EXTRACTION AND CHARACTERIZATION OF HALAL GELATIN FROM WASTE BY-PRODUCT (BLACK TILAPIA SKIN)

NORHASIKIN BINTI ISMAIL

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Universiti Tun Hussein Onn Malaysia

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To my precious Allah S.W.T.,
who gave me new life, hope and purpose of life.

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ABSTRACT

Gelatin is one of the most widely used biopolymers, derived from collagen of mammalian species that commonly apply in food and non-food industries. Since the porcine and bovine are mostly sources used from the total of the production, there is significant interest in the Halal or Kosher market. Thus, fish and other marine sources are being exploited simultaneously. However, fish gelatin has certain inferiorities compared to mammalian gelatin in terms of low gel strength and viscosity. Therefore, this study aims to extract gelatin from Black Tilapia fish skins, with a focus on extraction methods for producing high quality gelatin properties includes gel strength, viscosity, melting temperature and wettability. Gelatin was extracted by using hydrochloric acid (HCl) as a pre-treatment and thermal extraction with controlling acid concentration (0.03, 0.05, 0.1, 0.15 and 0.2 M), pre-treatment time (4, 16 and 24 h), thermal extraction temperature (45, 65 and 85 °C) and extraction time (4, 16 and 24 h). The effects of ultraviolet (UV) light on the properties of gelatin has been studied. Gelatin has been exposed to Ultraviolet A (UVA) and Ultraviolet C (UVC) for 0.5 to 2 h. The gelatin yield, gel strength, viscosity, melting temperature and amino acid composition were determined. From the obtained results, the suitable condition for pre-treatment is at 0.03 M HCl with 4 h duration. The gelatin yield, gel strength, viscosity and melting temperature of gelatin extracted at 45 °C for 4 h were found 26.35 ± 0.11 %, 286.7 ± 2.52 g, 8.43 ± 0.3 cP and 29.5 ± 0.8 °C, respectively. Gelatin has been successfully extracted from Black Tilapia skin with acid extraction. Gelatin treated with UVA and UVC has improved the gel strength (22.67 %) and viscosity (18.03 %) simultaneously. Results indicated that employing UV treatment can enhance the properties of gelatin.

ABSTRAK

Gelatin merupakan salah satu biopolymer yang paling banyak digunakan yang berasal dari kolagen spesis mamalia yang biasanya digunakan dalam industri makanan dan bukan makanan. Oleh kerana kebanyakan sumber yang digunakan dari pengeluaran gelatin adalah daripada babi dan lembu, terdapat keperluan yang besar di pasaran Halal dan Kosher. Oleh itu, ikan dan sumber laut dieksploitasi secara serentak. Walau bagaimanapun, gelatin ikan mempunyai kekurangan yang tertentu berbanding gelatin mamalia dari segi sifat seperti kekuatan gel dan kelikatan yang rendah. Oleh itu, tujuan kajian ini adalah untuk mengekstrak gelatin dari kulit ikan tilapia hitam, dengan memberi fokus kepada kaedah pengekstrakan untuk menghasilkan sifat gelatin yang berkualiti tinggi merangkumi kekuatan gel, kelikatan, suhu lebur dan kebolehbasahan. Gelatin diekstrak dengan menggunakan asid hidroklorik (HCl) sebagai pra-rawatan dan pengekstrakan terma dengan pengawalan kepekatan asid (0.03, 0.05, 0.1, 0.15 dan 0.2 M), masa pra-rawatan (4, 16 dan 24 jam), suhu pengekstrakan (45, 65 dan 85 °C) dan masa pengekstrakan (4, 16 dan 24 jam). Kesan sinar ultraungu (UV) terhadap sifat gelatin telah dikaji. Gelatin telah di dedahkan pada UVA dan UVC selama 0.5 hingga 2 jam. Keputusan hasil gelatin, kekuatan gel, kelikatan, suhu lebur dan komposisi asid amino telah ditentukan. Dari hasil yang diperolehi, keadaan yang sesuai untuk pra-rawatan adalah pada 0.03 M HCl dengan jangkamasa 4 jam. Hasil gelatin, kekuatan gel, kelikatan dan suhu lebur gelatin yang diekstrak pada 45 °C selama 4 jam didapati masing-masing menunjukkan 26.35 ± 0.11 %, 286.7 ± 2.52 g, 8.43 ± 0.3 cP dan 29.5 ± 0.8 °C. Gelatin telah berjaya di ekstrak daripada kulit ikan tilapia hitam dengan pengekstrakan asid. Gelatin yang dirawat dengan UVA dan UVC dapat meningkatkan kekuatan gel (22.67 %) dan kelikatan (18.03 %) secara serentak. Hasil menunjukkan bahawa dengan menggunakan rawatan UV dapat meningkatkan sifat gelatin.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percentage
°C	-	Degree Celcius
AOAC	-	Association of Official Analytical Chemists
ATR	-	Attenuated Total Reflectance
BSE	-	Bovine Spongiform Encephalopathy
BSI	-	British Standard Instituition
$C_2H_4O_2$	-	Acetic Acid
$C_3H_6O_3$	-	Lactic Acid
$C_6H_8O_7$	-	Citric Acid
CaO	-	Calcium Oxide
$Ca(OH)_2$	-	Lime solution
CH_3COOH	-	Acetic Acid
COOH	-	Carboxyl
cm	-	Centimeter
cP	-	Centipoise
EDX	-	Energy Dispersive X-Ray Spectroscopy
FAO	-	Food and Agriculture Organization
FMD	-	Foot and Mouth Disease
FTIR	-	Fourier Transform Infrared Spectroscopy
g	-	Gram
gL^{-1}	-	Gram per liter
GME	-	Gelatin Manufactures of Europe
GMIA	-	Gelatin Manufactures Institute of America
h	-	hour
H_2O_2	-	Hydrogen Peroxide
H_2SO_4	-	Sulphuric Acid
H_3PO_4	-	Phosporic Acid

