SYNTHESIS OF HYDROXYAPATITE FROM CHICKEN BONE WASTE USING THERMAL DECOMPOSITION METHOD

This report is submitted in accordance with requirement of the University Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Hons.)

by

AHMAD AMIRUL AIZAD BIN SAMSURI

FACULTY OF INDUSTRIAL AND MANUFACTURING
TECHNOLOGY AND ENGINEERING

2024

DECLARATION

I hereby, declared this report entitled “SYNTHESIS OF HYDROXYAPATITE FROM CHICKEN BONE WASTE USING THERMAL DECOMPOSITION METHOD” is the result of my own research except as cited in references.

Signature : ……

Author’s Name : AHMAD AMIRUL AIZAD BIN SAMSURI

Date : 19 JANUARY 2024

APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for Degree of Manufacturing Engineering (Engineering Materials) (Hons). The member of the supervisory committee are as follow:

.....

(Dr. Adiban Haneem Binti Mohamad Dom)

ABSTRAK

Sisa makanan global, yang terdiri daripada 76% bahan yang tidak boleh dimakan seperti tulang haiwan, menghadirkan cabaran yang besar. Memanfaatkan bahan buangan seperti tulang haiwan untuk pengekstrakan sebatian berharga adalah penting. Kajian ini mencadangkan kaedah lestari untuk mensintesis hidroksiapatit (HA), sebuah biomaterial yang serupa dengan mineral tulang. HA telah lama digunakan sebagai biomaterial dalam aplikasi ortopedik dan pergigian untuk membaiki atau menggantikan tisu keras, serta sistem penghantaran ubat. HA diperoleh daripada sisa tulang ayam melalui proses penguraian haba, yang melibatkan pembersihan, pengeringan, pengisaran, dan pemanasan pada suhu tinggi. Prekursor kaya kalsium fosfat yang terhasil berubah menjadi HA melalui proses kalsinasi yang dijalankan pada beberapa suhu antara 700 hingga 1100 °C selama dua jam. Selepas itu, pencirian akan dilakukan melalui SEM-EDX, PSA dan XRD untuk mengesahkan bahan yang disintesis iaitu HA. Serbuk HA dengan ketulenan tinggi berjaya disintesis daripada sisa tulang ayam melalui kaedah penguraian haba ini. Melalui eksperimen ini, didapati bahawa suhu kalsinasi yang lebih tinggi membawa kepada peningkatan keterkristalan hidroksiapatit (HA) yang diperoleh daripada serbuk tulang drumstick dan femur menggunakan analisis XRD. Dari analisis SEM dan PSA, saiz kristalit dan saiz zarah untuk kedua-dua jenis tulang dengan purata 20.157µm hingga 21.449µm untuk tulang drumstick dan 52.481µm hingga 363.078µm untuk tulang femur telah dikaji dan ketulenan untuk kedua-dua sampel tulang telah disiasat menggunakan analisis EDX. Kaedah mesra alam ini bukan sahaja menangani sisa tulang ayam tetapi juga menawarkan kaedah pengeluaran HA yang kos efektif, menunjukkan potensinya dalam aplikasi bioperubatan.

ABSTRACT

Global food waste, comprising 76% inedible materials like animal bones, presents a significant challenge. Utilizing waste materials, such animal bones, for valuable compound extraction is vital. This study proposes a sustainable method for synthesizing hydroxyapatite (HA), a biomaterial akin to bone minerals. HA has long been employed as a biomaterial in orthopaedic and dental applications to repair or replace hard tissues, as well as drug delivery systems. HA is obtained from chicken bone waste through a thermal decomposition process, which involves cleaning, drying, milling, and heating at elevated temperatures. The resulting calcium phosphate-rich precursor transforms into HA through calcination process conducted at several temperature ranging from 700 to 1100 ℃ for two hours. After that, characterization will be done through SEM-EDX, PSA and XRD to confirm the synthesized material which is HA. High purity HA powder was succesfully synthesised from chicken bones waste through this thermal decomposition method. By the experiment , we found that higher calcination temperatures led to increased crystallinity of hydroxyapatite (HA) derived from both drumstick and femur bone powders by using XRD analysis . From SEM and PSA analysis , the crystallite size and particle size for both type of bones with the average of 20.157µm to 21.449µm fo drumstick bones and 52.481µm to 363.078µm for femur bones has been studied and the purity for both type of bone samples has been investigated by using EDX analysis. This eco-friendly method not only addresses chicken bones waste but also offers a cost-effective HA production method, showcasing its potential in biomedical applications.

DEDICATION

Only

my beloved father, Samsuri Talip

my beloved mother, Kamariah Singah

my adored sister and brothers, Daus, Naim and Lily

for giving me moral support, money, cooperation, encouragement and also understandings

Thank You So Much & Love You All Forever

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious, the Most Merciful, I extend my deepest gratitude and praise to Allah for guiding me to successfully complete this final year project without difficulty.

I am immensely thankful to my respected supervisor, Dr. Adibah Haneem Binti Mohamad Dom, for her kindness, unwavering patience, and invaluable mentorship that guided me throughout the entire process. Her easily understood explanations and open-minded approach allowed me to grow and learn, shaping me into a better researcher.

Special thanks are due to my best friends, who provided immense motivation and mental support in completing this report. Their critical suggestions and comments significantly contributed to my research, and I am grateful for their enduring friendship.

Finally, I extend my thanks to everyone who played a crucial role in this FYP report. I apologize for not being able to personally mention each one of you due to the constraints of space on this single page. Your support and contributions are truly appreciated.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

XRD	-	X-ray diffraction
SEM	-	Scanning electron microscope
PSA	-	Particle Size Analyzer
EDX	-	Energy-dispersive X-ray spectroscopy
HA	-	Hydroxyapatite

LIST OF SYMBOLS

cm	-	Centimetre
m	-	Metre
%	-	Percent
g/cm^3	-	Grams per centimetre cube
Ca/P	-	Calcium to phosphorous ratio
HA/TCP	-	Tricalcium phosphate/hydroxyapatite
T	-	Temperature
pH	-	Potential of hydrogen
Kg	-	Kilogram
µm	-	Micrometer
kV	-	Kilo Volt
mm/s	-	Millimeters per second
cm^{-1}	-	Energy unit equal to the energy of a photon

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Even as time passes and the field of sciences gets wilder and more varied each day, the repair of bone defects caused by diseases or traumas remains a tough challenge for clinicians. The restoration of bone defects usually needs the use of synthetic biomaterial. As stated by A. Esmaeilkhanian et al , (2019) that nowadays, the limited availability of autologous bones has led to a widespread usage of synthetic biomaterials or xenografts, which are created from animal bone segments. This is because the chemical and crystallographic structures of human bone tissues and HA are quite comparable. As the autologous bone define that it has similar properties to human bones, this what makes it a popular choice to use .

Even though it can increase osteoconductivity to encourage bone formation and build a physiochemical link with the bone, it is also readily available in large quantities and requires less processing money than alternative methods including sol-gel, hydrothermal, and mechanomechanical method . S.-L. Bee et al (2019) stated that the animal bone first undergoes a deproteination process before it undergoes calcination at elevated temperatures in order to fully remove the organic components and destroy pathogens and the ash left only contains the mineral component of the bones . Therefore, a complete conversion of bovine ash to HA [Ca10 (PO4)6 (OH)2] can be obtained . A. Esmaeilkhanian et al (2019) also stated that because of its highly promising qualities, HA

has been used in dental implants and orthopedic procedures because of its excellent biocompatibility, osteoconductivity, bioactivity, and non-toxic, non-inflammatory, and non-immunogenic nature.Therefore, when compared to stoichiometric HA, it is known that the inclusion of these naturally occurring elements in the crystal structure imposes defects in the apatite lattice, reducing its crystallinity and thus boosting its solubility, bioactivity, and resorbability as mentioned by S.-L. Bee et al (2019) .

D. Gomes et al (2019) also described that nowadays, there are several ways HA can be produced such as chemical precursors, mainly calcium and phosphorus, employing various methods including, dry, wet, thermal, or a mixture of these approaches . Instead of employing chemical production, HA can also be recovered from natural sources, such as animal bones and scales which are rich in HA content. As the number of populations in Malaysia is reaching 33.57 million peoples , around 17007 tonnes (as for 2021) of food waste generated daily with 76% of it were inedible like bones. Due to the significant use of chicken by the Malaysian, therefore this kind of waste commonly come from by-product from food and agriculture industries are getting higher and usually disposed away without fully utilizing it. Therefore, in this study, an attempt to synthesis natural-HA from chicken femur bone and drumstick bone waste based on a simple thermal annealation approach is demonstrated and characterize using several characterization techniques such as SEM-EDX, PSA and XRD. The use of waste chicken bone employ in this study will be beneficial to the environment asit can reduced the waste output.

1.2 PROBLEM STATEMENT

Hydroxyapatite (HA) is a ceramic material which forms the mineral phase of bone. Currently, hydroxyapatite is utilized as scaffolds, granules, and blocks for bone regeneration and repair. It can also be used as a composite material with polymers or other ceramics, or as a coating for orthopedic or dental implants. As in the 21st century, Hydroxyapatite can be chemically synthesized by numerous approaches like sol-gel, hydrothermal and sonochemical. Besides, due to the multiple cons of sol-gel like high cost of the raw materials, large volume shrinkage and cracking during drying and Organic chemistry often uses confusing terminology , the heat treatment process has to be profound to be used. However, there's plenty of existing factors that disturb the result of heat treatment process that need to be cope making the difficulties to achieve precise control performance when using heat treatment process is high.

As the number of populations in Malaysia is reaches 33.57 million people , around 17007 tonnes (as for 2021) of food waste generated daily with 76% of it were inedible like bones as stated by Mohideen Abdul Kader (2022). Due to the significant use of chicken as in the research conducted by Statista in 2022, chicken consumption in Malaysia was the highest among other type of meat , therefore this kind of waste commonly come from by-product from food and agriculture industries are getting higher and usually disposed away without fully utilizing it. Due to the high calcium content in chicken bones, which makes them a good raw material for the preparation of calcium hydroxyapatite (HA), the large amount of chicken bone waste can be use to synthesis hydroxyapatite . Therefore in this study, HA will be produced from chicken bone waste through thermal treatment method. Several characterisation techniques such as SEM, EDX,XRD and PSA analysis will be employed to analyse the physical ang chemical properties of the synthesised HA.

1.3 OBJECTIVES

These are the objectives need to be achieve throughout the research:

i. To synthesis hydroxyapatite (HA) by using Heat Treatment Process from chicken waste.

ii. To characterize the physical and chemical properties of the hydroxyapatite (HA) synthesized by using SEM-EDX, PSA and XRD analysis.

iii. To compare the physical and chemical properties of the synthesized hydroxyapatite (HA) obtained from two different part of chicken bone waste.

1.4 SCOPES

The scopes of research are as follows:

This study is focuses on the synthesis of Hydroxyapatite (HA) from waste chicken bone . The bone parts proposed in this research are Drumstick and Femur bone. The Hydroxyapatite will only be produce by using the heat treatment process. This study will only covering on the characterizing the physical and chemical properties of the hydroxyapatite (HA) synthesized by using SEM, EDX, XRD and PSA and the comparison that will be done only to compare physical and chemical properties of the synthesized hydroxyapatite (HA) obtain from two different part of chicken bone waste.

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF HYDROXYAPATITE

Hydroxyapatite, denoted as HA (with the chemical formula Ca10(PO4)6(OH)2), maintains thermodynamic stability in its crystalline form within body fluids and shares a composition closely resembling that of bone mineral. As stated by Mohd Pu'ad et al (2019) , HA demonstrates the ability to mix in harmony with bone tissue without causing toxicity, inflammation, or reactions to foreign bodies on a local or systemic level. Consequently, HAp has found extensive use in various biomedical applications, notably in orthopedics, dentistry, and as a coating material for metallic implants. According to Ratner (2004), The use of hydroxyapatite (HA) in the field of regenerative science has its origins in the 1950s, during the era when bioceramics served as inert scaffolds for filling bone defects. The history of calcium orthophosphates, dating back to 1770, covers the period until 1950. As stated by G. Ma (2019) in the 1970s, following the successful production of hydroxyapatite (HAP) , there was an initiation of research and application of apatite as an artificially synthesized material. The focus is on landmark research in clinical sciences, reflecting changing perspectives on material interaction with living tissues.

Initially employed for grafting without eliciting reactions from adjacent tissues, HA's role evolved over time. It transitioned to a reactive nature,designating it as second generation, in which the substance serves as a scaffold that conducts bone formation.Consequently, the field of bioceramics has changed as a result of recent developments in production technology, which are fueled by nanotechnology and a deeper

comprehension of regenerative science.As added by G.Ma (2019) , Throughout the years, scientists have consistently modified the associated preparation techniques and procedures for producing nanometer-scale hydroxyapatite and its composite materials featuring diverse characteristics.

HA, among various bioceramics, stands out as a plentiful regenerative graft material in the market. With a close association with the bony apatite structure and being an inorganic component of bones, HA is embedded in the organic matrix alongside other mineral trace elements. In the context of bone-related diseases and ablative surgeries involving bone resections or partial removal, reconstruction becomes essential. As stated by N. Agbeboh et al (2020) the predominant component of the human bone structure is hydroxyapatite, which forms tooth enamel and accumulates in small quantities in certain regions of the brain.Because of this, HA is becoming a more important replacement material in regenerative science, especially when used in conjunction with autografts.This paper thoroughly examines the role of HA in regenerative science from its historical beginnings.

