

# UNIVERSITI PUTRA MALAYSIA

# HALAL COLLAGEN EXTRACTION FROM YOUNG AND SPENT RABBIT

NURUL FAZLIANA ABDUL GHANI

FP 2016 121

# HALAL COLLAGEN EXTRACTION FROM YOUNG AND SPENT

RABBIT



NURUL FAZLIANA BINTI ABDUL GHANI

DEPARTMENT OF ANIMAL SCIENCE

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR DARUL EHSAN

2015/2016

# HALAL COLLAGEN EXTRACTION FRO M YOUNG AND SPENT

BY

# NURUL FAZLIANA BINTI ABDUL GHANI

A project report submitted to the Faculty of Agriculture, Universiti Putra Malaysia, in fulfilment of the requirement of SHW 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture (Animal Science)

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR DARUL EHSAN

2015/2016

## CERTIFICATION

This report Halal collagen extraction from young and spent rabbit was prepared by Nurul Fazliana binti Abdul Ghani and submitted to the Faculty of Agriculture in fulfilment of the requirement of SHW 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture (Animal Science).

Student's name:	Student's signature
NURUL FAZLIANA BINTI ABDUL GHANI	
170461	
Certified by:	Date:
PROF. DR. DAHLAN ISMAIL	
Project Supervisor,	
Department of Animal Science,	
Faculty of Agriculture,	
Universiti Putra Malaysia,	
Serdang, Selangor Darul Ehsan	

G

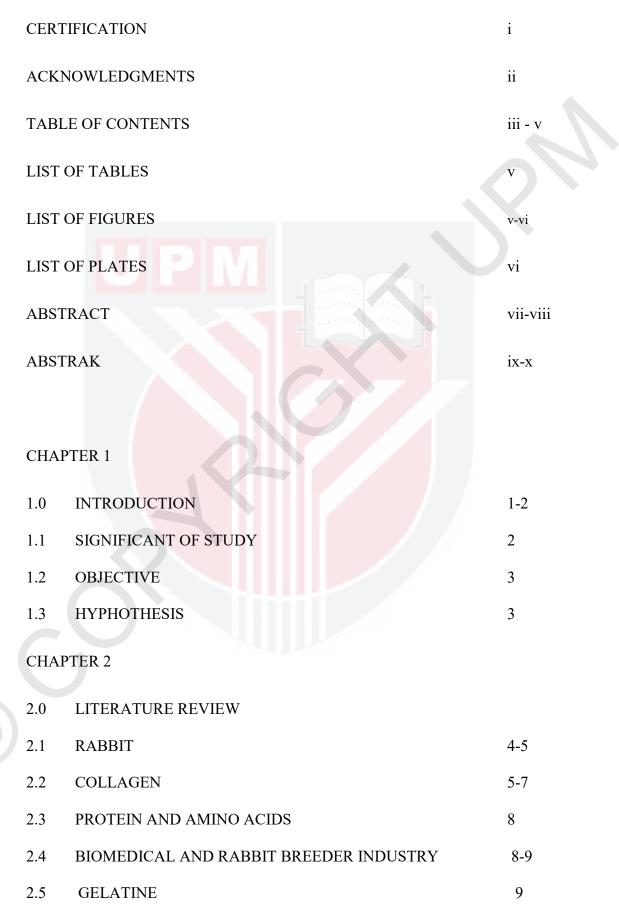
#### ACKNOWLEDGMENTS

Bismillahirrahmanirrahim, Alhamdulillah, with Allah S.W.T blessing has given me the strength and finally I able to complete this Final Year Project.

First and foremost, I would like to express my gratitude and appreciation to all those who gave me the possibility to complete this report. A special thanks to our Final Year Project coordinator and my supervisor, Professor Dr. Dahlan Ismail, who help in stimulating suggestions and encouragement, helped me to coordinate my project especially in writing this report. I also would like to grant a sincere attitude to all staff in Department of Animal Science Faculty of Agriculture, Mr Saparin, Miss Rahaida and Mr Anuar for their help and guidance throughout the duration of the research. A special thanks goes to all my friends especially my course mate (ANSCI7), who help me with my project and report. Last but not least, special thanks goes to my beloved parents, Mr. Abdul Ghani bin Abdullah and Mrs Patimah @ Zainun binti Ibrahim and all my beloved family for their understanding and endless love through the period of my project.