HCl	-	Hydrochloric Acid
HPLC	-	High Performance Liquid Chromatography
JAKIM	-	Jabatan Kemajuan Islam Malaysia
kDa	-	kilodaltons
kg	-	Kilogram
kV	-	kilo Volts
L	-	Liter
M	-	Molar
min	-	Minute
mm	-	Millimeter
mM	-	Millimolar
MPa	-	Mega Pascal
$MgSO_4$	-	Magnesium Sulfate
N	-	Normality
NaCl	-	Sodium Chloride
NaOH	-	Sodium Hydroxide
NCR	-	No Carbon Required
NH_2	-	Amino
nm	-	Nanometer
pH	-	Potential of hydrogen
rpm	-	Revolutions per Minute
SEM	-	Scanning Electron Microscopy
T	-	Temperature
TGA	-	Thermogravimetric Analysis
UK	-	United Kingdom
USA	-	United States of America
UTHM	-	Universiti Tun Hussein Onn Malaysia
UV	-	Ultraviolet
UVA	-	Ultraviolet A
UVB	-	Ultraviolet B
UVC	-	Ultraviolet C
WCA	-	Water contact angle
w/v	-	Weight per volume
µL	-	Microliter

μm	-	Micrometer
μM	-	Micromolar

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Gelatin is one of the biopolymers that widely used in food, cosmetic, pharmaceutical and photographic industries. It has the characteristic of being colourless, translucent, brittle, edible and flavourless (Arpi *et al.*, 2018). In the food industry, gelatin is mainly used in confections that provide chewiness, texture and foam stabilization while in the pharmaceutical industry, it is used in the manufacture of capsules including hard and soft capsules (Karim and Bhat, 2009). Most of the worldwide gelatin production is contributed to the food industry which consists of about 70% while 15% is used in the pharmaceutical industry, thus 10% is taken by the photographic industry and the rest is used for other industry such as cosmetics and technical applications (Ofari, 1999).

According to Tkaczewska *et al.*, 2018, the usage of gelatin in industrial applications has raised the global demand for gelatin enormously in recent years, with an expected gelatin output of 450,000 tons in 2018. Currently, only 1.5 % of gelatin is produced from fish collagen-containing raw materials, while 41 % is produced from pig-skin, 28.5 % from bovine hides and 29.5 % from bovine bones (Milovanovic, 2018). However, the use of gelatin extracted from porcine and bovine sources is restricted due to the religious issue and consider on serious risk for human health effect such as foot-and-mouth disease (FMD) and outbreaks of bovine spongiform encephalopathy (BSE) called “mad cow disease” (Ratnasari *et al*., 2013).

