UNIVERSITI PUTRA MALAYSIA

EXTRACTION AND CHARACTERIZATION OF ACETIC ACID-SOLUBLE COLLAGEN AND PAPAIN-SOLUBLE COLLAGEN HYDROLYSATE FROM POULTRY EGGSHELL MEMBRANE

ROAZITA MA

IPPH 2016 5

EXTRACTION AND CHARACTERIZATION OF ACETIC ACID-SOLUBLE COLLAGEN AND PAPAIN-SOLUBLE COLLAGEN HYDROLYSATE FROM POULTRY EGGSHELL MEMBRANE

By

ROAZITA MA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

August 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EXTRACTION AND CHARACTERIZATION OF ACETIC ACID-SOLUBLE COLLAGEN AND PAPAIN-SOLUBLE COLLAGEN HYDROLYSATE FROM POULTRY EGGSHELL MEMBRANE

By

ROAZITA MA

August 2016

Chairman : Associate Professor Dzulkifly Bin Mat Hashim
Faculty : Halal Products Research Institute

Collagens have unique biocompatible and biodegradable properties that are widely used in food, cosmetics, and biomedical applications. However, collagen derived from land-based animals pose problems as a risk to transmissible disease as Bovine Spongiform Encephalopathy (BSE) and foot-and-mouth diseases, the risk of autoimmune and allergic reaction in its biomedical applications, and skepticism amongst faith-based niche market particularly for Muslims and Jewish consumers. Collagen extracted from eggshell membrane was thus proposed as an alternative. Extraction efficiency and characterization of collagen of acetic acid-soluble collagen (ASC) and papain-soluble collagen hydrolysates (PSCH) from eggshell membrane were investigated. Studies were carried out under three variables (enzyme concentration, temperature, and pH), it was found that the most efficient conditions for ASC was at 15 °C, pH 3, with a yield of 0.23% on a dry weight basis, while PSCH was at 15 °C, pH 3 and enzyme concentration of 300K U/g of raw material with a yield of 0.55% on a dry weight basis. The isoelectric points of ASC and PSCH were found to be at pH 6.61 and 6.89, respectively. The FTIR spectra of both ASC and PSCH demonstrated similar absorption bands to that of porcine. The maximum transition temperature of ASC and entropy were observed at 61.84°C and 0.139 J/g, whilst PSCH was at 59.12°C and 0.046 J/g, indicating such thermal profile was almost at the par of the mammalian thermal profile. The color determination of ASC revealed that it had bright intensity of lightness, low intensity of redness and yellowness, whilst the colour determination of PSCH revealed that it had a slightly higher intensity of redness. ASC in 0.5 M acetic acid had high solubility till pH 3 and then remain relatively stable till pH 10, whilst PSCH in 0.5 M acetic acid had high solubility till pH 4 then remain relatively stable to pH 10. For the effect of NaCl concentration on collagen solubility, ASC demonstrated a distinguishable decrease in solubility especially at concentrations above 2% and then decreased slightly at above 4%, whilst PSCH demonstrated drastic decrease at 3% and remain steady at above 4%. The odor patterns of ASC displays a

low intensity, PSCH revealed that it had considerable intensity of odor which require careful consideration in its application.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

EKSTRAK DAN PENCIRIAN KOLAGEN LARUT ASID ASETIK DAN HIDROLISAT KOLAGEN LARUT PAPAIN DARI MEMBRAN KULIT TELUR POULTRI

Oleh

ROAZITA MA

Ogos 2016

Pengerusi : Professor Madya Dzulkifly Bin Mat Hashim
Fakulti : Institut Penyelidikan Produk Halal

Kolagen mempunyai sifat yang unik daripada segi biologi dalam keserasian dan mudah diuraikan daripada sebatian yang kompleks kepada bentuk lebih ringkas yang digunakan secara meluas dalam bidang makanan, kosmetik dan aplikasi bioperubatan. Walaubagaimanapun, kolagen adalah diterbitkan daripada haiwan darat yang mempunyai risiko dalam penyebaran penyakit sebagai contoh Bovine Spongiform Encephalopathy (BSE), penyakit kuku dan mulut. Manakala, dalam bidang aplikasi bioperubatan mempunyai risiko dalam autoimunisasi dan tindak balas alergi. Ia juga memberikan keraguan pasaran khususnya kepada pengguna Islam dan Yahudi. Secara alternatifnya, kolagen boleh diekstrak daripada membran kulit telur. Keberkesanan pengekstrakan, pencirian kolagen larut asid (ASC) dan juga hidrolisat kolagen larut papain (PSCH) dari membran kulit telur dikenalpasti. Di bawah tiga pembolehubah (kepekatan enzim, suhu dan pH asid asetik), didapati bahawa keadaan paling berkesan bagi ASC adalah 15 °C, pH 3 dengan hasil yang diperolehi adalah 0.23% berdasarkan berat kering. Manakala, keadaan paling berkesan bagi PSCH adalah 15 °C, pH 3 dan kepekatan enzim 300 K U/g bahan mentah dengan hasil yang diperolehi adalah 0.55% berdasarkan berat kering. Titik isoelektrik ASC dan PSCH masing-masing pada pH 6.61 dan 6,89. Spectrum FTIR ASC dan PSCH menunjukkan jalur penyerapan serupa dengan porcine. Suhu peralihan maksimum ASC dan entropi diperhatikan pada 61.84° C dan 0.139 J/g, manakala, PSCH adalah 59.12° C dan 0.046 J/g, menunjukkan profil haba tersebut adalah hampir setanding kepada profil haba haiwan darat. Penentuan warna ASC menunjukkan bahawa ia mempunyai keamatan terang ringan, keamatan rendah kemerahan dan kekuningan, manakala penentuan warna PSCH mendedahkan bahawa ia mempunyai keamatan kemerahan lebih tinggi. ASC dalam asid asetik 0.5 M mempunyai keterlarutan tinggi hingga pH 3 dan kemudian menurun hingga stabil pada pH 10, manakala PSCH dalam asid asetik 0.5 M mempunyai keterlarutan tinggi hingga pH 4 kemudian menurun hingga stabil pada pH 10. Untuk kesan kepekatan NaCl pada kelarutan kolagen, ASC menunjukkan penurunan keterlarutan pada kepekatan melebihi 2% dan kemudian menurun sedikit di atas 4%, manakala PSCH

menunjukkan penurunan drastik sebanyak 3% dan tetap kekal pada melebihi 4%. Profil bau ASC memaparkan keamatan yang rendah, manakala PSCH memaparkan keamatan yang lebih tinggi. PSCH menunjukkan bahawa keamatan baunya yang boleh digunakan namun pengaplikasiannya memerlukan beberapa pertimbangan yang teliti.

ACKNOWLEDGEMENTS

All praises be to Allah, the Most Magnificent, the Glorious and the Most Merciful, who has given me strength, guidance and patience to accomplish this study. In this opportunity, I would like to express my gratitude, particularly to my esteemed supervisor Associate Professor Dzulkifly Mat Hashim for his wisdoms, guidance, constructive ideas and supports. My heartfelt thanks to my co-supervisor Prof. Dr. Jamilah Bakar for her expertise and to Dr. Puziah Hashim for dedicated supports. Special thanks go to lab assistants of Halal Products Research Institute and Faculty of Food Science who have tremendously help my lab work, particularly Enc. Azman, Suraya, Faisal, Kak Faizan, and Mafuza .

