UNIVERSITI TEKNIKAL MALAYSIA MELAKA

EXTRACTION OF HYDROXYAPATITE USING CALCINATION METHOD FROM WASTE FISH BONES FOR BIOMEDICAL APPLICATION

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in fulfillment of the requirements for the degree of
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DECLARATION

I declare that this thesis entitled " Extraction of Hydroxypatite Using Calcination Method from Waste Fish Bones 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 : *Hilmi*

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Date : 11 JANUARY 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 (Automotive) with Honours.

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DEDICATION

I would like to dedicate this paper work to my project supervisor and co. supervisor, Ts. Azman Bin Ibrahim and Mr. Mohamed Saiful Firdaus Bin Hussin respectively, for their continuous guidance, advice, idea, comment and support given towards the accomplishment of this project. Next I would like to express my gratitude to my fellow friends that help me through thick and thin while preparing this project. I would also like to express my gratitude to my family that have supported me during my time of preparing this project. Last but not least, I would also like to express my sincere thanks to all my lectures who have directly or indirectly gave support, encouragement, idea and guidence towards completing this project.

ABSTRACT

This project aims to investigate the properties of natural hydroxyapatite extracted from waste fish bones through the calcination technique for biomedical applications. Hydroxyapatite has unique physical characteristics and is one of the natural materials commonly used in tissue engineering applications as it offers a biocompatibility feature that can enhance bone regeneration. Hydroxyapatite has been widely used in clinical practice, serving as fillers and scaffolds for orthopedic surgery. Most sources of hydroxyapatite are usually obtained from porcine, bovine, and cattle bones. However, these mammalian hydroxyapatite sources are rejected by some consumers due to religious beliefs or health concerns. The purpose of this study is to review an alternative source of hydroxyapatite, namely fish. This study seeks to establish an effective methodology of extracting hydroxyapatite with the help of prior published literature between 2010 and 2020 and characterize the natural hydroxyapatite properties through functional groups, crystalline phase, morphology characteristics, and chemical composition. The pure hydroxyapatite obtained from waste fish bones (Sardina Pilchardus) through calcination at 600 °C resulted in the production of carbonated natural hydroxyapatite. Powder samples exhibit high biocompatibility and proper morphology for use in biomedical applications through (FTIR) analysis. (XRD) analysis revealed the presence of hydroxyapatite peaks, while (SEM) analysis data demonstrated spherical morphology with an average particle size of ~300 nm which indicates great potential in biomedical application. However, the (Ca/P) ratio determined by (EDS) analysis was less than the theoretical hydroxyapatite stoichiometry ratio, and the powder physical form is described as white with a slight black shade. Therefore, the pure hydroxyapatite synthesis from waste sardine bones from this research has the potential used as an implant material substitute in bone tissue engineering as well as managing bio-waste fish by-products.

ABSTRAK

Projek ini bertujuan untuk menyiasat sifat hidroksiapatit semula jadi yang diekstrak daripada sisa tulang ikan melalui teknik pengkalsinan untuk aplikasi bioperubatan. Hydroxyapatite mempunyai ciri fizikal yang unik dan merupakan salah satu bahan semula jadi yang biasa digunakan dalam aplikasi kejuruteraan tisu kerana ia menawarkan ciri biokompatibiliti yang boleh meningkatkan pertumbuhan semula tulang. Hydroxyapatite telah digunakan secara meluas dalam amalan klinikal, berfungsi sebagai pengisi dan perancah untuk pembedahan ortopedik. Kebanyakan sumber hidroksiapatit biasanya diperoleh daripada babi, lembu, dan tulang lembu. Walau bagaimanapun, sumber hidroksiapatit mamalia ini ditolak oleh sesetengah pengguna kerana kepercayaan agama atau kebimbangan kesihatan. Tujuan kajian ini adalah untuk meninjau sumber alternatif hidroksiapatit iaitu ikan. Kajian ini bertujuan untuk mewujudkan metodologi yang berkesan untuk mengekstrak hidroksiapatit dengan bantuan literatur yang diterbitkan sebelum ini antara 2010 dan 2020 dan mencirikan sifat hidroksiapatit semula jadi melalui kumpulan berfungsi, fasa kristal, ciri morfologi, dan komposisi kimia. Hidroksiapatit tulen yang diperoleh daripada sisa tulang ikan (Sardina Pilchardus) melalui pengkalsinan pada 600 °C menghasilkan penghasilan hidroksiapatit semula jadi berkarbonat. Sampel serbuk mempamerkan biokompatibiliti yang tinggi dan morfologi yang sesuai untuk digunakan dalam aplikasi bioperubatan melalui analisis (FTIR). Analisis (XRD) mendedahkan kehadiran puncak hidroksiapatit, manakala data analisis (SEM) menunjukkan morfologi sfera dengan saiz zarah purata ~ 300 nm yang menunjukkan potensi besar dalam aplikasi bioperubatan. Walau bagaimanapun, nisbah (Ca/P) yang ditentukan oleh analisis (EDS) adalah kurang daripada nisbah stoikiometri hidroksiapatit teori, dan bentuk fizikal serbuk digambarkan sebagai putih dengan sedikit warna hitam. Oleh itu, sintesis hidroksiapatit tulen daripada sisa tulang sardin daripada penyelidikan ini berpotensi digunakan sebagai pengganti bahan implan dalam kejuruteraan tisu tulang serta menguruskan produk sampingan bio-sisa ikan.