2.1.1 STRUCTURE OF HYDROXYAPATITE

Hydroxyapatite, also known as hydroxylapatite (HA), is chemically represented as Ca5(PO4)3(OH), commonly denoted by N. M. Pu'ad et al (2020) as Ca10(PO4)6(OH)2 instead of Ca5(PO4)3(OH), as it incorporates two units within a single crystal. As the hydroxyl end member of the complex apatite group, hydroxyapatite has replaceable OH- ions that can be changed to fluorapatite or chlorapatite by substituting carbonate, chloride, or fluoride. As stated by N.I. Agbeboh et al (2020) , HA serves as a substitute for bone due to its chemical resemblance to natural bone . N.I. Agbeboh et al (2020) also stated that ,Bone primarily constituting A non-stoichiometric type of hydroxyapatite makes up around 70% of the weight and more than half of the volume of human bone, along with an organic matrix (22 weight percent) and water (9 weight percent). Bone, a significant calcified tissue in animals, is an intricately structured ceramic–organic bionanocomposite. The typical formula for HA is Ca10(OH)2(PO4)6, which is a near match to an inorganic

component of the bone matrix. This striking resemblance has prompted a great deal of study on HA as a potential bone replacement.

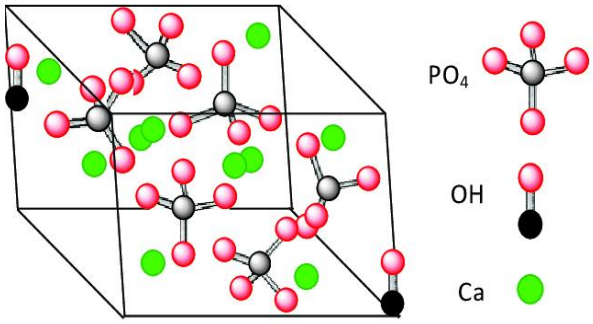


Figure 2.1: The structure of hydroxyapatite crystals (Sawittree Rujitanapanich et al, 2014)

HA is among the most stable and less-soluble calcium phosphate bioceramics, boasting a Ca/P ratio of 1.67 . Pure HA powder appears white, while naturally occurring HA can exhibit brown, yellow, or green hues, akin to discolorations seen in dental fluorosis. In biological systems, HA serves as the principal inorganic constituent in normal calcifications like bone, teeth, fish enameloid, and certain shell species, as well as in pathological calcifications such as dental and urinary calculus and stones . As mention by N.I. Agbeboh et al (2020), As research progresses to address these concerns, a range of materials is being created for diverse applications, with a significant portion belonging to the category of calcium phosphate (CaP).

2.1.2 PROPERTIES OF HYDROXYAPATITE

The mechanical properties of HA are influenced by factors such as porosity, density, sinterability, crystal size, and phase composition. The bending, compressive, and tensile strength of HA ceramics fall within the ranges of 38–250, 120–150, and 38–300 MPa, respectively as stated by Abere Dv et al (2019). Young's modulus depends on residual porosity and impurities and ranges from 35 to 120 GPa as stated by Y. Swapna et al (2022). L. Vaiani et al (2023) also stated that for dense HA ceramics, Weibull's modulus, a measure of brittleness, ranges from 5 to 18. Y. Swapna et al (2022) also mentioned that the Vicker's hardness of dense HA ceramics ranges from 3–7 GPa. Crucially, microstructure and sintering ability are directly related to the mechanical properties of HA bioceramics;

densely sintered bodies with finer grains are more robust and resilient than porous ones with bigger grains.

HA favorable biological characteristics, such as biocompatibility, bioaffinity, bioactivity, osteoconduction, osteointegration, and, in some cases, osteoinduction, HA bioceramics are widely used as artificial bone substitutes. The only ions in HA are calcium and phosphate, and it has no known harmful local or systemic toxicity. Through an apatite layer low in calcium and carbonated at the interface between the implant and the bone, it promotes the direct binding of freshly produced bone when implanted. The surface of HA promotes the activities of osteoblastic cells and acts as a vehicle for the delivery of cytokines, which concentrates bone morphogenetic proteins in vivo.

The interaction of apatite with biological tissues is crucial for regeneration, and evolving concepts in mineralization and tissue interaction are influenced by changes in production technology and material properties. HA's ability to bond directly with bone, its osteoinductivity, and biodegradation characteristics are highlighted. Bioactive materials like HA, with a Ca/P ratio of 1.0–1.7, are considered non-toxic and mimic fracture healing. The mechanism of bone induction by synthetic materials involves factors such as microporosity, surface area, geometry, and topography. HA's biodegradation is initiated by changes in biofluids, and the dissolution process depends on various factors, including surface area, fluid convection, acidity, and temperature. Bioresorption is mediated by osteoclast cells, and its kinetics vary based on the Ha/TCP ratio. Bioactive materials form chemical bonds, with roughness and porosity being critical factors for bonding. These insights contribute to the ongoing advancements in the bioactive or bioresorbable production of HA through nanotechnology.

2.1.3 APPLICATION OF HYDROXYAPATITE

Since its introduction in the mid-1980s, Hydroxyapatite (HA) has been extensively studied for its clinical applicability in addressing various bone defects. Its great short-term success rate and excellent initial osseointegration have been noted by numerous investigations. Implants coated with HA have shown variable survival rates. Different

forms of HA, derived from sources such as bovine HA and synthetic HA, serve various purposes, with varying success rates reported. As stated by K. Zhang et al (2019) , in the recent studies A composite scaffold containing hydroxyapatite (HA) and loaded with zoledronic acid is being suggested for the repair of bone defects induced by tumors.As stated by D.-E. Radulescu et al (2023) ,Synthetic HA, known for its biocompatibility, bioactivity, and osteoconductivity,finds extensive use as a scaffold in tissue engineering, covering for metallic implants, drug delivery carrier, and substitute for bone. Various types of HA, including porous and solid blocks, granules, paste, cement, and coatings, are exploited for biomedical purposes. Despite its advantageous properties, a notable drawback of HA is its low strength, limiting its use for high load-bearing implants.The main building block of bones and teeth, nanocrystalline HA, is used in grafting procedures for sinus augmentation, ridge reconstructions, and bone defect repair.

2.2 SYNTHESIS TECHNIQUES FOR HYDROXYAPATAITE POWDER

Various techniques can be employed for the synthesis of synthetic hydroxyapatite (HA). As mentioned by Javadinejad et al. the methods can be broadly categorized as dry, wet, and high-temperature approaches. Mohd Pu'ad et al. (2020) found that each of these approaches produces distinct sizes, shapes, and various crystalline phases of calcium phosphate, in addition to pure crystalline HA. Consequently, the properties of HA, such as bioactivity, mechanical strength, and biological attributes, are significantly influenced. These inherent characteristics play a crucial role in determining the biomedical applications of HA. Hence, there is a keen interest in developing synthesis methods that offer control over the morphology, crystallinity, size, and chemical composition of synthetic HA.

2.2.1 DRY METHOD

Solid-state and mechanochemical procedures are the two different ways that hydroxyapatite (HA) is synthesized using the dry method. The precursor chemicals

(phosphate and calcium), which are already dry, are combined in this dry technique to create HA. They are therefore ideal for the large-scale manufacture of powders because to this feature.

2.2.1.1 SOLID-STATE METHOD

The solid-state reaction involves the decomposition of mixed solid reactants through heating to generate new solids and gases. Javadinejad et al. (2021) stated that the commonly used solid-state method for producing HA involves mechanical alloying followed by calcination at elevated temperatures. In this method, chemical precursors containing calcium and phosphate are milled and calcined to produce hydroxyapatite (HA).Solid diffusion of ions from calcium and phosphate precursors is the mechanism, and the reaction is triggered by a high-temperature process. It was verified by X-ray diffraction (XRD) that the solid-state technique produces several calcium phosphate phases, including calcium-deficient HA and monetite. To enhance kinetics performance, the mechanochemical technique is suggested as an alternative due to its ability to improve ion diffusion during the reaction. The utilization of the Solid-State Method for hydroxyapatite (HA) synthesis offers several advantages, as highlighted by Dorozhkin et al (2019). This method is characterized by straightforward procedures and scalability. Additionally, as emphasized by Jarmila et al, 2019, it boasts high purity, minimizing solvent residue and impurities that are prevalent in solution-based techniques, ultimately yielding a purer HA product. Despite these merits, the Solid-State Method has its drawbacks, notably a high energy demand. The requisite high temperatures for calcination, typically ranging from 1000-1300°C, result in substantial energy consumption, rendering it less energy-efficient compared to certain solution-based methods (Jarmila et al., 2019). Furthermore, the Solid-State Method exhibits slower reaction kinetics in comparison to solution methods, where reactions occur in the liquid phase. Solid-state reactions are often impeded by diffusion limitations, leading to prolonged processing times (Dorozhkin, et al, 2019), thus diminishing its suitability for certain applications.

2.2.1.2 MECHANOCHEMICAL METHOD

N. Amirthalingam et al (2019) stated the pressure-induced disturbance of surface-bonded species is used in mechanochemical powder production, a solid-state technique, to enhance the thermodynamic and kinetic interactions between solid materials. Mechanochemical techniques involve compression, shear, or friction, often utilizing ball-milling or planetary mills at specific speeds.During mechanochemical reactions, high impact compression and elevated local temperatures support chemical transformations and improve diffusion processes. To solve problems in managing air and moisture-sensitive compounds, mechanochemical procedures are often conducted in sealed vessels made of materials like stainless steel, agate, or zirconia. M. V. Khvostov et al (2021) found that in contrast to techniques involving the synthesis of hydroxyapatite (HA) in a liquid environment, the solid-state mechanochemical approach eliminates the need to regulate the medium's acidity, filter, or anneal the produced product. Furthermore, during the mechanochemical synthesis, side products do not need to be removed. The inserted ion concentrations stay constant as a result, negating the need for elemental analysis.The application of the Mechanochemical Method in the synthesis of hydroxyapatite (HA) presents numerous advantages, as emphasized by Kumar et al. (2019). This technique is environmentally friendly, often eliminating the necessity for harmful solvents and high temperatures, thereby reducing its environmental impact. Additionally, Dhivya et al. (2019) noted that this process can be easily scaled up for industrial production compared to certain wet chemical methods. However, it does come with drawbacks, such as a contamination risk, as highlighted by Dhivya et al. (2019). The use of grinding tools and containers may introduce impurities into the final product, necessitating careful selection and cleaning processes. Consequently, it is not highly recommended for the synthesis of HA.

2.2.2 WET METHOD

The wet synthesis method for hydroxyapatite (HA) involves utilizing an aqueous solution in the synthesis process.Hydrolysis, hydrothermal procedures, and chemical precipitation are common wet methods for extracting haemophilus aureus. One benefit of

using wet procedures is that the powder's morphology and mean size can be controlled. Notwithstanding these advantages, wet techniques have several disadvantages as well, such as the possibility that HA will show low crystallinity as a result of the low processing temperatures.

2.2.2.1 CHEMICAL PRECIPITATION METHOD

The chemical precipitation method is a widely employed technique for synthesizing hydroxyapatite (HA) due to its cost-effectiveness and high yield. M. Sirait et al (2020) stated that In this approach, the synthesis of hydroxyapatite required only around two minimal precursor materials and the process involves mixing calcium and phosphate-containing reagents, adjusting the pH to an alkaline level, and stirring the solution for aging.The precipitates that are left behind are dried, cleaned, and filtered before being ground into a powder. Numerous investigations have looked into how to modify the properties of the synthesized HA by adjusting variables including reaction temperature, acid addition rate, and heat-treatment temperature. The method has been successful in producing spherical and rod-like HA morphologies with varying crystallinity. The chemical precipitation method requires careful consideration of parameters like molar ratio, pH, and processing conditions to ensure optimal HA production. I.-H. Lee et al (2020) also found that the crystallinity, size, and shape of the product could be influenced by various processing parameters, especially the reaction temperature, aging time, and rates of reactant addition. As Dorozhkin et al. (2019) noted, the procedure is relatively uncomplicated and utilizes readily available and affordable precursors. This renders it a scalable and cost-efficient approach for the large-scale production of hydroxyapatite, especially when compared to alternative methods like hydrothermal synthesis. However, it lacks precise control over porosity, as indicated by Sun et al. (2019). Chemical precipitation typically yields non-porous hydroxyapatite, and since porosity is vital for bone ingrowth and vascularization in biomedical applications, additional synthesis steps or post-processing techniques may be necessary to introduce porosity. This aspect diminishes its suitability for specific applications.

2.2.2.2 HYDROTHERMAL METHOD

The hydrothermal method involves synthesizing hydroxyapatite (HA) in an aqueous environment at elevated pressure and temperature, typically within an autoclave or pressure vessel. D. F. Fitriyana et al (2019) stated that Hydrothermal techniques involve employing reagents that are typically insoluble under normal conditions but become soluble at elevated temperatures (up to 1000 °C) and high pressures (hundreds of bar). This enables the production of unique products that may not be attainable through alternative methods. The high pressure and temperature enhance reactivity, leading to chemical bond formation and nucleation. As mentioned by Fitriyana et al (2019) Hydrothermally synthesized HAp nanoparticles exhibit a relatively stoichiometric and highly crystalline nature, and their purity can be regulated by elevating the hydrothermal temperature. High crystalline materials can be produced by the combined effects of pressure and temperature without the need for extra post-treatment. Various studies have employed the hydrothermal method, manipulating parameters such as temperature and duration to control the size and morphology of the resulting HA. Modifiers like surfactants and chelating agents have been used to enhance control over particle morphology. Compared to the chemical precipitation approach, it has been demonstrated that the hydrothermal process produces HA with higher crystallinity. Additionally, it offers the advantage of synthesizing HA with controllable size and morphology by incorporating specific modifiers. As noted by Sun et al. (2019), hydrothermal synthesis generally yields highly crystalline and pure hydroxyapatite (HA) with minimal secondary phases. This favorable outcome is a result of the controlled reaction conditions maintained at elevated temperatures and pressures, promoting well-defined crystal growth. However, a drawback, highlighted by Zhou et al. (2019), is the necessity to heat and pressurize the reaction vessel during the process, resulting in relatively high energy consumption compared to certain alternative synthesis methods. This raises concerns about the sustainability of production. Consequently, it is not strongly recommended for the synthesis of HA.

