EXTRACTION OF HYDROXYAPATITE USING HEAT DECOMPOSITION OF FISH BY PRODUCT FOR BIOMEDICAL APPLICATION

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Faculty of Mechanical and Manufacturing Engineering Technology

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AFIQ HAMIZAN BIN SYAHARUDIN

A thesis submitted
in fulfillment of the requirements for the degree of
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DECLARATION

I declare that this thesis entitled “Extraction Of Hydroxyapatite Using Heat Decomposition Of Fish By Product For Biomedical Application” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : *Afiq Hamizan*

Name : Afiq Hamizan Bin Syaharudin

Date : 10/1/2022

APPROVAL

I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the Bachelor of Mechanical Engineering Technology (Air Conditioning And Refrigeration System) with Honours.

Signature : ______________________________

Name : Azrin Bin Ahmad

Date : 10/1/2022

DEDICATION

I humbly dedicated this piece of work to my loving and supportive parents, Syaharudin Bin Mohd Ali, Rubinah @ Rubiah Binti Raman for their endless love, sacrifices, prayer and support, to my relatives for their financial support.

I also dedicate this to my supervisor, Mr. Azrin Bin Ahmad and Mr. Mohamed Saiful Firdaus Bin Hussin for encouragement and guidance.

Above all, to Almight God, ALLAH who always give me strength, knowledge and wisdom to everything I do.

ABSTRACT

This project aims to investigate and study fish bones for the production of hydroxyapatite $(Ca_{10})(PO_4)_6(OH)_2$. This material may replace the method of bone making, bone grafting, injecting biomaterial for bone surgery and strengthening the tooth layer. Hydroxyapatite is an essential ingredient for normal bones and teeth. Hydroxyapatite can provide rigidity to bones and teeth. The purpose of this study is to analyze the potential of Sardine (*sardinella longiceps*) for bone tissue regeneration by using bibliometric studies to investigate studies that have been done 10 years before, to investigate hydroxyapatite using calcination process method with temperature 700 °C and characterize hydroxyapatite using Electron Scanner Microscopy (SEM) to observe the morphology and particle size, an Energy Scattering X-Ray Spectroscopy (EDS) to analyze the element composition, X-Ray Diffraction Analysis (XRD) to analyze and revealed presence of hydroxyapatite peak and Fourier Transform Infrared Spectroscopy (FTIR).

ABSTRAK

Projek ini bertujuan untuk menyiasat dan mengkaji tulang ikan untuk penghasilan hidroksiapatit (Ca_{10}) $(PO_4)_6(OH)_2$*. Bahan ini mungkin menggantikan kaedah membuat tulang, cantuman tulang, menyuntik biomaterial untuk pembedahan tulang dan menguatkan lapisan gigi. Hydroxyapatite adalah bahan penting untuk tulang dan gigi normal. Hidroksiapatit boleh memberikan ketegaran kepada tulang dan gigi. Tujuan kajian ini adalah untuk menganalisis potensi Sardin (sardinella longiceps) untuk pertumbuhan semula tisu tulang dengan menggunakan kajian bibliometrik untuk menyiasat kajian yang telah dilakukan 10 tahun sebelum ini, untuk mengkaji hidroksiapatit menggunakan kaedah proses kalsinasi dengan suhu 700 °C dan mencirikan hidroksiapatit menggunakan Elektron. Mikroskopi Pengimbas (SEM) untuk memerhati morfologi dan saiz zarah, Spektroskopi X-Ray Penyebaran Tenaga (EDS) untuk menganalisis komposisi unsur, Analisis Difraksi Sinar-X (XRD) untuk menganalisis dan mendedahkan kehadiran puncak hidroksiapatit dan Spektroskopi Inframerah Transformasi Fourier (FTIR).*

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LIST OF SYMBOLS AND ABBREVIATION

°C	-	Celcius
cm	-	Centimeter
%	-	Percentage
nm	-	Nanometer
CPC	-	Calcium Phosphate Cement
Cl	-	Chloride
FB	-	Fish Bone
F	-	Fluoride
OH	-	Hydroxide
HA	-	Hydroxyapatite
PMMA	-	Polymethyl Methacrylate
FTIR	-	Fourier-transform Infrared Spectroscopy
SEM	-	Scanning Electron Microscopy
XRD	-	X-Ray Diffraction Analysis
EDS	-	Energy Dispersive X-Ray Spectroscopy

