# OPTIMIZATION AND CHARACTERIZATION OF HALAL GELATIN FROM FISH SKIN USING HIGH PRESSURE PROCESSING (HPP)

BY

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy Engineering

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FEBRUARY 2020

# ABSTRACT

Gelatin is an essential component in the food industry mainly originated from porcine and bovine's skin and bones. Due to religious and health concerns, alternative sources of gelatin were explored, including fish skin. However, the current extraction method is time consuming and the chemicals used bring adverse implications to the environment. Besides, fish gelatin is known to have weaker properties as compared to the mammalian gelatin. Chemical treatment is often used to overcome the problem. However the gelatin extract usually has high chemical residues. As such, the High Pressure Processing (HPP) is a novel, efficient and environmental friendly method for gelatin extraction due to reduction in processing time and chemical waste while enhancing the gelatin properties and increasing the gelatin yield. This research studied the performance of the HPP during extraction process. First, HPP was applied either during pretreatment or during thermal hydrolysis for four types of fishes, namely red tilapia (*Oreochromis niloticus*), black tilapia (*Oreochromis mossambicus*), grouper (*Epinephelus areolatus*) and the threadfin fish (*Nemipterus tambuloides*). Based on the yield of HPP during the pretreatment, red tilapia skin produced the highest amount of extract, followed by grouper, black tilapia and threadfin skin, with 32.1%, 30.1%, 21.75% and 15.75, respectively. In contrast, HPP applied during the thermal hydrolysis produced an insignificant amount of gelatin extract. Thus, red tilapia skin and HPP during pretreatment were chosen for optimization study while parameters of pressure, the pressure holding time, ratio skin to acid and extraction time were chosen as optimization parameters. Results from OFAT and FCCCD in RSM suggested that the optimum parameters for gelatin extracted from red tilapia fish skin pretreated with HPP are 250 MPa pressure, 10 min pressure holding time, 1:7.5 ratio solid to acid and 12 hours extraction time, with maximum protein concentration (19.51 mg/ml) and highest gelatin yield (320.4 mg/g). Later, characterization of the produced gelatin were done. Gel strength and thermal stability of the HPP-treated gelatin have increased significantly as compared to conventional gelatin, caused by the modification of the gelatin structure during pressure treatment. On the other hand, the amount of amino acid content declines when excessive pressure was applied because it damaged the protein structure. Other gelatin's properties display similar results with the conventional gelatin, showing the optimum pressurization preserved the properties of the gelatin protein. For kinetic modelling study, Power Law Model was selected as the best model with the 0.93 regression coefficient, 0.07 RMSD and 3.45% P. Lastly, the performance of the gelatin-treated with HPP in the food product has been tested using Texture Profile Analysis (TPA) vis-à-vis commercial fish gelatin. Six textural parameters i.e., hardness, adhesiveness, gumminess, chewiness, cohesiveness and springiness were evaluated. Results showed jelly prepared with HPP treated gelatin is preferred because the gummy is more rigid, firm and adhesive. In conclusion, application of HPP in gelatin extraction is effective in enhancing the production and properties of the fish gelatin. These findings are very beneficial to the food industry, especially in the halal food production due to its ability to increase the production and to enhance the properties of halal gelatin, which may enable the HPP-gelatin to replace the use of mammalian gelatin in the industry.

