Optimization of Protein Extraction for Slaughtered and Non Slaughtered Broiler Chicken Meat Authentication by Biogenic Silver Nanoparticles Interaction

by

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A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

Institute of Nano Electronic Engineering

UNIVERSITI MALAYSIA PERLIS

2016

ACKNOWLEDGEMENTS

In the name of Allah, the most Benevolent, the most Merciful. First of all, I wish to record an immeasurable gratitude and thankfulness to Allah, the One and the Almighty Creator for giving me the determination to complete this research.

I would like to express my sincere gratitude and indebtedness to my supervisor Dr. Zarina Zakaria, co-supervisor Professor Uda Hashim for their excellent ideas, invaluable guidance, and constant support in making this research possible over the years. My deepest admiration goes to Dr. Zarina Zakaria, who thinks every moment as an 'academic quarter of an hour' associated with it, and always had an open door to let me pick her brain about research area. Her profound technical insight and vision have helped in giving the proper shape to this thesis. I am deeply indebted to the University Malaysia Perlis (UniMAP), Malaysia for partially supporting this research.

I would like to special gratitude to my brother Associate Professor Dr. Fazlul Bari and my husband Dr. Md Samsuzzaman and thanks all of my friends and co-researchers especially Miss Aziera Rasib, Miss Sharul Aida, and staffs of the School of Bioprocess Laboratory, Institute of Nano Electronic Engineering (INEE) Laboratory, all staff of Institute of Nano Electronic Engineering (INEE) and Faculty of Engineering Technology, UniMAP, who helped me in many ways especially during my research work.

Last, but not least, I owe special gratitude to my parents, my brothers, my sister, other family members, friends and relatives for their encouragement throughout my study.Without the continual support from my family, there is no way I would be where I am today. It is my great pleasure to dedicate the thesis to my parents.

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LIST OF ABBREVIATIONS

ATP	Aadenosine Triphosphate
BCA	Bicinchoninic Acid
BSA	Bovin Serum Albumin
CC	Column Chromatography
DMF	N-dimethylformamide
DSC	Differential Scanning Calorimetry
ELISA	Enzyme-linked immunosorbent assay
FPPM	Filter Paper Press Method
FTIR	Fourier transform infrared spectroscope
GLM	General Linear Model
HMM	Heavy Meromyosin
HPH	High-Pressure Homogenization
HPLC	High-performance liquid chromatography
ITC	Isothermal Titration Calorimetry
LMM	Light Meromyosin
NPs	Nanoparticles
ORD	Optical Rotary Dispersion
PS	Properly Slaughtered
SPR	Surface Plasmon Resonance
TEM	Transmission electron microscope
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
WC	Water Content
ANOVA	Analysis of Variance

LIST OF SYMBOLS

˜	Combining Tilde Overlay
−	Minus Sign
"	Quotation Mark
%	Percent Sign
&	Ampersand
:	Colon
;	Semicolon
+	Plus Sign
⁄	Division Slash
<	Less Than Sign
=	Equals Sign
>	Greater Than Sign
±	Plus-Minus Sign
×	Multiplication
≤	Less Than or Equal To
≥	Greater Than or Equal To
→	Rightwards Arrow
↓	Downwards Arrow
°	Degree
C	Centigrade
α	Alpha
β	Beta
γ	Gamma
Δ	Delta
ε	Epsilon
έ	Epsilon With Oxia
ε_0	Epsilon Not
λ	Lamda
μ	Mu
π	Pi
σ	Sigma

Optimization of Protein Extraction for Slaughtered and Non Slaughtered Broiler Chicken Meat Authentication by Biogenic Silver Nanoparticles Interaction