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LIST OF SYMBOLS AND ABBREVIATIONS

℃	-	Degree Celcius
$	-	Dollar
%	-	Percentage
min	-	Minute
h	-	Hour
g	-	Gram
nm	-	Nanometer
NaOH	-	Sodium Hydroxide
HCl	-	Hydrochloric Acid
Na	-	Sodium
K	-	Potassium
Mg	-	Magnesium
Zn	-	Zinc
Sr	-	Strontium
KBr	-	Potassium Bromide
Ca/P	-	Stoichiometry Ratio
FTIR	-	Fourier-Transform Infrared Spectroscopy
XRD	-	X-Ray Diffraction
SEM	-	Scanning Electron Microscopy
EDS	-	Energy-dispersive X-ray spectroscopy
IR	-	Infrared radiation
Ca3(PO4)2	-	Bete-Tricalcium Phosphate
Ca5(PO4)3(OH)	-	Hydroxyapatite
CaO	-	Calcium Oxide

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CHAPTER 1

INTRODUCTION

1.1 Background

Modern world today has experienced a wide range of technologies, from industrial technology in the cities to the agricultural technology in the countryside. Vast technology has surround mankind throughout the world. No doubt, technology involvement has significantly help evolved human research and findings, especially in the medical industries. Rebuilding or regenerating damaged bone tissue caused by trauma, infection, tumours, or inherent genetic disorders has become a serious health concern in the modern era. (Gareta, et al., 2015). Currently, countries worldwide face an unprecedented demand for functional bone grafts (Amini, et al., 2012). Over $45 billion is spent annually on the treatment of nearly 15 million people suffering bone diseases, ranging from 1.6 million fractures traumatic injuries to 2 million osteoporotic bone abnormalities (O'Keefe & Mao, 2011). The recent emergence of bone problems has prompted the development of more effective treatments to accommodate this demand, particularly for the aging population (Tang, et al., 2016).

Bone grafting can be used to supplement bones that have been lost through trauma, natural or pathologic processes. This technique is commonly presented through autogenous bone, allogenic bone, xenogeneic bone, bone substitutes, and alloplasts (Tucker, et al., 2010). Bone grafts bridge gaps, offer support, and might even assist the defect heal biologically. (Calori, et al., 2011). Autologous bone or autograft remains the clinical "gold standard" and the most successful way of bone regeneration (Gareta, et al., 2015). However, constraints on

availability, high morbidity, and disease transmission risk have encourage alternative approaches such as bone tissue engineering (Chen, et al., 2015).

The fundamental goal of bone tissue engineering is to develop synthetic substitute of bone graft with a medium for scaffolding, functional cells and growth hormones. Biocompatible scaffolds garner substantial interest because of their capability to provide not only structural support to cells but also a cellular-adherence, differentiation and proliferation environment (Chen, et al., 2015).

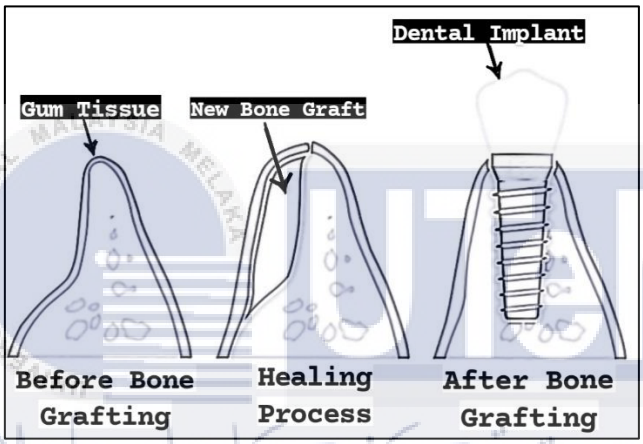

Figure 1.1 Bone Grafting Process

These biomaterial scaffolds with a high affinity for cells and osseointegrative properties are capable of creating an environment conducive to cell development that supports, reinforce, and organises bone tissue, thereby facilitating spontaneous bone regeneration and renewal. (Polo-Corrales, et al., 2014).