My deepest appreciation goes to my parents and family for enormous amount of love, support, and sacrifice. Not forgotten all my friends whom have facilitated this project. Be it Aishah Ma Ping, Intan Amin, Fida, Balqes Al-Areqi, Mohammed Jumaah, Haniff Hanafi, Menahil Mofdal, Zahraa Dahham Qadawi, Najat Al-Odaini, Randa Abdelkareem, Aini Adnan, Rozhin, Samah Kamil Hussein, Ahmed Mediani, Arifa, Nicholas, Ashraf and others whose name have not been mentioned. And of course, the beloved colleagues of Food 5’s protein lab that always spices up the day!

And I am grateful to Malaysia’s Ministry of Higher Education who has awarded me with Malaysia International Scholarship (MIS). Lastly, thanks to all my family for their understanding, patience and continuous supports.

Without all of your supports, cooperation, advices and kindness, I do not think I would have achieved till this day. I hope this project will be beneficial to all.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Dzulkifly Bin Mat Hashim, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Jamilah Binti Bakar, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Puziah Binti Hashim, PhD
Associate Fellow Researcher,
Halal Products Research Institute
Universiti Putra Malaysia
(Member)

BUJANG BIN. KIM. HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ________________________ Date: ___________________

Name and Matric No.: Roazita Ma, GS 23175

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: ______________________________

Name of Chairman of Supervisory Committee: Dzulkifly Bin Mat Hashim, PhD

Signature: ______________________________

Name of Member of Supervisory Committee: Jamilah Binti Bakar, PhD

Signature: ______________________________

Name of Member of Supervisory Committee: Puziah Binti Hashim, PhD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	Degree Celsius
AABA	α-Aminobutyric acid
ASC	Acetic acid-soluble collagen
DSC	Differential Scanning Calorimetry
EDTA	Ethylenedinitrilotetraacetic acid
ESM	Eggshell membrane
Et al.	and others
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
kDa	Kilo Dalton
L	Liter
M	Molar
mL	Milliliter
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
PAGE	Polyacrylamide gel electrophoresis
PRC	Porcine collagen
PSCH	Papain-soluble collagen hydrolysates
SDS	Sodium dodecyl sulphate
TCA	Trichloroacetic Acid
Td	Denaturation temperature of collagen sample
Tris-HCl	Tris-Hydrochloric Acid
ΔH	Heat enthalpy

CHAPTER I

INTRODUCTION

A class of protein that is most occurring in both animals and humans is collagen. It is composed of linear, fibre-like proteins. Its biocompatible and biodegradable properties are extensively used in biomedical, cosmetic, and food applications, which include binding-capacity, network-forming capacity, anchoring-function, water-bonding, film-forming and low-immunogenicity (Li, Jia, and Yao, 2009). Type I collagen, out of 27 different types of collagens, occurs widely, predominantly found in bone, skin, tendons, and connective tissue and that are conventionally isolated from land-based sources such as pigs or cows (Gomez-Guillen et al., 2011).

However, collagen originated from such sources does offer several problems which can cause restriction on the collagen trade. This includes the occurrence of transmissible spongiform encephalopathy (TSE), foot-and-mouth disease (FMD) and bovine spongiform encephalopathy (BSE) (Martínez-Ortiz et al., 2015). Its application in cosmetic and biomedicine may pose a risk of allergic and autoimmune reaction upon the population of 2-3% that is believed to be allergic. Furthermore, its source of origin is prohibited for edible consumption to certain religious niche market, particularly the Muslims and the Jews (Karim and Bhat, 2008). For the Muslims, their lives are guided by Islamic law that is comprised of Shariah law that is founded upon the Qur‟an (the Holy Book of Islam), Al-Hadith and Al-Sunnah (actions, habits, approval, and sayings of Prophet Mohammad), Ijama‟ (consensus of Islamic scholars) and Qiyas (analogy) based upon numerous Islamic Schools of Thought (Mazhab) or fatwa recognized by the relevant Islamic Authority (Che Man and Latif, 2002). The Holy Qur‟an and Sunnah not only guides Muslims in determining Halal (permissible) or Haram (impermissible) status of food, but also promotes the concept of quality and wholesomeness. Allah has specifically mentioned the concept of “Halalan Thoiyyiban” or permissible and wholesome in many occasions in the Quran, such as in the following verses:

“O mankind, eat from whatever is on earth (that is) lawful and pure” [Holy Quran, 2:168].

“O you who have believed, eat from the pure things which we have provided you” [Holy Quran, 2:172].

“So eat of the sustenance which God has provided for you, lawful and good; and be grateful for the favours of God, if it is He Whom ye serve.” [The Quran 16:114]

These above verses reflect that Halal is a quality credence that attributes to the nature or original state of the food products. Its processing method, which entail similarities with organic foods, give importance to animal welfare and sustainability issues that ensure that food products are maintained in its natural state (Bonne and Verbeke, 2008). Within this realm, alternative collagens that are compliant to this niche market are essential.

Many alternative sources of collagen have been explored and many studies of which have been focused on aquatic sources. However, its limitation is their amino acids composition whereby their melting point and gelling temperature are lower than that of land-based animals that restricted in many uses especially when it is kept at room temperature (Veeruraj et al., 2013).

Eggshells are common industrial wastes that can be exploited as the alternative source of collagen. Eggshell membrane lies between the inner surface of eggshell and egg white that can give a yield to 16-35% of the total weight of the egg (Takahashi et al, 1996), of which 35% is comprised of collagen (Long et al., 2008). It contains a high percentage of amino acids such as valine, glutamic acid, arginine, and methionine (DeVore and Long, 2013). Moreover, the occurrence of hydroxyproline in the membrane layers of egg shell suggested that it contained collagen (Wong et al., 1984). The collagen is of type I and can be used in various applications including cosmetic, pharmaceutical, biomedical and functional foods (King‟ori, 2011). According to Food and Agriculture Organization of the United Nations‟ report, the world egg production is estimated to be 65 million tons, whilst Asia produces at 38.1 million tons in 2013 (Anonymous, 2013).

Malaysia’s egg production was at 664 thousand tons by 2013 and is increasing annually (Department of Statistics Malaysia, 2014). Such large quantities pose its waste eggshells to be one of the vast by-products of food processing and manufacturing plants that are stockpiled on-site without any pretreatment, causing its odor gas emission during its biodegradation to seriously pollute the environment (Zeng et al., 2015). Thus, such a common industrial waste can be utilized as a viable source for collagen. As aforementioned earlier, eggshell membrane is believed to have type I, V and X collagen, to be autoimmune and have very low allergic reaction (King‟ori, 2011). It also has other useful functional properties in cosmetic applications such as in improving skin hydration and skin condition, and as well as improving wound healing of skin laceration lesion an ulceration for medical application (Long et al., 2008). Thus, eggshell membrane collagen can be served as a potential alternative to mammalian collagen especially pig- based.

With limited numbers of literatures on collagen of eggshell membrane, extraction and characterization of collagen from eggshell membrane using acetic acid and papain were studied. Acetic acid- soluble collagen and papain-soluble collagen hydrolysates were characterized with sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE), Fourier transforms infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), solubility, odor and colour determinations.

1.1 General Objectives

To study the acetic acid-soluble collagen and papain-soluble collagen hydrolysates from poultry eggshell membrane.