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CHAPTER 1

INTRODUCTION

1.1 Background

Disease and trauma frequently damage human tissues, and some diseases, as well as physical force, can result in bone fractures and dangerous complications, particularly in the elderly. Overuse or repetitive motions will fatigue muscles and increase bone pressure. When a bone is fractured, it is common to hear a crack or a scratching noise as the injury occurs. Swelling, bleeding, or tenderness will occur around the affected spot. Transverse, compression, oblique, displaced, greenstick, and comminuted are some of the different types of bone fractures.

To overcome these challenges, bioengineering techniques have been developed combining biomaterials and cells to accelerate bone regeneration restoring normal function. In the last several years, biomaterials based on calcium phosphate especially hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, (HAP) have been widely utilized to repair bone fractures (S. Samavedi et al, 2013) because of its capacity to physiochemically bond to the bone and increase osteoconductivity and osteoinductivity, hydroxyapatite has been recognized as one of the most potent implant materials for bone engineering applications to enhance the development of bones (A. Sugawara et al, 2013).

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, popularly known as HA, this biomaterial utilized in bone implants, bone cements, and dental pastes and cements are examples of medical uses (P.O. Weeraphat, 2016) since it has chemical characteristics that are identical mammalian bones and hard tissues contain the same mineral component. Hydroxyapatite is a hexagonal crystal form of the mineral calcium apatite, which contains calcium, phosphorous, and oxygen. The hue of pure hydroxyapatite is white.

Hydroxyapatite can be produced using one of two methods a synthetic technique based on calcium and phosphate sources or a biological resource extraction technique. In this study, many synthetic ways for producing hydroxyapatite have been developed like spray pyrolysis, microwave irradiation, ultrasound irradiation, sol-gel crystallization, electrodeposition, hydrothermal treatment, calcination and alkaline hydrolysis and combination method. A chemical reaction between calcium and phosphate ions is used in the synthetic processes. The shape, crystallinity, and stoichiometric composition (1.67) of produced hydroxyapatite may all be easily regulated utilizing these methods (S. Kannan et al, 2016). These approaches, on the other hand, are difficult, time consuming, and expensive, especially as HA has a low bioactivity. Hydroxyapatite have been discover from bovine bone, sea corals, egg shells, fish bones, and fish scales (N. Mustafa et al, 2015), according to several research.

Every year, China generates a large amount of fish waste as a huge freshwater fish country, with fish scales accounting for around 15% of it, which is a cost effective and readily available biological resource. (Mahboob et al, 2015). In Southeastern Australia, an estimate over every year, 20 million kilogram of fish waste are generated. During the preparation of fish, most seafood markets only keep the fillet. The remaining two-thirds of the overall weight, which comprises of fish heads, intestines, bones, and skin, is discarded. This fish waste may be moved from the processing site to be rendered but it rare happen, but it is mostly thrown in landfills. As a result, these businesses are facing significant backlash as a result of their insufficient waste disposal methods, which generate environmental issues.