Recently, hydroxyapatite has been used in a range of biomedical applications, including drug release controlling matrix and biomaterials for bone tissue engineering. Hydroxyapatite, with the stoichiometric formula [Ca10(PO4)6(OH)2], is a calcium phosphate mineral phase that naturally exist in bone. It is frequently used in a variety of medicinal applications due to its biocompatibility and bioactivity for bone regeneration. (Shi, et al., 2018). Hydroxyapatite

is a well-known bio-ceramic material due to its inorganic composition, which resembles of the natural bones and teeth. Both compact and porosity hydroxyapatite have been extensively explored as implant materials for orthopaedic and dental purposes, and both have demonstrated superior bioactivity, osteoconductivity, and osteoinductivity. (Boutinguiza, et al., 2012).

Due to the ease in which hydroxyapatite can be manufactured or extracted from natural sources, its stability and morphology allow porcine and bovine hydroxyapatite widely used in a variety of tissue engineering applications. However, the manufacturing costs of hydroxyapatite are ridiculously expensive, limiting its use in treatments. (Granito, et al., 2018). Therefore, this project will extract natural hydroxyapatite from waste fish bones, which will reduce manufacturing costs, make the structure biologically safe for humans, acceptable for future medical applications, and halal for Muslims worldwide.

Each year, almost 91 million tonnes of fish and shellfish are caught worldwide. According to the Food and Agricultural Organization (FAO), only around 50%–60% of this catch is utilised for human consumption, while the remainder is deemed waste. Countless by-products and the remainder of the raw materials are wasted, resulting in a negative environmental impact (Boutinguiza, et al., 2012). Hence, a variety of parameters will be considered in the bibliometrics research and analysis conducted as part of this project in order to enhance the efficiency of hydroxyapatite characterization from waste fish product for biological activities in bone tissue engineering.

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1.2 Problem Statement

Hydroxyapatite is a well-known bioceramic substance that is widely employed in a variety of biomedical applications as a biomaterial. Hydroxyapatite is a calcium and phosphorous-based mineral found in human bone and teeth. When natural and synthetic hydroxyapatite are compared, the latter demonstrated better metabolic activity as well as a more dynamic response to the environment than the former (Boutinguiza, et al., 2012).

The disadvantages of synthetic hydroxyapatite powder are that it requires extra chemical to increase its mechanical strength. Furthermore, the process demands an excessive number of substances to maintain the structures in the human body. The synthetic approach is somewhat involved and requires a massive initial investment, in comparison to the expense of extracting natural hydroxyapatite, which is simpler and less expensive (Mustafa, et al., 2014).

On a similar note, it is critical to emphasise that significant quantities of fish scales are generated by fish processing units in coastal cities worldwide. This is because fish is a vital nutritional source and provides a variety of nutrients that are helpful to human health. However, according to reports from the Food and Agricultural Organization (FOA), approximately 40%–50% of total catch is deemed waste. The degradation of these wastes has a harmful effect on the environment, resulting in offensive odours and contamination for the general public. (Yamamura, et al., 2018). Recently, much emphasis has been given to produce hydroxyapatite from biowastes such as marine coral, starfish, and fish bones.

Other than that, due to the high expense of the chemicals used in hydroxyapatite syntheses, many researchers are increasingly turning to natural resources such as animal bones to extract hydroxyapatite. Natural hydroxyapatite was most frequently found in animal bones

such as pig and cattle bones. However, animal diseases such as mad cow disease and food and mouth disease (FMD) have restricted the use of these raw materials. Also, this issue involved halal problems. Thus, religious beliefs were a significant factor in the researcher's quest for other alternatives (Aiza Jaafar, et al., 2017).

Therefore, this research will examine the easy and inexpensive method presented for manufacturing hydroxyapatite from fish bones via calcination. Occasionally, fish skin is still consumed and processed, although fish bones are frequently discarded as waste. Thus, by utilising fish bones as a source of hydroxyapatite, we may be able to minimise waste while producing a valuable fish product.