1.2 Specific Objectives

1. To determine the most extraction efficient conditions for maximum yield and characterization of acetic acid-soluble collagen from poultry eggshell membrane

2. To determine the most extraction efficient conditions for maximum yield and characterization of papain-soluble collagen hydrolysates from poultry eggshell membrane

1.3 Significance Of The Research

Determining the extraction efficiency not only helps to obtain the most efficient condition for highest yield, purity and structural integrity of collagen, but also helps to determine the cost-efficient and time-saving conditions in its applications. Through characterization, collagen extracted from the poultry eggshell membrane may exhibit similar physico-chemical properties as that to mammalian collagen, particularly porcine collagen, which can be proposed as alternative source of collagen.

REFERENCES

Abraham, G. A., Carr , Robert M. , J., Kemp, P. D., & Mercer, R. (1999). Chemical treatment, without detergents or enzymes, of tissue to form an acellular, collagenous matrix. (U. S. Patent, Ed.). United States.

Ahmed, H. (2005). Electrophoretic analyses of protein . In Principles and reactions of protein extraction, purification and characterization (pp. 71–132). Florida: CRC Press LLC.

Alijotas-Reig, J., Fernández-Figueras, M. T., & Puig, L. (2013). Inflammatory, immune-mediated adverse reactions related to soft tissue dermal fillers. Seminars in Arthritis and Rheumatism, 43(2), 241–258.

Angele, P., Abke, J., Kujat, R., Faltermeier, H., Schumann, D., Nerlich, M., Kinner, B., Englert, C Ruszczak, Z, Mehrl, R.,& Mueller, R. (2004). Influence of different collagen species on physico-chemical properties of crosslinked collagen matrices. Biomaterials, 25(14), 2831–2841.

Anonymous. (2013). GLOBAL POULTRY TRENDS: World Egg Production Sets a Record Despite Slower Growth. Sheffield, England. Retrieved from http://www.thepoultrysite.com/articles/2653/global-poultry-trends-world-egg-production-sets-a-record-despite-slower-growth

AOAC. (2000). Official Method of Analysis. Washington, DC: Associations of offficial chemist.

Balch, D. A., & Cooke, R. A. (1970). A study of the composition of hen‟s eggshell membranes. Ann. Biol. Anim. Biochim. Biophys., 10, 13–25.

Barth, A., & Zscherp, C. (2002). What vibrations tell us about proteins. Quarterly Reviews of Biophysics. United Kingdom: Cambridge University Press.

Bella, J., Brodsky, B., & Berman, H. M. (1995). Hydration structure of a collagen peptide. Structure, 3(9), 893–906.

Berisio, R., De Simone, A., Ruggiero, A., Improta, R., & Vitagliano, L. (2009). Role of side chains in collagen triple helix stabilization and partner recognition. Journal of Peptide Science, 15(3), 131–140.

Berkelman, T., Brubacher, M. G., & Chang, H. (2004). Important Factors Influencing Protein Solubility for 2-D Electrophoresis. BioRadiations, 114, 30–32.

Bilgen, G., Oktay, G., Tokgoz, Z., & Guner, G. (1999). Collagen Content and Electrophoretic Analysis of Type I Collagen in Breast Skin of Heterozygous Naked Neck and Normally Feathered Commercial Broilers. Tr. J. of Veterinary and Animal Sciences, 23, 483–487.

Bisswanger, H. (2011). Practical Enzymology, Second Edition. General Aspects of Enzyme Analysis. Weinheim: Wiley-VCH Verlag & Co. KGaA.

Bonne, K., & Verbeke, W. (2008). Religious values informing halal meat production and the control and delivery of halal credence quality. Agriculture and Human Values, 25, 35–47.

Brodsky, B., & Ramshaw, J. A. M. (1997). The collagen triple-helix structure. Matrix Biology, 15, 545–554.

Cao, H., & Xu, S. T. (2008). Purification and characterization of type II collagen from chick sternal cartilage. Food Chemistry, 108, 439–445.

Chalamaiah, M., Dinesh kumar, B., Hemalatha, R., & Jyothirmayi, T (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. Food Chemistry, 135, 3020–3038.

Che Man, Y. B., & Latif, M. A. (2002). Halal and cultural aspects of Livestock Production and Marketing . A workshop on research and development strategies for the livestock sector in South East Asia through national and international partnership. Bangkok.

Cheng, M., Takenaka, S., Aoki, S., Murakami, S., & Aoki, K. (2009). Purification and characterization of an eggshell membrane decomposing protease from Pseudomonas aeruginosa strain ME-4. Journal of Bioscience and Bioengineering, 107(4), 373–378.

Chi, C-F., Cao, Z-H., Wang, B., Hu, F-Y., Li, Z-R., Zhang, B. (2014). Antioxidant and functional properties of collagen hydrolysates from Spanish mackerel skin as influenced by average molecular weight. Molecules, 19, 11211-11230.

Choi, S. S., & Regenstein, J. M. (2000). Physicochemical and sensory characteristics of fish gelatin. Journal of Food Science, 65(2), 194–199.

Cordeiro, C. M. M., & Hincke, M. T. (2011). Recent Patents on Eggshell: Shell and Membrane Applications. Recent Patents on Food, Nutrition & Agriculture, 3, 1–8.

Damrongsakkul, S., Ratanathammapan, K., Komolpis, K., & Tanthapanichakoon, W. (2008). Enzymatic hydrolysis of rawhide using papain and neutrase. Journal of Industrial and Engineering Chemistry, 14, 202–206.

Dehsorkhi, A., Castelletto, V., Hamley, I. W., Adamcik, J., & Mezzenga, R. (2013). The effect of pH on the self-assembly of a collagen derived peptide amphiphile. Soft Matter, 9(26), 6033–6036.

Demircan, M., Cicek, T., & Yetis, M. I. (2015). Preliminary results in single-step wound closure procedure of full-thickness facial burns in children by using the collagen–elastin matrix and review of pediatric facial burns. Burns, (0), -.

Department of Statistics Malaysia. (2014). Selected Agricultural Indicators 2014. Malaysia.

DeVore, D. P., & Long, F. D. (2013). Anti-inflammatory activity of eggshell membrane and processed eggshell membrane preparations. (U. S. Patent, Ed.). United States.

Doerschera, D. R., Briggs, J. L., & Lonergan, S. M. (2003). Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar protein gels. Meat Science, 66, 181–188.

Duan, R., Zhang, J., Li, J., Zhong, X., Konno, K., & Wena, H. (2012). The effect of the subunit composition on the thermostability of collagens from the scales of freshwater fish. Food Chemistry, 135, 127–132.

Duan, X., Liu, L. L., Ren, G. Y., & Zhu, W. X. (2013). Microwave Freeze Drying of Type I Collagen from Bovine Bone. Drying Technology, 31(13-14), 1701– 1706.

Eyre, D. R., & Wu, J.-J. (2005). Collagen Cross-Links. Topics in Current Chemistry, 247, 207–229.

Fischer, J. (2015). Malaysian diaspora strategies in a globalized Muslim market. Geoforum, 59(0), 169–177.

Friess, W. (1998). Collagen – biomaterial for drug delivery. European Journal of Pharmaceutics and Biopharmaceutics, 45, 113–136.

Friess, W., & Lee, G. (1996). Basic thermoanalytical studies of insoluble collagen matrices. Biomaterials, 17, 2289–2294.

Gelse, K., Pöschl, E., & Aigner, T. (2002). Collagens – structure, function, and biosynthesis. Advanced Drug Delivery Reviews , 55, 1531–1546.

