.

Bardhan, R., Mahata, S., & Mondal, B. (2011). Processing of natural resourced hydroxyapatite from eggshell waste by wet precipitation method. *Advances in Applied Ceramics*, *110*(2), 80–86.

Bas, M., Daglilar, S., Kuskonmaz, N., Kalkandelen, C., Erdemir, G., Kuruca, S. E., Tulyaganov, D., Yoshioka, T., Gunduz, O., Ficai, D., & Ficai, A. (2020). Mechanical and biocompatibility properties of calcium phosphate bioceramics derived from salmon fish bone wastes. *International Journal of Molecular Sciences*, *21*(21), 1–14.

Bee, S. L., & Hamid, Z. A. A. (2020). Hydroxyapatite derived from food industry bio-wastes: Syntheses, properties and its potential multifunctional applications. *Ceramics International*, 46(11), 17149–17175.

Begam, H., Kundu, B., Chanda, A., & Nandi, S. K. (2017). MG63 osteoblast cell response on Zn doped hydroxyapatite (HAp) with various surface features. *Ceramics International*, *43*(4), 3752–3760.

Bennett, M. B. (2018). *Skeletal system OP - AccessScience*. McGraw-Hill Education.

Boulenger, G. A. F. R. S. (1907). *Zoology of Egypt ; The Fishes of the Nile*. Hugh Rees.

Boutinguiza, M., Pou, J., Comesaña, R., Lusquiños, F., Carlos, A. De, & León, B. (2012). Biological hydroxyapatite obtained from fi sh bones. *Materials Science & Engineering C*, *32*(3), 478–486.

Cacciotti, I., Bianco, A., Lombardi, M., & Montanaro, L. (2009). Mg-substituted hydroxyapatite nanopowders: Synthesis, thermal stability and sintering behaviour. *Journal of the European Ceramic Society*, *29*(14), 2969–2978.

Cai, Y., & Tang, R. (2008). *Calcium phosphate nanoparticles in biomineralization and biomaterials †*. 3775–3787.

Campanella, L., Grimaldi, F., Angeloni, R., Dell'Aglio, E., & Reale, R. (2020). Improvements in bones and stones consolidating processes by vacuum system method. *Natural Product Research*, *0*(0), 1–8.

Canillas, M., Rivero, R., García-Carrodeguas, R., Barba, F., & Rodríguez, M. A. (2017). Processing of hydroxyapatite obtained by combustion synthesis. *Boletin de La Sociedad Espanola de Ceramica y Vidrio*, *56*(5), 237–242.

Catros, S., Guillemot, F., Lebraud, E., Chanseau, C., Perez, S., Bareille, R., Amédée, J., & Fricain, J. C. (2010). Physico-chemical and biological properties of a nano-hydroxyapatite powder synthesized at room temperature. *Irbm*, *31*(4), 226–233.

Chen, F., Huang, P., Zhu, Y., Wu, J., & Cui, D. (2012). Multifunctional Eu 3 þ / Gd 3 þ dual-doped calcium phosphate vesicle-like nanospheres for sustained drug release and imaging. *Biomaterials*, *33*(27), 6447–6455.

Coelho, C. C., Grenho, L., Gomes, P. S., Quadros, P. A., & Fernandes, M. H. (2019). Nano-hydroxyapatite in oral care cosmetics: characterization and cytotoxicity assessment. *Scientific Reports*, *9*(1), 1–10.

Coelho, T. M., Nogueira, E. S., Weinand, W. R., Lima, W. M., Steimacher, A., Medina, A. N., & Baesso, M. L. (2013). *Thermal properties of natural nanostructured hydroxyapatite extracted from fish bone waste Thermal properties of natural nanostructured hydroxyapatite extracted from fish bone waste*. *084701*(2007).

Cozza, N., Monte, F., Bonani, W., Aswath, P., Motta, A., & Migliaresi, C. (2018). Bioactivity and mineralization of natural hydroxyapatite from cuttlefish bone and Bioglass® co-sintered bioceramics. *Journal of Tissue Engineering and Regenerative Medicine*, *12*(2), e1131–e1142.

Cui, F. Z., Li, Y., & Ge, J. (2007). Self-assembly of mineralized collagen composites. *Materials Science and Engineering R: Reports*, *57*(1–6), 1–27.

da Cruz, J. A., Weinand, W. R., Neto, A. M., Palácios, R. S., Sales, A. J. M., Prezas, P. R., Costa, M. M., & Graça, M. P. F. (2020). Low-Cost Hydroxyapatite Powders from Tilapia Fish. *Jom*, *72*(4), 1435–1442.

Deb, P., & Deoghare, A. B. (2019). Effect of Acid, Alkali and Alkali–Acid Treatment on Physicochemical and Bioactive Properties of Hydroxyapatite Derived from Catla catla Fish Scales. *Arabian Journal for Science and Engineering*, *44*(9), 7479–7490.

Dorozhkin, S. V., & Epple, M. (2002). Biological and Medical Significance ofCalcium Phosphates. *Angewandte Chemie - International Edition*, *41*(17), 3130–3146.

Dorozhkin, S. V. (2009). *Calcium Orthophosphate Cements and Concretes*. 221–291.

Dorozhkin, S. V. (2009b). *Nanodimensional and Nanocrystalline Apatites and Other Calcium Orthophosphates in Biomedical Engineering, Biology and Medicine*. 1975–2045.

Dunz, A. R., & Schliewen, U. K. (2013). Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as “Tilapia.” *Molecular Phylogenetics and Evolution*, *68*(1), 64–80.

El-Sayed A. F. M. (2006). Tilapia Culture. CABI Publishing Series.

Eppell, S. J., Tong, W., Lawrence Katz, J., Kuhn, L., & Glimcher, M. J. (2001). Shape and size of isolated bone mineralites measured using atomic force microscopy. *Journal of Orthopaedic Research*, *19*(6), 1027–1034.

FAO. (2020). The State of World Fisheries and Aquaculture (SOFIA). In *Nature and Resources* (Vol. 2020). FAO.