1.3 Research Objective

The objective of this project is:

- To analyse the potential of hydroxyapatite from fish bone for bone tissue regeneration using bibliometric study.
- To prepare natural hydroxyapatite from fish by product through calcination process.
- To characterize the hydroxyapatite properties using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) analysis.

1.4 Significant of Study

The research is about investigating the suitable method or technique to extract hydroxyapatite from waste fish bones that have certain physical characteristics that resemble the mammalian hydroxyapatite for use in bone tissue regeneration applications. The analysis

involves scientometric and bibliometric analysis as well as discussion about various parameters that could potentially be used for hydroxyapatite extraction. If the project objective is met, a more suitable hydroxyapatite product that applies to all background communities can be made. Plus, fish waste products in the aquaculture industries can be reduced while making full use of their potential substance content. Lastly, reducing the cost of demand for bone tissue regenerating applications.

1.5 Scope of Study

In order to achieve this objective, the scope of study is prepared as shown below:

1. Analyse the trend in early articles and citations concerned with quantitative features and characteristics of science to find the formula for fish bone hydroxyapatite extraction.
2. Perform bibliometric analysis across Scopus database through keyword, author and country research while utilizing Vos viewer software to abstract quantitative paper relating to hydroxyapatite research in biomedical application.
3. Review all possible methods involved regarding the process of hydroxyapatite extraction from different kinds of fish bones at different times and temperatures to conclude the recommended condition.
4. Study the characterization of natural hydroxyapatite extracted from fish by product through various calcination temperature using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Scientometrics Study

Scientometrics is a study that focuses on the quantitative aspects and characteristics of science, science communication, and science policy. Research methods encompass quantitative aspects of scientific knowledge production, transmission and utilisation with a goal of enhancing the knowledge of scientific research mechanisms as a social activity (Chellappandi & Vijayakumar, 2018). The major aim of the Scientometrics Study is to measure and understand the effect of research publications and academic journals (Leydesdorff & Milojević, 2012). Scientometrics is interconnected and shares similar interests with bibliometrics, informetrics, altmetrics, and webometrics (Chellappandi & Vijayakumar, 2018).

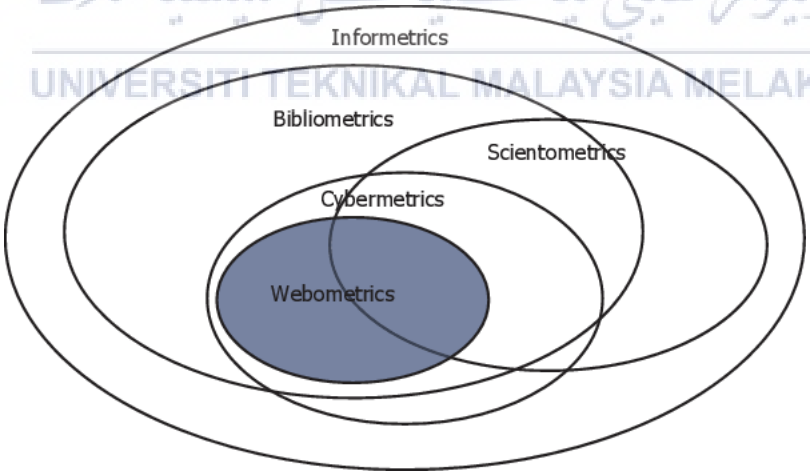

Figure 2.1 Björneborn and Ingwersen (2004, p. 1217)

2.1.1 Scientometrics Terminology

There are several different forms in the domain of Scientometrics co-citation analysis. The Document Co-citation Analysis (DCA) is one of the most prominent since it helps us to understand the relationship between various documents. DCA helps to comprehend the relationships among various papers when compared to Author Co-citation Analysis (ACA), because ACA does not consider the citation content. Others include Country Co-Citation (CCA) and Journal Co-Citation Analysis (JCA) research. These co-citation techniques can then be used to build groups and clusters to find significant groupings of connected articles, authors, journals, etc. The results can be validated using the network built by means of two types of measurements; temporal metrics and structural metrics.

2.2 Bibliometrics Study

The Bibliometrics study reveals key articles and objectively displays the connections among books, articles and other publications on a given research theme or field by mathematically assessing how many times other published articles co-cited them (Fetscherin & Usunier, 2012). Bibliometrics can be defined as a quantitative assessment of the science and technological performance of a journal in any field, country, or region (Utap Anyi, Zainab, & Anuar, 2009). The scientific communication between researchers through scientific literature, such as books, articles, research papers, and academic journals, is fundamental to these studies. The output and impact are key elements for bibliometric analysis evaluated through publications and citations.