Gomez-Guillen, M. C., Gimenez, B., Lopez-Caballero, M. E., & Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocolloids, 25, 1813–1827.

Greene, D. M. (2003). Use of Poultry Collagen Coating and Antioxidants as Flavor Protection for Cat Foods Made with Rendered Poultry Fat. Food Science and Technology. Virginia Polytechnic Institute and State University, Virginia.

Gunasekaran, S. (1998). Preparation of collagen using papain and a reducing agent. (U. S. Patent, Ed.). United States.

Gunasekaran, S. (2003). Purifying type I collagen using two papain treatments and reduce and delipidation agents. (U. S. Patent, Ed.). United States.

Gustavson, K. H. (1956). The chemistry and reactivity of collagen. New York: Academic Press Inc.

Hammarstrom, P., & Jonsson, B.-H. (2007). Protein denaturation and the denatured state. In M. M. Cox & G. N. Philips (Eds.), Handbook of proteins structure, function and methods (Vol. 1). England: John Wiley and Sons. Ltd.

Harper, B. A., Barbut, S., Lim, L.-T., & Marcone, M. F. (2012). Microstructural and textural investigation of various manufactured collagen sausage casings. Food Research International, 49(1), 494–500.

Huda, N., Seow, E. K., Normawati, M. N., Nik Aisyah, N. M., Fazilah, A., & Easa, A. M. (2013). Effect of duck feet collagen addition on physicochemical properties of surimi. International Food Research Journal, 20(2), 537–544.

Ino, T., Hattori, M., Yoshida, T., Hattori, S., Yoshimura, K., & Takahashi, K. (2006). Improved Physical and Biochemical Features of a Collagen Membrane by Conjugating with Soluble Egg Shell Membrane Protein. Biosci. Biotechnol. Biochem., 70(4), 865–873.

Ionescu, A., Aprodu, I., & Pascaru, G. (2008). Effect of papain and bromelain on muscle and collagen proteins in beef meat. . The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology, 2(31), 9–16.

Irwandi, J., Faridayanti, S., Mohamed, E. S. M., Hamzah, M. S., Torla, H. H., & Che Man, Y. B. (2009). Extraction and characterization of gelatin from different marine fish species in Malaysia. International Food Research Journal, 16, 381–389.

Jafari, J., & Scott, N. (2014). Muslim world and its tourisms. Annals of Tourism Research, 44(0), 1–19.

Jamilah, B., Umi, H., R., M., Mat Hashim, D., & Sazili, A. Q. (2013). Properties of collagen from barramundi (Lates calcarifer) skin. International Food Research Journal, 20(2), 835–842.

Jeevithan, E., Wu, W., Nanping, W., Lan, H., & Bao, B. (2014). Isolation, purification and characterization of pepsin soluble collagen isolated from silvertip shark (Carcharhinus albimarginatus) skeletal and head bone. Process Biochemistry, 49(10), 1767–1777.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2005). Isolation and characterization of collagen from bigeye snapper (Priacanthus macracanthus) skin. Journal of the Science of Food and Agriculture, 85(7), 1203–1210.

Jongjareonrak, A., Rawdkuen, S., Chaijan, M., Benjakul, S., Osako, K., & Tanaka, M. (2010). Chemical compositions and characterisation of skin gelatin from farmed giant catfish (Pangasianodon gigas). LWT - Food Science and Technology, 43, 161–165.

Kaewdang, O., Benjakul, S., Kaewmanee, T., & Kishimura, H. (2014). Characteristics of collagens from the swim bladders of yellowfin tuna (Thunnus albacares). Food Chemistry, 155, 264–270.

Kaewruang, P., Benjakul, S., & Prodpran, T. (2014). Effect of phosphorylation on gel properties of gelatin from the skin of unicorn leatherjacket. Food Hydrocolloids, 35(0), 694–699.

Karim, A. A., & Bhat, R. (2008). Gelatin alternativs for the food industry: recent developments, challenges and prospects. Trends in Food Science and Technology, 19, 644–656.

Kiew, P. L., & Mat Don, M. (2013). Screening of significant factors in collagen extraction from hybrid Clarias sp. using a statistical tool. International Food Research Journal, 20(4), 1913–1920.

King‟ori, A. M. (2011). A review of uses of poultry eggshells and shell membranes. International Journal of Poultry Science, 10(11), 908–912.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Kishimura, H., & Shahidi, F. (2010). Isolation and Characterisation of collagen from the skin of brownbanded bamboo shark (Chiloscyllium punctatum). Food Chemistry, 119(4), 1519–1526.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M. (2005). Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (Priacanthus tayenus). Food Chemistry, 89, 363–372.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., & Shahidi, F. (2010). Isolation and characterization of collagen from the cartilages of brownbanded bamboo shark (Chiloscyllium punctatum) and blacktip shark (Carcharhinus limbatus). LWT - Food Science and Technology, 43(5), 792– 800.

Kumosinski, T. F., & Farrell, H. M. (1993). Determination of the global secondary structure of proteins by Fourier transform infrared (FTIR) spectroscopy. Trends in Food Science & Technology, 4, 169–175.

Lab, H. (2008). Hunter L,a,b Color Scale Application Notes. (H. Lab, Ed.). Virginia.

Lauritzen, N. J., Shimp, L. A., & Mitchell, B. (2008). Collagen products and methods for producing collagen products. (U. S. P. A. Publicatipn, Ed.). United States.

Lee, C. H., Singla, A., & Lee, Y. (2001). Biomedical applications of collagen. International Journal of Pharmaceutics, 221, 1–22.

Leikina, E., Mertts, M. V, Kuznetsova, N., & Leikin, S. (2002). Type I collagen is thermally unstable at body temperature. Proceedings of the National Academy of Sciences of the United States of America, 99(3), 1314–1318.

Lewis, C. A. (2007). Chromatographic techniques. In M. M. Cox & G. N. Philips (Eds.), Handbook of proteins structure, function and methods (Vol. 2, pp. 861–867). England: John Wiley & Sons Ltd.

Li, D., Yang, W., & Li, G. Y. (2008). Extraction of native collagen from limed bovine split wastes through improved pretreatment methods. Journal of Chemical Technology and Biotechnology, 83(7), 1041–1048.

Li, F., Jia, D., & Yao, K. (2009). Amino acid composition and functional properties of collagen polypeptide from Yak (Bos grunniens) bone. LWT - Food Science and Technology, 42(5), 945–949.

Li, G., Fukunaga, S., Takenouchi, K., & Nakamura, F. (2005). Comparative study of the physiological properties of collagen, gelatin and collagen hydrolysate as cosmetic materials. International Journal of Cosmetic Science, 27, 101–106.

Li, S.-T. (2000). Biologic Biomaterials:Tissue-Derived Biomaterials (Collagen). (D. J. Bronzino, Ed.)The Biomedical Engineering Handbook (Vol. 1). Heidelberg: Springer-Verlag GmbH & Co. KG.

Li, Y., Lu, R., & Wang, J. (2013). Optimization of conditions for extraction of collagen from pig hide by response surface methodology. In 32nd Congress of the International Union of Leather Technologists and Chemist Societies, IULTCS 2013.