ABSTRACT

The slaughtering of broiler chicken is considered as a key factor to reduce the blood volume in the meat. Today, consumer demands low fat, safe, healthy and fresh meat. In order to fulfill above demands, meat should have low levels of blood, which can readily be achieved by the proper slaughtering of animals. Currently, there is no proper identifying method available to differentiate between slaughtered and non-slaughtered broiler chicken meat. Therefore, distinguish between slaughtered and non-slaughtered meat is an important element in meat industries for a healthy and pious living. In addition, proteins are essential components of meat tissue and they participate in every process within cells. The protein extractability, protein solubility, and water content measurement of slaughtered and non-slaughtered meat were conducted in this research. The silver nanoparticles were prepared by simple, capable, and eco-friendly biosynthesis method using plant extracts. The highest protein extraction for phosphate buffer was obtained at pH 8.0, with a value of 92.80 mg/g. In addition, the significant protein extractability was found for non-slaughtered meat at pH 8.0 with a value of 95.43 mg/g compare to slaughtered meat value was 81.42 mg/g and the optimum protein extractability was found for non-slaughtered meat with a value of 96.40 compare to slaughtered meat value 87.23 at 25° C and NaCl was best protein extractant for non-slaughtered meat at 1.4 molarity. It was observed that the significant protein solubility was found at pH 8.0 for Na_2SO_4 of non-slaughtered meat with a value of 95.03 g/100g and optimum temperature was found at 25° C with 95.50 g/100g. Moreover, pH 5.5 was responsible for higher water content of non-slaughtered meat with value of 52.49 %. This thesis focuses on the interaction of silver nanoparticles and protein for distinguishing between slaughtered and non-slaughtered meat. This study also easy and time effective than other electrical methods. The interaction of the protein with biogenic silver nanoparticles in aqueous solution was studied through UV–Vis spectral changes, FTIR spectroscopy, and TEM analysis. The UV-Vis absorbance band of the interacted silver nanoparticles with a non-slaughtered meat protein was higher than slaughtered protein. The high absorbance peak may be attributed to the non-slaughtered protein was more aggregate on the nanoparticle surfaces compare to slaughtered protein. The conformational changes of proteins upon interaction with silver nanoparticles may be due the slaughtering time. The FTIR spectroscopy study revealed the changed functional group of non-slaughtered and slaughtered meat protein after interaction with silver nanoparticles. The TEM study of the interacted silver nanoparticles was also carried out to shown the interaction changes. This study revealed that the non-slaughtered protein was more aggregate on nanoparticles surfaces than slaughtered protein. From another point of view, the slaughtered protein interaction showed the presence of a protein layer surrounding the silver nanoparticles, on the other hand, the surrounding protein layers on silver nanoparticles surfaces were absence for non-slaughtered protein. This study established that it is possible to distinguish between slaughtered and non-slaughtered broiler chicken meat using biogenic silver nanoparticles.

Pengoptimuman pengekstrakan Protein untuk disembelih dan tidak disembelih Broiler Ayam Daging Pengesahan oleh biogenik Silver nanopartikel interaksi

