PREPARATION AND CHARACTERIZATION OF SYNTHETIC HYDROXYAPATITE FROM FISHBONE WASTE

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

by

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I hereby, declared this report entitled "Preparation and Characterization of Synthetic Hydroxyapatite from Fishbone Waste" is the result of my own research except as cited in references.

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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 (Hons). The member of the supervisory committee is as follow:

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(ADIBAH HANEEM BINTI MOHAMAD DOM)

ABSTRAK

Tulang ikan mempunyai bahan komposit seperti HA berkarbonat, kolagen jenis 1, protein bukan kolagen dan air yang mudah didapati di seluruh dunia sebagai sumber hidroksiapatit semula jadi (HA). HA terkenal dengan sifat biokompatibiliti yang sangat baik kerana mempunyai komposisi kimia yang serupa dengan tisu manusia. Beberapa tahun kebelakangan ini, banyak kajian telah dilakukan untuk mensintesis HA untuk aplikasi dalam biobahan. Oleh itu, kajian ini melaporkan penggunaan sisa tulang ikan (*Skipjack tuna*) untuk membentuk HA menggunakan kaedah kalsinasi pada suhu antara 800°C hingga 1000°C. Semua sampel telah menjalani pencirian fizikal, kimia dan mekanikal untuk memerhatikan morfologi permukaan, penghabluran, kumpulan berfungsi dan kekerasan. Pembelauan Sinar-X digunakan untuk memerhatikan pengaruh suhu kalsinasi pada komposisi fasa HA ketika HA yang dikalsinasi stabil pada 1000°C kerana corak menunjukkan pantulan utama HA dan pantulan CaP yang lebih sedikit, sementara struktur ikatan molekul yang dibentuk oleh HA dihasilkan menggunakan Fourier transform infrared (FTIR) spektroskopi menunjukkan fosfat dan sedikit puncak karbonat dalam sampel yang dirawat. HA yang dihasilkan dari suhu sintesis yang berbeza mempunyai ukuran kristal 44.91-63.17 nm yang dikenal pasti menggunakan persamaan Scherer. Kekerasan HA juga ditentukan oleh penguji kekerasan Vickers, menunjukkan nilai kekerasan tertinggi 0.64 GPa pada suhu 1000°C, yang mana nilai kekerasan berada dalam jarak tulang kortikal femoral manusia yang sebenar. Struktur mikro dan morfologi HA dalam keadaan terkumpul ditentukan dengan menggunakan Mikroskop elektron imbasan. Apabila suhu kalsinasi meningkat, zarah HA membentuk zarah yang padat dan mengakibatkan peningkatan ukuran zarah. Ia dapat disimpulkan bahawa 1000°C dicapai sebagai suhu kalsinasi yang sesuai dengan masa yang tetap bagi tulang ikan untuk membentuk hidroksiapatit.

ABSTRACT

Fishbone is a composite material made of carbonated HA, type 1 collagen, non-collagen protein and water that is available worldwide as a source of hydroxyapatite (HA). HA was known for its excellent biocompatibility properties due to the similar chemical composition of human hard tissues. Recent years, extensive research and studies have been conducted to synthesise HA for applications in biomaterials. Therefore, this study reported the use of fishbone waste (*Skipjack tuna*) to form HA using calcination method at temperatures ranging from 800°C to 1000°C. All samples have undergone physical, chemical and mechanical characterisation in order to observe surface morphology, crystallinity, functional group and hardness. X-ray diffraction is used to observe the influence of the calcination temperature on the HA phase composition where the calcined HA was stable up to 1000°C because the pattern shows major HA-corresponding reflections and fewer CaP-reflections, while the molecular bond structure formed by HA was produced using Fourier transform infrared spectroscopy (FTIR) demonstrates the phosphate and few carbonate peaks in the sample treated. HA produced from different synthesis temperatures has a crystal size of 44.91-63.17 nm identified by Scherer equation. The hardness of the HA was also determined using the Vickers hardness tester, which showed the highest hardness value of 0.64 GPa at 1000°C, where the hardness value is within the range of the actual human femoral cortical bone. The microstructure and morphology of HA in agglomerated condition was determined using a scanning electron microscopy. As the calcined temperature increases, the HA particle formed a dense particle and resulted in increased particle size. It is concluded that 1000°C is attained as suitable calcination temperature with constant time for fishbone to obtain synthetic hydroxyapatite.