Li, Z.-R., Wang, B., Chi, C., Zhang, Q.-H., Gong, Y., Tang, J.-J., Luo, H-Y & Ding, G. (2013). Isolation and characterization of acid soluble collagens and pepsin soluble collagens from the skin and bone of Spanish mackerel (Scomberomorous niphonius). Food Hydrocolloids, 31(1), 103–113.

Liang, Q., Wang, L., Sun, W., Wang, Z., Xu, J., & Ma, H. (2014). Isolation and characterization of collagen from the cartilage of Amur sturgeon (Acipenser schrenckii). Process Biochemistry, 49(2), 318–323.

Lin, Y. K., & Liu, D. C. (2006). Comparison of physical-chemical properties of type I collagen from different species. Food Chemistry, 99, 244–251.

Lin, Y. K., & Liu, D. C. (2006). Effects of pepsin digestion at different temperatures and times on properties of telopeptide-poor collagen from bird feet. Food Chemistry, 94, 621–625.

Liu, C., Peng, D., Yang, J., Li, Y., & Li, J. (2010). Anti-oxidative and anti-aging activities of collagen hydrolysate. In Proceedings - 2010 3rd International Conference on Biomedical Engineering and Informatics, BMEI 2010 (Vol. 5, pp. 1981–1984).

Liu, D. C., Lin, Y. K., & Chen, M. T. (2001). Optimum Condition of Extracting Collagen from Chicken Feet and its Characteristics. Asian Australas. J. Anim. Sci, 14(11), 1638–1644.

Liu, D., Wei, G., Li, T., Hu, J., Lu, N., Regenstein, J. M., & Zhou, P. (2015). Effects of alkaline pretreatments and acid extraction conditions on the acid-soluble collagen from grass carp (Ctenopharyngodon idella) skin. Food Chemistry, 172(0), 836–843.

Liu, D., Zhang, X., Li, T., Yang, H., Zhang, H., Regenstein, J. M., & Zhou, P. (2015). Extraction and characterization of acid- and pepsin-soluble collagens from the scales, skins and swim-bladders of grass carp (Ctenopharyngodon idella). Food Bioscience, 9(0), 68–74.

Liu, D., Zhou, P., Li, T., & Regenstein, J. M. (2014). Comparison of acid-soluble collagens from the skins and scales of four carp species. Food Hydrocolloids, 41(0), 290–297.

Loden, M. (2009). Hydrating substances. In O. A. Barel, M. Paye, & H. . Maiback (Eds.), Handbook of cosmetic science and Technology (Third, pp. 107– 121). New York: Informa Healthcare USA, Inc.

Long, F. D., Adams, R. G., DeVore, D. P., & Franklin, M. R. (2008). Therapeutic, nutraceutical and cosmetic applications for eggshell membrane and processed eggshell membrane preparations. (U. S. P. A. Publication, Ed.). United States.

Martínez-Ortiz, M. A., Hernández-Fuentes, A. D., Pimentel-González, D. J., Campos-Montiel, R. G., Vargas-Torres, A., & Aguirre-Álvarez, G. (2015). Extraction and characterization of collagen from rabbit skin: partial characterization. CyTA – Journal of Food, 13(2), 253–258.

Matmaroh, K., Benjakul, S., Prodpran, T., Encarnacion, A. B., & Hideki, K. (2011). Characteristics of acid soluble collagen and pepsin soluble collagen from scale of spotted golden goatfish (Parupeneus heptacanthus). Food Chemistry, 129, 1179–1186.

McClain, P. E., & Wiley, E. R. (1972). Differential scanning calorimetry studies of the thermal transitions of collagen. The Journal of Biological Chemistry, 247, 692–697.

McLellan, T. (1982). Electrophoresis buffers for polyacrylamide gels at various pH. Analytical Biochemistry, 126, 94–99.

Meena, C., & Mengi, S. . (1999). Biomedical and industrial applications of collagen. Proc Indian Acad Sci (Chem Sci), 111(2), 319–329.

Menicagli, C. (1990). Process for the preparation of collagen and obtained product. (U. S. Patent, Ed.). United States.

Miles, C. A., & Bailey, A. J. (2001). Thermally labile domains in the collagen molecule. Micron, 32, 325–332.

Muralidhar, R. V, & Panda, T. (1999). Useful products from human placenta. Bioprocess Engineering, 20, 23.25.

Mursyidi, A. (2013). The Role of Chemical Analysis in the Halal Authentication of Food and Pharmaceutical Products . Journal of Food and Pharmaceutical Sciences, 1, 1–4.

Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (Lates niloticus). Food Chemistry, 86, 325–332.

Nagai, T., Izumi, M., & Ishii, M. (2004). Fish scale collagen. Preparation and partial characterization. International Journal of Food Science and Technology, 39, 239–244.

Nagai, T., & Suzuki, N. (2000). Isolation of collagen from fish waste material - skin, bone and fins. Food Chemistry, 68, 277–281.

Nakano, T., Ikawa, N.I., & Ozimek, L. (2003). Chemical composition of Chicken Eggshell and Shell Membranes. Poultry Science, 82, 510 – 514.

Nalinanon, S., Benjakul, S., Kishimura, H., & Osako, K. (2011). Type I collagen from the skin of ornate threadfin bream (Nemipterus hexodon):Characteristics and effect of pepsin hydrolysis. Food Chemistry, 125(2), 500–507.

Nalinanon, S., Benjakul, S., Visessanguan, W., & Kishimura, H. (2007). Use of pepsin for collagen extraction from the skin of bigeye snapper (Priacanthus tayenus). Food Chemistry, 104, 593–601.

Odermatt, E., Risterli, J., Van Delden, V., & Timpl, R. (1983). Structural diversity and domain composition of a unique collagenous fragment (intima collagen) obtained from human placenta. Biochem. J., 211, 295–302.

Orphal, J. (2007). Fourier transform infrared. In M. M. Cox & G. N. Philips (Eds.), Handbook of proteins structure, function and methods (Vol. 2, pp. 1037– 1040). England: John Wiley and Sons, Ltd.

Hashim, P., Mohd Ridzwan, M. S. & Bakar, J. (2014). isolation and characterization of collagen from chicken feet. International Journal of Biological, Veterinary, Agricultural and Food Engineering, 8(3).

Parenteau-Bareil, R., Gauvin, R., & Berthod, F. (2010). Collagen-Based Biomaterials for Tissue Engineering Applications. Materials, 3, 1863–1887.

Pati, F., Adhikari, B., & Dhara, S. (2010). Isolation and characterization of fish scale collagen of higher thermal stability. Bioresource Technology, 101, 3737–3742.

Patrick, R. S., & Cooper, D. (2007). Collagen sourced from sheep is equivalent or superior to traditional collagen sources. Colltech Australia Conference. Perth, Australia.

Play, D., Bonneau, M., Merieux, C., Herbage, D., & Comte, P. (1985). Process for the industrial prepartion of collageneous materials from human placental tissues, human collageneous materials obtained and their application as biomaterial. (U. S. Patent, Ed.). United States.

Ramshaw, J. A. M. (1998). Gly-X-Y tripeptide frequencies in collagen: a context for host-guest triple-helical peptides. Journal of Structural Biology, 122, 86– 91.

Ramshaw, J. A. M., Peng, Y. Y. P., Glattauer, V., & Werkmeister, J. A. (2009). Collagens as biomaterials. J Matter Sci: Mater Med , 20, S3–S8.