Fathi, M. H., & Zahrani, E. M. (2009). Fabrication and characterization of fluoridated hydroxyapatite nanopowders via mechanical alloying. *Journal of Alloys and Compounds*, *475*(1–2), 408–414.

Ficken, M. D., Barnes, H. J., & Qureshi, M. A. (1991). Acute Airsacculitis in Turkeys Inoculated with Cell-free Culture Filtrate of Pasteurella multocida. *Veterinary Pathology*, *28*(1), 46–54.

Figueiredo, M., Fernando, A., Martins, G., Freitas, J., Judas, F., & Figueiredo, H. (2010). Effect of the calcination temperature on the composition and microstructure of hydroxyapatite derived from human and animal bone. *Ceramics International*, *36*(8), 2383–2393.

Fihri, A., Len, C., Varma, R. S., & Solhy, A. (2017). Hydroxyapatite: A review of syntheses, structure and applications in heterogeneous catalysis. *Coordination Chemistry Reviews*, *347*, 48–76.

Fitzsimmons, K. (2008). Tilapia Product Quality and New Product Forms for International Markets. *8 Th International Symposium on Tilapia in Aquaculture 2008*, *January 2008*, 1–10.

Freitag, R., & Hilbrig, F. (2012). *Isolation and purification of recombinant proteins , antibodies and plasmid DNA with hydroxyapatite chromatography*. *10*, 90–102.

Fytianos, G., Rahdar, A., & Kyzas, G. Z. (2020). Nanomaterials in cosmetics: Recent updates. *Nanomaterials*, *10*(5), 1–16.

Gayathri, B., Muthukumarasamy, N., Velauthapillai, D., Santhosh, S. B., & Asokan, V. (2018). Magnesium incorporated hydroxyapatite nanoparticles: Preparation, characterization, antibacterial and larvicidal activity. *Arabian Journal of Chemistry*, *11*(5), 645–654.

Gerlier, D., & Thomasset, N. (1986). Use of MTT colorimetric assay to measure cell activation. *Journal of Immunological Methods*, *94*(1–2), 57–63.

Gopi, D., Kanimozhi, K., Bhuvaneshwari, N., Indira, J., & Kavitha, L. (2014). Novel banana peel pectin mediated green route for the synthesis of hydroxyapatite nanoparticles and their spectral characterization. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *118*, 589–597.

Goto, T., Kim, I. Y., Kikuta, K., & Ohtsuki, C. (2012). Hydrothermal synthesis of composites of well-crystallized hydroxyapatite and poly(vinyl alcohol) hydrogel. *Materials Science and Engineering C*, *32*(3), 397–403.

Govindaraj, D., & Rajan, M. (2016). Synthesis and Spectral Characterization of Novel nano-Hydroxyapatite from Moringaoleifera Leaves. *Materials Today: Proceedings*, *3*(6), 2394–2398.

Grande, C. J., Torres, F. G., Gomez, C. M., & Carmen Bañó, M. (2009). Nanocomposites of bacterial cellulose/hydroxyapatite for biomedical applications. *Acta Biomaterialia*, *5*(5), 1605–1615.

Guo, X., Yan, H., Zhao, S., Li, Z., Li, Y., & Liang, X. (2013). Effect of calcining temperature on particle size of hydroxyapatite synthesized by solid-state reaction at room temperature. *Advanced Powder Technology*, *24*(6), 1034–1038.

Habibie, S., Santosa Wargadipura, A. H., Gustiono, D., Herdianto, N., Riswoko, A., Nikmatin, S., & Clarke, S. (2017). Production and Characterization of Hydroxyapatite Bone Substitute Material Performed from Indonesian Limestone. *International Journal of Biomedical Engineering and Science*, *4*(1), 11–23.

Hammood, A. S., Hassan, S. S., Alkhafagy, M. T., & Jaber, H. L. (2019). Effect of calcination temperature on characterization of natural hydroxyapatite prepared from carp fish bones. *SN Applied Sciences*, *1*(5).

Hariani, P. L., Muryati, M., Said, M., & Salni, S. (2020). Synthesis of nano-hydroxyapatite from snakehead (Channa striata) fish bone and its antibacterial properties. *Key Engineering Materials*, *840 KEM*, 293–299.

Harris, S. A., Enger, R. J., Riggs, L. B., & Spelsberg, T. C. (1995). Development and characterization of a conditionally immortalized human fetal osteoblastic cell line. *Journal of Bone and Mineral Research*, *10*(2), 178–186.

Herliansyah, M. K., Hamdi, M., Ide-ektessabi, A., Wildan, M. W., & Toque, J. A. (2009). The in fl uence of sintering temperature on the properties of compacted bovine hydroxyapatite. *Materials Science & Engineering C*, *29*(5), 1674–1680.

Herliansyah, M. K., Nasution, D. A., Hamdi, M., Ide-Ektessabi, A., Wildan, M. W., & Tontowi, A. E. (2007). Preparation and characterization of natural hydroxyapatite: A comparative study of bovine bone hydroxyapatite and hydroxyapatite from calcite. *Materials Science Forum*, *561–565*(PART 2), 1441–1444.

Ho, W. F., Hsu, H. C., Hsu, S. K., Hung, C. W., & Wu, S. C. (2013). Calcium phosphate bioceramics synthesized from eggshell powders through a solid state reaction. *Ceramics International*, *39*(6), 6467–6473.

Hoyer, B., Bernhardt, A., Heinemann, S., Stachel, I., Meyer, M., & Gelinsky, M. (2012). Biomimetically mineralized salmon collagen scaffolds for application in bone tissue engineering. *Biomacromolecules*, *13*(4), 1059–1066.

Huang, B., Caetano, G., Vyas, C., Blaker, J. J., Diver, C., & Bártolo, P. (2018). Polymer-ceramic composite scaffolds: The effect of hydroxyapatite and β-tri-calcium phosphate. *Materials*, *11*(1).

Huang, G., Lu, C. H., & Yang, H. H. (2018). Magnetic Nanomaterials for Magnetic Bioanalysis. In *Novel Nanomaterials for Biomedical, Environmental and Energy Applications*. Elsevier Inc.