DEDICATION

I dedicate this research report to
my father, Abdul Jabar bin Tomin
my mother, Sakinah binti Hussein
my sisters and brother,
Thank you for your loving support of my dream and supporting me during my hard time.

ACKNOWLEDGEMENT

By the Name of Allah, the Most Merciful and Gracious

I am using this opportunity to express my deepest gratitude and special thanks to my supervisor, Madam Adibah Haneem binti Mohamad Dom who despite being extraordinarily busy with her duties, took time out to hear, guide and keep me on the correct path throughout this semester and allowing me to carry out the project at their esteemed organisation. Therefore, I consider myself as a very lucky individual as I have the opportunity of associating with her.

I am also very grateful to our assistant engineers who helped me set up the project and gave me practical insights. It is my radiant sentiment to place on record my best regards, deepest sense of gratitude to my family for their careful and precious guidance which were extremely valuable for my study both theoretically and practically. Not forgetting the panels, my friends and the people who helped, supported and contributed to the completion of this research.

I perceive this research as a big milestone in my career development. I will strive to use gained skills and knowledge in the best possible way and I will continue to work on their improvement, to attain desired career objectives.

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LIST OF ABBREVIATIONS

ASTM HA	-	American Society for Testing and Materials (Standard HA)
A800	-	Calcined temperature at 800˚C
B900	-	Calcined temperature at 900˚C
C1000	-	Calcined temperature at 1000˚C
C-HA	-	Commercial hydroxyapatite
Cu Kα	-	Copper K-alpha
DCPA	-	Dicalcium Phosphate Anhydrous
EDX	-	Energy dispersive x-ray
FB	-	Fish bone
FTIR	-	Fourier-transform infrared spectroscopy
FWHM	-	Full width at half maximum
HA	-	Hydroxyapatite
IR	-	Infrared radiation
JCPDS	-	Joint Committee on Powder Diffraction Standards
PSA	-	Particle size analyser
PVA	-	Polyvinyl alcohol
R-FB	-	Raw fish bone
SEM	-	Scanning electron microscope
SST	-	sea surface temperature
TCP	-	Tri-calcium phosphate
TTCP	-	Tetracalcium Phosphate
XRD	-	X-ray diffraction

LIST OF SYMBOL

λ	-	Wavelength (Lambda)
°	-	Degree
θ	-	Angle Diffraction
%	-	Percentage
°/min	-	Degree per minute
˚C	-	Degree Celsius
µm	-	Micrometre
cm^{-1}	-	Reciprocal Centimetre
drop/min	-	Drop per minute
g	-	Gram
kgf	-	Kilogram-force
kV	-	Kilovolt
mA	-	Milliampere
mm	-	Millimetre
nm	-	Nanometre
pH	-	Potential of Hydrogen
psi	-	Pound per square inch
rpm	-	Revolution per minute
GPa	-	Giga Pascal
M	-	Moles
MPa	-	Mega Pascal
X	-	Magnification
β-TCP	-	β- Tricalcium Phosphate
Al	-	Aluminum
AlO_2	-	Aluminum Oxide
Ba	-	Barium
C-H	-	Carbon hydrogen bond

C-N	-	Carbon nitrogen bond
C=O	-	Carbon oxygen double bond
C-C	-	Carbon carbon bond
C-O	-	Carbon oxygenbond
CO_2	-	Carbon dioxide
CO_3^{2-}	-	Carbonate
Ca	-	Calcium
CaO	-	Calcium Oxide
$CaCO_3$	-	Calcium Carbonate
$Ca(OH)_2$	-	Calcium hydroxide
CaP	-	Calcium Phosphate
HCl	-	Hydrochloric Acid
HPO_4^{2}-	-	Hydrogen Phosphate
H_3PO_4	-	Phosphoric acid
K	-	Potassium
KH_2PO_4	-	Potassium Phosphate
Mg	-	Magnesium
Na	-	Sodium
NH_4OH	-	Ammonium hydroxide Solution
OH-	-	Hydroxide
OH	-	Hydroxyl
Pb	-	Lead
PO_4^{3-}	-	Phosphate
Si	-	Silicon
Sr	-	Strontium
Ti	-	Titanium
Zr	-	Zirconium