Porcine and bovine gelatin sources are commonly used around the world, accounting for 98.5 % of the world's gelatin supply (Sow and Yang, 2015). These existing gelatins in Islamic countries do not comply with the criteria for the Halal market as for use or consumption of the pork-related product is prohibited (Sanaei *et al.*, 2013; Riquelme *et al.*, 2015). Issues of non-Halal gelatin in packed food and cosmetic products have recently brought up sensitive conflicts among Malaysians. Malaysia is a multiracial country in which Islam is the largest community followed by Buddhism, Christianity, Hinduism and other religion. Muslims are prohibited to consume pork-related products while Hindus and Buddhists are forbidden to consume any cow-related products due to their belief. Hence, both porcine and bovine gelatins have limitations of consumption and utilization due to religious outlook.

Due to that issue, many researchers nowadays explore other sources that can produce gelatin such as marine by-products (fish) including skins, scales and bones which can be acceptable, especially for Islam and also can be used with minimally restricted for Judaism and Hinduism (Nik Aisyah *et al.*, 2014, in *et al.* 2017). Thus, collagen and gelatin extracted from marine by-products, especially from fish waste give a better advantage and increase the economic return for the fish-processing industry (Regenstein & Zhou, 2006). Fish gelatin is free from disease and is also recognized by all religions in Malaysia as Halal gelatin.

Fish resources in Malaysia are well-known categorized into freshwater and saltwater. More than 100 million tons of various species of fish have been harvested annually throughout the world. From the data reported, tilapia fish was second harvesting about 6 million tons per year throughout the world (FAO, 2018). Fish-based industries in Malaysia, such as canned fish and fillet processing, are rapidly growing due to high consumer demands. These fish processing industries produce a high quantity of fish waste as much as 70 – 85 % of the total catch, which leads to environmental issues (Kristinsson and Rasco, 2000). Since fish skins and bones contribute almost 30 % of the total fish weight, the use of these wastes from the fishery industry is excellent for the preparation of high protein food especially, gelatin. Conversion of these wastes into value-added products has both economic and waste management benefits for the fish industry (Choi and Regenstein, 2000). The production of gelatin from fish wastes has gained considerable attention recently (Etxabide *et al.*, 2016).

Table 1.1 shows several types of sources that can be extracted to produce collagen and gelatin. There are three types of sources of marine collagen and gelatin: marine invertebrates, sea mammals and fishes.

Table 1.1: Sources of marine collagen and gelatin (Regenstein and Zhou, 2006)

Types of Marine Species	**Species**	**Tissues**
Invertebrate	Cuttlefish, octopus and squid Jellyfish Sea urchin Sea cucumber	Outer skin and cartilaginous tissue Exumbrella and mesogela Body wall Test (or shell) Body wall
Sea mammal	Seal and whale	Skin
Fish	Lawless fish Cartilaginous fish Bony fish	Skin, notochord and cartilaginous tissues Skin and cartilage Skin, scale, bone and swim bladder

This study was focused on the Black Tilapia fish which is categorized as a freshwater fish to extract the gelatin. The skins of fish were used as sources of gelatin focusing on the method of extraction and the characterization of the gelatin produced. The aim of this study is to produce a better quality of gelatin with effective methods.

1.2 Problem Statements

Gelatin extracted from fish is one of the alternative sources besides porcine and bovine. Gelatin from fish has an advantage as they are not associated with the risk of FMD and BSE. Besides, fish gelatin is acceptable for Muslims and also for Judaism and Hinduism. For example, in Malaysia, there was an issue regarding the Cadbury chocolate that contains non-halal gelatin from porcine which affected to the business and reputation of the company. Due to those issues, the demand for halal foods has created fish gelatin which can apply to related industries.

Badii and Howell, (2006) reported that the industry of fish processing produced a bulk of waste including skin, bones and scales that were cause waste and pollution (Badii and Howell, 2006). The processing of fish leads to enormous amounts of waste. About 25% of the total caught fish weight was used for the filleting industry and the rest was considered discard (Gomez-Guillen *et al*., 2002).