2.2.2.3 HYDROLYSIS METHOD

Hydrolysis, a wet method less commonly used for synthesizing hydroxyapatite (HA),entails ionizing water, which leads to the diffusion of hydroxide and hydrogen ions. Despite the ability to produce high-purity HA, hydrolysis methods generally require extended processing hours. As indicated by Kumar et al. (2019), hydrolysis is known for its high purity, often yielding high-purity hydroxyapatite (HAp) as it lacks foreign ions or impurities typically introduced in alternative synthesis methods such as solid-state reactions. However, it exhibits slower reaction rates compared to methods like sol-gel or precipitation, as noted by Chen et al. (2019). This characteristic results in extended processing times, diminishing its suitability for certain applications.

2.2.3 HIGH TEMPERATURE METHOD

Hydroxyapatite (HA) can be produced through a high-temperature approach involving the decomposition of materials. This method includes combustion and pyrolysis techniques. However, these approaches are infrequently utilized in HAp synthesis due to challenges in controlling processing parameters and the tendency to generate secondary aggregates.

2.2.3.1 COMBUSTION METHOD

The combustion technique involves rapid exothermic reactions in an aqueous phase, initiated by heating followed by sudden cooling, leading to nucleation and prevention of further particle growth. Various studies utilized this method with different calcium and phosphate sources and organic fuels. H. A. Batista et al (2020) stated that by using this method to synthesis, it does involve aliquots of the combustion solution, a crucible, with varying conditions such as temperatures (500 to 800 °C), time (10 to 30 min), pH (1 to 2), and fuels (glycine and urea). The expected formation of hydroxyapatite is based on the

stoichiometry of the raw materials and the specified reaction equations. As noted by Kashif et al. (2019), in comparison to traditional approaches such as solid-state reactions, combustion presents notably accelerated synthesis times, often completed within minutes. This rapidity can be advantageous for large-scale production or applications necessitating swift processing. Additionally, according to Diban et al. (2019), the elevated temperatures (>1500°C) achieved during combustion contribute to the creation of highly pure hydroxyapatite (HA) with minimal secondary phases or impurities, a critical factor for biocompatibility and the attainment of desired mechanical properties. However, the considerable energy input required for the high temperatures in combustion renders it less energy-efficient than certain alternative methods. This may raise concerns, particularly for large-scale production or applications prioritizing environmental considerations.

2.2.3.2 PYROLYSIS METHOD

The pyrolysis technique incorporates the spraying of precursor materials into an electric furnace's hot zone, resulting in the conversion of gas into particle or liquid into particle by the aerosol decomposition process. Unlike the combustion method, pyrolysis doesn't require mixing fuel with reactants and is easily scalable for continuous production of HA particles.Precursor droplets are allowed to evaporate completely before the nucleation and development of nanoparticles in the gas phase. Overall, pyrolysis is a versatile method for synthesizing HA, offering control over particle morphology and size. According to Kumar et al. (2019), pyrolysis entails the controlled heating of easily available precursors such as eggshells, bones, or calcium carbonate. This eliminates the requirement for intricate chemical reagents or costly equipment, rendering it a feasible choice for large-scale production. Moreover, by adjusting parameters such as heating temperature, time, and atmosphere, the crystallinity, grain size, and morphology of the synthesized hydroxyapatite (HA) can be manipulated, enabling the customization of material properties for specific applications (Geng et al., 2019). However, as highlighted by Geng et al. (2019), in comparison to alternative synthesis methods, HA derived from pyrolysis may contain impurities like calcium oxide, magnesium oxide, and residual carbonates. These impurities have the potential to impact the biocompatibility and

mechanical properties of the material, making this process less suitable for certain applications.

2.3 EXTRACTION OF HYDROXYAPATITE FROM NATURAL RESOURCES

Synthetic biomaterials are often required for restoration, especially due to limited autologous bone supply and the risk of infection from allograft use. As stated by N. M. Pu'ad et al (2019) Hydroxyapatite (HA) can be produced through chemical synthesis or obtained from natural sources. .Xenografts, derived from different animal species like bovine, sheep, pig, or fish bones, offer a viable alternative. N. M. Pu'ad et al (2019) also stated that Among mammalian origins, the retrieval of HA from bovine bone has been commonly documented in literature. The cortical section of the femoral bone is typically selected due to its morphological and structural resemblance to human bone contain trace beneficial ions, and are cost-effective. The process involves deproteination and calcination to remove organic components and pathogens, yielding bone ash, which can be fully converted to hydroxyapatite (HA). This HA contains valuable trace ions that aid bone regeneration. While ion-substituted HA is more expensive, it mimics mammalian hard tissue. Research explores various biological resources, such as eggshells, seashells, animal bones, and plants, to synthesize HA with properties closely resembling those of natural bone.

2.3.1 FOOD WASTE IN MALAYSIA

Food waste is a significant challenge in the global food system, encompassing all edible materials intended for human consumption but left uneaten. This waste occurs throughout the food supply chain, including farms, processing plants, and kitchens. Roughly 1.3 billion tonnes of food are produced each year for human consumption, or one-third of it is lost or squandered. Approximately 33 percent of food in Southeast Asia is

wasted; a typical Malaysian household throws out 0.5 to 0.8 kg of uneaten food every day. As stated by D. Zainal et al (2019) ,While Malaysia maintains an acceptable level of food security, the prevalent issue of food wastage habits among its citizens has gained significance.Out of the 15,000 tonnes of food that are wasted in Malaysia, over 3,000 tonnes are edible and shouldn't be thrown away. This amount typically increases by 15% to 20% over the holidays.. This rate of reusing and recycling food waste in Malaysia is relatively low at 5%, contrasting with higher rates for paper (60%) and plastic (15%). 76% of the food were inedible like bones. Owing to Malaysians' extensive usage of chicken, this type of waste mostly originates from agricultural and food industry byproducts, which are increasing and typically thrown away before being used to their full potential.

As the issue is expected to escalate with economic development, population growth, and urbanization. Globally, food wastage adversely impacts food availability, contributing to starvation and malnutrition affecting 868 million people. Beyond economic and environmental concerns, food waste is recognized as an ethical problem, prompting increased attention and efforts at international, regional, and national levels to reduce it.

In this study, an attempt to synthesis natural-HA from chicken femur bone and drumstick bone waste based on a simple thermal annealation approach is demonstrated in this literature where the amount of HA produced will be recorded and the comparison of the HA produced by the differ parts of bone which are femur bone and the drumstick bone will be made and may reducing the amount of food waste by fully utilizing it.

2.3.2 PREVIOUS STUDIES ON SYNTHESIS OF HYDROXYAPATITE FROM ANIMAL BONES

As the time moves forward and the fields of science has been widely discovered, there has been a lot of studies on the Synthesis of Hydroxyapatite From Animal Bones. As example, the Synthesis of Hydroxyapatite From Cow Bones.

The bovine, a commonly consumed animal by humans, offers a notable source of bone waste with potential applications. As stated by F. Afriani et al (2020) Bovine bones comprise approximately 58.3% calcium phosphate, making them suitable for hydroxyapatite synthesis, given that hydroxyapatite primarily consists of calcium and phosphate compounds. Figure 2.2 displays the surface morphology of hydroxyapatite derived from cow bones under scanning electron microscopy and comparative analysis of x-ray diffraction patterns for hydroxyapatite synthesized from various animal bones, including cow bones, goat bones, and fish bones (Osphronemus goramy and Euthynnus affinis).

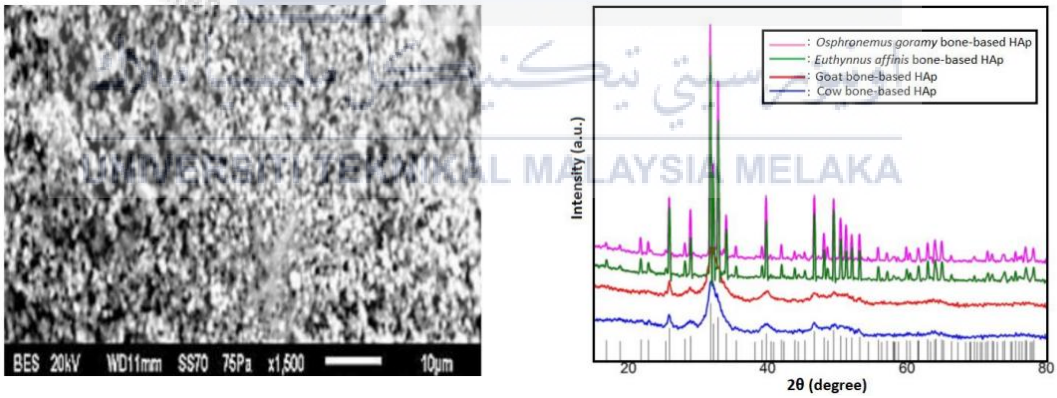


Figure 2.2: Surface morphology of hydroxyapatite derived from cow bones under scanning electron microscopy and comparative analysis of x-ray diffraction patterns for hydroxyapatite synthesized from various animal bones, including cow bones, goat bones, and fish bones (Afriani et al, 2020).

F. Afriani et al (2020) also stated that Various techniques, such as wet methods, dry methods, and precipitation methods, can be used to produce hydroxyapatite from cow bones.

Besides, there are also Studies on the Synthesis of Hydroxyapatite from Chicken Bones have also been conducted. As a byproduct of the food and agriculture industries, chicken bone waste is usually thrown away before it is completely used. In contrast to the well-researched biogenic bones—like fish or cow bones—there is little data regarding the process by which chicken bone debris is transformed into hydroxyapatite (HA). This study describes an attempt to use a simple thermal annealing technique to create natural-HA from chicken femur bone waste. It demonstrates the examination of changes in crystallinity, morphology, and composition of the synthesized HA as influenced by various calcination temperatures.

In this study, chicken drumstick bone waste was gathered from Malaysia.S.-L. Bee et al (2019) stated that the bones underwent a thorough cleaning process to remove skin, meat, and adhered proteins. Subsequently, trabecular bone and bone marrow were extracted to obtain only cortical bone. After de-greasing and drying, the bones were ground into powder using a porcelain mortar and pestle. The pre-treated bone powder was then subjected to calcination at different temperatures (500°C, 700°C, and 900°C) using an electric furnace. The resulting calcined samples, labeled as 500-HA, 700-HA, and 900-HA, were sieved for further characterization.

S.-L. Bee et al (2019) also stated that Using a Simultaneous Thermal Analyzer, the thermal dissociation of uncalcined chicken bone was investigated, and particle size changes during thermal treatment were evaluated using Particle Size Analyzer (PSA) spectroscopy. Crystallographic properties were determined by X-ray diffraction (XRD), and scanning electron microscopy (SEM) was utilized to observe morphological transformations post-calcination. X-ray fluorescence spectrometry was employed to identify the elemental composition of the bone samples. The characterization methods included heating the samples under oxidizing conditions, PSA analysis, XRD with Cu-Kα radiation, and SEM imaging after coating with a thin layer of platinum for conductivity.

2.4 MATERIAL CHARACTERISATIONS OF HYDROXYAPATITE

The material characterizations of hydroxyapatite (HA) involve various analytical techniques to assess its physical, chemical, and structural properties. The common method are by using SEM , XRD , PSA and EDX.

2.4.1 SCANNING ELECTRON MICROSCOPE (SEM)

The scanning electron microscope (SEM) stands out as a crucial tool for examining the microstructural features as stated by N. Ural (2021). Its exceptional resolution is the key factor in its effectiveness for hydroxyapatite microstructure. Consequently, SEM has played a pivotal role in the exploration of hydroxyapatite. Through SEM analysis, we can discern the bonding structure between additives and hydroxyapatite particles. F. Miculescuet al (2020) stated that Particles within all studied size ranges displayed intricate geometries and were devoid of agglomerations. An analysis of the morphology of the powders revealed the existence of particles with rounded and faceted edges, irregular, elongated forms, and rough surfaces. The particle sorting sieves' mesh sizes correspond to the maximum particle sizes. In kinds that have the smallest and largest sizes, a constant dimensional distribution is visible. On the other hand, a mix from a wider dimensional range is found for powders smaller than 40 μm.

2.4.2 X-RAY DIFFRACTION (XRD)

X-ray Diffraction (XRD) is a commonly used technique for characterizing hydroxyapatite (HA). The basis for X-ray Diffraction (XRD) is the way X-rays interact with crystalline materials. When X-rays are directed towards a crystalline substance such as hydroxyapatite, they engage with the crystal lattice, causing constructive interference. M. Rabiei et al (2020) stated that X-rays have the capability to penetrate the size of the crystal, enabling the extraction of information. As a result, the determination of size is associated

with the crystals rather than the individual particles. This interaction produces diffraction patterns that can be examined to infer details about the crystal structure.

2.4.3 ENERGY-DISPERSIVE X-RAY SPECTROSCOPY (EDS OR EDX)

Energy-Dispersive X-ray Spectroscopy (EDS or EDX) is a technique commonly employed for the elemental analysis of materials, including hydroxyapatite (HA). The foundation of EDX is the idea that X-rays released during a high-energy electron bombardment of a material can be detected. As stated by Y. F. AlFawaz et al (2020), The examination of adhesives incorporating hydroxyapatite (HA) and graphene oxide (GO) particles at a concentration of 2% (HA-GO 2%) using Energy-Dispersive X-ray Spectroscopy (EDX) revealed the presence of carbon (C) in conjunction with calcium (Ca) and phosphorus (P). Therefore, the elemental composition of the sample can be ascertained by examining the energy and intensity of the distinctive X-rays that each element emits.