Regenstein, J. M., & Wang, Y. (2009). Effect of EDTA, HCl, and Citric Acid on Ca Salt Removal from Asian (Silver) Carp Scales Prior to Gelatin Extraction. Journal of Food Science, 74(6), 426–431.

Rochdi, A., Foucat, L., & Renou, J.-P. (2000). NMR and DSC studies during thermal denaturation of collagen. Food Chemistry, 69, 295–299.

Rydziel, S., & Canalis, E. (1989). Analysis of Hydroxyproline by High Performance Liquid Chromatography and its Application to Collagen Turnover Studies in Bone Cultures. Calcified Tissue International, 44, 421– 424.

Sadowska, M., & Kołodziejska, I. (2005). Optimisation of conditions for precipitation of collagen from solution using j-carrageenan. Studies on collagen from the skin of Baltic cod (Gadus morhua). Food Chemistry, 91, 45–49.

Said, M., Hassan, F., Musa, R., & Rahman, N. A. (2014). Assessing Consumers' Perception, Knowledge and Religiosity on Malaysia's Halal Food Products. Procedia - Social and Behavioral Sciences, 130(0), 120–128.

Sankarana, S., Khota, L. R., & Panigrahi, S. (2012). Biology and applications of olfactory sensing system: A review. Sensors and Actuators B: Chemical, 171-172, 1–17.

Santos, M. H., Silva, R. M., Dumont, V. C., Neves, J. S., Mansur, H. S., & Heneine, L. G. D. (2013). Extraction and characterization of highly purified collagen from bovine pericardium for potential bioengineering applications. Materials Science and Engineering C, 33(2), 790–800.

Schrieber, R., & Gareis, H. (2007a). From collagen to gelatin. In Gelatine Handbook: Theory and industrial practise (pp. 45–117). Weinheim: Wiley-VCH Verlag & Co. KGaA.

Schrieber, R., & Gareis, H. (2007b). Practical aspects: collagen in cosmetic applications. In Gelatine Handbook: Theory and industrial practise (pp. 277–281). Weinheim: Wiley-VCH Verlag & Co. KGaA.

Secchi, G. (2008). Role of protein in cosmetics. Clinics in Dermatology, 26, 321– 325.

See, S. F., Hong, P. K., Ng, K. L., Wan Aida, W. M., & Babji, A. S. (2010). Physicochemical properties of gelatins extracted from skins of different freshwater fish species. International Food Research Journal, 17, 809–816.

Silvipriya, K. S., Krishna Kumar, K., Bhat, A. R., Dinesh Kumar, B., John, A., & Lakshmanan, P. (2015). Collagen: Animal sources and biomedical application. Journal of Applied Pharmaceutical Science, 5(3), 123–127.

Singh, P., Benjakul, S., Maqsood, S., & Kishimura, H. (2011). Isolation and characterisation of collagen extracted from the skin of striped catfish (Pangasianodon hypophthalmus). Food Chemistry, 124, 97–105.

Škaljac, S., Petrović, L., Tasić, T., Ikonić, P., Jokanović, M., Tomović, V., Džinić, N, Šojić, B., Tjapkin, A & Škrbić, B. (2014). Influence of smoking in traditional and industrial conditions on polycyclic aromatic hydrocarbons content in dry fermented sausages (Petrovská klobása) from Serbia. Food Control, 40(1), 12–18.

Skehel, M.J. (2004). Preparation of extracts from animal tissues. In P. Cutler (Ed.), Protein Purification Protocols (2nd Edition, pp 15-20). New Jersey: Humana Press Inc., Skierka, E., & Sadowska, M. (2007). The influence of different acids and pepsin on the extractability of collagen from the skin of Baltic cod (Gadus morhua). Food Chemistry, 105, 1302–1306.

Smith, D. M. (2004). Protein separation and characterization procedures. In S. S. Nielson (Ed.), Food Analysis (3rd Edition, pp. 247–265). New York: Wolters Kluwer Law & Business.

Song, W., Chen, W., Yang, Y., & Li, C. (2014). Extraction optimization and characterization of collagen from the lung of soft-shelled turtle Pelodiscus sinensis. International Journal of Nutrition and Food Sciences, 3(4), 270–278.

Subhan, F., Ikram, M., Shehzad, A., & Ghafoor, A. (2014). Marine Collagen: An Emerging Player in Biomedical applications. Journal of Food Science and Technology.

Swatschek, D., Schattona, W., Kellermannd, J., Mu¨llerb, W. E. G., & Kreuter, J. (2001). Marine sponge collagen: isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum. European Journal of Pharmaceutics and Biopharmaceutics, 53, 107–113.

Takahashi, K., Shirai, K., Kitamura, M., & Hattori, M. (1996). Soluble eggshell membrane protein as a regulating material for collagen matrix reconstruction. Japan Society for Bioscience, Biotechnology, and Agrochemistry, 60(8), 1299–1302.

Veeruraj, A., Arumugam, M., Ajithkumar, T., & Balasubramanian, T. (2015). Isolation and characterization of collagen from the outer skin of squid (Doryteuthis singhalensis). Food Hydrocolloids, 43(0), 708–716.

Veeruraj, A., & Muthuvel Balasubramanian, T. A. (2013). Isolation and characterization of thermostable collagen from the marine eel-fish (Evenchelys macrura). Process Biochemistry, 48, 1592–1602.

Voss, A., Witt, K., Fischer, C., Reulecke, S., Poitz, W., Kechagias, V., Figulla, H. R. (2012). Smelling heart failure from human skin odor with an electronic nose. 34th Annual International Conference of the IEEE EMBS. San Diego, California USA.

Waller, J. M., & Maibach, H. I. (2009). A quantitative approach to age and skin structure and function: protein, glycosaminoglycan, water, and lipid content and structure. In A. O. Barel, M. Paye, & H. I. Maibach (Eds.), Handbook of cosmetic science and Technology 3rd edition. New York: Informa Healthcare USA, Inc.

Wang, L., Yang, B., & Du, X. (2009). Extraction of acid-soluble collagen from grass carp (Ctenopharyngodon Idella) Skin. Journal of Food Process Engineering, 32, 743–751.

Wang, L., Yang, B., Wang, R., & Du, X. (2008). Extraction of pepsin-soluble collagen from grass carp (Ctenopharyngodon idella) skin using an artificial neural network. Food Chemistry, 111(3), 683–686.

Wilson, A. D., & Baietto, M. (2011). Advances in Electronic-Nose Technologies Developed for Biomedical Applications: Review. Sensors and Actuators B: Chemical, 11, 1105–1176.

Wong, M., Hendrix, M. J. C., von der Mark, K., Little, C., & Stern, R. (1984). Collagen in the egg shell membranes of the hen. Developmental Biology, 104(1), 28–36.

Woo, J.-W., Yu, S.-J., Cho, S.-M., Lee, Y.-B., & Kim, S.-B. (2008). Extraction optimization and properties of collagen from yellowfin tuna (Thunnus albacares) dorsal skin. Food Hydrocolloids, 22, 879–887.

Yan, M., Li, B., Zhao, X., & Qin, S. (2012). Effect of concentration, pH and ionic strength on the kinetic self-assembly of acid-soluble collagen from walleye pollock (Theragra chalcogramma) skin. Food Hydrocolloids, 29, 199–204.

Yi, F., Guo, Z.-X., Zhang, L.-X., Yu, J., & Li, Q. (2004). Soluble eggshell membrane protein: preparation, characterization and biocompatibility. Biomaterials, 25, 4591–4599.

