Impact of Halal Slaughtering on Quality and Shelf-life of Broiler Chicken Meat

Aneesa Addeen

A Thesis Submitted in Partial Fulfillment of the Requirement for the Degree of Master of Science in Food Science and Technology

Prince of Songkla University

2014

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ชื่อวิทยานิพนธ์ ผลของการเชือดแบบฮาลาลต่อคุณภาพและอายุการเก็บรักษาเนื้อไก่กระทง
ผู้เขียน นางสาวอานีสาห์ อัดดีน
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บทคัดย่อ

จากการศึกษาผลของวิธีการเชือดต่างๆต่อคุณภาพและความคงตัวของเนื้อไก่ส่วนหน้าอก พบว่าเนื้อไก่วิธีการเชือดแบบฮาลาล (Islamic slaughtering method; IM) วิธีการเชือดแบบตัดศีรษะ (Decapitation method; DM) วิธีการเชือดแบบดั้งเดิม (Conventional neck cut method; CM) และไม่ผ่านการเชือด (Un-bled sample UN) มีปริมาณเหล็กที่อยู่ในรูปฮีมเท่ากับ 2.41, 2.35, 2.56 และ 3.41 มิลลิกรัม/100 กรัม และปริมาณเหล็ก 10.09, 12.47, 14.21 และ 18.10 มิลลิกรัม/กิโลกรัม ตามลำดับ โดยพบว่าเนื้อไก่ที่ผ่านการเชือดมีปริมาณเหล็กที่อยู่ในรูปฮีมและปริมาณเหล็กที่ไม่อยู่ในรูปฮีมใกล้เคียงกัน ระหว่างการเก็บรักษาที่อุณหภูมิ 4 องศาเซลเซียส นาน 8 วัน พบว่าตัวอย่าง IM มีค่า Peroxide value (PV) และ Thiobarbituric acid reactive substances (TBARS) ต่ำที่สุดภายในสี่วันแรกของการเก็บรักษาเมื่อเทียบกับเนื้อไก่ที่ได้จากวิธีการเชือดอื่น ($P<0.05$) และไม่มีความแตกต่างของรูปแบบโปรตีนระหว่างเนื้อไก่ที่ได้จากวิธีการเชือดที่แตกต่างกัน อีกทั้งพบว่าเนื้อไก่ที่ได้จากการเชือดด้วยวิธีการแบบฮาลาลมีปริมาณกรดไขมันไม่อิ่มตัวสูงในปริมาณที่สูงสุดระหว่างการเก็บรักษาเป็นเวลา 8 วัน ปริมาณแบคทีเรียที่ชอบอุณหภูมิปานกลาง (MBC) และปริมาณแบคทีเรียที่ชอบอุณหภูมิต่ำ (PBC) มีค่าสูงสุดในเนื้อไก่ที่ไม่ผ่านการเชือด ($P<0.05$) และเนื้อไก่ที่ไม่ผ่านการเชือดมีค่า a^* ΔE^*และ ΔC^* สูง อีกทั้งพบว่าค่า L^* และ a^* มีการลดลงภายหลังการเก็บรักษานาน 8 วัน

การศึกษาคุณภาพของเนื้อไก่ขึ้นรูปสุกจากไก่ที่ผ่านการเชือดด้วยวิธีการที่แตกต่างกันระหว่างการเก็บรักษาที่ 4 องศาเซลเซียสเป็นระยะเวลา 12 วัน พบว่าเนื้อไก่ขึ้นรูปสุกที่ได้จากการเชือดแบบฮาลาลมีค่า PV และ TBARS ต่ำกว่าเนื้อไก่ขึ้นรูปสุกที่ได้จากเนื้อไก่ที่ผ่านการเชือดด้วยวิธีการอื่น ($P<0.05$) ซึ่งสอดคล้องกับปริมาณของเฮกซานาลและออกทานาลที่ต่ำสุด ภายหลังการเก็บรักษานาน 12 วัน เนื้อไก่ขึ้นรูปสุกที่ได้จากการเชือดแบบฮาลาลให้ค่า a^* ต่ำและค่า L^* สูงกว่ารวมทั้งมีค่าความแข็งสูงกว่าเมื่อเทียบกับเนื้อไก่ขึ้นรูปสุกที่ได้จากไก่ที่ผ่านการเชือดด้วยวิธีการอื่น ($P<0.05$) เมื่อพิจารณาสมบัติทางประสาทสัมผัส พบว่าเนื้อไก่ขึ้นรูปสุกจากตัวอย่าง IM มีคะแนนความชอบสูงสุด ($P<0.05$) นอกจากนี้ปริมาณแบคทีเรีย MBC และ PBC ต่ำสุดเมื่อเทียบกับเนื้อไก่ขึ้นรูปสุกที่ได้จากการเชือดด้วยวิธีการอื่น ($P<0.05$).