ABSTRAK

Penyembelihan ayam daging dianggap sebagai faktor utama untuk mengurangkan jumlah darah dalam daging. Hari ini, pengguna menuntut rendah lemak, selamat, sihat dan daging segar. Bagi memenuhi permintaan di atas, daging harus mempunyai tahap yang rendah dalam darah, yang mudah boleh dicapai dengan penyembelihan yang betul haiwan. Pada masa ini, tidak ada kaedah mengenal pasti betul disediakan untuk membezakan antara daging ayam daging yang disembelih dan tidak disembelih. Oleh itu, membezakan antara disembelih dan daging yang tidak disembelih adalah elemen penting dalam industri daging untuk hidup sihat dan soleh. Di samping itu, protein adalah komponen penting dalam tisu daging dan mereka yang mengambil bahagian dalam setiap proses dalam sel. The extractability protein, kelarutan protein, dan pengukuran kandungan air daging yang disembelih dan bukan disembelih telah dijalankan dalam kajian ini. Nanopartikel perak telah disediakan dengan kaedah biosintesis mudah, mampu, dan mesra alam menggunakan ekstrak tumbuhan. Pengekstrakan protein tertinggi bagi penimbal fosfat telah diperolehi pada pH 8.0, dengan nilai 92.80 mg / g. Di samping itu, extractability protein yang signifikan untuk daging bukan disembelih pada pH 8.0 dengan nilai 95.43 mg / g berbanding dengan nilai daging disembelih 81.42 mg / g dan extractability protein optimum ditemui kerana tidak disembelih daging dengan nilai daripada 96,40 berbanding dengan nilai daging yang disembelih 87,23 pada 25 ° C dan NaCl adalah extractant protein terbaik bukan disembelih daging pada 1.4 kemolaran. Ia adalah diperhatikan bahawa kelarutan protein yang signifikan pada pH 8.0 untuk Na2SO4 daging tidak disembelih dengan nilai 95,03 g / 100g dan suhu optimum ditemui pada 25 ° C dengan 95.50 g / 100g. Selain itu, pH 5.5 bertanggungjawab untuk kandungan air yang lebih tinggi daripada daging yang tidak disembelih dengan nilai 52.49%. Tesis ini memberi tumpuan kepada interaksi nanopartikel perak dan protein untuk membezakan antara daging yang disembelih dan tidak disembelih. Kajian ini juga mudah dan masa yang berkesan daripada kaedah elektrik yang lain. Interaksi protein dengan nanopartikel perak biogenik dalam larutan akueus dikaji melalui perubahan UV-Vis spektrum, FTIR spektroskopi, dan analisis TEM. The band kuantiti UV-Vis daripada nanopartikel perak berinteraksi dengan protein daging yang tidak disembelih adalah lebih tinggi daripada protein disembelih. Puncak kuantiti yang tinggi boleh dikaitkan dengan protein bukan disembelih lebih agregat nanoparticle permukaan berbanding dengan protein disembelih. Perubahan conformational protein kepada interaksi dengan partikel perak mungkin disebabkan masa penyembelihan. The FTIR kajian spektroskopi mendedahkan kumpulan berfungsi berubah protein bukan disembelih dan menyembelih daging selepas interaksi dengan nanopartikel perak. Kajian TEM daripada nanopartikel perak berinteraksi juga telah dijalankan untuk menunjukkan perubahan interaksi. Kajian ini membuktikan bahawa protein bukan disembelih lebih agregat nanopartikel permukaan daripada protein disembelih. Dari satu sudut pandangan, interaksi protein yang disembelih menunjukkan kehadiran lapisan protein sekitar nanopartikel perak, di sisi lain, lapisan protein sekitar pada permukaan nanopartikel perak adalah ketiadaan protein bukan disembelih. Kajian ini menetapkan bahawa ia adalah mungkin untuk membezakan antara daging ayam daging disembelih dan tidak disembelih menggunakan nanopartikel perak biogenik.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Poultry meat contributes substantially to the human diet. In the whole world, poultry meat is an important, low-cost source of animal protein. This encourages the consumption of poultry products by a large number of consumers. The consumption of poultry meat has increased by most of the people in these countries. Currently, poultry plants are slaughtering between 140 to 180 broilers per minute; sometimes animals are not slaughter, which makes the manual slaughtered necessary. Traditionally, slaughter practices have dealt with factors that affect wholesomeness and quality of meat. For instance, the meat for Muslim consumption is required to be halal and thoyyib (meaning acceptable and wholesome). The industry aims at achieving customer acceptability through the development and control of processes in order to produce wholesome products with high quality and safety (Castro-Giráldez, Dols, Toldrá, & Fito, 2011), while consumers expect meat products to have the expected nutritional value, wholesomeness, and freshness; all of which are influenced by the animal production system. Slaughtering is such a vital step in the production chain for not only animal welfare, but also meat quality and safety.

To optimize bleed out at slaughter and reduce carcass and meat defects is a major goal of the meat processing industry, as improved bleeding can improve the quality of the

meat during storage (Ali, Abdalla, & Mahgoub, 2011). Inefficient and improper bleeding may cause more blood to be retained in the meat. Blood favours multiplication of spoilage microorganisms and acts as a carrier for food borne pathogens (Lerner, 2009). Additionally, residual blood in the meat equates to retention of more haemoglobin. Haemoglobin is a powerful promoter of lipid oxidation (Alvarado, Richards, O'Keefe, & Wang, 2007). Lipid oxidation constitutes a major cause of non-microbial meat spoilage, especially under pro-oxidative conditions such as storage and cooking. It can also occur during refrigeration and frozen storage (Soyer, Özalp, Dalmış, & Bilgin, 2010).