Young, G. S. (1998). Thermodynamic characterization of skin, hide and similar materials composed of fibrous collagen. Studies in Conservation, 43(2), 65– 79.

Zayas, J. F. (1997). Solubility of proteins. Funtionality of proteins in foods. Berlin: Springer-Verlag.

Zeng, D., Zhang, Q., Chen, S., Liu, S., Chen, Y., Tian, Y., & Wang, G. (2015). Preparation and characterization of a strong solid base from waste eggshell for biodiesel production. Journal of Environmental Chemical Engineering,3(1), 560–564.

Zhang, J., Duan, R., Huang, L., Song, Y., & Regenstein, J. M. (2014). Characterisation of acid-soluble and pepsin-solubilised collagen from jellyfish (Cyanea nozakii Kishinouye). Food Chemistry, 150, 22–26.

Zhang, J., Hatakeyama, J., Eto, K., & Abe, S. (2014). Reconstruction of a seminiferous tubule-like structure in a 3 dimensional culture system of re-aggregated mouse neonatal testicular cells within a collagen matrix. General and Comparative Endocrinology, 205(0), 121–132.

Zhang, Z., Li, G., & Shi, B. (2006). Physicohcemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. Journal of the Society of Leather Technologists and Chemists, 90, 23–28.

Zhao, Y.-H., & Chi, Y.-J. (2009). Characterization of collagen from eggshell membrane. Biotechnology, 8(2), 254–258.

Zhu, B. W., Dong, X. P., Zhou, D. Y., Gao, Y., Yang, J. F., Li, D. M., … Yu, C. (2012). Physicochemical properties and radical scavenging capacities of pepsin-solubilized collagen from sea cucumber Stichopus japonicus. Food Hydrocolloids, 28(1), 182–188.

Zubay, G. L. (1998). Methods for characterization and purification of proteins. In Biochemistry: Forth Edition. US: Wm. C. Brown Publishers.

Appendix A

Flow Chart

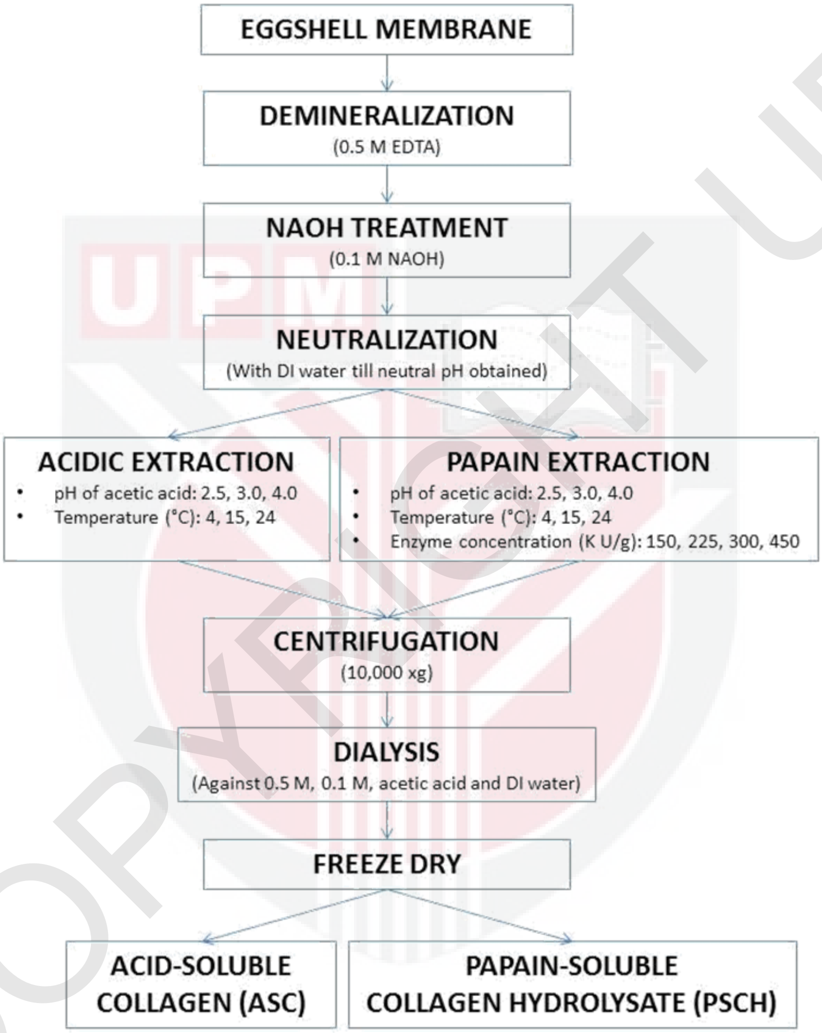

Figure A1: Flow chart of acetic acid-soluble collagen (ASC) and papain-soluble collagen hydrolysates (PSCH) extraction from eggshell membrane.

Appendix B

HPLC Amino Acid Analysis

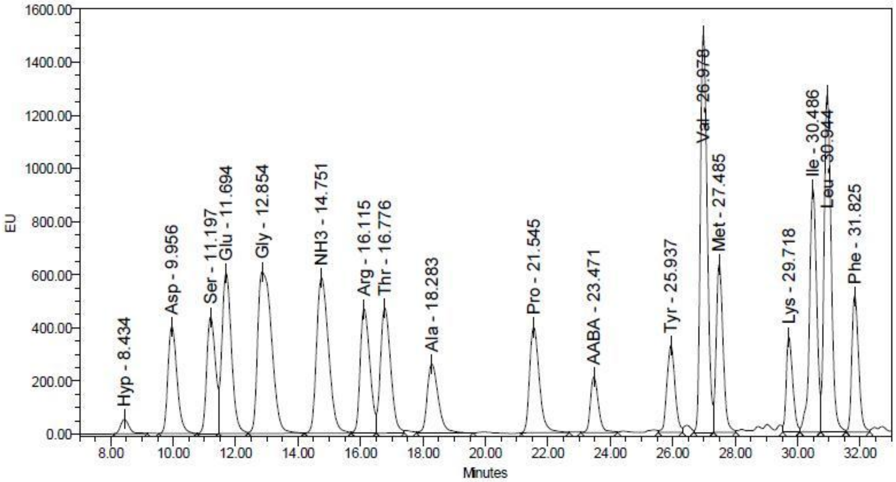

Figure A2: HPLC amino acid analysis of ASC from eggshell membrane.

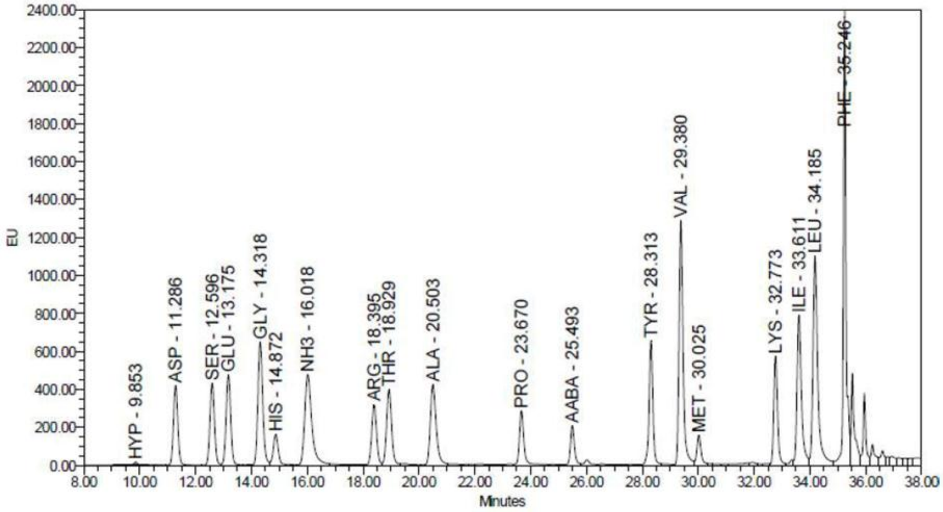

Figure A3: HPLC amino acid analysis of PSCH from eggshell membrane.