จากการศึกษาผลของฮีโมโกลบินในรูปของออกซีฮีโมโกลบิน (OxyHb) และเมทฮีโมโกลบิน (MetHb) ต่อการเกิดออกซิเดชั่นของเนื้อไก่บดที่ผ่านการล้างระหว่างการเก็บรักษาที่ 4 องศาเซลเซียส เป็นระยะเวลา 8 วันพบว่า เนื้อไก่บดที่ผ่านการล้างและเติม OxyHb และ MetHb ให้ค่า PV และ TBARS ที่สูงกว่าเมื่อเทียบกับชุดควบคุม ($P<0.05$) และออกซีฮีโมโกลบินเป็นตัวเร่งการเกิดออกซิเดชั่นที่

ดีกว่าเมทฮีโมโกลบินโดยให้ค่า PV และ TBARS ที่สูงกว่า (P<0.05) อีกทั้งสารประกอบอัลดีไฮด์ที่ระเหยได้มีค่าสูงสุดในเนื้อไก่บดที่ผ่านการล้างและเติมออกซีฮีโมโกลบินเมื่อเปรียบเทียบกับเนื้อไก่ในชุดควบคุมและเนื้อไก่ที่มีการเติมเมทฮีโมโกลบิน ในวันที่ 0 ของการเก็บรักษาเนื้อไก่ที่ผ่านการล้างและเติม OxyHb ให้ค่า a* สูงกว่าแต่ให้ค่า b* และ L* ต่ำกว่าเมื่อเทียบกับชุดควบคุม และพบว่าค่า a* ในตัวอย่างที่มีการเติม OxyHb มีค่าลดลงภายหลังการเก็บรักษาที่อุณหภูมิ 4 องศาเซลเซียส นาน 8 วัน

เมื่อศึกษาผลของเลือดต่อการเจริญของเชื้อแบคทีเรียก่อโรคและเชื้อแบคทีเรียที่ก่อให้เกิดการเน่าเสียในระหว่าการเก็บรักษาที่ 4 องศาเซลเซียสเป็นระยะเวลา 8 วันพบว่าในอาหารเลี้ยงเชื้อที่มีการเติมเลือด 5 ไมโครโมล/มิลลิลิตร ให้ผลการเจริญของเชื้อ *Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella* Typhimurium และ *Campylobacter jejuni* สูงที่สุดเมื่อเทียบกับอาหารเลี้ยงเชื้อที่ไม่มีการเติมเลือด (P<0.05) ในเนื้อไก่บดที่มีการเติมเลือด 10 ไมโครโมล/กรัม พบว่ามีการเจริญของเชื้อสูงสุด (P<0.05) MBC และ PBC ของเนื้อไก่บดที่มีการเติมเลือด 10 ไมโครโมล/กรัม มีปริมาณเพิ่มจาก 4.04 เป็น 9.49 และจาก 3.54 เป็น 9.84 log CFU/g ภายหลังการเก็บรักษานาน 8 วัน โดย MBC และ PBC ของเนื้อไก่บดที่มีการเติมเลือด 10 ไมโครโมล/กรัม ให้ผลการเจริญที่มากกว่าเมื่อเทียบกับเนื้อไก่บดที่มีการเติมเลือดที่ระดับอื่นๆ (P<0.05) เมื่อเปรียบเทียบเนื้อไก่บดที่ได้จากการเชือดด้วยวิธีการที่แตกต่างกันพบว่าตัวอย่าง CM มีปริมาณเชื้อแบคทีเรียก่อโรคและเชื้อแบคทีเรียที่ก่อให้เกิดการเน่าเสียสูงกว่าตัวอย่าง IM (P<0.05) ดังนั้นการเชือดแบบฮาลาลสามารถลดปริมาณเลือด ส่งผลให้ลดการเกิดออกซิเดชั่นของไขมัน การเปลี่ยนแปลงสี และชะลอการเจริญของจุลินทรีย์ในเนื้อไก่ระหว่างการเก็บรักษาที่อุณหภูมิต่ำ