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Hydroxyapatite (HA) or known as synthetic HA is specifically found in the form of granular particles, porous blocks, powder and different sintered form as a source of calcium for teeth and important compounds to repair bones described by Afriani *et al*. (2019). The molecular formula of HA is $Ca_5(PO_4)_3OH$ but frequently written as $Ca_{10}(PO_4)_6(OH)_2$ (Pokhrel, 2018). The material is one of the most important minerals in the group calcium phosphate (CaP). CaP was found in bone basically in the form of a nanometre-sized needle of crystals. However, it has a poorly crystallized non-stoichiometric apatite phase (Gomes *et al*., 2019). Dynamically, Ghiasi *et al*. (2019) stated that HA is the most stable based on physiological conditions as compared to other CaP compounds. Hence, HA is focusing on biomedical applications with properties closer to living organisms. Based on chemical properties, synthetic HA is a rare material due to high bioactivity, high biocompatibility and non-toxicity of bone and tooth in living organisms, which is not harmful to the body (Martin *et al*., 2019).

The application enhances from HA has been applied widely in industries which, for example, replacement concretes material, fertilizer activities, limestone agent and production of tile. HA extracted from chemical synthesis have been intensively studied in recent years. However, the study of use natural sources has recently increased. Recently, many researchers reported that HA from natural sources specifically fishbone has excellent potential in health applications that used as a biomaterial for bone implant (Mustafa *et al.*, 2015; Shi *et al.,* 2018; Pu'ad *et al.,* 2019). Indeed, HA compound from fishbone has been used as human body implant materials such as prosthetic implants and coating (Pokhrel, 2018; Khiri *et al*., 2019).

The aquaculture industry is one of the leading industry and eventually to become the source of Malaysia’s economy. Consequently, Malaysia is known as a maritime nation because of the strategic location with an abundant supply of land and water suit to aquaculture activities. Fish is known as one of the primary protein sources that can be found easily from aquaculture activities. There are 22,000 known species of fish in global. *Skipjack tuna* is one of the species which known as *Katsuwonus pelamis* is a perciform fish in the tuna family. The major increase in tuna landings was due to the expansion of commercial tuna fishing of Malaysia's exclusive economic zone (EEZ) waters, especially around the South China Sea. It is expected that the production of tuna in Malaysia will continue to show significant growth in the future. With a growing population of people, the estimation of the annual demand for fishes will increase. Thus, the need for fishes has increased the amount of fish waste specifically fishbone. Fishbone is a composite material made of carbonated HA, type 1 collagen, non-collagenous protein and water. It is available in worldwide, low-cost source to obtain natural HA (Shi *et al.*, 2018). Many research have been carried to reuse waste bones for both environmental and economic benefits.

Many researchers have studied the method to synthesise HA. HA can be synthesised in various ways such as dry method, wet chemical precipitation reaction and hydrothermal technique (Khandelwal and Prakash, 2016). The alkaline method is able to produce pure HA, but with lower crystallinity as compared to the calcination method. In addition, the alkaline method is one of the expensive preparation technique used by the laboratory to synthesize HA is limited because it requires a large quantity of material to form it**.** Therefore, the method of synthesising HA is crucial to the production of pure HA with high crystallinity. The preparation of synthetic HA with specific characteristics remains challenging for every researcher due to the possibility of developing toxic products during the synthesis process (Pu’ad *et al.*, 2019). Thus, this research is focusing on synthesised and characterised synthetic HA from fishbone powder by using x-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Vickers hardness tester and the scanning electron microscopy (SEM).

1.2 Problem Statement

Bone is a skeleton organ system known as calcified tissue that protects soft tissues and organs, provides the body with a mechanical support and restores mineral homeostasis. Bone consists of approximately 60% of minerals with hydroxyapatite (HA) being one of the mineral phases, 30% of organic by mass and 10% of water. Recent years, HA is well known for bone regeneration through conduction or by acting as a scaffold for repairing the defects. As a consequence, HA plays a significant role in bone replacement or bone tissue regeneration. Due to the limited supply of natural bones for bone replacement, there is a growing need for synthetic bones to replace bones with the same biological properties as natural bones. This has given rise to importance in the development of artificial materials as bone graft substitutes.