Huang, Y. H., Shih, Y. J., & Cheng, F. J. (2011). Novel KMnO 4-modified iron oxide for effective arsenite removal. *Journal of Hazardous Materials*, *198*, 1–6.

Huang, Y., Hsiao, P., & Chai, H. (2011). Hydroxyapatite extracted from fish scale : Effects on MG63 osteoblast-like cells. *Ceramics International*, *37*(6), 1825–1831.

Ismail, N., & Abdullah, H. Z. (2016). Effects of hydrochloric acid concentration on the properties of black tilapia fish skin gelatin. *Materials Science Forum*, *840*, 165–169.

Indira, J., & Malathi, K. S. (2021). Comparison of template mediated ultrasonic and microwave irradiation method on the synthesis of hydroxyapatite nanoparticles for biomedical applications. *Materials Today: Proceedings*, 51(xxxx), 1765–1769.

Itatani, K., Tsugawa, T., Umeda, T., Musha, Y., Davies, I. J., & Koda, S. (2010). Preparation of submicrometer-sized porous spherical hydroxyapatite agglomerates by ultrasonic spray pyrolysis technique. *Journal of the Ceramic Society of Japan*, *118*(1378), 462–466.

Jaber, H. L., Hammood, A. S., & Parvin, N. (2018). Synthesis and characterization of hydroxyapatite powder from natural Camelus bone. *Journal of the Australian Ceramic Society*, *54*(1).

Jablonská, E., Horkavcová, D., Rohanová, D., & Brauer, D. S. (2020). A review of: In vitro cell culture testing methods for bioactive glasses and other biomaterials for hard tissue regeneration. *Journal of Materials Chemistry B*, *8*(48), 10941–10953.

Jamarun, N., Azharman, Z., Arief, S., Sari, T. P., Asril, A., & Elfina, S. (2015). Effect of temperature on synthesis of hydroxyapatite from limestone. *Rasayan Journal of Chemistry*, *8*(1), 133–137

Jang, J. H., Oh, B., & Lee, E. J. (2021). Crystalline hydroxyapatite/graphene oxide complex by low-temperature sol-gel synthesis and its characterization. *Ceramics International*, 47(19), 27677–27684.

Jena, P. (2007). Synthesis and Characterization of Hydroxyapatite. In *Synthetic Metals*. National Institute of Technology Rourkela.

Jensen, A. C. S., Ibsen, C. J. S., Sutherland, D., & Birkedal, H. (2014). Transparent aggregates of nanocrystalline hydroxyapatite. *Crystal Growth and Design*, *14*(12), 6343–6349.

Ji, D., Kuo, T., Wu, H., Yang, J., & Lee, S. (2012). A novel injectable chitosan / polyglutamate polyelectrolyte complex hydrogel with hydroxyapatite for soft-tissue augmentation. *Carbohydrate Polymers*, *89*(4), 1123–1130.

Joint Committee on Powder Diffraction Standards (JCPDS) Card No. 6-0453. (1994).

Joint Committee on Powder Diffraction Standards (JCPDS) Card No. 9-169. (1994).

Joint Committee on Powder Diffraction Standards (JCPDS) Card No. 9-432. (1994).

JoVE Science Education Database (2022). Materials Engineering. X-ray Diffraction. JoVE, Cambridge, MA. Retrieved from https://www.jove.com/v/10446/x-ray-diffraction

Kalita, S. J., Bhardwaj, A., & Bhatt, H. A. (2007). Nanocrystalline calcium phosphate ceramics in biomedical engineering. *Materials Science and Engineering C*, *27*(3), 441–449.

Kamalanathan, P., Ramesh, S., Bang, L. T., Niakan, A., Tan, C. Y., Purbolaksono, J., Chandran, H., & Teng, W. D. (2014). Synthesis and sintering of hydroxyapatite derived from eggshells as a calcium precursor. *Ceramics International*, *40*(PB), 16349–16359.

Kanno, C. M., Sanders, R. L., Flynn, S. M., Lessard, G., & Myneni, S. C. B. (2014). Novel apatite-based sorbent for defluoridation: Synthesis and sorption characteristics of nano-micro-crystalline hydroxyapatite-coated-limestone. *Environmental Science and Technology*, *48*(10), 5798–5807.

Khiri, M. Z. A., Matori, K. A., Zainuddin, N., Abdullah, C. A. C., Alassan, Z. N., Baharuddin, N. F., & Zaid, M. H. M. (2016). The usability of ark clam shell (Anadara granosa) as calcium precursor to produce hydroxyapatite nanoparticle via wet chemical precipitate method in various sintering temperature. *SpringerPlus*, *5*(1).

Kim, H. S., Kim, J. T., Jung, Y. J., Ryu, S. C., Son, H. J., & Kim, Y. G. (2007). Preparation of a porous chitosan/fibroin-hydroxyapatite composite matrix for tissue engineering. *Macromolecular Research*, *15*(1), 65–73.

Kim, H. W., Knowles, J. C., & Kim, H. E. (2005). Hydroxyapatite porous scaffold engineered with biological polymer hybrid coating for antibiotic Vancomycin release. *Journal of Materials Science: Materials in Medicine*, *16*(3), 189–195.

Kokubo, T., & Takadama, H. (2006). How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials*, *27*(15), 2907–2915.

Kokubo, T., Kim, H.-M., & Kawashita, M. (2003). Novel bioactive materials with different mechanical properties. *Biomaterial*, *37*(1), 665–668.

Koutsopoulos, S. (2002). Synthesis and characterization of hydroxyapatite crystals : A review study on the analytical methods. *February*, 31–34.

Kye, S. B., & Park, S. N. (2016). Morphology and mechanical properties through hydroxyapatite powder surface composite. *Applied Chemistry for Engineering*, *27*(3), 299–306.

Li, J., Chen, Y., Tseng, Y., Mozumdar, S., & Huang, L. (2010). Biodegradable calcium phosphate nanoparticle with lipid coating for systemic siRNA delivery. *Journal of Controlled Release*, *142*(3), 416–421.