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ABSTRACT

Effect of different slaughtering methods on quality and stability of chicken meat was investigated. Chicken breast meat from Halal or Islamic slaughtering method (IM), decapitation method (DM), conventional neck cut method (CM) and un-bled sample (UN) contained haem iron contents of 2.41, 2.35, 2.56 and 3.41 mg/100g sample with Fe content of 10.09, 12.47, 14.21 and 18.10 mg/kg, respectively. Similar haem and non-haem iron contents were found amongst bled samples. During the storage at 4ºC for 8 days, IM sample showed the lower peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) within the first four days of storage, compared with others ($P<0.05$). There were no differences in protein patterns of chicken meat obtained from different slaughtering methods. PUFA content of chicken meat from IM sample was higher than that of samples bled with other methods after 8 days of storage. Higher mesophilic bacteria count (MBC) and psychrophilic bacterial count (PBC) were observed in UN sample, as compared to the slaughtered samples ($P<0.05$). Un-bled samples had the higher a*, ΔE*and ΔC* values than bled counterparts, and L* and a* values decreased after 8 days of storage ($P<0.05$).

Quality of cooked patties from chicken obtained from various slaughtering methods was monitored during 12 days of refrigerated storage. Cooked patties from IM chicken showed the lower PV and TBARS, compared with others ($P<0.05$). This was coincidental with the lowest abundance of the hexanal and octanal. After 12 days of storage, cooked patties from IM chicken had the lower a* but higher L* value and exhibited the higher hardness in comparison with other samples. For sensory property, the higher likeness score was observed for cooked patties from IM chicken ($P<0.05$). Additionally, MBC and PBC were lower in cooked patties from IM chicken ($P<0.05$).

Impact of oxyhaemoglobin (OxyHb) and methaemoglobin (MetHb) on lipid oxidation and color of washed chicken mince during 8 days of refrigerated storage was investigated. Washed chicken mince added with oxyHb and metHb showed the higher PV and TBARS, compared with the control sample ($P<0.05$). Oxy-form was more pro-oxidative than met-form as evidenced by the higher PV and TBARS ($P<0.05$). Volatile aldehydes were also formed at higher extent in the washed chicken mince added with oxyHb, compared with

the control and that added with metHb. At day 0 of storage, washed chicken mince added with oxyHb showed the higher a* value but lower L* and b* value when compared with the control sample. The decrease in a*value was more pronounced in the washed chicken mince containing oxyHb at day 8 of refrigerated storage.

The effect of blood on growth of pathogen and spoilage bacteria of chicken mince during storage of 8 days at 4°C was studied. Broth containing 5 µmol/mL blood showed the higher growth of *Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella* Typhimurium and *Campylobacter jejuni*, when compared to that without blood ($P<0.05$). The highest microbial count was found in chicken mince added with blood at a level of 10 µmol blood/g ($P<0.05$). MBC and PBC of chicken mince containing 10 µmol blood/g increased from 4.04 to 9.49 and from 3.54 to 9.84 log CFU/g, respectively, after 8 days of storage. The higher MBC and PBC were observed in the chicken mince with 10 µmol blood/g, in comparison with those with other blood concentrations ($P<0.05$). When chicken mince from different slaughtering methods were stored for 8 days, both pathogen and spoilage bacteria in the CM sample method showed the higher bacterial counts than IM sample ($P<0.05$). Therefore, Islamic slaughtering method could be a means to reduce blood, in which lipid oxidation, discoloration and microbial growth in chicken meat could be retarded during refrigerated storage.

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CHAPTER 1
INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Islam is the world's second largest religion, with the fastest growing rate. The worldwide Muslim population is 2.1 billion (Religious population, 2012). Islam is not only a religion of rituals, but also it is a way of life. Rules and manners govern the life of the individual Muslim. In Islam, eating is considered a matter of worshiping God, like ritual prayers. Islamic law prescribes a set of dietary rule, called "Halal" (legal, permitted by Allah) which lists the permitted food and prohibit the consumption of meat not obtained according to Islamic rules, covering livestock handling before and during slaughter (Regenstein *et al*., 2003; Bonne, 2008). Muslims must follow the Islamic dietary code and the foods, which meet that code are called halal (lawful or permitted). Muslims are supposed to make an effort to obtain halal food of good quality. It is their religious obligation to consume only halal food. For non-Muslim consumers, halal foods are often perceived as specially selected and processed to achieve the halal standards of quality.