Thanks and may Allah bless all of you.

## **TABLE OF CONTENTS**



# CHAPTER 3

3.0	MATERIALS AND METHODS		
3.1	EXPERIMENT DESIGN	10	
3.2	PREPARATION OF SAMPLES	11	
3.3	CARCASS ANALYSIS	11	
3.4	PROXIMATE ANALYSIS	12-14	
3.5	EXTRACTION METHOD	15-19	
3.6	ANALYSIS OF GELATINE QUALITY	20	
3.7	STATISTICAL METHOD	20	
CHAP	TER 4		
4.0	RESULT		
4.1	DRESSING PERCENTAGE	21	
4.2	CARCASS COMPOSITION	22	
4.3	CHEMICAL COMPOSITION OF RAW SAMPLES	23	
4.4	EXTRACTION OF COLLAGEN	24-27	
4.5	CHEMICAL COMPOSITION OF COLLAGEN	28-31	
4.6	GELATINE PHYSICAL QUALITY AND CHARACTERISTICS	32-33	
CHAPTER 5			
5.0	DISCUSSION		
5.1	COLLAGEN EXTRACTION	34-35	
5.2	CHEMICAL COMPOSITION OF COLLAGEN	35-36	
5.3	GELATINE PHYSICAL QUALITY AND CHARACTERISTICS	36	
CHAPTER 6			
6.0	CONCLUSION AND RECOMMENDATION	37	

	REFERENCES	38-42		
	APPENDICES	43-44		
	APPENDICES (STATISTICAL ANALYSIS)	45-66		
LIST OF TABLE				
	Table 1: Dressing percentage of New Zealand and Dutch rabbit			
	at different age	5		
	Table 2: Types of collagen	5		
	Table 3: Experimental design	10		
	Table 4: Mean and Standard Deviation of live weight, weight			
	after slaughter and dressing percentage	21		
	Table 5: Mean, Standard Deviation and Coefficient of Variation			
	of Dressing percentage	21		
	Table 6: chemical composition of raw samples	23		
	Table 7: Colour and texture of gelatine	32		
LIST OF FIGURES				
	Figure 1: Flow chart of collagen extraction in method 1	16		
	Figure 2: Flow chart of collagen extraction in method 2	18		
	Figure 3: Composition of muscle, bone and fat in young and spent rabbit	22		
	Figure 4: Mean collagen extraction in muscle and legs bone			
	of young rabbit in method 1 and method 2	24		

Figure 5: Mean collagen in muscle and legs bone of spent rabbit	
in method 1 and method 2	25
Figure 6: Mean collagen in young and spent rabbit muscle in	
method 1 and method 2	26
Figure 7: Mean collagen in young and spent rabbit legs	
bone in method 1 and method 2	27
Figure 8: Moisture percentage in collagen	28
Figure 9: Protein percentage in collagen	29
Figure 10: Ether extraction percentage in collagen	30
Figure 11: Ash percentage in collagen	31
LIST OF PLATES	
Plate 1 Halal rabbit slaughter	43
Plate 2 Debone process	43
Plate 3 Extraction process	43
Plate 4 Centrifugation process	43
Plate 5 Wet collagen before drying	43
Plate 6 Freeze drying process	43
Plate 7 Dry collagen	44
Plate 8 Gelatine of rabbit under microscope	44

# vi

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in the fulfilment of the

requirement for the degree of Bachelor of Agriculture (Animal Science)

#### HALAL COLLAGEN EXTRACTION FROM YOUNG AND SPENT RABBIT

By

#### NURUL FAZLIANA BINTI ABDUL GHANI

2015/2016

Keywords: Halal, Rabbit Collagen, gelatine, extraction method, collagen purity

#### ABSTRACT

A Research was conducted to determine the percentage of collagen in young and spent rabbit muscles and legs bone. Nowadays, there are a lots of collagen production in industries but the main issues is the halal status of the collagen. A lots of collagen production in industries nowadays are the non- halal collagen from non- halal animals. Rabbit is the halal animal that have potential in collagen production. Biomedical industry have used young rabbit to extract hormone and enzyme while the carcass of rabbit will throw away. Rabbit breeder industry have thrown the spent rabbit carcass that do not suitable to eat any more. As alternative to improve and produce the halal collagen, these waste product from biomedical industry and rabbit breeder industry can be used in industry of collagen and indirectly will maximize the profit of both industries. This is because the rabbit carcass can be used as collagen sources instead of throw it away. The samples of young rabbit (2-3 months of age) and spent rabbit (> 1 year of age) were randomly selected. Collagen extraction was compared between the method 1 and method 2. Method 1 of collagen extraction was using Acetic Acid