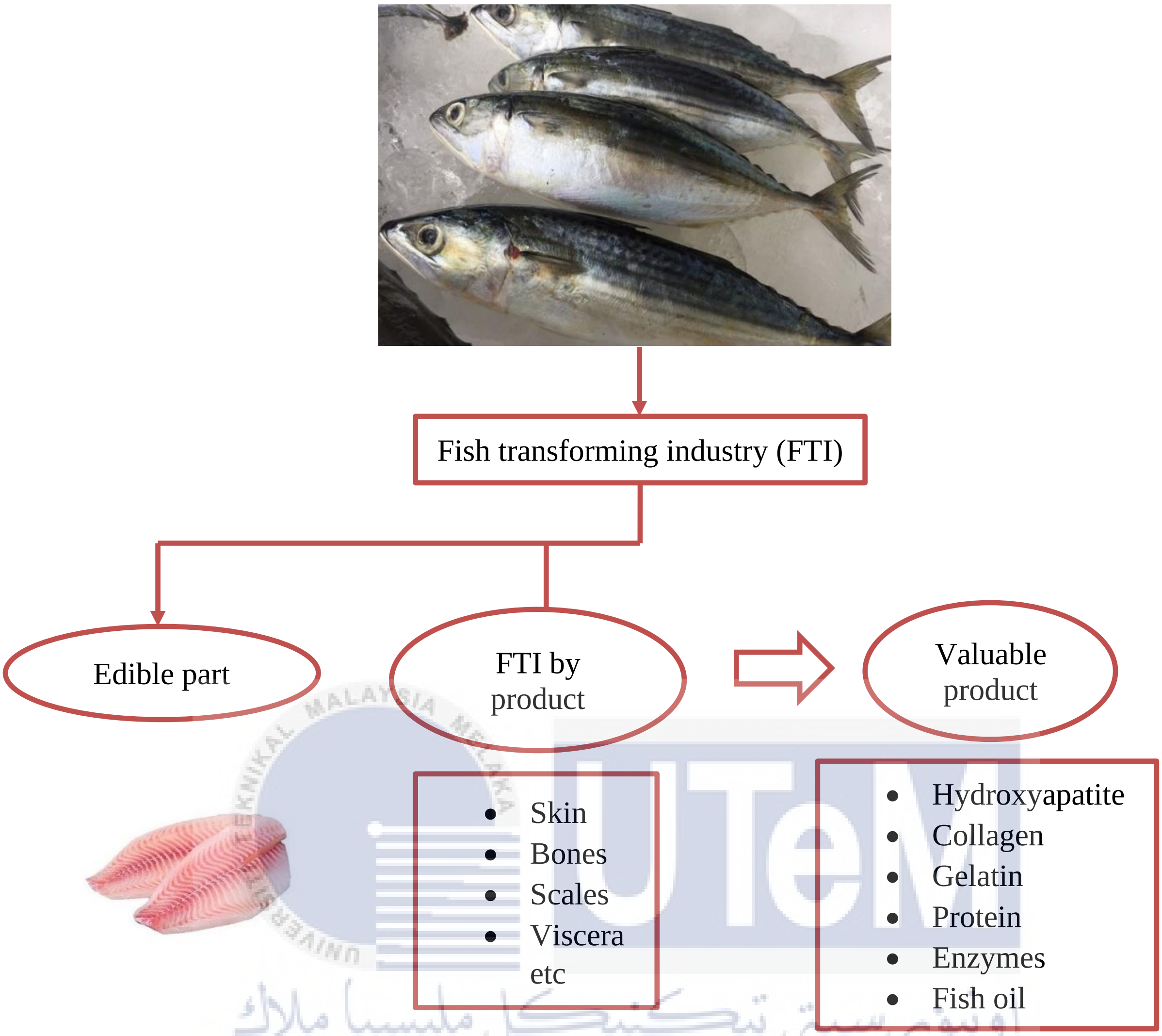

Figure 1.1 Fish tranforming industry

The rapid expansion of fish processing industries around the world has resulted in massive annual production of solid fish waste. Globally, over 91 million tons of fish are caught each year. Approximately 50–60% of the entire fish harvest is consumed by humans, while the remaining by-products are discarded as trash during processing. Resulting in one of many wastes management and environmental issues. The conversion of fish bio-waste particularly bones and scales, into useful products such as hydroxyapatite is one of the research directions that has obtained a lot of attention (J.A. Da Cruz et al, 2020). Natural hydroxyapatite made from fish bio-waste is preferred over coral and mammalian sources because it is more sustainable and safer, with low risks of disease transmission to humans (M. Boutinguiza et al, 2012). Furthermore, unlike hydroxyapatite derived from bovine or porcine sources, hydroxyapatite derived from fish sources have no issue by religious or cultural beliefs (M.C. Gomez-Guillen et al, 2011).

The fast expansion of the aquatic products business, a significant quantity of trash is generated during the processing of fish with fish scales accounting for around 4% of these wastes (N. Muhammad, 2017). Hydroxyapatite makes up 38–46% of the gross weight of fish scales. It has gained a lot of interest in the medical industry because of its biological compatibility, bone conductivity, and biological nontoxicity. Paul S et al used high-temperature calcination to make hydroxyapatite powder from fish scales. They concluded that at 1200°C, hydroxyapatite powder contains a high concentration of magnesium and strontium ions and causes no cytotoxicity (S. Paul et al, 2017).