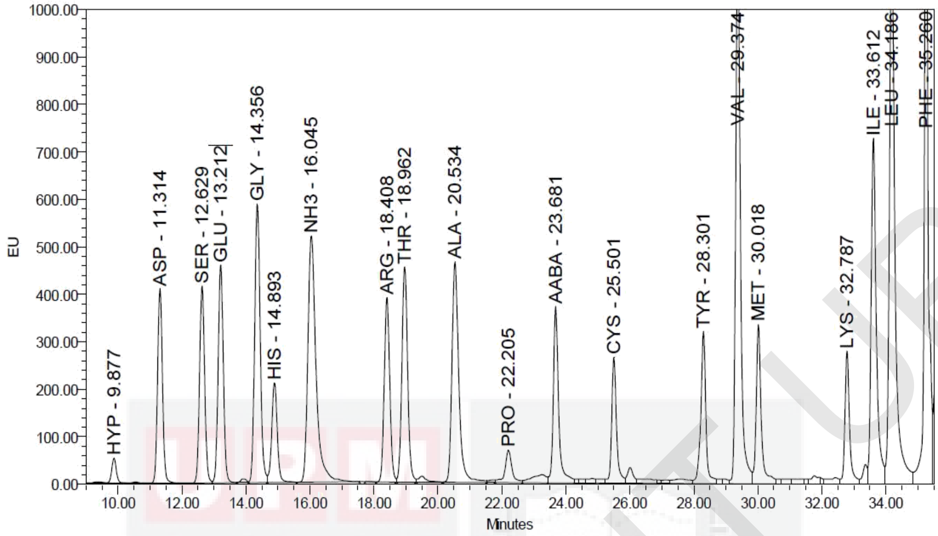

Figure A4: HPLC amino acid analysis of eggshell membrane.

Table A5: HPLC amino acid analysis of acetic acid-soluble collagen (ASC) and papain-soluble collagen hydrolysates (PSCH) in residues/1000 residues of eggshell membrane

Amino Acid	ESM		ASC		PSCH	
Hydroxyproline	7	± 2[a]	13	± 1[a]	30	± 4[b]
Aspartic Acid	104	± 3[a]	86	± 0[b]	85	± 1[b]
Serine	54	± 2[a]	66	± 3[a]	59	± 1[b]
Glutamic Acid	144	± 7[a]	115	± 2[b]	116	± 2[b]
Glycine	11	± 5[a]	76	± 2[b]	76	± 2[b]
Histidine	80	± 4[a]	50	± 1[b]	50	± 2[b]
Arginine	67	± 9	78	± 0	81	± 2
Threonine	65	± 6	58	± 0	58	± 0
Alanine	20	± 6[a]	37	± 0[b]	33	± 5[ab]
Proline	52	± 3[a]	71	± 3[b]	80	± 1[b]
Tyrosine	35	± 1[a]	51	± 3[b]	47	± 5[b]
Valine	69	± 0[a]	60	± 1[b]	61	± 0[b]
Methionine	36	± 0	33	± 1	33	± 2
Lysine	44	± 4	48	± 1	44	± 1
Isoleucine	45	± 2[a]	39	± 2[b]	37	± 1[b]
Leucine	42	± 1[a]	61	± 4[b]	51	± 2[a]
Phenylalanine	38	± 1[a]	28	± 0[b]	24	± 2[c]
Cystine	ND		ND		ND	
Total amino acid	913	± 6	970	± 0	964	± 1
Imino Acid	59	± 3	84	± 4	109	± 3

Values were means ± standard deviation of three replicates;
* Values with the different superscripts within each row were significantly different ($p<0.05$)
ND: not determined

Appendix C

SDS-PAGE Reagent And Gel Preparation

Reagent And Gel Preparation For Laemmli SDS Polyacrylamide Gel Electrophoresis

I. **Stock Reagent Preparation**
   a. **Acrylamide/Bis**
   b. **1.5 M Tris-HCl, pH 8.8**
   c. **0.5 M Tris-HCl, pH 6.8**
   d. **10% (w/v) SDS**
   e. **10% Ammonium Persulfate (w/v)**
      i. Dissolve 100 mg ammonium presulfate in 1 ml distilled water
   f. **Sample buffer**

Distilled water	1.84 ml
0.5 M Tris-Hcl, pH 6.8	2.00 ml
Glycerol	2.00 ml
10% SDS	1.96 ml
β-Mercaptoethanol	1.00 ml
0.5% (w/v) bromophenol blue (in water	0.40 ml

Heat at 95ºC for 4 minutes.

   g. **5x Electrode (Running) Buffer** (1x = 23 mM Tris, 192 mM glycine, 0.1% SDS, pH8.3)

Tris base	45.0 g
Glycine	216.0 g
SDS	15.0 g

Distilled water to 3 L, store at 4ºC and warm to 37ºC before use if precipitation occurs. Dilute 300 ml 5x stock with 1.2 L distilled water for one electrophoresis run.

II. Gel Preparation

Formulation for SDS-PAGE Separating and Stacking Gels

	Separating Gel	Stacking Gel
Monomer Concentration	12%	4%
30% Acrylamide/bis	4.00 ml	0.67 ml
Distilled water	3.35 ml	3.00 ml
1.5 M Tris-HCl, pH 8.8	2.50 ml	-
0.5 M Tris-HCl, pH 6.8	-	1.25 ml
10% (w/v) SDS	0.10 ml	0.05 ml
10% ammonium persulfate	50 µl	25 µl
TEMED	8 µl	7 µl

BIODATA OF STUDENT

Born in Bangkok, Thailand in March 26, 1984. She graduated with 2nd class honor in Bachelor of Science in Food Science and Technology at Mahidol University International College, Thailand in 2006. She has been awarded with Malaysia International Scholarship from Malaysia‟s Minister of Higher Education in furthering her study in Msc. Halal Products Development at the Halal Products Research Institute, UPM, Malaysia.

PUBLICATION

Ma, R, Mat Hashim, D. Bakar, J., Hashim, P. (2016). Isolation and characterization of acid-soluble collagen from eggshell membrane. Journal of the Science of Food and Agriculture– submitted.

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : ________________

TITLE OF THESIS / PROJECT REPORT :

EXTRACTION AND CHARACTERIZATION OF ACETIC ACID-SOLUBLE COLLAGEN AND PAPAIN-SOLUBLE COLLAGEN HYDROLYSATE FROM POULTRY EGGSHELL MEMBRANE

NAME OF STUDENT: ROAZITA MA

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from ____________ (date) until ____________ (date)

Approved by:

________________________ (Signature of Student)
New IC No/ Passport No.:

Date :

________________________ (Signature of Chairman of Supervisory Committee)
Name:

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]