 $\mathbf{O}$ 

and Sodium Hydroxide while extraction of collagen in method 2 was using amyl alcohol, acetic acid and sodium chloride. The comparison of muscle and leg bone collagen content was conducted. The sample was compare to determine which sample produce more collagen. Besides, chemical analysis which is proximate analysis was conducted to evaluate the purity of the collagen and the chemical content on fresh samples. There were significantly different (P<0.05) for collagen extraction in both methods. The young rabbit bone in both method showed lower percentage of collagen extraction then spent rabbit bone. The percentage of young rabbit bone collagen are (13.59%) in method 1 and (9.0%) in method 2 while spent rabbit bone collagen are (15.35%) in method 1 and (9.39%) in method 2. The young rabbit muscle collagen have lower percentage of collagen then spent rabbit muscle collagen in both methods. The percentage of young rabbit muscle collagen in method 1 is (18.04%) and spent rabbit muscle collagen is (18.21%) while young rabbit muscle collagen in method 2 is (12.74%) and spent rabbit muscle collagen is (14.12%). The time consume by method 1 to finish the extraction process is about 5 days while method 2 about 7 days and above. The protein percentage in collagen from method 2 higher than the collagen from method 1 while the ether extraction percentage in method 1 is lower than method 2. That's means, the collagen extraction from method 1 have lower percentage of fat. The lower the fat percentage, the high the purity of the collagen. Besides, the gelatine quality and characteristics test shown that there have no significant different in colour of gelatine. All the gelatine have yellowish in colour. The texture test shown that the gelatine come from muscle are more soft and sticky while gelatine from bone are more coarse and sticky. Based on the results, spent rabbit and muscle part have more collagen yield then young rabbit and bone part. However, extraction method 1 is more efficient to extract more collagen and have short time period of extraction than method 2.

Abstrak tesis yang telah dikemukakan kepada senat Universiti Putra Malaysia dalam memenuhi keperluan penganugerahan Ijazah Bacelor Pertanian (Sains Ternakan)

# PENGEKSTRAKTAN KOLAGEN HALAL DARIPADA ARNAB MUDA DAN

## ARNAB PENCEN

Oleh:

## NURUL FAZLIANA BINTI ABDUL GHANI

2015/2016

Kata kunci: Halal, Kolagen arnab, gelatin, cara ekstrak, ketulinan kolagen

#### ABSTRAK

Sebuah kajian telah dijalankan untuk mengenalpasti peratusan kolagen di dalam otot dan tulang arnab muda dan arnab pencen. Pada masa kini, terdapat banyak pengeluaran kolagen dalam industri tetapi isu utama adalah status halal kolagen tersebut. Terdapat banyak kolagen yang di hasilkan dalam industri pada masa kini adalah kolagen yang tidak halal daripada sumber haiwan yang tidak halal. Arnab adalah haiwan yang halal dan mempunyai potensi dalam produksi kolagen. Industri bioperubatan telah menggunakan arnab muda untuk menekstrak hormon dan enzim manakala karkas arnab akan dibuang. Industri arnab pembaka akan membuang karkas arnab pencen yang tidak sesuai untuk dimakan. Sebagai alternative untuk meningkatkan produksi kolagen Halal, sisa dari industri bioperubatan dan arnab pembiak boleh digunakan untuk penghasilan kolagen dan secara tidak langsung memberi untung maksima kepada kedua-dua industri. Ini kerana karkas arnab boleh digunakan untuk menghasilkan kolagen daripada hanya dibuang begitu sahaja. Sempel daripada arnab muda (berumur 2-3 bulan) and arnab pencen (berumur > 1 tahun) telah dipilih secara rawak.