Fish bones were gathered and utilized to extract hydroxyapatite as a follow-up to previous experiments. For future applications, the shape, content, and porosity of the produced hydroxyapatite were studied. Furthermore, the recovery procedure allows for the use of all biomolecules included in the fish by-products. Furthermore, chemical research suggests that these materials are rich in calcium phosphate and might be utilized to make hydroxyapatite at a low cost. Furthermore, these elements would be perceived as food industry by products (biowaste).

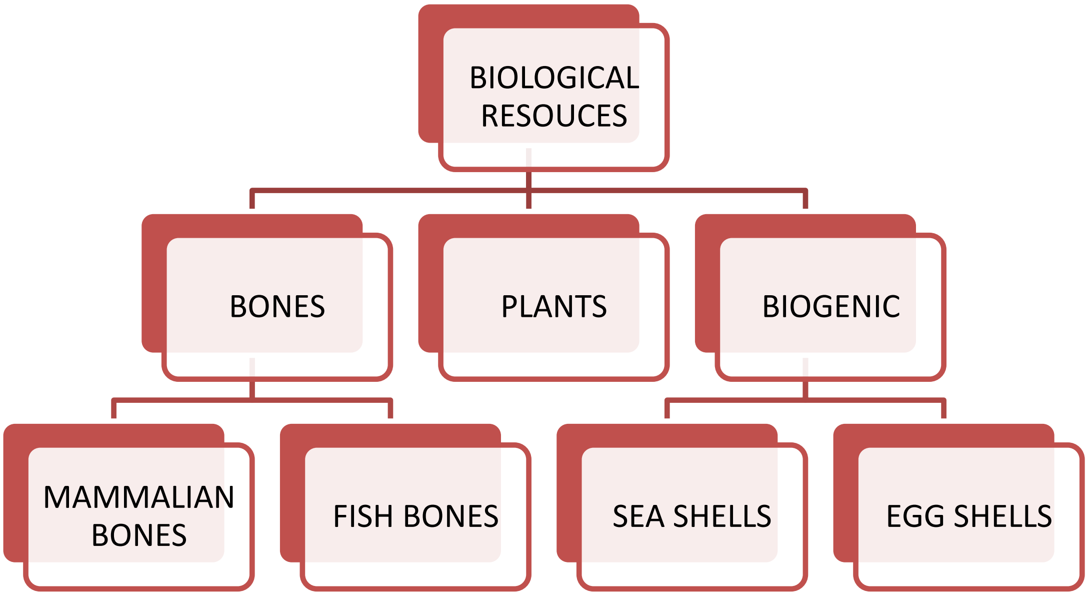

Figure 1.2 Biological resources

The conversion of such biowaste would be one of the advantages of solid waste management since it would have a lower environmental effect. There are two benefits to the rehabilitation process. It results in a cost-effective and appealing product, as well as a reduction in trash output in the fishing sector.

1.2 Problem Statement

Human tissues are frequently damaged by illnesses or traumas, necessitating a sequence of complex and regulated processes including cell proliferation, differentiation, matrix creation, and natural structural remodeling (F.J. O'Brien, 2011). Tissue engineering is a proven treatment for repairing and regenerating damaged tissues or organs. Tissue engineering is a complicated process that uses appropriate models and technology to restore defective or damaged tissues to their original condition and function (R. Cortesini, 2005). However, there are certain disadvantages or limitation to apply tissue engineered bone right away. Injectable biomaterials, on the other hand are better prepared to adjust to the defect shape and create better bone-biomaterial interaction, even for complicated defects. Moreover, there are a few injectable biomaterials that have been used for medical applications such as calcium phosphate cement (CPC), polymethyl methacrylate (PMMA) and also hydroxyapatite (HA). Heat generation, lack of biodegradation, fast deterioration, and loss of strength are some of the downsides of PMMA and CPC (Y. Zhang et al, 2015). Besides, hydroxyapatite be able to change old method which is bone graft. Bone graft from old method is taking other part of the bone in body and craft it to replace the broken bone. This method is very expensive and taking long time to operate. So, hydroxyapatite a commonly utilized biomaterial in biomedical applications, degrades slowly and can last for a few years in the body, forming a strong connection with bone and soft tissue.