2.4.4 PARTICLE SIZE ANALYZER

Particle size analysis (PSA), particularly using the laser diffraction technique, plays a vital role in characterizing hydroxyapatite (HA) for various applications. It's a key technique in particle size analysis (PSA), is essential for understanding hydroxyapatite (HA). Researchers suspend HA particles in a liquid and shine a laser beam through it. By measuring how the light scatters at different angles, the PSA reveals the size distribution of the HA particles. This information is critical because, as highlighted in studies by Goyanes et al., (2020) on HA nanoparticles, particle size significantly impacts HA properties.. For instance, smaller particles might be more biocompatible for bone regeneration. Additionally, size affects how well the particles fuse during heat treatment (sintering behavior) and their potential to deliver drugs (capacity and release rate). Therefore, PSA offers a fast and non-destructive way to analyze HA, allowing researchers and manufacturers to tailor this material for specific applications..

CHAPTER 3

METHODOLOGY

3.1 OVERVIEW

This research paper explains the synthesis of the hydroxapitate by using natural resources through thermal treatment. As for this research, the natural resources proposed are to use chicken bones based on two differ parts which are femur and drumsticks. Besides, the characterization of the physical and chemical properties will also be identified in this research. The methodology part shows and explain all of the equipment , raw materials , machines and the procedures involved in order to gain the results that we are expecting. All of the information of the procedures and equipments are all been studied through articles, journals and resources neiter from the UTeM's library or online. Information and knowledge that suitable for the study criteria and citations are included.

3.2 MATERIALS AND EQUIPMENTS

The most significant materials that needed in this research are the chicken bones. Based on the objectives stated in Chapter 1, there are two parts of chicken bones will be needed, the femur and the drumsticks bones. In addition, this research will also be needing the use of blender and ball milling in order to change the bulk size of the bones to the fine powder size. Furthermore, the usage of the furnace in eliminating unwanted substances from the bones also vitally needed. Moreover, the use of multiples machines such as x-ray

diffraction (XRD), Particle Size Analyzer (PSA), scanning electron microscope (SEM) and Energy dispersive X-Ray (EDX) will also be needed.

3.3 EXPERIMENTAL

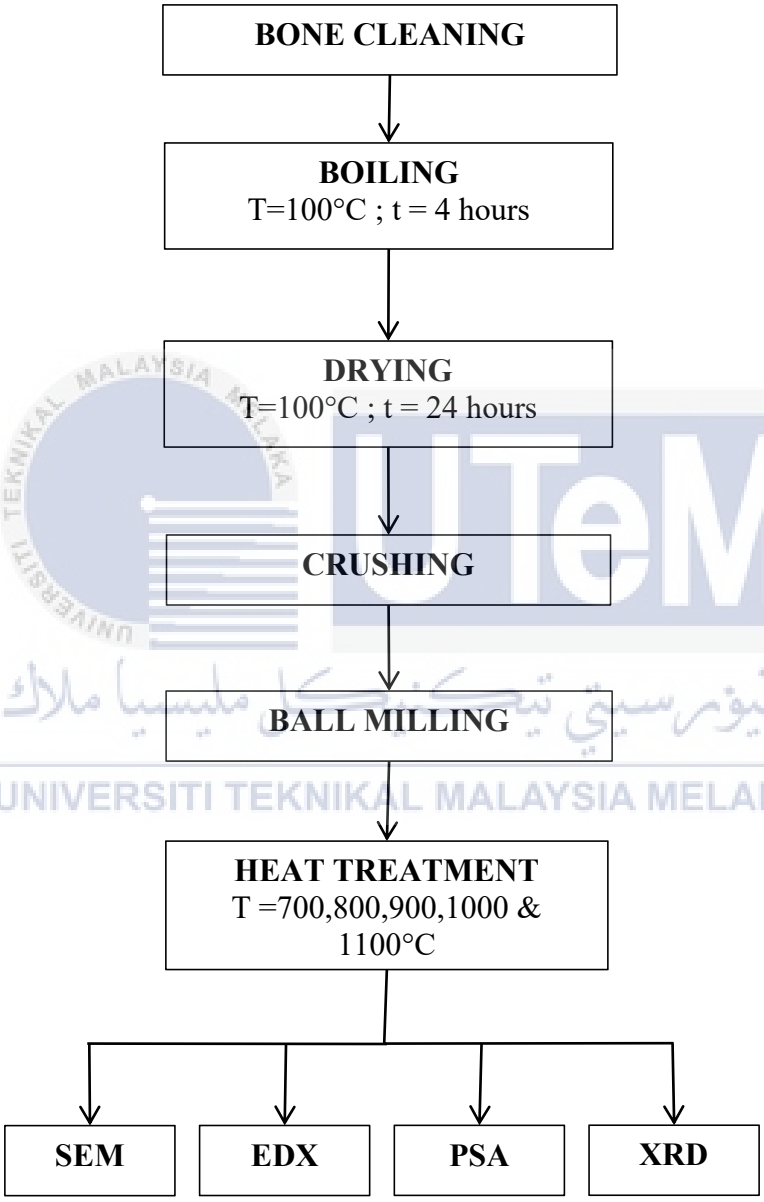


FIGURE 3.1 Process Flow Of Simple Thermal Decomposition Of Chicken Bones

Hydroxyapatite (HA), a valuable biomaterial used in bone regeneration, can be derived from chicken bone waste through a multi-step process. This approach offers a two-fold advantage which are it utilizes a sustainable source material and promotes waste reduction.

The process begins with meticulous cleaning of the chicken bones to remove any adhering meat, fat, or contaminants. This ensures a pure starting material for the subsequent steps, crucial for the quality of the final HA product.

Following cleaning, the bones undergo a four-hour boiling process. This step serves two important purposes. First, it facilitates the extraction of calcium and other essential minerals from the bone structure, providing the building blocks for HA formation. Second, boiling helps sterilize the material, minimizing the risk of bacterial contamination in the final product.

After boiling, the bones are dried at a controlled temperature of 100°C for 24 hours by using drying oven. This drying step removes any moisture content that could hinder the later stages of the process. Excess moisture can affect the efficiency of converting bone material into HA and potentially compromise the final properties of the biomaterial.

Next, the dried bones are crushed into smaller particles by using pastel and mortar. This increases the surface area of the material, allowing for better interaction with the chemicals used in subsequent steps. The crushed bone particles are then subjected to ball milling, a process that further refines the particle size and ensures a more uniform distribution. This uniformity is essential for achieving consistent properties in the final HA product.

The final stage involves heat treatment, where the milled bone powder is exposed to various temperatures ranging from 700°C to 1100°C. This crucial step induces a transformation within the bone material, converting calcium compounds into hydroxyapatite, the desired biomaterial.

3.3.1 CHARACTERISATION OF THE SYNTHESISED HA

3.3.2.1 SCANNING ELECTRON MICROSCOPE (SEM)

A scanning electron microscope (SEM) is a powerful tool used for high-resolution imaging of surfaces. When studying hydroxyapatite (HA) with SEM, researchers are often interested in observing the morphology, size, and distribution of hydroxyapatite crystals.

A scanning electron microscope (SEM) helps in Quantitative Analysis where it can be use quantitative analysis, such as particle size distribution and elemental composition. This is due to it has Software tools are available for image analysis and measurement. In addition, the SEM can be combined with energy-dispersive X-ray spectroscopy (EDS) to determine the elemental compositions. The analysis was performed using Hitachi SU5000 FESEM (Figure 3.2) with 15 kV electron acceleration voltages.

Figure 3.2 Hitachi SU5000 FESEM

3.3.2.2 ENERGY DISPERSIVE X-RAY (EDX)

Energy Dispersive X-ray Spectroscopy (EDS or EDX) is amethod for determining a sample's elemental composition by observing distinctive X-rays released when the sample is exposed to an electron beam.When examining hydroxyapatite (HA) at 10 kV to learn

more about the distribution of components in the sample, this method can be quite helpful. The two main components of hydroxyapatite, calcium and phosphorus, can be verified for existence and distribution using this method. Besides, it also can detect trace elements present in hydroxyapatite, providing additional compositional informations.

3.3.2.3 X-RAY DIFFRACTION (XRD)

X-ray diffraction (XRD) is extensively employed for assessing synthetic hydroxyapatite (HA). Hydroxyapatite, a calcium phosphate compound found in natural bone and teeth, forms the mineral basis. When produced artificially, it's crucial to examine its crystal structure, phase purity, and crystalline qualities, among other attributes, all of which can be evaluated through XRD analysis.

XRD helps in the characterization of synthetic hydroxyapatite in phase identification which XRD is used to determine the phase composition of synthetic HA. It helps in confirming whether the synthesized material matches the desired hydroxyapatite phase or if there are any impurities or additional phases present. Besides it's also be used in Crystal Structure Analysis and Crystallinity Assessment. XRD provides information about the crystal structure of synthetic hydroxyapatite. The diffraction pattern obtained from XRD helps in understanding the arrangement of atoms within the crystal lattice of HA, such as the unit cell parameters, crystal orientation, and atomic spacing. Beside It allows the evaluation of the material's crystallinity, which refers to the degree of structural order within the crystal lattice. Higher crystallinity is often desirable in materials intended for biomedical applications due to its similarity to natural bone.

The phase characterization of the produced HA pwoders was carried out essentially utilising X-ray diffraction Miniflex X-ray diffractometer (HyPix-400 MF) as figure 3.3. Fitted with an X'celerator to detect Si and using Cu K radiation (λ=1.5418A). The diffractometer was at 45 kV and 40 mA for a 2 angle range of 20°-60°, with 0.03 step size and scan rate 0.2 second exposure.

Figure 3.3 X-ray Diffractometer HyPix-400 MF

3.3.2.4 PARTICLE SIZE ANALYZER (PSA)

Particle size analysis is a crucial technique used in various industries to measure the size distribution of particles in a sample. One prominent technique employed for this purpose is Particle Size Analyzer (PSA). PSAs encompass a range of methods, but a common and versatile approach is laser diffraction..

Laser diffraction, a common PSA technique, is well-suited for analyzing HA nanoparticles. In this method, a focused laser beam illuminates a suspension of HA particles in a liquid. As the light interacts with the particles, it scatters at angles related to their size. Detectors measure the scattered light intensity at various angles. By analyzing this data, the PSA calculates the size distribution of the HA particles. This rapid, non-destructive technique allows for measuring particles across a wide range, typically from a few nanometers to several micrometers, which encompasses the relevant size range for bone regeneration applications.

Figure 3.4 PSA 1190

CHAPTER 4

RESULT AND DISCUSSION

4.1 X-RAY DIFFRACTION (XRD) ANALYSIS OF HYDROXYAPATITE SYNTHESIZED FROM CALCINED CHICKEN BONE SAMPLES.

The degree of crystallinity of HA increased significantly as the temperature increased. The structural analysis of the bone powder and samples heated at different temperatures was performed using X-ray diffraction (XRD). As shown in Figure 4.1, the XRD profiles of the extracted HA from drumstick bone calcinated at temperatures ranging from 700°C to 1100°C reveal the formation of hydroxyapatite (HA) at specific planes. These observations align with findings reported by Cote et al. (2020), who observed a similar trend of increasing crystallinity with calcination temperature for HA nanoparticles derived from bovine bone. The presence of additional peaks in the profiles also suggests the formation of tricalcium phosphate (TCP) as a decomposition product at higher temperatures.

The XRD profile of the bone powder undergoes calcination at 700°C is represented by the black line. This profile indicates poor semicrystalline nature of Ha with small peak of decomposition of Ha into tricalcium phosphate in the bone powder compared to other calcination tempature's result.

The crystalline nature becomes clear on thermal decomposition at 800°C. The XRD profile represented by the red colour line corresponds to a semicrystalline nature of HA

with shaper peaks compared to 700°C. This is due to the increasing removal of organic impurities and presence of a slight amount of the carbonate group in the bone ash.

The diffraction profile shown by the blue lines indicates the calcination at 900°C, which offer sharper in comparison with curves for 700°C and 800°C and points out better crystallinity and declining order of organic impurities as the temperature increase. Besides, the thermal decomposition peaks of HA into alpha tricalcium phosphate (α-TCP) and beta tricalcium phosphate (β-TCP) are getting harper in comparison with curves for 700°C and 800°C due to at higher temperatures, hydroxyapatite undergoes thermal decomposition, leading to the increasing formation of TCP phases which provide sufficient energy to break chemical bonds within the Ha lattice. Therefore resulting in the release of water and hydroxyl groups which indicates the decomposition of Ha.

The sharp and clear peak positions observed by the green lines which indicates the calcination at 1000°C. The diffraction profile for this temperature offer higher peaks compared to 900°C, 800°C and 700°C due to the phase purity and higher degree of crystallinity as the temperature of calcination increasing. Higher temperatures can help anneal the material, reducing defects and allowing impurities to diffuse out or become more uniformly distributed. This purification and defect reduction process enhances the material's crystallinity and is reflected in taller XRD peaks. This also goes to the decomposition peaks of HA.

The diffraction profile shown by the purple lines indicates the calcination at 1100°C, which offer sharper in comparison to all of the curves ranging from 700°C to 1000°C, which points out the highest crystallinity and declining order of organic impurities as the temperature increase. Besides, the thermal decomposition peaks of HA into alpha tricalcium phosphate (α-TCP) and beta tricalcium phosphate (β-TCP) are also the sharpest among all of the curves. Both HA formation peaks and decomposition peaks set to be the highest due to Higher temperatures promote crystal growth and stability, resulting in larger and more well-defined crystals. The TCP peaks in XRD analysis become taller as the crystals grow in size and become more ordered, leading to stronger diffraction signals. Additionally, the elimination of defects and strain within the TCP crystal lattice at higher temperatures contributes to the increased peak intensity.