There have been increasing research and development activities of HA due to its high demand as a promising biomaterial. One of the factors contributing to the increase in the global stoichiometric HA market is the excessive applicability of HA in the medical sector. Additionally, the demand for HA in dental implants is predicted to increase over the forecast period (Thirumalai, 2018). Nevertheless, the low mechanical properties of HA are expected to restrain market growth. In order to reduce the dependency on stoichiometric HA materials for industries, efforts have been made to incorporate by-products and wastes from different natural sources as alternatives in obtained HA. Therefore, fishbone currently is found to be the best candidate as the alternative material as they lead to the best results producing HA (Pu'ad *et al.*, 2019). The formation of HA using fishes waste can be reduced the production cost and the price of HA in the global market. Waste should be recycled and reused toward the production of value-added products to sustain product development. Previous studies by Venkatesan *et al.* (2015) have generally focused on the thermal method of synthesising HA from the fishbone to form HA. However, it was found that the presence of tricalcium phosphate (TCP) phase at $1200^{\circ}C$. Hence, the calcination temperature is crucial to the production of HA. This study focuses on the use of fishbone waste (*Skipjack tuna*) as a source for the synthesis and characterisation of highly pure HA powder. This research will helps meet the objective by raising awareness of possible ways to develop economic return from natural waste, especially in fishbone.

1.3 Objectives

The objectives of this study are as follows:

a) To synthesise hydroxyapatite powder from fishbone via calcination method.
b) To characterise and analyse the physical, chemical and mechanical properties of fishbone.
c) To investigate the suitable calcination temperature with constant time for fishbone to obtain hydroxyapatite.

1.4 Scope of the Research

The scopes of research are as follows:

a) Study the physical properties of fishbone via the purification process and dried in oven for one hour. The amount of fishbone powder was prepared by crushing it into small parts and go through the pulverizing process.
b) Identify the best amount of fishbone for the preparation of three samples that were subjected in the sintering process which all samples in three different condition temperatures of thermal method.
c) Investigate the best temperature that produces synthesis HA from fishbone, which identified via the X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR).
d) Observe the effect shape of the HA particles in different synthetic temperature in considering the crystallinity of HA powders and improved synthesis method to produce HA from fishbone.

CHAPTER 2

LITERATURE REVIEW

This chapter consists of two sections that explored the theory and experimental work performed by different researcher years ago. Related knowledge from previous studies refers to the book and some source of internet relevance to the scope of the study included background of natural hydroxyapatite and characterisation techniques.

2.1 Biological Apatite

Biological apatite is known as inorganic calcium phosphate (CaP) has many characteristics differing either from mineral apatite or synthetic apatite while Vallet-Regí (2016); Siddiqi and Azhar (2020) said that biological apatite specifically in human bone is containing 4 to 8 weight% of carbonate ion in lattice substitutions which occur in two groups known as A-site for hydroxyl ion and B-site for phosphate ion. It is found as apatite structure, but some of crystallized CaP phases (Whitlockite, Brushite and Octacalcium Phosphate) may form in unknown pathologic clarifications. CaP is stated as one of the essential minerals for both bones (skeletal system and homeostasis of mineral balance in body) and teeth (Upadhyay, 2017). CaP has been performed as filler in biological tissues where it is important to the physicochemical characteristic of any materials. Haider *et al.* (2017) reported that biological apatite structure is identical to the tissues present in the bone which is bioactive and applied as a substitute for the bone construction in periodontology, oral as well as orthopaedic processes. The bones were used for the reconstruction of the bone defect. Biological apatite is an excellent mineral dependent on structure and work in and applications of biological tissues which many researcher had performed the investigation of its physiochemical and biological properties (Eliaz and Metoki, 2017; Kołodziejska *et al.*, 2020).