# خلاصة البحث

الجيلاتين هو عنصر أساسي في صناعة الأغذية. المصادر الرئيسية للجيلاتين هي جلود وعظام البقر والخنزير. بسبب المشاكل الدينية والصحية، تم اكتشاف مصادر بديلة للجيلاتين، بما في ذلك جلد السمك. ومع ذلك، فإن طريقة القاعدة الحمضية التقليدية تستغرق وقتا طويلا وتسبب مشكلة بيئية بسبب المادة الكيميائية المستخدمة. بالإضافة إلى ذلك، من المعروف أن جيلاتين السمك له خصائص أضعف بالمقارنة مع جيلاتين الثدييات. يتم استخدام العلاج الكيميائي في كثير من الأحيان للتغلب على المشكلة. ومع ذلك، فإن المواد الكيميائية لديها إمكانية عالية للبقاء في مستخلص الجيلاتين. المعالجة بالضغط العالي (HPP) هي طريقة جديدة صديقة للبيئة لاستخراج الجيلاتين. يمكن تطبيق HPP في استخراج الجيلاتين في أقل وقت، وذلك يقلل من النفايات الكيميائية ويعزز خصائص الجيلاتين مع زيادة إنتاجه. في هذه الدراسة، تم التحقق من أداء HPP أثناء عملية الاستخراج. في البداية، تم تطبيق HPP إما خلال المعالجة المسبقة أو خلال التحلل الحراري لأربعة أنواع من الأسماك، وهي: البلطي الأحمر (Oreochromis niloticus)، البلطي الأسود (Oreochromis mossambicus)، الهامور (Epinephelus areolatus) وسمك التراب الخيطي (Nemipterus tambuloides). استناداً إلى المحصول، أنتج جلد السمط البلطي الأحمر كمية أعلى من مستخلص الجيلاتبن، يليها الهامور، البلطى الأسود والبلطى الخيطي، مع 32.1٪ و 30.1٪ و 21.75٪ و 15.75 على التوالي. إلى جانب ذلك ، HPP ثبت أن التحلل الحراري ليست مهماً لاستخراج الجيلاتين. وهكذا، تم اختيار جلد البلطي الأحمر و HPP أثناء المعالجة من أجل دراسة أمثلية مع أربعة معايير مستقلة، والتي كانت: الضغط، ووقت الضغط، ونسبة الجلد إلى الحمض ووقت الاستخراج. اقترحت النتائج من OFAT و FCCCD في RSM أن المؤشرات المثلى لاستخراج الجيلاتين من جلد أسماك البلطي الأحمر المعالجة مسبقًا مع HPP هي 250 ميغا باسكال للضغط، و 10 دقائق من وقت الضغط، و 1: 7.5 نسبة المواد الصلبة إلى الحامض و 12 ساعة من وقت الاستخراج، مع الحد الأقصى من البروتين التركيز (19.51 مجم / مل) وأعلى محصول للجيلاتين (320.4 ملجم / جم). تم وصف الجيلاتين الأمثل بالمقارنة مع الجيلاتين التقليدي. زادت قوة الجل والاستقرار الحراري للجيلاتين المعالجة بـ HPP بشكل ملحوظ مقارنة بالجيلاتين التقليدي. تعديل ارتفاع ضغط هيكل الجيلاتين. تم تعزيز البلمرة الجزيئية أثناء الضغط من خلال تجلط الدم المكون البروتيني، مما يجعل محتوى مكونات الجزيئات العالية للجيلاتين يزداد. ينخفض محتوى الأحماض الأمينية عند تطبيق الضغط الزائد، من 601.6 ملجم /جم عند 250 ميغاباسكال إلى 546.08 ملجم / جم عند 350 ميغا باسكال بسبب الضغط الذي يتجاوز الحد الذي يضر بنية البروتين. وتظهر خصائص الجيلاتين الأخرى نتائج مماثلة مع الجيلاتين التقليدي، والتي تبين التأثيرات الإيجابية لتكنولوجيا HPP على نشاط الاستخراج. بالنسبة لدراسة النمذجة الحركية، تم اختيار موديل Power Law كأفضل نموذج بمعامل انحدار 0.93 ، RMSD 0.07 و 3.45٪ .P تم اختبار أداء الجيلاتين المعالج بـ HPP في المنتج الغذائي باستخدام Texture Profile Analysis (TPA) إلى الجيلاتين السمك التجاري. تم تقييم ستة معايير نصية، والتي كانت صلابة، الالتصاق، التعلك، المضغ، التماسك والمرونة. أظهرت النتائج أن الهلام المحضر مع الجيلاتين المعالج من HPP هو الأفضل لأن الصمغ يكون أكثر صلابة وثباتًا ولصقًا. وفي الختام، فإن تطبيق تقنية HPP في استخراج الجيلاتين له تأثير كبير على إنتاج وخصائص جيلاتين الأسماك. هذه النتائج مفيدة جدًا للصناعات الغذائية، وخاصة في إنتاج الغذاء الحلال. هذا لأنه يمكن أن يزيد من إنتاج الجيلاتين الحلال وتحسين خصائص تسمح ل HPP-gelatin أن يحل محل استخدام الجيلاتين المستخلص من الثدييات في هذه الصناعة.