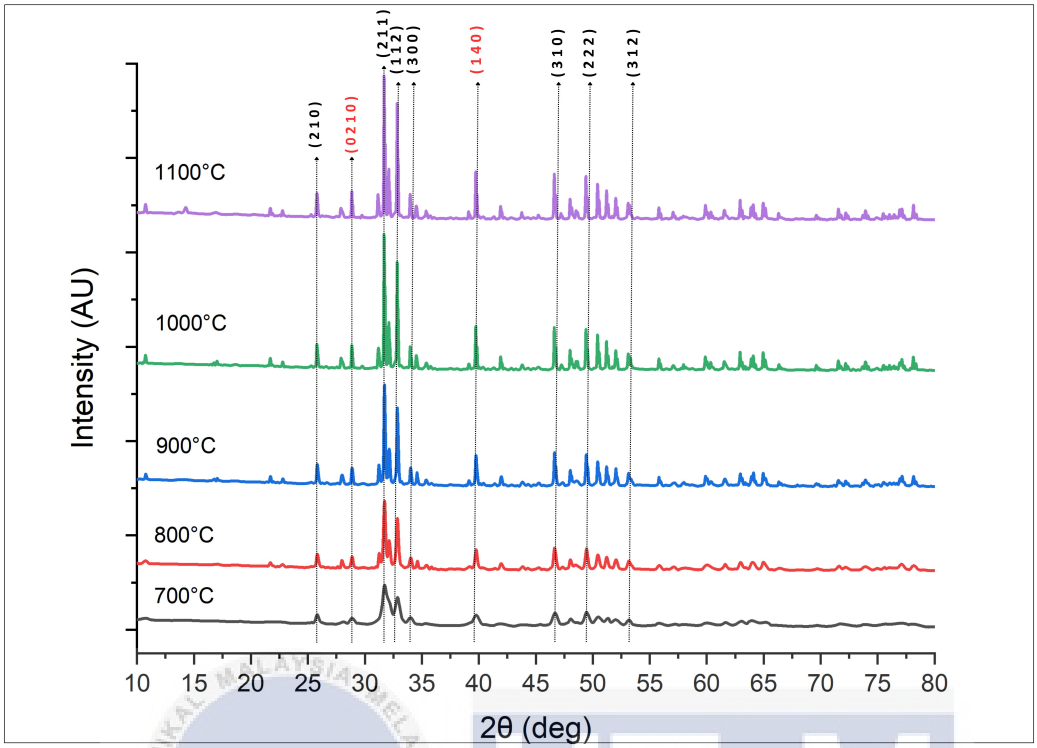


Figure 4.1: XRD patterns of drumsticks bone powder, the black lines representing the powder calcined at 700°C for 2h, the red line representing the powder calcined at 800°C for 2h, the blue line representing the powder calcined at 900°C for 2h, the green line representing the powder calcined at 1000°C for 2h and purple line representing the powder calcined at 1100°C for 2h.

For the femur bone, the XRD profiles of the extracted HA through calcination from 700°C to 1100°C are shown in Figure 4.2. The XRD profile of the bone powder calcined at 700°C, depicted by the black line, indicates a poorly semicrystalline nature of HA, with small peaks indicating the decomposition of HA into tricalcium phosphate. This aligns with observations reported by Goyanes et al. (2020) for bovine femur-derived HA nanoparticles, where lower calcination temperatures resulted in lower crystallinity. This result is less pronounced compared to higher calcination temperatures.

At 800°C, represented by the red line reveal a progressive improvement in crystallinity, evident from the sharper and more distinct peak positions as studied by Cote et al., (2020) who observed a similar increase in crystallinity with calcination temperature for HA derived from bovine bone. This improvement is due to the increased removal of organic impurities and a slight presence of carbonate groups in the bone ash.

The diffraction profile depicted by the blue lines represents the calcination at 900°C, showing sharper peaks compared to the profiles at 700°C and 800°C. This indicates

improved crystallinity and a decrease in organic impurities as the temperature rises. Additionally, the thermal decomposition peaks of HA into alpha tricalcium phosphate (α-TCP) and beta tricalcium phosphate (β-TCP) become more pronounced at 900°C, highlighting the effects of the higher temperature.

The sharper and distinct peak positions indicated by the green lines represent calcination at 1000°C. The diffraction profile at this temperature shows higher peaks compared to 900°C, 800°C, and 700°C, reflecting greater phase purity and a higher degree of crystallinity as the calcination temperature increases.

The diffraction profile shown by the purple lines represents calcination at 1100°C, which offers sharper peaks compared to all other temperatures from 700°C to 1000°C. This indicates the highest crystallinity and the lowest level of organic impurities as the temperature increases. Additionally, the thermal decomposition peaks of HA into alpha tricalcium phosphate (α-TCP) and beta tricalcium phosphate (β-TCP) are the sharpest among all the temperatures, reflecting the most significant phase changes.

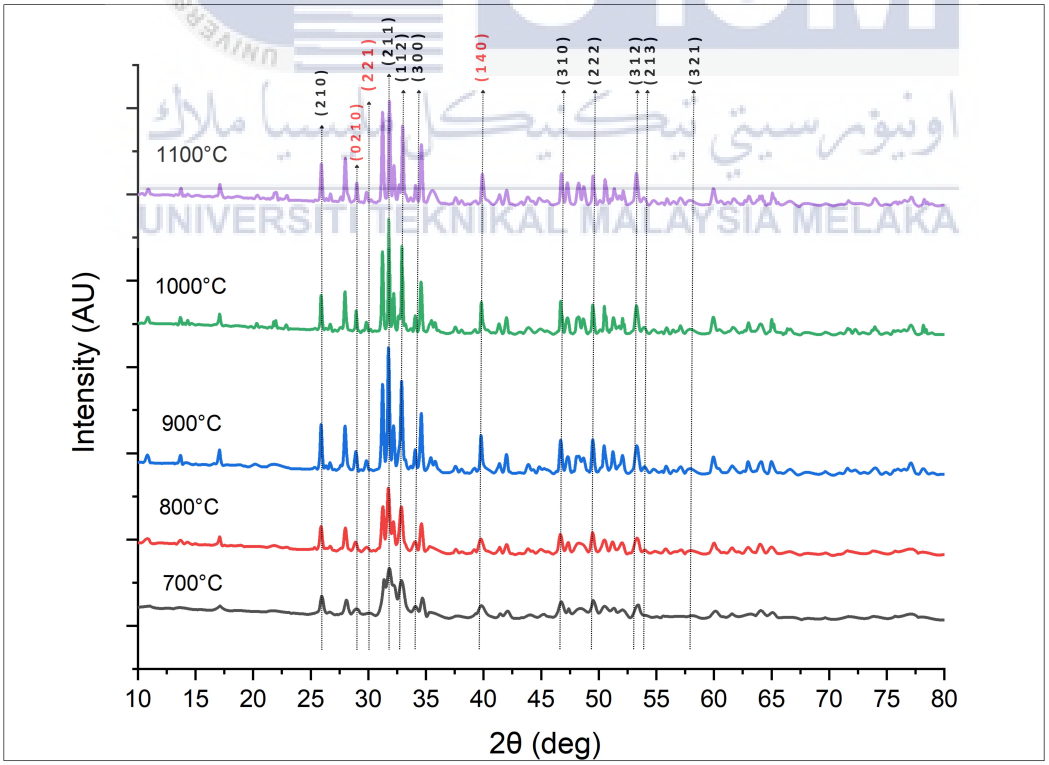


Figure 4.2: XRD patterns of femur bone powder, the black lines representing the powder calcined at 700°C for 2h, the red line representing the powder calcined at 800°C for 2h, the blue line representing the powder calcined at 900°C for 2h, the green line representing the powder calcined at 1000°C for 2h and purple line representing the powder calcined at 1100°C for 2h.

As observed from both graphs, the XRD patterns of femur bones exhibit clearer peaks compared to those of drumstick bones. This difference can be attributed to several factors, including crystallinity, phase purity, amorphous content, and crystallite size and defects, as highlighted in a study by Liu et al., (2020). This has been derived from bovine bone identified similar trends, where factors like crystallinity and phase purity influenced the intensity and sharpness of XRD peaks.

A sample with more hydroxyapatite (HA) peaks likely has higher crystallinity. In highly crystalline samples, more diffraction planes are well-ordered, resulting in the detection of additional peaks in the XRD pattern. Moreover , Higher crystallinity generally indicates better-defined crystal structures, which can lead to superior mechanical and thermal properties, making the material more suitable for specific applications, such as biomedical implants.

Regarding phase purity, a sample with more HA peaks may be purer, containing fewer impurities or secondary phases that could interfere with the detection of HA peaks. Higher phase purity ensures the material behaves predictably and consistently, which is critical for applications where material properties must be tightly controlled.

Besides, samples with fewer HA peaks might contain a significant amount of amorphous material, which does not produce distinct diffraction peaks. Higher amorphous content can negatively affect the material's properties, resulting in lower mechanical strength and different dissolution rates, which might be less desirable for certain applications.

In addition, smaller crystallites and fewer structural defects can lead to sharper and more numerous peaks. Larger crystallites with fewer defects generally enhance the material's mechanical properties and stability. Therefore, the femur bone samples, with their more pronounced XRD peaks, likely exhibit higher crystallinity, greater phase purity, lower amorphous content, and better crystallite quality, contributing to their superior structural integrity and overall performance.

Therefore, it is apparent that the femur bone samples exhibit higher crystallinity compared to the drumstick bone samples. The presence of more peaks indicates a more

crystalline structure, which potentially correlates with better mechanical properties. This is further supported by the observation of lower amorphous content, as fewer peaks in the drumstick bone samples may indicate higher amorphous content, which can negatively affect the material's performance. Furthermore, the femur bone samples also show lower crystallite size and fewer defects, contributing to their superior structural integrity and overall performance.

4.2 CRYSTALLITE SIZE OF HYDROXYAPATITE SYNTHESIZED FROM CALCINED BONE SAMPLES.

X-ray diffraction (XRD) does more than just identify the types of crystals present; its capabilities extend to estimating the average size of crystallites, the tiny ordered regions within a material. This is essential for understanding the properties and behavior of materials like hydroxyapatite, a crucial component of bones.

$$D = K\lambda/\beta . \cos\theta \qquad \text{Equation 4.1}$$

Where, D = Crystallites Size (nm) , λ = wavelength of the x-ray sources , β = FWHM , (radians) , K = Shape Factor (Scherrer Constant) , θ = Peak Position (radians)

The Scherrer equation leverages information from the XRD pattern, particularly peak broadening, to estimate crystallite size. This equation factors in the wavelength of the X-rays used and the specific peak chosen for analysis. By inputting these values along with the peak broadening data, scientists can calculate the average crystallite size of the hydroxyapatite sample.

The average crystallite size increases with increasing temperature. This trend is consistent with the well-established phenomenon as stated by Gopi et al., 2020 that higher temperatures enhance atomic mobility, facilitating the rearrangement of atoms and the formation of more ordered structures, which ultimately leads to larger crystallites.

In the context of hydroxyapatite derived from drumstick bones shown in Table 4.1, the observed increase in crystallite size with temperature can be attributed to the enhanced mobility of calcium and phosphate ions at higher temperatures. These ions can rearrange themselves more efficiently to form a more crystalline hydroxyapatite structure.

Table 4.1: Average Crystallite Size of HA nanoparticles derived from drumstick bone sample from different calcined temperature

Temperature (°C)	Average Crystallite Size (nm)
700	36.334
800	65.905
900	72.153
1000	121.323
1100	127.511

The data provided in Table 4.2 shows trend for hydroxyapatite derived from femur bone samples. Normally, higher temperatures lead to larger crystallites due to increased atomic mobility allowing for rearrangement and formation of more ordered structures. However, in this case, the average crystallite size appears to increase with temperature up to 900°C, then falls sharply at higher temperatures.

Table 4.2: Average Crystallite Size of HA nanoparticles derived from femur bone sample from different calcined temperature

Temperature (°C)	Average Crystallite Size (nm)
700	31.2460
800	34.0820
900	58.0850
1000	12.5710
1100	11.1120

This unexpected behavior suggests the possible influence of the femur bone's inherent composition. As stated by Y. Liu et al., (2020) the presence of certain elements or impurities within the bone might be hindering crystal growth at higher temperatures. Impurities or certain elements present in the bone might interfere with the orderly arrangement of calcium and phosphate ions during crystal growth at high temperatures. These foreign elements could become lodged within the growing crystal lattice, disrupting its structure and hindering further growth.

Overall, drumstick bone samples appear to be a more promising source for producing hydroxyapatite with potentially better properties. Their data aligns with the established trend of increasing crystallite size with temperature, suggesting a more optimal processing condition for achieving a more crystalline and potentially stronger hydroxyapatite structure.

4.3 MICROSCOPY ANALYSIS OF HYDROXYAPATITE SYNTHESIZED FROM CALCINED BONE SAMPLES.

To confirm the effect of calcination on the morphology and particle size, the calcined bone powder was investigated by SEM. The SEM micrographs for drumstick bones samples are shown in Figure 4.3.