Halal or Islamic slaughtering process is implemented for the production of halal chicken. It must be executed by a throat cut in order to bring the animal to a quick death without suffering. This leads to more bleeding and rapid speed of blood flow in the blood vessels before clotting. Slaughtering methods can be associated with composition and post-mortem quality of chicken meat, mediated by varying blood retained. From a health point of view, proper animal slaughter causes a rapid and thorough bleeding process producing a healthier and less contaminated meat (D'Agata, Russo, & Preziuso, 2010; Hanzaee & Ramezani, 2011). On the other hand, from religion point of view, consuming properly slaughtered meat is a must for Jews and Muslims (López et al., 2008). Therefore, the distinction between slaughtered and non-slaughtered meat is an important element in meat industries for a healthy and pious living.

Meat is a biological tissue that supplies the human body with protein necessary for growth. Meat with high blood content, however, is considered as an unhealthy meat because the blood retained in the meat could potentially become a growth medium for hazardous microorganisms and bacteria (Nurdeng, 2009; Regenstein, Chaudry, & Regenstein, 2003). To obtain the healthy meat, it is recommended to drain out as much

blood as possible from the animal during slaughter. Therefore, a proper animal slaughtering process that causes a rapid and thorough bleeding out must be used.

Silver nanoparticles are clusters of silver atoms in the size range of 1–100 nm. "Nano" is a Greek word synonymous to dwarf meaning extremely small. Besides, application of silver nanoparticles finds limited use in meat technology. From tiny and sophisticated projects of distinguishing slaughtered and non-slaughtered meat, the nanoparticles application is one of the most potential research topics. Silver nanoparticles are important materials that have been studied extensively. They can be synthesized by several physical, chemical and biological methods (Sharma, Yngard, & Lin, 2009; Zhang, Peng, Huang, Zhou, & Yan, 2008).

On many of the silver nanoparticles application, the silver nanoparticles using such as a nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine (Jain, Huang, El-Sayed, & El-Sayed, 2008; Lee & El-Sayed, 2006; Nair & Laurencin, 2007). Wherever the silver nanoparticles (AgNPs) come in contact with a living organism, physical and chemical interactions take place between the surfaces of the AgNPs and bio matter, in particular, proteins. When AgNPs are exposed to biological fluids, an adsorption layer of proteins, a "protein corona" forms around the AgNPs (Röcker, Pötzl, Zhang, Parak, & Nienhaus, 2009). Consequently, macromolecules interact with the protein-coated AgNPs. To anticipate biological responses to AgNPs, we thus require comprehensive knowledge of the interactions at the bio–nano interface. In recent years, a wide variety of biochemical techniques has been employed to elucidate mechanistic aspects of AgNPs–protein interactions. Understanding the formation and persistence of the protein corona is a complex task and of great importance for the elucidation, interpretation, and assessment of the biological effects of AgNPs. The

formation process is essentially a competition of proteins and other biomolecules for binding to the AgNPs surface (Cedervall et al., 2007).

1.2 Problem Statement

Meat is considered as a source of high-quality protein for humans. One of the most effective parameters which influence the quality of meat is the residual blood in the meat after slaughtering the animal. Blood is a good medium for microorganisms to grow and poison or deteriorate the meat. The proper slaughtering of animals is the key factor to reduce the blood volume in the meat. Muslims have genuine concerns regarding the origin of the meat at the market. On the other hand, several Muslim countries import meat whose origin is doubtful and bearing an unverifiable halal-label. This is because there is no proper method available to check either meat is slaughtered or not. Slaughtered meat has positive health and hygiene implications because low blood content present in that meat since blood is a very good medium for the growth and multiplication of microorganisms, it can have a very dangerous effect on human health and causes a visual problem for the consumer (Rosen, 2004). So far, study on various frequencies showed that dielectric constant for slaughtered chicken meat was lower than that of non-slaughtered chicken meat (Adam & Nasukha, 2011). Studied on dielectric properties also showed similar results that properly slaughtered chicken meat showed lower dielectric properties than the non-properly slaughtered chicken meat. In terms of colour, there was a clear difference, where the properly slaughtered chickens were a light red and the non-properly slaughtered chickens were more reddish (Rabih, Rawther, Bin Ibrahim, & Burhanudin, 2011). Those methods are an electrical test, where they used the sophisticated and expensive equipment that are not cost effective and also not an easy method. On the other hand, the biochemical

test of identifying slaughtered meat is an easy procedure, time effective and also cost-effective. Moreover, it has a high potential for using in the quality control of animal tissues (Damez, Clerjon, Abouelkaram, & Lepetit, 2008).