# APPROVAL PAGE

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# DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Signature ..... Date .....

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By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

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# ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious, The Most Merciful.

Alhamdulillah, praises and thanks to Allah the Almighty who has given me the strength and determination to complete this study and salam to His messenger, Prophet Muhammad S.A.W.

I would like to express my sincere gratitude to my advisor Prof. Irwandi Jaswir for the continuous support of my Ph.D study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. Besides my advisor, I would like to thank the rest of my thesis committee: Prof. Parveen Jamal and Prof. Mohammad Saedi Jami for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives.

It is my utmost pleasure to dedicate this work to my family, especially my dear parents, Yusof bin Ismail and Hamidah Kamai for their unending support, love, pray and belief throughout writing this thesis and my life in general.

I also wish to express my appreciation and thanks to those who provided their time, effort and support for this project, especially the member of Molecular Laboratory of IIUM.

Finally, special acknowledgement to Ministry of Higher Education for financial support under MyBrain PhD program.

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# LIST OF SYMBOLS

| | |
|---|---|
| %P | Mean relative percentage deviation |
| °C | Degree (temperature) |
| µl | Micro-liter |
| ΔH | Enthalpy |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| b | Billion |
| Adj R-Squared | Adjusted R-Squared |
| ANOVA | Analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| APS | Ammonium per sulphate |
| BME | 2-mercaptoethanol |
| BSA | Bovine serum albumin |
| BSI | British Standards Institution |
| BT | Black tilapia fish |
| $C_2H_4O_2$ | Sulfuric acid |
| $C_6H_8O_7$ | Citric acid |
| CAGR | Cumulative Annual Growth Rate |
| cm | Centimetre |
| CV | Coefficient of variance |
| CSNPs | Groups of hydroxyl and amino of chitosan |
| DNA | Deoxyribonucleic acid |
| DSC | Differential Scanning Calorimetry |
| DSM | Departments of Standards Malaysia |
| etc | Etcetera |
| FCCCD | Faced Centered Central Composite Design |
| FESEM | Field Emission Scanning Electron Microscopy |
| FTIR | Fourier transform infrared spectroscopy |
| g | Gram |
| Gly | Glycine |
| GMIA | Gelatin Manufacturers Institute of America |
| GR | Grouper fish |
| HCl | Hydrochloric acid |
| HDC | Halal Industry Development Corporation |
| HFCE | Halal Food Council of Europe |
| HHP | High Hydrostatic Pressure |
| HPE | High Pressure Extraction |
| HPLC | High Performance Liquid Chromatography |
| HPP | High Pressure Processing |
| hrs | Hours |
| Hyp | Hydroxyproline |
| IIUM | International Islamic University Malaysia |
| IMF | International Monetary Fund |
| INHART | International Institute for Halal Research and Training |
| JAKIM | Department of Islamic Development Malaysia *(Jabatan Kemajuan Islam Malaysia)* |
| kDa | Kilo dalta |
| kg | Kilogram |
| MBE | Mean Bias Error |
| mg | Milligram |
| min | Minute |