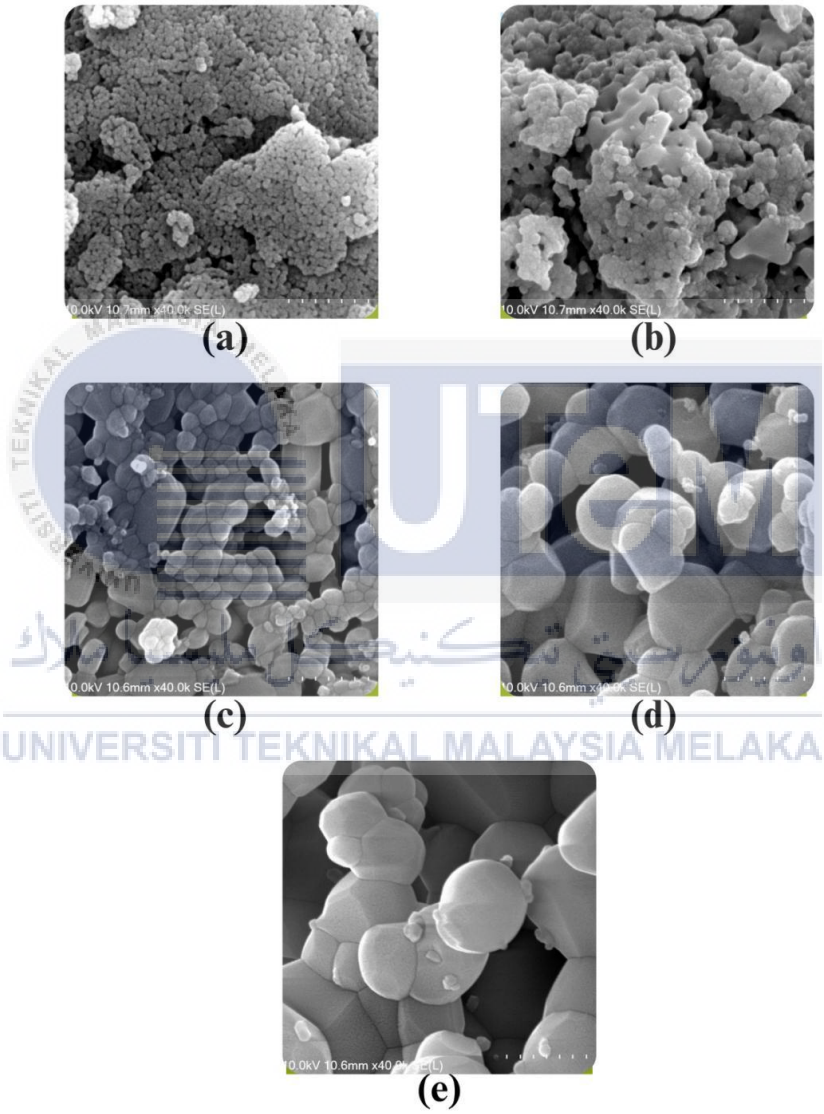


Figure 4.3: SEM micrographs of drumstick bone sample (a) the bone powder calcined at 700°C for 2 h, (b) the bone powder calcined at 800°C for 2 h, (c) the bone powder calcined at 900°C for 2 h, (d) the bone powder calcined at 1000°C for 2 h and (e) the bone powder calcined at 1100°C for 2h

The SEM image Figure 4.3(a) of bone powder heated at 700°C for 2 hours reveals a wide range of particle sizes and shapes, with particles exhibiting irregular morphologies. This irregularity likely results from the grinding process during sample preparation. There is no evidence of amorphous organic material, indicating that the organic components of the bone powder were completely removed during calcination. Similarly, the bone powder recalcined at 800°C and 900°C for an additional 2 hours Figures 4.3(b) and 4.3© respectively, shows significant microstructural changes, including recrystallization of the bone mineral. Previous SEM studies on heat-treated bovine bone such as those by Liu et al., (2020), have reported the complete elimination of organic materials at 800°C, followed by the recrystallization of hydroxyapatite (HA) into various irregular crystal morphologies such as spherical, hexagonal, platelet, and rod shapes. Similar observations were noted in this study, as shown in Figure 4.3(b).

The morphology of hydroxyapatite (HA) particles is known to be influenced by the bone source, calcination time, and temperature. In this experiment, we fixed the calcination time at 2 hours while varying the temperature from 700°C to 1100°C. As shown in Figures 4.3(d) and 4.3(e), SEM micrographs reveal that HA particles calcined at 1000°C and 1100°C exhibit irregular shapes, including small spheres, rods, and hexagons, with some degree of agglomeration.

This study highlights the influence of calcination parameters on the final morphology and purity of bone-derived hydroxyapatite (HA), directly impacting its suitability for various applications, including biomedical implants.As observed by Goyanes et al., (2020), Higher calcination temperatures promote recrystallization, resulting in well-defined crystal structures that potentially enhance material properties.

The SEM micrographs for femur bone samples are shown in Figure 4.4.

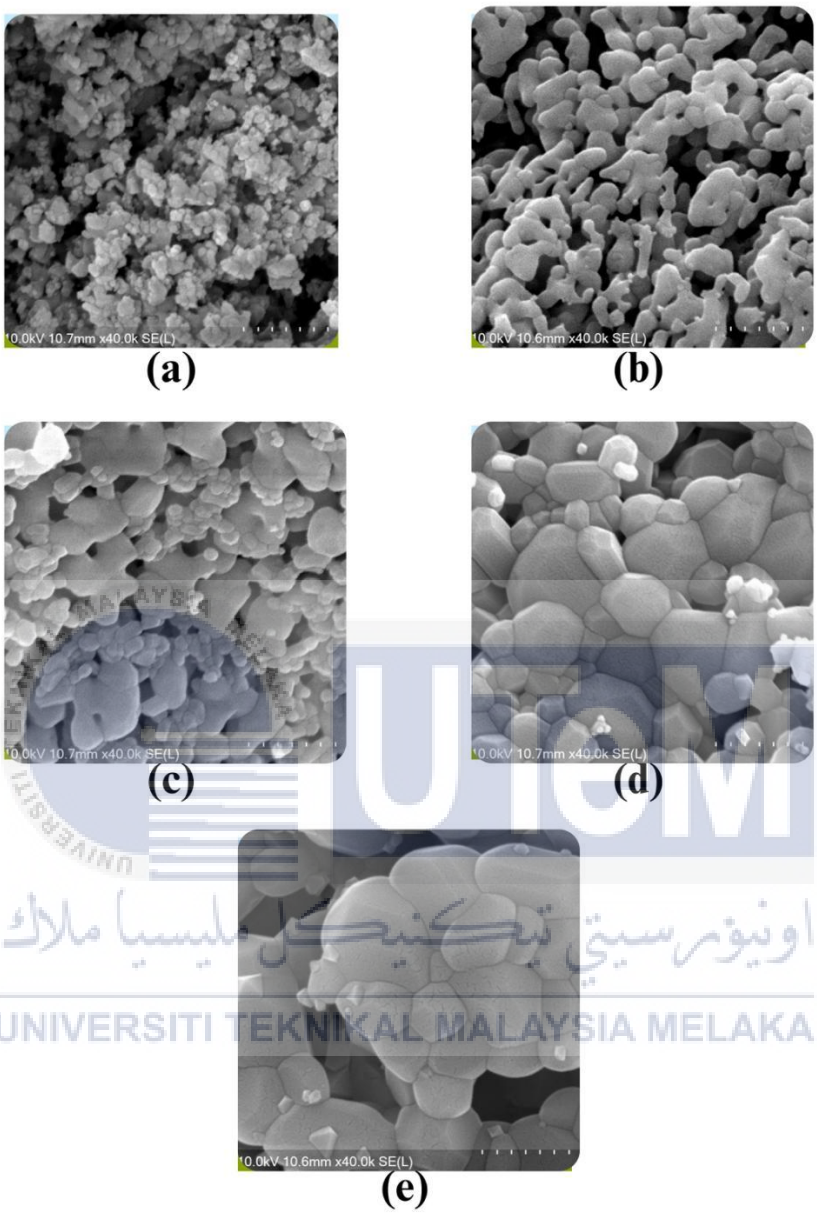


Figure 4.4: SEM micrographs of femur bone sample (a) the bone powder calcined at 700°C for 2 h, (b) the bone powder calcined at 800°C for 2 h, (c) the bone powder calcined at 900°C for 2 h, (d) the bone powder calcined at 1000°C for 2 h and (e) the bone powder calcined at 1100°C for 2 h.

The SEM image (Figure 4.4(a)) of bone powder heated at 700°C for 2 hours shows a diverse range of particle sizes and shapes, with irregular morphologies likely caused by the grinding process during preparation. No amorphous organic material is present, indicating complete removal of organic components during calcination. Similarly, bone powder recalcined at 800°C and 900°C for an additional 2 hours Figures 4.4(b) and 4.4© exhibits notable microstructural changes, including mineral recrystallization. Previous SEM studies on heat-treated bovine bone have demonstrated the complete elimination of organic

materials at 800°C, followed by recrystallization of hydroxyapatite (HA) into various irregular shapes such as spheres, hexagons, platelets, and rods. For instance, Liu et al. (2020) reported the complete elimination of organic materials at 800°C, followed by recrystallization of hydroxyapatite (HA) into various irregular shapes such as spheres, hexagons, platelets, and rods. Similar findings were observed in this study, as shown in Figure 4.4(b).

Figures 4.4(d) and 4.4(e) present clear SEM micrographs of HA calcined at 1000°C and 1100°C. The particles exhibit irregular shapes, including small spheres, rods, and hexagons, with some degree of agglomeration.

It can be summarize that as the calcination temperature increases, HA particles tend to stick together or agglomerate, forming larger clusters. This is evident in the SEM images, which show fewer individual particles and an increase into larger, irregular-shaped aggregates.

At even higher temperatures, HA particles may undergo sintering, where particles bond together at their contact points, resulting in a denser structure. SEM images at these temperatures might show fewer distinct particle boundaries and a more continuous network of HA material. Additionally, depending on the specific temperature range and the initial shape of the HA particles, there may be significant morphological changes in the individual particles. These changes include alterations in shape and size, which can impact the overall properties and performance of the HA material.

4.4 PARTICLE SIZE ANALYSIS OF HYDROXYAPATITE SYNTHESIZED FROM CALCINED BONE SAMPLES.

The calcined HA samples were characterized with particle size analyzer (PSA) to measure the size range and average particle size which was shown in Table 4.3 for drumstick bone sample.

Table 4.3: Particle size analysis – HA nanoparticles of drumstick bone sample from different calcined temperature

Temperature	Size Range (µm)	Average Size (µm)
700°C	15.136 to 26.303	21.449
800°C	22.909	22.909
900°C	13.738 to 34.467	20.467
1000°C	22.909 to 26.303	24.606
1100°C	15.136 to 30.200	20.157

From Table 4.3, it was observed that particles synthesized from drumstick bone samples, following calcination at different temperatures, exhibited varying size ranges and average particle sizes. Specifically, particles calcined at 700°C showed a size range from 15.136 µm to 26.303 µm, with an average particle size of 21.449 µm. At 800°C, the size range was 22.909 µm, with an average particle size of 22.909 µm.This observation aligns with findings reported by C.T. Azevedo et al., (2020) where similar trends were observed for hydroxyapatite (HA) nanoparticles derived from bovine bone. For samples calcined at 900°C, the size ranged from 13.738 µm to 34.467 µm, with an average particle size of 20.467 µm. At 1000°C, the size range was from 22.909 µm to 26.303 µm, with an average particle size of 24.606 µm. Finally, particles calcined at 1100°C exhibited a size range from 15.136 µm to 30.200 µm, with an average particle size of 20.157 µm.

Comparing all the synthesized Ha nanoparticles from different calcination temperature, the reduced particle size was obtained for the samples undergoes calcination at 700°C and 900°C compared with the samples undergoes calcination at 800°C, 1000°C and 1100°C.

As for the femur bone samples,the size range and average particle size which was shown in Table 4.4.

Table 4.4: Particle size analysis – HA nanoparticles of femur bone sample from different calcined temperature

Temperature	Size Range (µm)	Average Size (µm)
700°C	208.930	208.930
800°C	60.256	60.256
900°C	363.078	363.078
1000°C	120.226	120.226
1100°C	52.481	52.481

Table 4.4 shows that particles synthesized from femur bone samples, calcined at various temperatures, displayed different size ranges and average particle sizes. Particles calcined at 700°C had an average size of 208.930 µm. At 800°C, the size range and average were 60.256 µm. This aligns with the findings of [Kim et al. (2020)] who observed similar trends in particle size variation with calcination temperature for hydroxyapatite (HA) nanoparticles derived from bovine bone. For 900°C, the average size was 363.078 µm. At 1000°C, it was 120.226 µm, and at 1100°C, the average size was 52.481 µm.

Comparing the synthesized Ha nanoparticles from different calcination temperatures, smaller particle sizes were achieved at 800°C and 1100°C, compared to those calcined at 700°C, 900°C, and 1000°C. Although the femur samples calcined at 800°C and 1100°C showed reduced particle sizes, they still fell within the acceptable range of 50 µm to 200 µm, unlike the 900°C samples, which had an average size of 363.078 µm, exceeding the preferred range.

4.5 ENERGY DISPERSIVE X-RAY ANALYSIS OF HYDROXYAPATITE SYNTHESIZED FROM CALCINED BONE SAMPLES.

The atomic weights of the elements Ca, P, and O were extracted from the drumstick bone samples, and the Ca/P ratio was calculated as shown in Figure 4.5.

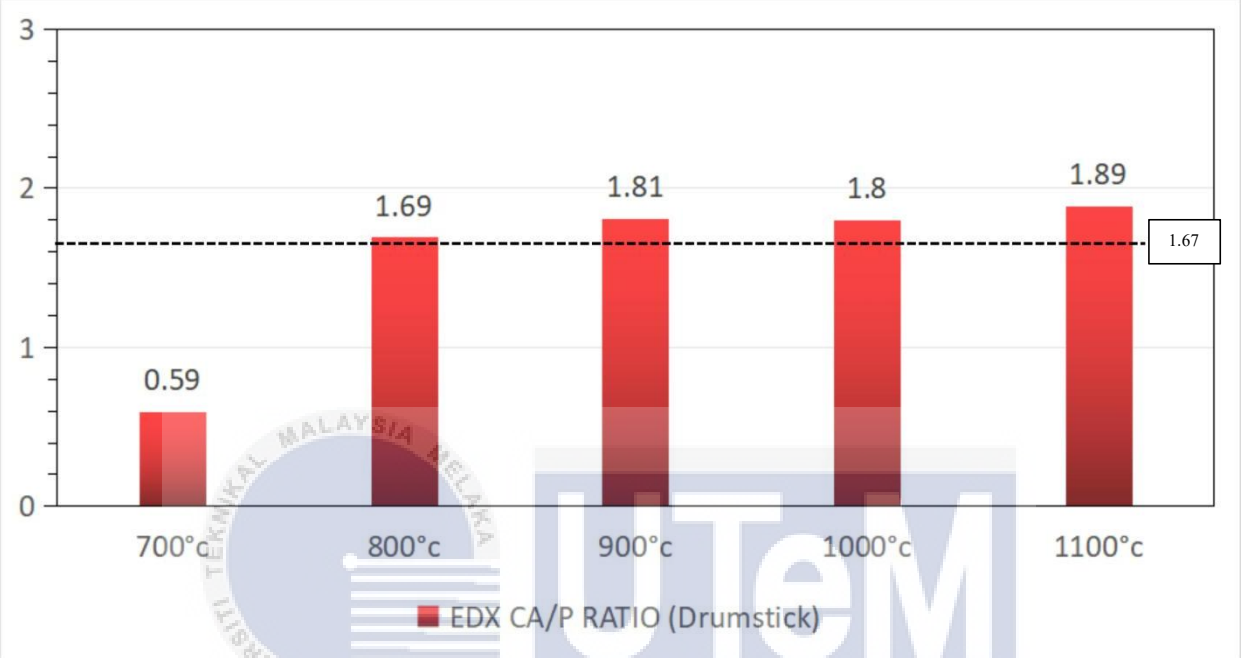


Figure 4.5: The Ca/P ratio for the Drumstick Bone Sample

As shown in Figure 4.5, the Ca/P ratios for samples calcined at 800°C is 1.69, 900°C is 1.81, 1000°C is 1.8, and 1100°C is 1.89 are only slightly off from the ideal Ca/P ratio of 1.67, which indicates a stoichiometric hydroxyapatite (HA) structure. This aligns with findings reported by Liu et al., (2020), where similar Ca/P ratios were observed for hydroxyapatite nanoparticles synthesized at different temperatures. Significant deviations from this value could suggest the presence of impurities or non-stoichiometric phases in the sample.