Pengekstraktan kolagen telah dibandingkan antara keadah 1 and keadah 2. Kaedah 1 mengunakan asid asetik dan sodium hidrosida manakala kaedah 2 menggunakan alkohol amyl, acid asetik dan sodium klorida. Perbezaan antara kolagen dalam otot dan tulang telah dijalankan. Sampel telah dibandingkan untuk mengenal pasti sample mana yang menghasilkan lebih banyak kolagen. Selain itu, analisa kimia iaitu analisa proximate telah dijalankan untuk mengenalpasti ketulinan kolagen dan kandungan kimia dalam sampel segar. Terdapat perbezaan ketara (P<0.05) untuk kedua-dua kaedah pengektraktan. Arnab muda dalam kedua-dua kaedah menunjukkan peratusan kolagen yang rendah berbanding arnab pencen. Peratusan kolagen dalam tulang arnab muda jalah (13.59%) dalam kaedah 1 dan (9.0%) dalam kaedah 2 manakala kolagen tulang arnab pencen ialah (15.35%) dalam kaedah 1 dan (9.39%) dalam kaedah 2. Otot arnab muda mengandungi peratusan kolagen yang rendah berbanding otot arnab tua dalam kedua-dua kaedah. Peratusan kolagen dalam otot arnab muda dalam kaedah 1 ialah (18.04%) dan otot arnab pencen ialah (18.21%) manakala otot arnab muda dalam kaedah 2 ialah (12.74%) dan otot arnab pencen ialah (14.12%). Masa yang diperlukan oleh kaedah 1 untuk siap proses estrak ialah lebih kurang 5 hari manakala kaedah 2 memerlukan masa 7 hari dan keatas. Peratusan protein dalam kolagen kaedah 2 lebih tinggi daripada kolagen kaedah 1 manakala peratusan ether ektraksi dalam kaedah 1 lebih rendah daripada kaedah 2. Ini bermaksud, kolagen yang di ekstrak dari kaedah 1 mengadungi peratusan lemak yang rendah. Semakin rendah peratusan lemak, semakin tinggi ketulinan kolagen tersebut. Selain itu, ujian kualiti dan ciri-ciri gelatin menunjukkan tidak ada perbezaan ketara dalam warna gelatin yang terhasil semua gelatin bewarna kekuningan. Ujian tekstur menunjukkan gelatin daripada otot lebih lembut dan belendir manakala gelatin daripada tulang lebih kasar dan belendir. Berdasarkan keputusan, arnab pencen dan bahagian otot mempunyai lebih banyak kolagen daripada arnab muda dan bahagian tulang. Walau bagaimana pun, kaedah ektraksi 1 lebih berkesan unutk mengekstrak lebih banyak kolagen dan memerlukan kurang masa berbanding kaedah ektraksi 2.

#### CHAPTER 1

#### **1.0 INTRODUCTION**

Collagen is a group of naturally occurring proteins. In nature, it is found in animals, especially in the flesh and connective tissues of mammals. It is the main component of connective tissue, and is the most abundant protein in mammals, making up about 25% to 35% of the whole body protein content. Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendon, ligament and skin, and is also abundant in cornea, cartilage, bone, blood vessels, the gut, and intervertebral disc. (Hussin, 2012). Collagen is very important in cosmetic and medicine industry.

There are too difficult to find the Halal collagen making for Muslim use. The Halal collagen can be extracted from halal animal that slaughter in Halal way. The Arabic word Halal means permissible, and the rules of slaughter are based on Islamic law. The animal has to be alive and healthy, a Muslim has to perform the slaughter in the appropriate ritual manner, and the animal's throat must be cut by a sharp knife severing the carotid artery, jugular vein and windpipe in a single swipe. Blood must be drained out of the carcass.

Mature bone is composed of proteins and minerals. Approximately 60% the weight of the bone is mineral, mainly calcium and phosphate. The rest is water and matrix, which is formed before the mineral is deposited, and can be considered the scaffolding for the bone. About 90% of the matrix proteins are collagen, which is the most abundant protein in the body. Collagen is very strong and forms bone, cartilage, skin, and tendons(Young, 2003). The side product from collagen extraction from bone is Di calcium phosphate which is also can be used in animal feed industry.