| | |
|---|---|
| mL | Mili-liter |
| MPa | Mega Pascal (SI derived unit of pressure or stress) |
| MS | Malaysia Standard |
| MT | Metric tan |
| N | Newton |
| NaOH | Sodium hydroxide |
| nm | Nanometer |
| OFAT | One-Factor-At-Time |
| P | Mean Relative Percentage Deviation |
| pI | Isoelectric point |
| Pred R-Squared | Predicted R-Squared |
| Pro | Proline |
| PVT LTD | Private Limited |
| $R^2$ | Regression coefficient |
| RM | Ringgit Malaysia |
| RMSD | Root Mean Square Deviation |
| RSM | Response Surface Methodology |
| rt | Room temperature |
| RT | Red tilapia fish |
| s | Second |
| S1 | Gelatin extracted using traditional acid-base method |
| S2 | Gelatin extracted using HPP during pre-treatment |
| S3 | Gelatin extracted using HPP during thermal hydrolysis |
| SDS | Sodium dodecyl sulphate |
| SDS PAGE | Sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| SME | Small and Medium Enterprises |
| SS | Soluble solid |
| t | Time |
| T | Temperature |
| Tf (DSC) | Endset temperature |
| To (DSC) | Onset temperature |
| Tp (DSC) | Peak temperature |
| TPA | Texture Profile Analysis |
| TR | Threadfin bream |
| UHP | Ultra-High Processing |
| UN | United Nation |
| USD | United State Dollar |
| UV | Ultra violet |
| V | Voltan |
| vs | Versus |
| w/v | Weight per volume |
| X1 | Pressure (MPa) |
| X2 | Amount of acid (ml) for 1g of skin, |
| X3 | Extraction time (Hours) |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

The integrity of halal products encompasses the whole supply chain, from farm to table, which means the integrity of the ingredients, the process, handling and the product distribution. Assurance the halal ingredient integrity is important since it is the first measures of ensuring the halalness of a product. Malaysia is a leader in the world's halal industry (Muhammad, 2017). The country has knowledge and capabilities related to this area and providing training and guidance to other nations regarding the halal and haram issue. International Islamic University Malaysia (IIUM) also playing a role in offering expertise and conducting researches concerning halal food (Jaswir & Ramli, 2018). These activities are carried out by leveraging the expertise through collaboration with various departments within IIUM, with the supervision of International Institute for Halal Research and Training (INHART) and Department of Islamic Development Malaysia (JAKIM). The expected outcomes from the association will strengthen the credibility of halal products, services and systems which will further support Malaysia as a global halal hub and governance.

Gelatin is an essential component in the industry nowadays. It is used widely in the food industry (confections, low-fat spread, dairy, baked goods, meat products, beverages) (Schrieber & Gareis, 2007), pharmaceutical and medical applications (Chapman, 2014; Winfield, 2014) and cosmetics (face mask, shampoo) (Hattrem & Draget, 2014). Nevertheless, issues related to religion and health has led researchers to

look for other alternatives to replace gelatin from pigs and unslaughtered bovine. It leads to the discovery of the latest source of gelatin, which are fish skins, scales and bones. However, disadvantages of gelatin from fish are it requires long production time and has low quality compared to mammalian gelatin (Zhang et al., 2016). Since then, numerous number of research has been done to overcome the shortcomings and to improve the gelatin structure and its properties.

High Pressure Processing (HPP), also known as High Hydrostatic Pressure Processing (HHP) or Ultra High-Pressure Processing (UHP), is a preservation method of food processing where food is subjected to elevate pressures (up to 87,000 pounds per square inch or approximately 6,000 atmospheres), with or without the addition of heat, to alter the food attributes and/or to achieve microbial inactivation in order to achieve consumer-desired qualities (Dumay et al, 2010). HPP is an emerging novel technology and has gained tremendous popularity in the food industry in recent years. Until recently, several food products and food ingredients prepared using HPP technology has been commercialized such as milk, fruit jams and fruit juices, as well as nano encapsulation of liposome products (Rao et al., 2014). However, application of HPP as a new practice in isolating active components from raw material is increasing (Huang et al., 2017). Recently, high pressure technique was introduced as a novel method for gelatin extraction from fish skin. Literatures have shown that the gelatin yield increased and the structure of the gelatin improved when HPP applied to the process (da Silva et al., 2011; Gómez-Guillén et al., 2005; Nan et al., 2018).

In this project, gelatin was extracted from fish skin, assisted by HPP methods. The effects of high pressure on the gelatin extract will be tested and analysed.