Besides, rapid advancements in animal agriculture and the food business have resulted in large-scale waste creation throughout the world. Bones, seafood shells, and eggshells, which are inedible by-products of the slaughtering, catering, baking, and food processing sectors and have no economic value, are commonly viewed as wastes and disposed non landfills without being completely used. Improper food waste management has a number of negative environmental repercussions, including the spread of hazardous pathogens, the production of objectionable odors, and the ability to leach chemicals into the environment. Statistics show carcasses (fish bones) problem an increase dramatically because it is estimated that between 0.97 to 2.7 trillion fish are captured and killed in the wild each year throughout the world (H. Yamamura et al, 2018). This does not include the billions of fish that are farmed. Usually, fish guts, heads, tails, fins, skin, bone, and crab shells are regularly discarded in marine waters by seafood processors. As a result of bacterial breakdown, fish wastes cause aesthetic issues and unpleasant smells if not properly kept or controlled. After that, this has become a big issue for Muslim and Hinduism, since the status of the ingredients used, and the production process may not fulfill the religion requirement. Extraction of hydroxyapatite from bovine and porcine may cause to transmission of disease and prohibition toward Muslim and Hinduism communities (Mursyidi, 2013). In additional, from religion point of view, the handling of these bovine bone need halal compliant hence making it viable for Muslim society acceptance of implant material manufactured from these animal bones. This issues need is very sensitive toward religion and communities. Last but not least, with growth rates ranging from 0.3 to 2 centimeters per year for huge corals and up to 10 centimeters per year for branching corals, it can take up to 10,000 years for a coral reef to emerge from a clump of larvae. Barrier reefs and atolls can take anywhere from 100,000 to 30,000,000 years to fully develop, depending on their size. Fish bone and scale is very important biomaterials for bone tissue engineering the internal structure of the fish scale is divided into two layers. The outer osseous layer is made up of hydroxyapatite crystals, and the inner or bottom layer is made up of a plywood structure with collagen fibers (S. Krishnan et al, 2012), which has a structure and components that are similar to the extracellular matrix of bone and has good biocompatibility (H. Feng et al, 2020). Furthermore, hydroxyapatite is the primary component of fish scales has become a study hotspot in the field of biological resources. As for hydroxyapatite, ability of supporting bone growth because of same properties to hard tissue. After that, synthetic hydroxyapatite powders were produced such as the wet chemical precipitation technique and natural hydroxyapatite powders derived from the fish scales. However, due to the high expense of chemical synthesis and the fact that it is physiologically dangerous, different options for a more environmentally friendly, easy to create, and economical procedure were studied. Natural biological sources for natural hydroxyapatite have been explored including eggshell, fish scale, fish bone, bovine bones, and bones and teeth of pig (A. M. Janus et al, 2008). In this research, Sardine (*sardinella longiceps*) fish bone is used due to low costs and easy to obtain the fish bone, fish bone has shown to be the greatest alternative source of hydroxyapatite. Calcination process has been investigated as a low-cost and quick extraction approach.

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1.3 Research Objective

The primary goal of this research is to analyze full potential of fish bone in hydroxyapatite. Specifically, the objectives are as follows:

a. To analyze the potential of fish bone for bone tissue regeneration using bibliometric study

b. To prepare natural hydroxyapatite from fish by product toward hydroxyapatite production

c. To characterize hydroxyapatite using XRD, SEM, EDS and FTIR

1.4 Scope of Research

There are two main highlight scope for this research which is bibliometric study by doing literature study on past journal in Scopus to get more understanding and trend about the research with VOSviewer software to analyze the past decade trend. After that, material characterization which is from Sardine (*sardinella longiceps*) fish bone need to be clean with tap water and boil for 1 hour with temperature 100 °C. Then, dried the fish bones in electric oven for 24 hours and calcinated the fish bones for 5 hours with temperature 700 °C. After the calcination process observed the colour appearance before and after calcination of temperature 700 °C of fish bone powder. Lastly, analyze the calcinated fish bones powder with X-Ray Diffraction Analysis (XRD), Scanning Electron Microscope (SEM), an Energy Dispersive X-Ray Spectroscopy (EDS) and Fourier-Transform Infrared Spectroscopy (FTIR).