Meat is a major source of proteins, essential amino-acids, complex-B vitamins, minerals, and other bioactive compounds. Recommended by nutritionists over other meats, rabbit meat is valued for its nutritional properties because is lean, rich in proteins of high biological value, low in cholesterol content and high in linolenic acid. Rabbit meat was richer in calcium (21.4 mg/100 g) and phosphorus (347 mg/100 g) than other types of meat and lower in fat (9.2 g/100 g) and cholesterol (56.4 mg/100 g) (Nistor et al., 2013).

Nowadays, there have biomedicine industries that use young rabbit for medicine purpose such as for vaccine production and for hormone extraction. They only use certain part of the rabbit and the carcass will be throw. Instead of throw the carcass, the carcass can be used for collagen making. On the other hand, the rabbit breeder industries will produce the spent rabbit which is the use of meat from this kind of rabbit among consumers is very low. So that, the carcass can be used for collagen extraction to avoid wasting.

## **1.1 SIGNIFICANT OF STUDY**

Nowadays, there are too difficult to find the collagen from halal sources. Most of the collagen in market come from swine or from animal that do not slaughter in Halal way. As alternative to improve and produce the halal collagen, the waste product from biomedical industry and rabbit breeder industry can be used in industry of collagen and indirectly will maximize the profit of both industry. This is because the rabbit carcass can be used as collagen source instead of throw it away.

### **1.2 OBJECTIVE**

General objective of this study is to extract the collagen from rabbit muscle and bone to produce Halal collagen.

The specific objective are:-

- To compare the percentage of collagen content in whole muscle and legs bone of young and spent rabbit in method 1(acetic acid and sodium hydroxide) and method 2(amyl alcohol, acetic acid and sodium chloride)
- 2. To identify the chemical composition of collagen in young and spent rabbit
- 3. To identify the gelatine physical quality and characteristics

## **1.3 HYPOTHESIS**

Spent rabbit expected to provide higher percentage of collagen then young rabbit while muscle part expected to provide higher percentage of collagen then bone part.

#### REFERENCE

A.O.A.C (2000). Official Methods of Analysis. 17 Ed. Association of official analytical chemist. Washington, D.C USA, Pages 69-88

Babraj, J., D.J. Cuthbertson, P. Rickhuss, W. MeierAugenstein, K. Smith, J. Bohe,

R.R. Wolfe, J.N.A. Gibson, C. Adams and M.J. Rennie (2002). Sequential extracts of human bone show differing collagen synthetic rates. Biochemical Society Transactions. 30(2): 61-65.

Batge, B. J. Diehold, H. Stein, M. Bodo and P.K. Muller (1992) Compositional analysis of The collagenous bone matrix: A study on adult normal and osteopenic bone tissue. European Journal of Clinical Investigation. 22: 805-812.

Cheryl H.M (2014). Exploitation to animal for human used. (Rabbit the most

exploited animal) 43: 60-71

Ding, Min zhang, Guoying Li (2015). International Journal of Biological

Macromolecules, Vol 80, Page 317-323.

Dahlan (1993). Animal Industry In Malaysia, Faculty of Vet.Med. and Animal Sc.

UniversitiPertanian Malaysia. Rabbit industry in Malaysia p 99-100

Gloria N, Micheal, John walsh (2002). Animal body protein and the composition of

Fatty acid. P19-23

Giridharan, (2000). Potential animal used in biomedical research (rabbit as animal

research). P 43-60

Hua Yang, Borderías J, Turnay J, Leyzarbe MA. (2013). Characterization of hake (MerlucciusmerlucciusL.) and trout (Salmo irideusGibb.) collagen. J Agric Food Chem 38(3):604-609.

Hussin, N. (2012). Extraction and Characterization of Collagen Extracted from the

Skin of Stripped Catfish (Pangasianodonhypophthalmus), (January), 13-20.

Johnston-Banks FA. 1990. Dressing percentage and the quality of the animal slaughter, P 233-289.