## 1.2 STATEMENTS OF THE PROBLEM

Production of the gelatin always facing halal issue due to its source. Generally, gelatin has been obtained from mammalian skin and bones. Almost 400,000 metric tonnes of collagen-gelatin protein were produced annually (Karim & Bhat, 2009) and the number increases every year due to high demand. However, nearly 75% of the extract obtained from non-halal source, such as pig and unslaughtered mammalians (Hu, 2012). This raise a critical halal issue for 1.67 b Muslim population worldwide (Kenny, 2015) as any food produced from pig and unslaughtered animals (except from marine source) are haram to be consumed by Muslim (Al-Qardawi, 2007). Studies have shown that fish-by-product can be an alternative source for gelatin and collagen extract (Thies, 2012) because product from fish are free from any religious, ethical and health issues. Nevertheless, the protein extracted from fish cover only 1% from the total amount of the commercial gelatin produced globally (Gudipati, 2013). Thus more studies are required to increase the production of gelatin from halal source.

Waste generated from the marine industry is increasing and creating an environmental problem (Srikanya et al., 2017). Reports shown that 30% from 75% of fish by-product consist of skin, scale and bone (Ehrlich, 2014). Prolonged discarded fish wastes contributed to insufficient littering space, bad odour issue and flies and maggots which can cause health problems if not controlled (Penven et al., 2013). Fish is a good source of protein (Sadiq et al., 2018), yet the removals occur continuously and contributed to human loss. Realizing the indefinite advantages of fish waste, scientist and researcher began their work to utilize the cheap marine source into valuable products (pharmaceuticals, food ingredients etc) (Kiew & Mat Don, 2012; Kumar et al., 2018). One of the solution is to generate gelatin protein from fish skin waste.

Traditional acid-base extraction method has several disadvantages. The conventional method is time-consuming (Kumar et al., 2017; Liqing et al., 2012). At least 24 hours are required to produce one batch of the fish protein. Besides, the gelatin extracted from fish has weaker properties compared to gelatin produced from mammalian, such as low gel strength and low melting point (Meng & Cloutier, 2014). These problems limit the use of fish gelatin in the industry. Chemical treatment often being used to enhance the properties of fish gelatin (Karim & Bhat, 2009; Kittiphattanabawon et al., 2016; Ktari et al., 2014; Manjula et al., 2015; Niu et al., 2013; Ratnasari et al., 2014; Tabarestani et al., 2014; Tabarestani et al., 2010; Yang & Shu, 2014; Zhang & Regenstein, 2017 ), but it is also possible to leave residues in the gelatin extract (da Silva & Pinto, 2012). The need for new procedure that could reduce the operation time while enhancing the fish gelatin properties is critical. Studies shown that HPP can reduced more that 50 percent of processing time, increasing the gelatin yield and enhancing the structure of the protein produced (Chang, Niu, Tang, & Wang, 2013; da Silva & Pinto, 2012; Gómez-Guillén et al, 2005; Liqing et al., 2012; Zhang et al., 2016; Zhang, Ma, & Shi, 2011).

Despite being used widely in the food industry (Kadam et al., 2011), there are limited numbers of works have done in extracting gelatin from fish skin assisted by high pressure (Gómez-Guillén et al., 2005). It leave rooms for more study on extracting protein from marine source assisted by HPP. Although developing countries such as Japan, United Kingdom, Europe and France have used the HPP technique broadly as a preservation tools, but less research has been done to study the effectiveness of HPP in extracting gelatin, especially fish gelatin (Patterson et al., 2006). It limit its application in the industry. Thus more research is needed regarding fish gelatin extraction assisted by the HPP. HPP device which is used in this study is the first ever HPP equipment in

Malaysia. Screening for gelatin extraction from various types of marine source using available HPP equipment is significant to determine which species of fish that will give better yield.

## 1.3 RESEARCH OBJECTIVES

The main purpose of the study is to extract gelatin from fish skin using High Pressure Processing (HPP) method for sustainable halal food products. The objectives were specified into four parts, which were:

1. To screen various types of fish for gelatin extraction by HPP machine.
2. To optimize the process parameters (pressure, HPP-time, extraction time, ratio of skin and acid) and to characterize the gelatin produced by the HPP method.
3. To evaluate the suitable kinetic models for extraction of gelatin assisted by HPP.
4. To examine the performance of the produced fish gelatin.