2.1.1 Characterisation of biological apatite

Based on chemical characterisation, biological apatite is regarded as HA which the result of CaP performed in apatite structure. CaP capable to absorb proteins into biomaterial surfaces that give benefit for tissue or material interface, which leading to expanded bone formation (Neacsu *et al.*, 2019). Among the apatite family, HA is the best material for improving any application involved. HA is known as one of the apatite family that plays a significant role in the biomaterials field (Haider *et al.*, 2017). From the previous research of Liu *et al*. (2013), they showed the composition and structure of HA which two different crystals formed had investigated known as hexagonal with the lattice parameters (Hexagonal HA) and monoclinic with the lattice parameters (Monoclinic HA). They found that both had the same elements and stoichiometric CaP ratio of 1.67. Besides, the direction of hydroxyl (OH) group is the main difference that occurs in their structure. For Hexagonal HA, the OH groups formed in the reverse directions, while for Monoclinic HA, the OH groups have the same direction among themselves (Saxena *et al.*, 2018). Table 2.1 below shows the properties of the crystal structure of HA which described the summary of both elements.

Table 2.1: The properties of crystal structure of HA (Saxena *et al.*, 2018)

Properties	**Hexagonal**	**Monoclinic**
Symmetry	$P6_3/m$	$P2_1/b$
Lattice parameter	a= b=9·432Å c= 6·881Å γ=120°	a=9·4214Å b=2a, c=6·8814 Å γ=120°
Orientation of Hydroxyl groups (OH)	Reversed direction.	Same direction.

For this reason, biological apatite basically is CaP minerals incorporated by various type of ions which is Calcium (Ca), Phosphorus (P) and Oxygen (O) are three major elements composing the CaP minerals. Carbon (C), Sodium (Na), Potassium (K), Fluorine (F), Magnesium (Mg), Aluminium (Al), Strontium (Sr), Chloride (Cl) and other elements also found as incorporated ions in the biological apatite structure which then influence the stability of biological apatite (Laskus and Kolmas, 2017). Their present may differ among sources which all the elements stated may be found in small amounts in biological apatite structure. Eliaz and Metoki (2017) reported that the Ca/P ratio of biological apatite was either lower than the Ca/P ratio of stoichiometric HA or close to the Ca/P ratio of stoichiometric HA which state as 1.67 based on research by Elliott (2013). However, the Ca/P ratio of biological apatite would be higher than Ca/P ratio of stoichiometric HA which different from the statement mentioned from Barakat *et al.* (2008). This statement may lead to the difference testing method, raw materials as well as detection error. The difficulty of evaluating chemical compositions is due to the ion absorptions. The effect on each crystal may be distinctive, which the structure of apatite allows for wide compositional variations due to the ability in obstruct different ions in different conditions (Eliaz and Metoki, 2017). In physiological characterization, CaP is partially dissolved caused by cellular activities and will increased the biological or physiological fluid. There are two aspect that biological apatite shows the unique chemical composition. The first aspect is the lacking of anticipatory hydroxyl group while second aspect is the existence of HPO_4^{2-}. It was reported by Liu *et al.* (2013) that hydroxyl group have only a least amount concentration detected in bone. It is because the multiple ionic substitutions which often occur in the lattice of biological apatite.

Other than that, the existence of HPO_4^{2-} also could be occur due to the ionic substitutions. However, due to the lack of accuracy of the analytical method, it is impossible to measure the number of chemical groups accurately. The external factor that can influenced the chemical groups were temperature, inorganic solvent, ionic effect and the pH scale. Biological apatite might lose carbonate groups by sintering it in temperature of 850˚C for two hours who was observed by Liu *et al.* (2013). This can cause a partial dissolution of biological apatite crystals for inorganic solvents such as acid and pure water. Based on crystal structure analysis, Liu *et al.* (2013) said that the biological apatite crystal size may reach more than 100 nm. They believed that the size of crystal as well as age of crystal highly depends on the age of mammal's bone. Kaflak *et al.* (2019) approved the statement in their research where they noticed a decrease in the percentage of amorphous minerals in rats from 69% to 36% when the animal age rose from five to 70 days.

2.1.2 Substitution of hydroxyapatite

In actual fact, biological apatite behaviour has been investigated since ages ago which then the researcher namely Horvath derived the solubility of this apatite specifically from bones and teeth (Horvath, 2006). Based on the research, the result of the solubility of