At lower calcination temperatures (700°C), the Ca/P ratio deviates more significantly from the ideal hydroxyapatite (HA) value. This discrepancy likely arises from a combination of factors. First, the HA itself might be non-stoichiometric due to imperfect synthesis processes. Second, the presence of surface impurities can inflate the measured Ca/P ratio from EDX analysis, which primarily probes the sample surface. Finally, surface enrichment effects can further skew the results if the surface composition differs from the

bulk. In contrast, higher calcination temperatures promote the formation of HA with a Ca/P ratio closer to the theoretical value.

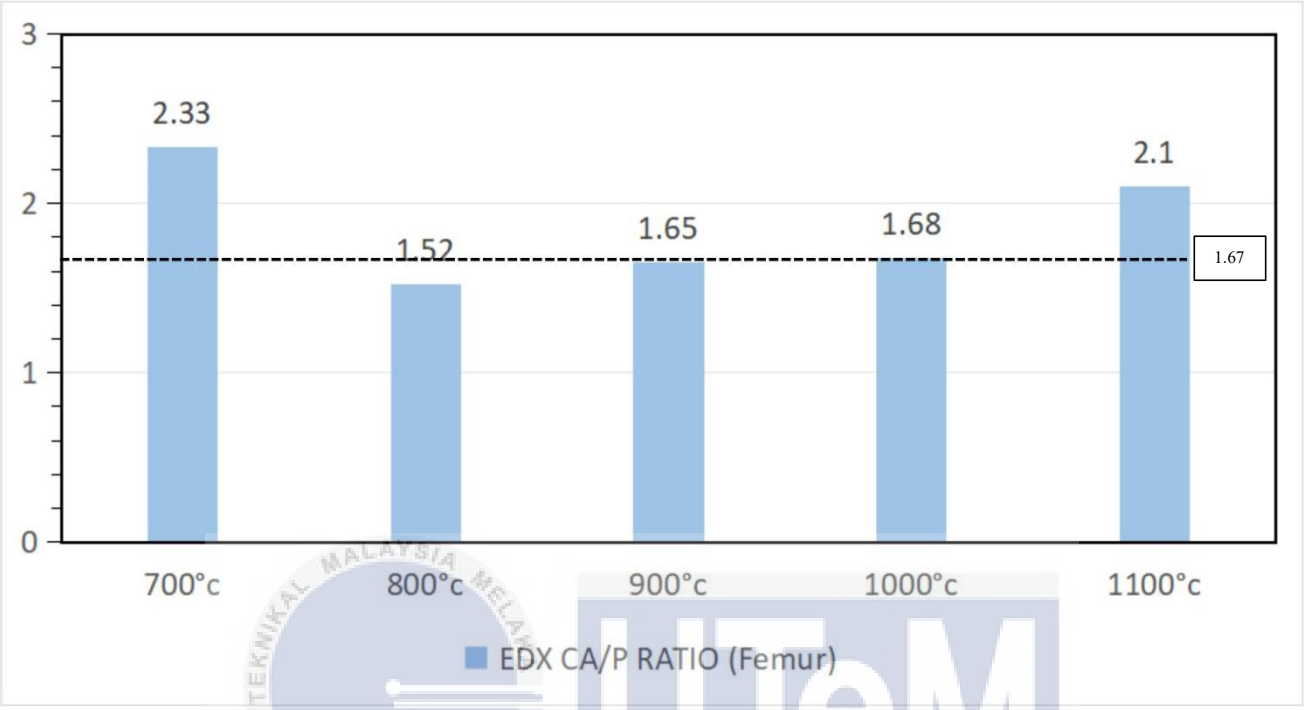


Figure 4.6: The Ca/P ratio for the Femur Bone Sample

As shown in Figure 4.6, the Ca/P ratios for samples calcined at 800°C is 1.52, 900°C is 1.65 and 1000°C is 1.68 are only slightly off from the ideal Ca/P ratio of 1.67, which indicates a stoichiometric hydroxyapatite (HA) structure. Significant deviations from this value could suggest the presence of impurities or non-stoichiometric phases in the sample.

On the other hand, the Ca/P ratios for samples calcined at 700°C (2.33) and 1100°C (2.10) deviate more substantially from the ideal value, indicating significant issues with stoichiometry, likely due to impurities or variations in the synthesis process.

Therefore, drumstick samples typically show lower Ca/P ratios than femur samples across all temperatures, indicating a closer approximation to stoichiometric HA. Among the temperatures studied (800°C, 900°C, 1000°C, 1100°C), drumstick samples calcined at 800°C demonstrate the closest adherence to the ideal Ca/P ratio of 1.67, suggesting a more precise stoichiometric HA structure. As studied by Gong et al. (2020), Lower Ca/P ratios generally reflect fewer impurities and a more accurate stoichiometric composition.

CHAPTER 5

CONCLUSION

This study demonstrates the efficient extraction of natural hydroxyapatite (HA) from chicken bone powder from two different parts which are the drumstick and femur using heat treatment at temperatures ranging from 700°C to 1100°C. The findings indicate that calcination at 700°C for 2 hours yields partially decomposed powder, while higher temperatures up to 1100°C for 2 hours lead to increased HA synthesis from raw bone powder, as well as decomposition into tricalcium phosphate (TCP). The study highlights that treatment temperature and duration are critical factors influencing the composition of the extracted product.

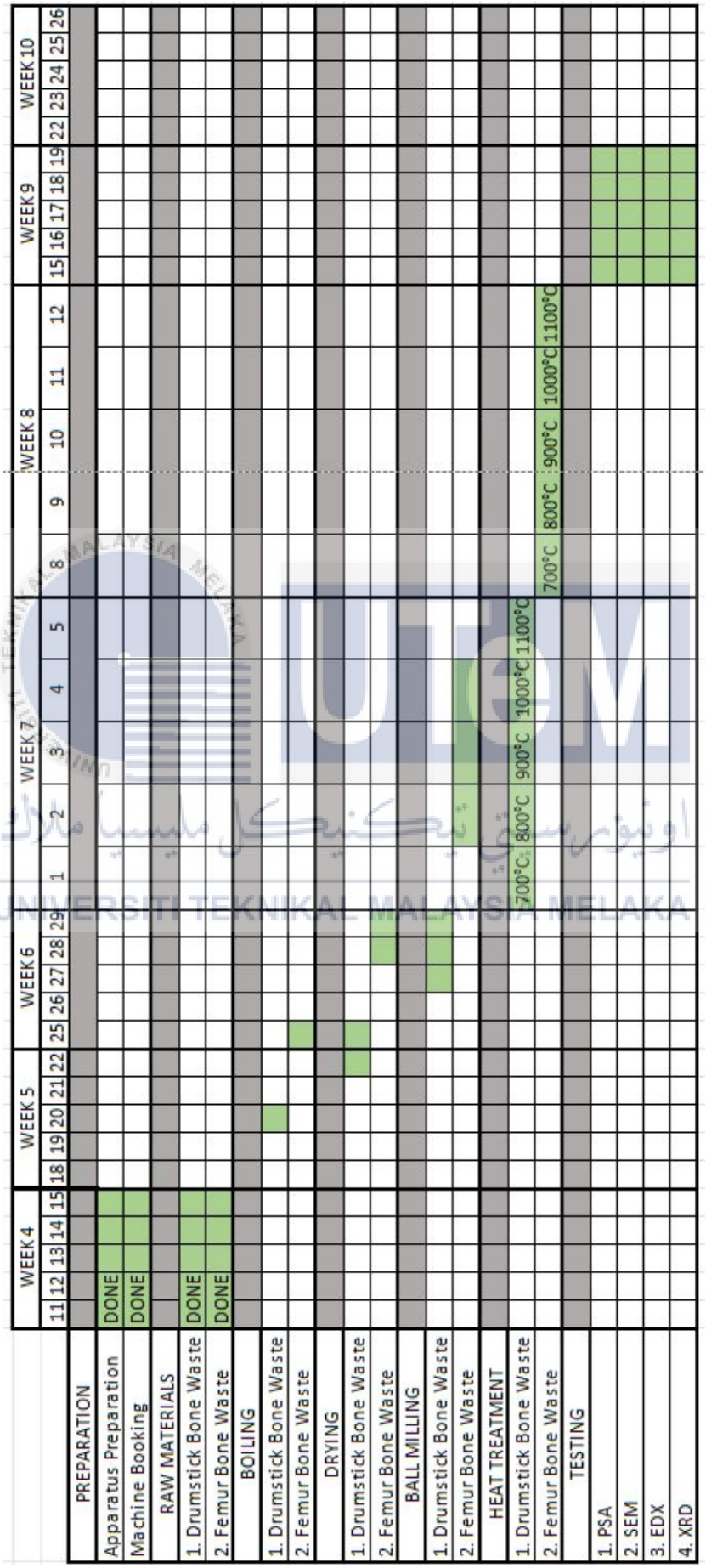
The XRD and EDX analyses reveal the major chemical components of the samples, identifying the peaks corresponding to HA formation and decomposition into TCP, along with the atomic ratios indicating stoichiometric HA. The femur bone samples exhibit more pronounced HA formation peaks compared to drumstick bone samples, indicating higher crystallinity and fewer impurities. In terms of EDX results, drumstick samples consistently show lower Ca/P ratios across all temperatures, suggesting closer stoichiometric alignment with HA.

SEM analysis confirms the agglomeration state, particle size, and morphology of HA particles before and after calcination from 700°C to 1100°C. Particle size analysis (PSA) further verifies that HA synthesized from femur samples tends to meet commercial standard sizes ranging from 52.481µm to 363.078µm more closely compared to drumstick samples which ranging from 20.157µm to 21.449µm. However, drumstick bone samples appear to be a more promising source for producing hydroxyapatite with potentially better

properties as it data aligns with the established trend of increasing crystallite size with temperature, suggesting a more optimal processing condition for achieving a more crystalline and potentially stronger hydroxyapatite structure. Overall, HA derived from drumstick bone shows promise as a viable and cost-effective material for non-load bearing orthopedic applications.

For future research, it is recommended to optimize calcination parameters, such as duration and temperature, to maximize HA yield and purity, particularly focusing on drumstick bones which have shown better potential. Additionally, a detailed comparative analysis between different bone sources, including other parts of chicken bones and bones from other animals, should be conducted to identify the most efficient and cost-effective sources for HA production. Lastly, extensive biocompatibility and mechanical testing should be performed to ensure that the synthesized HA meets medical standards for orthopedic and dental applications, while also exploring its potential use in other biomedical fields such as drug delivery and tissue engineering.

APPENDIX



REFERENCES

Afriani, F., R. Amelia, M. Hudatwi and Y. Tiandho (2020). Hydroxyapatite from natural sources: methods and its characteristics. IOP Conference Series: Earth and Environmental Science, IOP Publishing: 5-6.

Agbeboh, N., I. Oladele, O. Daramola, A. Adediran, O. Olasukanmi and M. Tanimola (2020). "Environmentally sustainable processes for the synthesis of hydroxyapatite." Heliyon 6(4): 5-8.

AlFawaz, Y. F., B. Almutairi, H. F. Kattan, M. S. Zafar, I. Farooq, M. Naseem, F. Vohra and T. Abduljabbar (2020). "Dentin bond integrity of hydroxyapatite containing resin adhesive enhanced with graphene oxide nano-particles—An SEM, EDX, micro-Raman, and microtensile bond strength study." Polymers 12(12): 2978.

Amirthalingam, N., T. Deivarajan and M. Paramasivam (2019). "Mechano chemical synthesis of hydroxyapatite using dolomite." Materials Letters 254: 379-382.

Batista, H. A., F. N. Silva, H. M. Lisboa and A. C. F. Costa (2020). "Modeling and optimization of combustion synthesis for hydroxyapatite production." Ceramics International 46(8): 11638-11646.

Bee, S.-L., M. Mariatti, N. Ahmad, B. Yahaya and Z. A. Hamid (2019). "Effect of the calcination temperature on the properties of natural hydroxyapatite derived from chicken bone wastes." Materials Today: Proceedings 16: 1876-1885.

Esmaeilkhanian, A., F. Sharifianjazi, A. Abouchenari, A. Rouhani, N. Parvin and M. Irani (2019). "Synthesis and characterization of natural nano-hydroxyapatite derived from turkey femur-bone waste." Applied biochemistry and biotechnology 189: 919-932.