Li, L., Pan, H., Tao, J., Xu, X., Mao, C., & Tang, R. (2008). Repair of enamel by using hydroxyapatite nanoparticles as the building blocks. 4079–4084.

Li, Y., Klein, C. P. A. T., Zhang, X., & de Groot, K. (1993). Relationship between the colour change of hydroxyapatite and the trace element manganese. *Biomaterials*, *14*(13), 969–972.

Lin, K., Liu, P., Wei, L., Zou, Z., Zhang, W., Qian, Y., Shen, Y., & Chang, J. (2013). Strontium substituted hydroxyapatite porous microspheres : Surfactant-free hydrothermal synthesis , enhanced biological response and sustained drug release. *Chemical Engineering Journal*, *222*, 49–59.

Liu, M., Zhou, G., Song, W., Li, P., Liu, H., & Fan, Y. (2012). *Nanoscale Effect of nano-hydroxyapatite on the axonal guidance growth of rat cortical*. 3201–3207.

Luthfiyah, S., Soegijono, B., Susetyo, F. B., & Notonegoro, H. A. (2022). Comparing Properties of Bovine Bone Derived Hydroxyapatite and Synthetic Hydroxyapatite. *Journal of Applied Science and Engineering (Taiwan)*, 25(6), 1045–1051.

Madupalli, H., Pavan, B., & Tecklenburg, M. M. J. (2017). Carbonate substitution in the mineral component of bone: Discriminating the structural changes, simultaneously imposed by carbonate in A and B sites of apatite. *Journal of Solid State Chemistry*, *255*, 27–35.

McGrouther, M (2019, February 15) *Fish scales*. The Australian Museum. https://australian.museum/learn/animals/fishes/fish-scales/

Mechay, A., Feki, H. E. L., Schoenstein, F., & Jouini, N. (2012). Nanocrystalline hydroxyapatite ceramics prepared by hydrolysis in polyol medium. *Chemical Physics Letters*, *541*, 75–80.

Mohd Pu'ad, N. A. S., Koshy, P., Abdullah, H. Z., Idris, M. I., & Lee, T. C. (2019). Syntheses of hydroxyapatite from natural sources. *Heliyon*, *5*(April), e01588.

Mohd Pu'ad, N. A. S., Abdul Haq, R. H., Mohd Noh, H., Abdullah, H. Z., Idris, M. I., & Lee, T. C. (2020). Synthesis method of hydroxyapatite: A review. *Materials Today: Proceedings*, *July*.

Mondal, B., Mondal, S., Mondal, A., & Mandal, N. (2016). Fish scale derived hydroxyapatite scaffold for bone tissue engineering. *Materials Characterization*, *121*, 112–124.

Mondal, S., Mahata, S., Kundu, S., & Mondal, B. (2010). Processing of natural resourced hydroxyapatite ceramics from fish scale. *Neurological Research*, *32*(8), 810–815.

Morrison, C. J., Gagnon, P., & Cramer, S. M. (2010). *Purification of Monomeric mAb From Associated Aggregates Using Selective Desorption Chromatography in Hydroxyapatite Systems*. *108*(4), 813–821.

Muhammad, N., Gao, Y., Iqbal, F., Ahmad, P., Ge, R., Nishan, U., Rahim, A., Gonfa, G., & Ullah, Z. (2016). Extraction of biocompatible hydroxyapatite from fish scales using novel approach of ionic liquid pretreatment. *Separation and Purification Technology*, *161*, 129–135.

Mustafa, N., Ibrahim, M. H. I., Asmawi, R., & Amin, A. M. (2015). Hydroxyapatite Extracted from Waste Fish Bones and Scales via Calcination Method. *Applied Mechanics and Materials*, *773–774*, 287–290.

Mustaffa, R., Mohd Yusof, M. R., & Abdullah, Y. (2015). A Novelty of Synthetic Hydroxyapatite from Cockle Shell and Characterization. *Advanced Materials Research*, *1087*, 429–433.

Naga, S. M., El-Maghraby, H. F., Mahmoud, E. M., Talaat, M. S., & Ibrhim, A. M. (2015). Preparation and characterization of highly porous ceramic scaffolds based on thermally treated fish bone. *Ceramics International*, *41*(10), 15010–15016.

Nakazato, T., Tsukui, S., Nakagawa, N., & Kai, T. (2012). Continuous production of hydroxyapatite powder by drip pyrolysis in a fluidized bed. *Advanced Powder Technology*, *23*(5), 632–639.

Nandi, S. K., Kundu, B., Mukherjee, J., Mahato, A., Datta, S., & Balla, V. K. (2015). Converted marine coral hydroxyapatite implants with growth factors: In vivo bone regeneration. *Materials Science and Engineering C*, *49*, 816–823.

Nayak, A. K. (2010). Hydroxyapatite Synthesis Methodologies: An Overview. *International Journal of ChemTech Research*, *2*, 903–907.

Nayar, S., & Guha, A. (2009). Waste utilization for the controlled synthesis of nanosized hydroxyapatite. *Materials Science and Engineering C*, *29*(4), 1326–1329.

Neto, J. da R. S., de Azevedo Silva Ribeiro, F., Gonçalves, A. A., & Coelho Emerenciano, M. G. (2019). Tilapia processing waste silage (TPWS): An alternative ingredient for Litopenaeus vannamei (Boone, 1931) diets in biofloc and clear-water systems. *Aquaculture and Fisheries*, *4*(5), 214–218.

Okabayashi, R., Nakamura, M., Okabayashi, T., Tanaka, Y., & Nagai, A. (2009). *Efficacy of Polarized Hydroxyapatite and Silk Fibroin Composite Dressing Gel on Epidermal Recovery From Full-Thickness Skin Wounds*. 641–646.

Okuda, M., Takeguchi, M., Tagaya, M., & Tonegawa, T. (2009). *Elemental distribution analysis of type I collagen fibrils in tilapia fish scale with energy-filtered transmission electron microscope*. *40*, 665–668.