Therefore, there is a need to develop a more reliable biochemical testing method to differentiate between properly slaughtered and non-slaughtered meat. Silver nanoparticles are a promising compound which can be used for this purpose. So, the aim of this thesis is to distinguish between slaughtered and non-slaughtered broiler chicken meat by using biogenic silver nanoparticles.

1.3 Objective of the Research

The main objective of this research is to differentiate slaughtered and non-slaughtered broiler chicken meat by using biogenic silver nanoparticles. The objectives of this research can be listed as follows:

1. To optimize the protein extraction of slaughtered and non-slaughtered broiler chicken meat.
2. To determine the protein solubility and water content of slaughtered and non-slaughtered broiler chicken meat.
3. To synthesis the silver nanoparticles by a biological method from selected plant.
4. To evaluate the protein-biogenic silver nanoparticles interaction in slaughtered and non-slaughtered broiler chicken meat.

1.4 Scope of Work

The main emphasis of this research was to differentiate slaughtered and non-slaughtered broiler chicken meat by biogenic silver nanoparticles interaction. In order to achieve that, the research had been divided into many parts; total protein content, protein extraction, protein solubility, the water content of meat, protein purification from slaughtered and non-slaughtered broiler meat, synthesis of biogenic silver nanoparticles and protein interaction with biogenic silver nanoparticles.

In order to start, a comprehensive review was covered to obtain knowledge according to the objectives. The total protein content, protein extraction and protein solubility were measured from slaughtered and non-slaughtered meat, because protein is the important macromolecule of meat muscle and conformational change of proteins depends on many factors. The meat proteins, approximately 20% of a muscle's weight, represent the main constituents that make up the structure of the meat. They undergo substantial changes on slaughtered condition and therefore the quality of the meat, which is mainly governed by the meat structure, also changes drastically after slaughtering. Besides, water content was also measured in this research because the meat muscle consists of 75% water, during the slaughtering time, most of the water released from the meat, so water content was varies to slaughtered and non-slaughtered meat. After that protein separated from slaughtered and non-slaughtered was conducted by column chromatography. Moreover, to synthesize the biogenic silver nanoparticles from fruits extract were obtained, this synthesized method is eco-friendly, easy and cost-effective. The proposed differentiate technique were using separated protein from slaughtered and non-slaughtered meat and biogenic silver nanoparticles interaction. The measurement of interaction was carried out using UV-Vis spectrophotometer, FTIR spectroscopy, and

TEM analyses. Finally, the comparison was made between slaughtered and non-slaughtered results then analysed and documented.

1.5 Hypothesis

The hypothesis of this research is that slaughtered and non-slaughtered broiler chicken meat is able to be differentiated by the interaction of the protein with biogenic silver nanoparticles.

1.6 Outline of the Thesis

This thesis is organized in five chapters as follows:

Chapter 1 presents the inception of the thesis. The problem statement and research objective are described in this chapter. The research scopes and the hypothesis of the thesis are also outlined in this chapter.

Chapter 2 describes the literature review for this study. Review of previous studies and overview of slaughter and non-slaughter method, as well as the differentiate technique of slaughtered and non-slaughtered meat, are discussed. The literature on synthesized biogenic silver nanoparticles and protein-nanoparticles interactions are also included.

Chapter 3 provides the methodology of this study including the protein extractability and protein solubility of slaughtered and non-slaughtered meat and separated the protein from meat. The technique to differentiate slaughtered and non-slaughtered meat by using biogenic silver nanoparticles and protein interaction are also

included. The flowchart of research methodology is described in detail with suitable equations and photographs.

Chapter 4 describes the total protein content measurement and protein extractability on slaughtered and non-slaughtered meat. In this chapter, protein solubility and water content of meat are presented. Then, protein purified from slaughtered and the non-slaughtered meat was also done. The silver nanoparticles synthesized had been based on the biological process which is eco-friendly and cost-effective. Besides, the slaughtered and non-slaughtered meat proteins, interaction with biogenic silver nanoparticles results are also presented. Finally, the results of the comparative analysis, along with discussions, are also included.

Chapter 5 presents the concluding remarks of the researches according to the objectives.