- Lodish, John micheal (2000). Screening for raw material of modified collagen in marineanimal skins caught in coastal offshore water in Korea. AgricChem Biotech. 39(2):134-139.
- Liu, D.C., Lin, Y. K., and Chen, M.T. (2001). Optimum Condition of Extracting
  Collagenfrom Chicken Feet and Its Characteristics. Asian-Aust. J. Anim. Sci.
  Vol 14. No 11: Pages 1638-1644

Lodish H, Berk A, Zipursky SL, et al (2000) Molecular Cell Biology. 4th edition.

New York: W. H. Freeman; 2000. Section 22.3, Collagen: The Fibrous Proteins of the Matrix. Available from:

http://www.ncbi.nlm.nih.gov/books/NBK21582/

Mhaske, Henrickson RL. (2010). Chemical, biochemical, functional, and nutritional characteristics of collagen in food systems. Advances in food research 28. London: Academic Press. P 232-372.

Muller, W.E.G. (2003). The Origin of Metazoan Complexity: Porifera as Integrated

Animals. Integrative & Comparative Biology. 43(1):03–10

- Micheal M, John, Hudson (2006). Collagen types and principle tissue distribution in animal tissue. 72(3): 4-5
- Mitchell ,Hafsteinsson H. 1976. Gelatin from cod skins as affected by chemical treatments. J Food Sci 62(1):37-39 47.
- Nistor, V. A. Bampidis, N. Păcală, M. Pentea, J. Tozer, H. Prundeanu.(2013). Nutrient Content of Rabbit Meat as Compared to Chicken, Beef and Pork Meat. 172-176. <u>doi:10.5455/japa.20130411110313</u>.
- Nilson, Sarah, M Pentea (2004). Rabbit production system, Slaughtering and dressing percentage of rabbit. 41(4): 21-2
- Nakamura, R., Sekoguchi, S. and Sato, Y. (1975). The contribution of intramuscular collagen to the tenderness of meat from chicken with different ages. Poultry Sci. 54:1604-1612

Orgel, J.P.R.O., T.C. Irving, A. Miller and T.J. Wess (2006). Microfibrillar structure

of type I collagen in situ. Proceedings of the National Academy of Sciences of the United States of America. 103(24): 9001–9005.

Qureshi, S., Mhaske, A., Raut, D., Singh, R., Mani, A., & Patel, J. (2010). Extraction

and Partial Characterization of Collagen from Different Animal Skins. *Recent Research in Science and Technology*, 2(9), 4. Retrieved from <a href="http://recent-science.com/index.php/rrst/article/view/4777">http://recent-science.com/index.php/rrst/article/view/4777</a>

Ramchandran, Orgel, . 1976. The chemistry and reactivity of collagen. New York:

Academic Press. P 102-202.

Rao, D.R., Chen, C.P., Sunki, G.R. and Johnson W.M., 1978. Effect of weaning and

slaughter ages upon rabbit meat production: II. Carcass Quality and Composition. Journal of Animal Science, 46(3) 578-583.

- Sadaf Quereshi\*, Ashok Mhaske, DishaRaut, Rupal Singh, Abin Mani and Jaswant Patel (2010). Extraction and partial characterization of collagen from different animal skins. Recent Research in Science and Technology 2010, 2(9): 28-31
- Samuel (2009). Isolation of native acid-solublecollagen from fish muscle. Nippon Suisan Gakkaishi 53(8):1431-1436.

Sirkorski DA. (2001). Collagen protein. In: Mitchell JR, Ledward DA, editors.

Functional properties of food macromolecules. London: Elsevier Applied Science. P 171-201.

Takeshi et al,. (2002). Collagen extraction from Diamondback Squid

(Thysanoteuthisrhombus) Outer Skin, 275, 271–275.

Veis, Bergman M. 1964. Process for the production of gelatin from fish skins. U.S.

patent 5,093, 474.

Veit, BH. (2006). Types of collagen and properties of different mammalian and fish

gelatins. Food Hydrocolloids 5:353-361.

Walsh, G. (2004). Protein structure. In: Proteins Biochemistry and Biotechnology

(ed.) G. Walsh. John Wiley and sons Ltd., England. pp. 01-47.

Young, M. F.(2003). Bone matrix proteins: their function, regulation, and

relationship to osteoporosis. OsteoporosInt 14 Suppl 3: S35-42.