Olszta, M. J., Cheng, X., Jee, S. S., Kumar, R., Kim, Y. Y., Kaufman, M. J., Douglas, E. P., & Gower, L. B. (2007). Bone structure and formation: A new perspective. *Materials Science and Engineering R: Reports*, *58*(3–5), 77–116.

Ozawa, M., & Suzuki, S. (2002). Microstructural development of natural hydroxyapatite originated from fish-bone waste through heat treatment. *Journal of the American Ceramic Society*, *85*(5), 1315–1317.

Pai, S., Kini, M. S., & Selvaraj, R. (2021). A review on adsorptive removal of dyes from wastewater by hydroxyapatite nanocomposites. *Environmental Science and Pollution Research*, *28*(10), 11835–11849.

Pal, A., Paul, S., Choudhury, A. R., Balla, V. K., Das, M., & Sinha, A. (2017). Synthesis of hydroxyapatite from Lates calcarifer fish bone for biomedical applications. *Materials Letters*, *203*, 89–92.

Panda, N. N., Pramanik, K., & Sukla, L. B. (2014). Extraction and characterization of biocompatible hydroxyapatite from fresh water fish scales for tissue engineering scaffold. *Bioprocess and Biosystems Engineering*, *37*(3), 433–440.

Patty, D. J., Nugraheni, A. D., Dewi Ana, I., & Yusuf, Y. (2022). Mechanical Characteristics and Bioactivity of Nanocomposite Hydroxyapatite/Collagen Coated Titanium for Bone Tissue Engineering. *Bioengineering*, 9(12).

Piccirillo, C., Pullar, R. C., Costa, E., Santos-Silva, A., Pintado, M. M. E., & Castro, P. M. L. (2015). Hydroxyapatite-based materials of marine origin: A bioactivity and sintering study. *Materials Science and Engineering C*, *51*, 309–315.

Piccirillo, C., Pullar, R. C., Tobaldi, D. M., Lima Castro, P. M., & Estevez Pintado, M. M. (2015). Silver-containing calcium phosphate materials of marine origin with antibacterial activity. *Ceramics International*, *41*(8), 10152–10159.

Piccirillo, C., Rocha, C., Tobaldi, D. M., Pullar, R. C., Labrincha, J. A., Ferreira, M. O., Castro, P. M. L., & Pintado, M. M. E. (2014). A hydroxyapatite-Fe2O3 based material of natural origin as an active sunscreen filter. *Journal of Materials Chemistry B*, *2*(36), 5999–6009.

Piccirillo, C., Silva, M. F., Pullar, R. C., Braga, I., Jorge, R., Pintado, M. M. E., & Castro, P. M. L. (2013). Extraction and characterisation of apatite- and tricalcium phosphate-based materials from cod fi sh bones. *Materials Science &Engineering C*, *33*(1), 103–110.

Pleshko, N., Boskey, A., & Mendelsohn, R. (1991). Novel infrared spectroscopic method for the determination of crystallinity of hydroxyapatite minerals. *Biophysical Journal*, *60*(4), 786–793.

Pon-On, W., Suntornsaratoon, P., Charoenphandhu, N., Thongbunchoo, J., Krishnamra, N., & Tang, I. M. (2016). Hydroxyapatite from fish scale for potential use as bone scaffold or regenerative material. *Materials Science and Engineering C*, *62*, 183–189.

Porter, A. E., Botelho, C. M., Lopes, M. A., Santos, J. D., Best, S. M., & Bonfield, W. (2004). Ultrastructural comparison of dissolution and apatite precipitation on hydroxyapatite and silicon-substituted hydroxyapatite in vitro and in vivo. *Journal of Biomedical Materials Research - Part A*, *69*(4), 670–679.

Prabakaran, K., & Rajeswari, S. (2006). *Development of Hydroxyapatite from Natural Fish Bone Through Heat Treatment*. *20*(1), 20–23.

Pramanik, S., Agarwal, A. K., Rai, K. N., & Garg, A. (2007). Development of high strength hydroxyapatite by solid-state-sintering process. *Ceramics International*, *33*(3), 419–426.

Rahaman, M. N. (2003). Ceramic Processing and Sintering (2nd ed.). CRC Press.

Rahavi, S. S., Ghaderi, O., Monshi, A., & Fathi, M. H. (2017). A comparative study on physicochemical properties of hydroxyapatite powders derived from natural and synthetic sources. *Russian Journal of Non-Ferrous Metals*, *58*(3), 276–286.

Ramis, J. M., Coelho, C. C., Córdoba, A., Quadros, P. A., & Monjo, M. (2018). Safety assessment of nano-hydroxyapatite as an oral care ingredient according to the EU cosmetics regulation. *Cosmetics*, *5*(3), 1–13.

Rao, R. R., Roopa, H. N., & Kannan, T. S. (1997). Solid state synthesis and thermal stability of HAP and HAP - β-TCP composite ceramic powders. *Journal of Materials Science: Materials in Medicine*, *8*(8), 511–518.

Rhee, S. H. (2002). Synthesis of hydroxyapatite via mechanochemical treatment. *Biomaterials*, *23*(4), 1147–1152.

Rho, J. Y., Kuhn-Spearing, L., & Zioupos, P. (1998). Mechanical properties and the hierarchical structure of bone. *Medical Engineering and Physics*, *20*(2), 92–102.

Río, J. G. del, Sanchez, P., Morando, P. J., & Cicerone, D. S. (2006). Retention of Cd, Zn and Co on hydroxyapatite filters. *Chemosphere*, *64*(6), 1015–1020.

Rocha, J. H. G., Lemos, A. F., Agathopoulos, S., Valério, P., Kannan, S., Oktar, F. N., & Ferreira, J. M. F. (2005). Scaffolds for bone restoration from cuttlefish. *Bone*, *37*(6), 850–857.

Rodr, I., Delgado-lo, J. M., Dura, M. A., Ia, M., Tampieri, A., Colangelo, D., Prat, M., & Go, J. (2013). *pH-Responsive Delivery of Doxorubicin from Citrate − Apatite Nanocrystals with Tailored Carbonate Content*.