The total world volume of fishing in 2018 is estimated at 179 million tons, and the figure is increasing every year (FAO, 2020). The waste from fish processing can increase up to 85 % of the total catch as estimated (Wasswa *et. al,* 2007 and Salvatore *et. al,* 2020). According to Ahmed *et. al,* 2020, a significant percentage of waste includes skin (about 30 %), bones and scales which contain a high of collagen. Therefore, the production and use of fish as a source of gelatin seem to be very promising.

Due to that problem, this research has used waste by-products of black tilapia fish skin as a source to become a valuable halal gelatin product that helps to eliminate harmful environment and improve the quality of the fish industry. Fish skin also has received attention as an alternative raw material that has the potential for producing high-quality gelatin.

1.3 Objectives

i. To identify suitable pre-treatment parameters (hydrochloric acid concentration and time) and thermal extraction parameters (temperature and time) for extraction of gelatin from black tilapia skin
ii. To investigate the influence of thermal extraction time and temperature on the properties of gelatin from black tilapia skin
iii. To determine the effects of UVA and UVC irradiation in enhancing the properties of gelatin from black tilapia skin

1.4 Scope of Study

The scopes of this research are:
The first stage involved the procedure on study the characteristics of the raw materials (Black Tilapia skins);

i. Raw material: waste by-product (Black Tilapia skin) from fillet industry [Trapia (M) Berhad].
ii. Surface morphology of raw material was undergoing Scanning Electron Microscopy (SEM).
iii. Proximate composition of raw material was analyzed according to AOAC (2000) methods.

iv. The thermal stability of raw material was analyzed by Thermogravimetric Analysis (TGA).

The second stage in this study involved the characterization of gelatin which includes the preparation of gelatin extraction;

i. Pre-treatment process by using hydrochloric acid with concentration of 0.03, 0.05, 0.1, 0.15 and 0.2 M at various times of 4, 16 and 24 hours.
ii. Thermal extraction of gelatin with temperatures of 45, 65 and 85°C and duration time of 4, 16 and 24 hours.
iii. Filtration process by using Whatman filter paper No. 4.
iv. Drying process for gelatin with oven at a temperature of 60°C for 48 h.
v. Color determination via visual observation.
vi. Gel strength of gelatin was determined according to British Standard (BS 757:1975).
vii. Viscosity of gelatin was determined according to Killekar *et al.*, 2012 method.
v. Melting temperature of gelatin was determined according to Koli *et al.*, 2012 method.
vi. Amino acid composition of gelatin was analyzed according to AOAC (2000) method.

The third stage in this study was involved the treatment to enhance the properties of gelatin, which includes;

i. Irradiation via Ultraviolet A (UVA) and Ultraviolet C (UVC)
ii. Irradiation time of 0.5, 1.0, 1.5 and 2.0 hours.

1.5 Significance of Study

This study is focused on extracting Halal gelatin which is not only acceptable to Muslim consumers, but also to all Malaysian. Fish gelatin can be used as an alternative to replace mammalian gelatin, especially gelatin that extracted from bovine, which we consider the issues of an outbreak of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD). BSE are progressive neurological disorders affecting

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VITA

The author was born on 1st May 1981 in Muar, Johor. She went for secondary education in Sekolah Menengah Teknik Johor Baru, Johor. She pursued her higher education at the Universiti Teknologi MARA (UiTM), Selangor, and graduated with the Diploma in Industrial Chemistry in 2000. Upon graduation, she continue her degree and graduated in B. Eng (Hons) in Chemical Engineering at the same institution in October 2005. After graduation, she worked as a Laboratory Executive in Cylin Cosmetic Sdn. Bhd., Muar and then moved to Kotra Pharma (M) Sdn. Bhd. in Melaka worked as a Chemist. In June 2009 she got the opportunity to work at Universiti Tun Hussein Onn Malaysia (UTHM), Johor as a tutor at Faculty of Mechanical & Manufacturing Engineering. Realized the importance of knowledge level as an academician, she then pursued her studies at the University Kebangsaan Malaysia (UKM), Selangor and she was awarded the M. Eng. in Mechanics in 2012. In June 2014, she decided to further pursue her studies at UTHM to complete her Ph.D. in Mechanical Engineering under Department of Materials Engineering and Design. Her field of studies is in biomaterials engineering which focusing in extraction and characterization of gelatin from fish wastes. During her Ph.D. journey, she had produces 3 technical papers. She is a currently a member of Board of Engineers Malaysia (BEM) and Institute of Engineers Malaysia (IEM).