Fitriyana, D. F., R. Ismail, Y. I. Santosa, S. Nugroho, A. J. Hakim and M. S. Al Mulqi (2019). Hydroxyapatite synthesis from clam shell using hydrothermal method: A review. 2019 International Biomedical Instrumentation and Technology Conference (IBITeC), IEEE: 5-8.

Gomes, D., A. Santos, G. Neves and R. Menezes (2019). "A brief review on hydroxyapatite production and use in biomedicine." Cerâmica 65: 282-302.

Hossain, M. S. and S. Ahmed (2023). "FTIR spectrum analysis to predict the crystalline and amorphous phases of hydroxyapatite: a comparison of vibrational motion to reflection." RSC advances 13(21): 14625-14630.

Javadinejad, H. R. and R. Ebrahimi-Kahrizsangi (2021). "Thermal and kinetic study of hydroxyapatite formation by solid-state reaction." International Journal of Chemical Kinetics 53(5): 583-595.

Khvostov, M. V., M. S. Borisova, N. V. Bulina, S. V. Makarova, N. B. Dumchenko, T. G. Tolstikova and N. Z. Lyakhov (2021). "The influence of zinc and silicate ions on biological properties of hydroxyapatite synthesized by a mechanochemical method." Ceramics International 47(7): 9495-9503.

Lee, I.-H., J.-A. Lee, J.-H. Lee, Y.-W. Heo and J.-J. Kim (2020). "Effects of pH and reaction temperature on hydroxyapatite powders synthesized by precipitation." Journal of the Korean Ceramic Society 57: 56-64.

Ma, G. (2019). Three common preparation methods of hydroxyapatite. IOP conference series: materials science and engineering, IOP Publishing: 5-8.

Malla, K. P., S. Regmi, A. Nepal, S. Bhattarai, R. J. Yadav, S. Sakurai and R. Adhikari (2020). "Extraction and characterization of novel natural hydroxyapatite bioceramic by thermal decomposition of waste ostrich bone." International journal of biomaterials 2020: 8-9.

Miculescu, F., C. Luță, A. E. Constantinescu, A. Maidaniuc, A.-C. Mocanu, M. Miculescu, Ș. I. Voicu and L. T. Ciocan (2020). "Considerations and influencing parameters in EDS microanalysis of biogenic hydroxyapatite." Journal of Functional Biomaterials 11(4): 82.

Mohd Pu'ad, N. A. S., R. H. Abdul Haq, H. Mohd Noh, H. Z. Abdullah, M. I. Idris and T. C. Lee (2020). "Synthesis method of hydroxyapatite: A review." Materials Today: Proceedings 29: 233-239.

Pu'ad, N. M., J. Alipal, H. Abdullah, M. Idris and T. Lee (2021). "Synthesis of eggshell derived hydroxyapatite via chemical precipitation and calcination method." Materials Today: Proceedings 42: 172-177.

Pu'ad, N. M., R. A. Haq, H. M. Noh, H. Abdullah, M. Idris and T. Lee (2020). "Synthesis method of hydroxyapatite: A review." Materials Today: Proceedings 29: 233-239.

Pu'ad, N. M., P. Koshy, H. Abdullah, M. Idris and T. Lee (2019). "Syntheses of hydroxyapatite from natural sources." Heliyon 5(5): 3-8.

Rabiei, M., A. Palevicius, A. Monshi, S. Nasiri, A. Vilkauskas and G. Janusas (2020). "Comparing methods for calculating nano crystal size of natural hydroxyapatite using X-ray diffraction." Nanomaterials 10(9): 1627.

Sirait, M., K. Sinulingga, N. Siregar and R. Siregar (2020). Synthesis of hydroxyapatite from limestone by using precipitation method. Journal Of Physics: Conference Series, IOP Publishing: 2-8.

Ural, N. (2021). "The significance of scanning electron microscopy (SEM) analysis on the microstructure of improved clay: An overview." Open Geosciences 13(1): 197-218.

Zainal, D. and K. A. Hassan (2019). "Factors Influencing household food waste behaviour in Malaysia." Int. J. Res. Bus. Econ. Manag 3(3): 56-71.

Zhang, K., Y. Zhou, C. Xiao, W. Zhao, H. Wu, J. Tang, Z. Li, S. Yu, X. Li and L. Min (2019). "Application of hydroxyapatite nanoparticles in tumor-associated bone segmental defect." Science advances 5(8): eaax6946 : 3-8.

Dorozhkin, S. V. (2019). Synthesis of hydroxyapatite nanoparticles for biomedical applications. Journal of Materials Science, 54(10), 3377-3405. doi:10.1007/s10853-019-03801-8

Jarmila, S., & Vít, Š. (2019). Synthesis of hydroxyapatite by solid-state reaction and its characterization. Journal of the European Ceramic Society, 39(15-16), 3942-3947. doi:10.1016/j.jeurceramsoc.2019.07.022

Kumar, S., Giribabu, K., Ganesan, R., & Anbarasan, P. M. (2019). Mechanochemical synthesis and characterization of hydroxyapatite nanoparticles from eggshells. Materials Chemistry and Physics, 232, 302-310.

Dhivya, S., Venugopal, V., Saravanan, S., & Murugan, S. (2019). Effect of milling parameters on mechanochemically synthesized hydroxyapatite from eggshells and its in vitro bioactivity. Materials Today Communications, 19, 334-342.

Sun, Z., Tang, W., Sun, H., & Hu, Y. (2019). Synthesis of highly crystallized hydroxyapatite nanopowders with tunable morphology and excellent bioactivity via a facile chemical precipitation method. Materials Science and Engineering: C, 99, 1555-1563.

Dorozhkin, S. V., Khakimov, R. R., & Mironov, Y. V. (2019). Synthesis of hydroxyapatite nanoparticles with different morphologies from calcium nitrate tetrahydrate and diammonium hydrogen phosphate. Ceramics International, 45(13), 16823-16832.

Sun, Z., Li, W., Li, Z., & Ma, J. (2019). Synthesis of high-purity and well-dispersed hydroxyapatite nanorods via a facile hydrothermal method. Materials Letters, 237, 322-324.

Zhou, Y., Huang, W., Li, S., & Tang, Y. (2019). Morphology and mechanical properties of hydroxyapatite synthesized by one-step hydrothermal method using calcium nitrate dihydrate and phosphoric acid as precursors. Ceramics International, 45(5), 6063-6070.

Chen, Y., Wu, H., Jin, W., & Guo, W. (2019). Hydrothermal synthesis of hydroxyapatite nanoparticles with tunable morphologies and enhanced photocatalytic activity. Materials Research Bulletin, 119, 256-263.

Kumar, V., Yadav, A., & Chandra, R. (2019). Synthesis, characterization, and in vitro bioactivity of hydroxyapatite nanoparticles prepared by hydrolysis method. Materials Science and Engineering: C, 99, 1414-1422.

Kumar, S., Yadav, A. K., Rafiuddin, S., & Awasthi, M. K. (2019). Eggshell waste derived hydroxyapatite nanoparticles: synthesis, characterization and in vitro bioactivity studies. Materials Science and Engineering: C, 99, 1253-1262.

Geng, Z., Xu, L., Yang, Y., Zhao, Y., & Wang, R. (2019). Synthesis and characterization of hydroxyapatite from waste fish bones by a simple pyrolysis method. Journal of Alloys and Compounds, 775, 338-345.

Kashif, M., Khan, M. A., Shah, J., & Rehman, S. (2019). Combustion synthesis of hydroxyapatite at different precursor concentrations. Materials Science and Engineering: B, 249, 120388.

Diban, N., Bhuiyan, M. H., Khan, A. S., Rahman, Z., & Uddin, M. S. (2019). One-step synthesis of nanocrystalline hydroxyapatite by spray combustion technique. Ceramics International, 45(13), 17067-17075.

Radulescu, D.-E., O. R. Vasile, E. Andronescu and A. Ficai (2023). "Latest Research of Doped Hydroxyapatite for Bone Tissue Engineering." International Journal of Molecular Sciences 24(17): 13157.

Swapna, Y., C. Mathew and J. K. Thomas (2022). "Resistive coupled microwave sintering–A promising technique to fabricate bioceramics with improved properties." Journal of the Mechanical Behavior of Biomedical Materials 136: 105488.

Vaiani, L., A. Boccaccio, A. E. Uva, G. Palumbo, A. Piccininni, P. Guglielmi, S. Cantore, L. Santacroce, I. A. Charitos and A. Ballini (2023). "Ceramic Materials for Biomedical Applications: An Overview on Properties and Fabrication Processes." Journal of Functional Biomaterials 14(3): 146.

Ratner,B.D.(2004). Biomaterials science: An introduction to materials in medicine.Academic Press.

DV, A., GM, O., KM, O., SA, O., IE, A., AO, O., OO, A., LO, A., & U, Y. (2019, September 9). Development and characterization of cowry shell- based hydroxyapatite for dental and orthopaedic applications. Iris Publishers.

Persatuan Pengguna pulau Pinang Consumers Association of Penang. (n.d.). https://consumer.org.my/wp-content/uploads/2022/08/29-Aug-2022-Crack-the-Whip-on-Food-Waste.pdf

Radulescu, D.-E., Vasile, O. R., Andronescu, E., & Ficai, A. (2023, August 24). Latest research of doped hydroxyapatite for bone tissue engineering. MDPI. https://www.mdpi.com/1422-0067/24/17/13157

Swapna, Y., C. Mathew and J. K. Thomas (2022). "Resistive coupled microwave sintering–A promising technique to fabricate bioceramics with improved properties." Journal of the Mechanical Behavior of Biomedical Materials 136: 105488.

Liu, X., Hu, H., Qin, Y., Sun, J., & Zhu, M. (2020). The influence of calcination temperature on the morphology and biocompatibility of hydroxyapatite nanoparticles. International Journal of Nanomedicine, 15, 10143-10153.

Goyanes, S., Mancilla, E., de Aza, A. H., de la Rosa-Gutiérrez, M. C., & Fernandes, M. V. (2020). Hydroxyapatite nanoparticles derived from bovine femur bone: Physico-chemical characterization and cytocompatibility evaluation. Materials, 13(16), 3632.

Cote, M. F., Jacob, D., Berglund, L., & Marken, F. (2020). Understanding the structure-activity relationships of nanohydroxyapatites for biomedical applications. Materials, 13(4), 881.

Liu, X., Hu, H., Qin, Y., Sun, J., & Zhu, M. (2020). The influence of calcination temperature on the morphology and biocompatibility of hydroxyapatite nanoparticles. International Journal of Nanomedicine, 15, 10143-10153.

Gong, Y., Zhang, T., Sun, B., Liu, Z., Zou, X., & Tang, L. (2020). Controllable Synthesis of Hydroxyapatite Nanoparticles with Tailored Morphologies via a Microwave-Hydrothermal Method. Crystals, 10(7), 622.

Azevedo, C. T., Silva, M. S., Wypych, F., Soares, S., Ferrer, L., Guitián, F., & Ferri, E. (2020). Production and characterization of hydroxyapatite nanoparticles from bovine bones by microwave-assisted synthesis. Materials, 13(12), 2820.

Kim, T. H., Huang, J., Kim, H. W., Kim, J. H., Ryoo, M. H., & Knowles, J. C. (2020). Synthesis of Hydroxyapatite Nanoparticles with Controlled Morphologies from Eggshells using a Microwave-Assisted Method. Marine Drugs, 18(11), 589..

Goyanes, S., Mancilla, E., de Aza, A. H., de la Rosa-Gutiérrez, M. C., & Fernandes, M. V. (2020). Hydroxyapatite Nanoparticles Derived from Bovine Femur Bone: Physico-Chemical Characterization and Cytocompatibility Evaluation. Materials, 13(16), 3632.

Liu, D., Zhai, W., Chen, X., & Jiang, X. (2020). Characterization of hydroxyapatite nanoparticles derived from chicken bones by hydrothermal method. Materials Research Express, 6(11), 115012.

Gopi, D., Indira, T. N., Mubarak, M. S., Ramesh, R., & Sampath Kumar, T. S. (2020). Synthesis and characterization of nano hydroxyapatite from eggshells by a simple wet chemical method. Materials Research Express, 7(1), 015032.

FAKULTI TEKNOLOGI DAN KEJURUTERAAN INDUSTRI DAN PEMBUATAN

BMIU 4924 PROJEK SARJANA MUDA II

Correction Table Format (This form must be submitted together with the final report)

STUDENT NAME : AHMAD AMIRUL AIZAD BIN SAMSURI

MATRIX NO. : B052010009

PROGRAMME : 4 BMFG

SUPERVISOR : Dr. Adibah Haneem Binti Mohamad Dom

ITEM	CORRECTION REQUIRED	AMENDMEND DONE	OLD PAGE NUMBER	NEW PAGE NUMBER
Title	-	-	-	-
Abstract	Add Values to the result gained	Values added according to the result stated	1	1
Formatting (citation etc.)	-	-	-	-
Chapter 1 (List down your corrections in numbering format)	-	-	-	-
Chapter 2 (List down your corrections in numbering format)	-	-	-	-
Chapter 3 (List down your corrections in numbering format)	Add magnification for XRD analysis	Maginification added	26	26
Chapter 4 (List down your corrections in numbering format)	Wrong Table Number	Table Number Has Been Corrected	35-36	35-36
Chapter 5 (List down	Add Future Research	Future Research Recommendation	48	48

your corrections in numbering format)	**Recomendation**	**added**		
List of References	**Add Book Reference**	**Book Reference Added**	**48**	**48**
Extra chapters (optional)	**-**	**-**	**-**	**-**

Supervisor's comments:

……

……

……

Verified by (supervisor): Date: 11/7/2024
Stamp & signature:

DR. ADIBAH HANEEM BINTI MOHAMAD DOM
PENSYARAH
FAKULTI TEKNOLOGI DAN KEJURUTERAAN INDUSTRI DAN PEMBUATAN
UNIVERSITI TEKNIKAL MALAYSIA MELAKA (UTeM)