Rohmadi, R., Harwijayanti, W., Ubaidillah, U., Triyono, J., Diharjo, K., & Utomo, P. (2021). In vitro degradation and cytotoxicity of eggshell-based hydroxyapatite: A systematic review and meta-analysis. *Polymers*, *13*(19).

Roopalakshmi, S., Ravishankar, R., Belaldavar, S., Prasad, R. G. S. V., & Phani, A. R. (2017). Investigation of Structural and Morphological Characteristic of Hydroxyapatite Synthesized by Sol-Gel Process. *Materials Today: Proceedings*, *4*(11), 12026–12031.

Rungsarit Koonawoot, Cherdsak Saelee, Sakdiphon Thiansem, S. P. (2012). Synthesis Control and Characterization of Hydroxyapatite Ceramic Using a Solid State Reaction. *1st Mae Fah Luang University International Conference 2012*, 1–9.

Sadat-Shojai, M., Khorasani, M. T., Dinpanah-Khoshdargi, E., & Jamshidi, A. (2013). Synthesis methods for nanosized hydroxyapatite with diverse structures. *Acta Biomaterialia*, *9*(8), 7591–7621.

Santhosh, S., & Balasivanandha Prabu, S. (2013). Thermal stability of nano hydroxyapatite synthesized from sea shells through wet chemical synthesis. *Materials Letters*, *97*, 121–124.

Santos, M. H., Oliveira, M. de, Souza, L. P. de F., Mansur, H. S., & Vasconcelos, W. L. (2004). Synthesis control and characterization of hydroxyapatite prepared by wet precipitation process. *Materials Research*, *7*(4), 625–630.

Sary, C., de Paris, L. D., Bernardi, D. M., Lewandowski, V., Signor, A., & Boscolo, W. R. (2017). Tilapia by-product hydrolysate powder in diets for Nile tilapia larvae. *Acta Scientiarum - Animal Sciences*, *39*(1), 1–6.

Sasikumar, S., & Vijayaraghavan, R. (2008). Solution combustion synthesis of *Ceramics International*, *34*(6), 1373–1379.

Sassoni, E. (2018). Hydroxyapatite And Other calcium phosphates for the conservation of cultural heritage: A review. *Materials*, *11*(4).

Schwarz, R. B. (1996). Introduction to the viewpoint set on: mechanical alloying. *Scripta Materialia*, *34*(1), 1–4.

Shafiu Kamba, A., Ismail, M., Tengku Ibrahim, T. A., & Zakaria, Z. A. B. (2013). Synthesis and characterisation of calcium carbonate aragonite nanocrystals from cockle shell powder (Anadara granosa). *Journal of Nanomaterials*, *2013*.

Shavandi, A., Bekhit, A. E. D. A., Ali, A., & Sun, Z. (2015). Synthesis of nano-hydroxyapatite (nHA) from waste mussel shells using a rapid microwave method. *Materials Chemistry and Physics*, *149*, 607–616.

Sheikh, L. (2015). Synthesis and Characterization of Stable Hydroxyapatite (Issue August).

Shen, T. D., Koch, C. C., Mc Cormick, T. L., Nemanich, R. J., Huang, J. Y., & Huang, J. G. (1995). The structure and property characteristics of amorphous/nanocrystalline silicon produced by ball milling. *Journal of Materials Research*, *10*(1), 139–148.

Shepherd, C. J., & Jackson, A. J. (2013). Global fishmeal and fish-oil supply: Inputs, outputs and marketsa. *Journal of Fish Biology*, *83*(4), 1046–1066.

Shu, C., Yanwei, W., Hong, L., Zhengzheng, P., & Kangde, Y. (2005). Synthesis of carbonated hydroxyapatite nanofibers by mechanochemical methods. *Ceramics International*, *31*(1), 135–138.

Sirait, M., Sinulingga, K., Siregar, N., & Siregar, R. S. D. (2020). Synthesis of hydroxyapatite from limestone by using precipitation method. *Journal of Physics: Conference Series,* 1462(1).

Silva, J. F. X., Ribeiro, K., Silva, J. F., Cahú, T. B., & Bezerra, R. S. (2014). Utilization of tilapia processing waste for the production of fish protein hydrolysate. *Animal Feed Science and Technology*, *196*, 96–106.

Sofronia, A. M., Baies, R., Anghel, E. M., Marinescu, C. A., & Tanasescu, S. (2014). Thermal and structural characterization of synthetic and natural nanocrystalline hydroxyapatite. *Materials Science and Engineering C*, *43*, 153–163.

Sotome, S., Uemura, T., Kikuchi, M., Chen, J., Itoh, S., Tanaka, J., Tateishi, T., & Shinomiya, K. (2004). Synthesis and in vivo evaluation of a novel hydroxyapatite/collagen- alginate as a bone filler and a drug delivery carrier of bone morphogenetic protein. *Materials Science and Engineering C*, *24*(3), 341–347.

Sridevi, S., Sutha, S., Kavitha, L., & Gopi, D. (2020). Physicochemical and biological behaviour of biogenic derived hydroxyapatite and carboxymethyl cellulose/sodium alginate biocomposite coating on Ti6Al4V alloy for biomedical applications. *Materials Chemistry and Physics*, *254*, 123455.

Steckiewicz, K. P., Barcinska, E., Malankowska, A., Zauszkiewicz–Pawlak, A., Nowaczyk, G., Zaleska-Medynska, A., & Inkielewicz-Stepniak, I. (2019). Impact of gold nanoparticles shape on their cytotoxicity against human osteoblast and osteosarcoma in in vitro model. Evaluation of the safety of use and anti-cancer potential. *Journal of Materials Science: Materials in Medicine*, *30*(2), 1–15.

Sternitzke, V., Kaegi, R., Audinot, J. N., Lewin, E., Hering, J. G., & Johnson, C. A. (2012). Uptake of fluoride from aqueous solution on nano-sized hydroxyapatite: Examination of a fluoridated surface layer. *Environmental Science and Technology*, *46*(2), 802–809.

Suguna A/P Jeganathan. (2020). *Synthesis of Hydroxyapatite From Fish Bone (Scomber Scombrus) Using Thermal Decomposition Method*. University of Malaya

Sunil, B. R., & Jagannatham, M. (2016). Producing hydroxyapatite from fish bones by heat treatment. *Materials Letters, 185*(August), 411–414.

Szcześ, A., Hołysz, L., & Chibowski, E. (2017). Synthesis of hydroxyapatite for biomedical applications. *Advances in Colloid and Interface Science*, *249*, 321–330.

Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, *285*(1–4), 146–158.

Tampieri, A., Sprio, S., Ruffini, A., Celotti, G., Lesci, I. G., & Roveri, N. (2009). From wood to bone: Multi-step process to convert wood hierarchical structures into biomimetic hydroxyapatite scaffolds for bone tissue engineering. *Journal of Materials Chemistry*, *19*(28), 4973–4980.

Teixeira, M. A. C., Piccirillo, C., Tobaldi, D. M., Pullar, R. C., Labrincha, J. A., Ferreira, M. O., L. Castro, P. M., & E. Pintado, M. M. (2017). Effect of preparation and processing conditions on UV absorbing properties of hydroxyapatite-Fe2O3 sunscreen. *Materials Science and Engineering C*, *71*,141–149.

Uskokovic, D. P. (2010). *Review Nanosized hydroxyapatite and other calcium phosphates : Chemistry of formation and application as drug and gene delivery agents*.

Venkatesan, J., Pallela, R., Bhatnagar, I., & Kim, S. K. (2012). Chitosan-amylopectin/hydroxyapatite and chitosan-chondroitin sulphate/hydroxyapatite composite scaffolds for bone tissue engineering. *International Journal of Biological Macromolecules*, *51*(5), 1033–1042.

Venkatesan, J., Rekha, P. D., Anil, S., Bhatnagar, I., Sudha, P. N., Dechsakulwatana, C., Kim, S. K., & Shim, M. S. (2018). Hydroxyapatite from Cuttlefish Bone: Isolation, Characterizations, and Applications. *Biotechnology and Bioprocess Engineering*, *23*(4), 383–393.

Walsh, P. J., Buchanan, F. J., Dring, M., Maggs, C., Bell, S., & Walker, G. M. (2008). Low-pressure synthesis and characterisation of hydroxyapatite derived from mineralise red algae. *Chemical Engineering Journal*, *137*(1), 173–179.

Wang, M. C., Chen, H. T., Shih, W. J., Chang, H. F., Hon, M. H., & Hung, I. M. (2015). Crystalline size, microstructure and biocompatibility of hydroxyapatite nanopowders by hydrolysis of calcium hydrogen phosphate dehydrate (DCPD). *Ceramics International*, *41*(2), 2999–3008.

Wang, P. E., & Chaki, T. K. (1993). Sintering behaviour and mechanical properties of hydroxyapatite and dicalcium phosphate. *Journal of Materials Science: Materials in Medicine*, *4*(2), 150–158.

Wang, Y., Zhang, S., Wei, K., Zhao, N., Chen, J., & Wang, X. (2006). Hydrothermal synthesis of hydroxyapatite nanopowders using cationic surfactant as a template. *Materials Letters*, *60*(12), 1484–1487.

Widiyastuti, W., Setiawan, A., Winardi, S., Nurtono, T., & Setyawan, H. (2014). Particle formation of hydroxyapatite precursor containing two components in a spray pyrolysis process. *Frontiers of Chemical Science and Engineering*, *8*(1), 104–113.

Wu, S. C., Hsu, H. C., Hsu, S. K., Chang, Y. C., & Ho, W. F. (2016). Synthesis of hydroxyapatite from eggshell powders through ball milling and heat treatment. *Journal of Asian Ceramic Societies*, *4*(1), 85–90.

Xiong, Z. C., Yang, R. L., Zhu, Y. J., Chen, F. F., & Dong, L. Y. (2017). Flexible hydroxyapatite ultralong nanowire-based paper for highly efficient and multifunctional air filtration. *Journal of Materials Chemistry A*, *5*(33), 17482–17491.

Xu, H., Chen, Y., & Zhang, S. F. (2011). Synthesis and characterization of microporous hydroxyapatite via hydrothermal method. *Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry*, *41*(1), 31–35.

Yang, F., He, D., Liu, Y., Li, N., Wang, Z., Ma, Q., & Dong, G. (2016). Conservation of bone relics using hydroxyapatite as protective material. *Applied Physics A: Materials Science and Processing*, *122*(4), 1–5.

Yang, L., Ning, X., Chen, K., & Zhou, H. (2007). Preparation and properties of hydroxyapatite filters for microbial filtration. *Ceramics International*, *33*(3), 483–489.

Yeong, K. C. B., Wang, J., & Ng, S. C. (2001). Mechanochemical synthesis of nanocrystalline hydroxyapatite from CaO and $CaHPO_4$. *Biomaterials*, *22*(20), 2705–2712.

Yu, J., Wang, Y., Dai, Z., Yang, F., Fallahpour, A., & Nasiri-Tabrizi, B. (2021). Structural features modeling of substituted hydroxyapatite nanopowders as bone fillers via machine learning. *Ceramics International,* 47(7), 9034–9047.

Yue, G., Lin, H., & Li, J. (2016). Tilapia is the Fish for Next-Generation Aquaculture Tilapia. *International Journal of Marine Science and Ocean Technology(IJMO), 3(1)*(March), 11–13. https://doi.org/10.19070/2577-4395-160003

Yunoki, S., Ikoma, T., Tsuchiya, A., Monkawa, A., Ohta, K., Sotome, S., Shinomiya, K., & Tanaka, J. (2006). Fabrication and Mechanical and Tissue Ingrowth Properties of Unidirectionally Porous Hydroxyapatite / Collagen Composite. 166–173.

Zainol, I., Alwi, N. M., Abidin, M. Z., Haniza, H. M. Z., Ahmad, M. S., & Ramli, A. (2012). Physicochemical properties of hydroxyapatite extracted from fish scales. *Advanced Materials Research*, *545*, 235–239.

Zainol, I., Adenan, N. H., Rahim, N. A., & Aiza Jaafar, C. N. (2019). Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment. *Materials Today: Proceedings,* 16, 1942–1948.

Zadpoor, A. A. (2014). Relationship between in vitro apatite-forming ability measured using simulated body fluid and in vivo bioactivity of biomaterials. *Materials Science and Engineering C, 35*(1), 134–143.

Zhang, G., Chen, J., Yang, S., Yu, Q., Wang, Z., & Zhang, Q. (2011). Preparation of amino-acid-regulated hydroxyapatite particles by hydrothermal method. *Materials Letters*, *65*(3), 572–574.

LIST OF PUBLICATION

1. Dermawan, S. K., Mohd Ismail, Z. M., Jaffri, M. Z., and Abdullah, H. Z. (2022). Extraction and Characterization of Natural Hydroxyapatite from Black Tilapia Fish Bone for Biomedical Applications. *Key Engineering Materials.* 908, 159-164

2. Dermawan, S. K., Mohd Ismail, Z. M., Jaffri, M. Z., and Abdullah, H. Z. (2022). Effect of the Calcination Temperature on the Properties of Hydroxyapatite from Black Tilapia Fish Bone. *Journal of Physics: Conference Series.* 2169, 012034.

3. Dermawan, S. K., Mohd Ismail, Z. M., Jaffri, M. Z., Mohd Pu'ad, N. A. S., and Abdullah, H. Z. (Submitted). Halal Hydroxyapatite (HAp) Derived from Black Tilapia Fish Bone via Calcination Method for Biomedical Applications. *The 3rd International Conference on Biosciences and Medical Engineering (iCBME2021).*

4. Dermawan, S. K., Mohd Ismail, Z. M., Jaffri, M. Z., Mohd Pu'ad, N. A. S., Idris, M. I., Lee, T. C., and Abdullah, H. Z. (Submitted). Characterization of Biological Hydroxyapatite as a Biomaterial Extracted from Waste Fish Bones. *International Conference on Mechanical and Manufacturing Engineering (ICME 2021).*

LIST OF CONFERENCES

1. Dermawan, S. K., Ismail, Z. M. M, Jaffri, M. Z., and Abdullah, H. Z. Extraction and Characterization of Natural Hydroxyapatite from Black Tilapia Fish Bone for Biomedical Applications. *The 10^{th} International Conference on X-Ray and Related Techniques in Research and Industry (ICXRI) 2021*. Virtual Conference: Universiti Pertahanan Nasional Malaysia. 2021.
2. Dermawan, S. K., Ismail, Z. M. M, Jaffri, M. Z., Pu'ad, N. A. S. M., and Abdullah, H. Z. Halal Hydroxyapatite (HAp) Derived from Black Tilapia Fish Bone via Calcination Method for Biomedical Applications. *The 3^{rd} International Conference on Biosciences and Medical Engineering (ICBME) 2021*. Virtual Conference: Universiti Teknologi Malaysia. 2021.
3. Dermawan, S. K., Ismail, Z. M. M, Jaffri, M. Z., Pu'ad, N. A. S. M., Idris, M. I., Lee, T. C., and Abdullah, H. Z. Characterization of Biological Hydroxyapatite as a Biomaterial Extracted from Waste Fish Bones. *The 11^{th} International Conference in Mechanical and Manufacturing Engineering (ICME) 2021*. Virtual Conference: Universiti Tun Hussein Onn Malaysia. 2021.
4. Dermawan, S. K., Ismail, Z. M. M, Jaffri, M. Z., and Abdullah, H. Z. Influence of Calcination Temperature on the Properties of Hydroxyapatite from Black Tilapia Fish Bone. *The 1^{st} International Conference on Green Materials, Processing and Manufacturing (ICoGMPaC 2021)*. Virtual Conference: Universiti Malaysia Perlis. 2021.

LIST OF COMPETITIONS PARTICIPATED AND AWARDS

1. 31st International Invention, Innovation & Technology Exhibition 2020 (ITEX 2020), International Level, 20th -21st November 2020, Kuala Lumpur, Malaysia.
Gold Medal

2. International Research and Innovation Symposium and Exposition 2020 (RISE) 2020, International Level, 2020, Virtual Platform: Universiti Tun Hussein Onn Malaysia (UTHM).
Silver Medal

3. International Conference on X-Ray and Related Techniques in Research and Industry (ICXRI) 2021, International Level, 18th – 19th August 2021, Virtual Platform: Universiti Pertahanan Nasional Malaysia (UPNM).
Best Poster Award

4. The 11th International Conference in Mechanical and Manufacturing Engineering (ICME) 2021, International Level, 25th -26th August 2021, Virtual Platform: Universiti Tun Hussein Onn Malaysia (UTHM).
Best Presenter Award

VITA

The author was born in Batu Pahat, Johor, on January 22, 1996. She finished her secondary school at Maktab Rendah Sains MARA (MRSM) Batu Pahat. She then resumed her tertiary education in Engineering Foundation at Universiti Teknologi MARA (UiTM) Puncak Alam and completed in 2015. She then continued her studies at Universiti Tun Hussein Onn Malaysia (UTHM), where she received her bachelor's degree in Mechanical Engineering with Second Class Upper with Honors in October 2019. She decided to continue her studies at UTHM in June 2019, with the aim of accomplishing her Master of Mechanical Engineering in the Materials Engineering and Design Department. Her area of study is biomaterials, and she focused on extracting and analysing halal natural hydroxyapatite (HAp) from black tilapia fish bone processing wastes. During her master's programme, she was also a researcher who published four technical publications. The Malaysia Engineering Council (BEM), the Institute of Engineers Malaysia (IEM), and the Institute of Materials Malaysia (IMM) are all professional organisations that the author belongs to (IMM).