Poultry production and processing involve a series of interrelated steps designed to convert domestic birds into ready-to-cook whole carcasses, cut-up carcass parts, or various forms of deboned meat product (Alan, 2001). However, poultry meat is perishable if it is not handled properly. Food safety and the shelf-life aspects of chicken meat are of important concerns in relation to the microbial growth. The focus is mainly on the absence or control of potentially pathogenic microbes such as *Salmonella* spp. and *Campylobacter* spp. (Ali *et al*., 2011). One of the most important factors that affects the level of contamination and enhance the extent of the deterioration is the amount of blood left within the carcass after bleeding (Ali *et al*., 2011). Blood is considered to be an excellent medium for the growth of bacteria due to its high nutritive value, its temperature, pH and relative humidity. The amount of blood bled by the animal depends on the slaughtering method (Ali *et al*., 2011). Blood components, especially hemoglobin, are powerful promoters of lipid oxidation and may decrease the shelf-life of meat products (Alvarado *et al*., 2007).

There are many slaughtering methods in the world; these include the Halal method, hanging method and stunning (electrical and CO_2) method. The Islamic method is a traditional method of slaughtering for halal foods. Stunning prior to slaughtering is not permitted. If the stunner's voltage is too high, the chicken would be dead before slaughtering. Sams (2001) reported that harsher electrical stunning results in higher incidence of hemorrhaging and broken bones. The chicken meat can be haram (forbidden to be eaten by Islamic law) (Directorate of veterinary public health, 2006). Moreover, chickens which are dead before slaughtering would cause the incomplete bleeding process. Halal slaughtering must be executed by a throat cut in order to bring the animal to a quick death without suffering, by reaction of carotid arteries, jugular veins, trachea and esophagus and absence of previous stunning, allowing a rapid and complete bleeding (Grandin and Regenstain, 1994). In Thailand, slaughtering process for chicken can be varied, depending on the belief or practice. However, halal method has been believed to render the complete bleeding, and it may be beneficial for shelf-life extension or quality maintenance of chicken meat. However, a little information about the effect of slaughtering methods on quality of post-mortem broiler chicken meat, particularly on lipid oxidation and the growth of spoilage bacteria or pathogens. Therefore, the objective of this study is to evaluate the impact of slaughtering method on quality and storage stability of raw and cooked chicken meat. The role of blood in chemical and microbiological deterioration of chicken meat model system will be also studied.

1.2 Review of Literature

1.2.1 Halal concept for production of meat and poultry

Islam is the way of life, guided by what is prescribed in the Quran (the divine Book from God to the Prophet Muhammad for whole of humanity) and by the traditions of the Prophet Muhammad (Peace be upon him) (ASIDCOM Association, 2008). Muslims must ensure that their food is Halal (permissible) (Riaz and chaudry, 2004). This is because food intake will boost the development of human wellness and behavior. On the other hand, Haram (non-permissible) food is explicitly prohibited in

the Quran, Sunnah (the life, actions, and teachings of Prophet Muhammad) and the consensus of the Muslim jurist (Ijma) (Fadzlilah *et al.,* 2011).

1.2.1.1 Halal Food laws and regulations

Halal is an Arabic term which means permitted, allowed, authorised, approved, sanctioned, lawful, legal, legitimate or licit. Guidelines for Halal are given by Allah in the Holy Quran (Nakyinsige *et al*., 2012). General Quranic guidance dictates that all foods are Halal except those that are specifically mentioned as Haram. All foods are made lawful according to the Muslim scripture the Quran:

"O you who have believed, eat from the good things which we have provided for you, and be grateful to Allah, if it is (indeed) him that you worship."

(Al-Baqarah, 2:172)

In Islamic law, Muslims are prohibited from eating the flesh of pork and its derivatives as it is a sin and impiety to do so. These rulings have been stated from islamic law as guidelines to all of mankind. Besides, eating of Haram materials and using it as an adulterant or additive in food products are also forbidden (Fadzlillah *et al*., 2011). This is clearly demonstrated in many verses of the Quran.

The Halal dietary laws determine which foods are lawful or permitted for Muslim. These laws are found in the Quran and in the Sunnah, the practice of the Prophet Muhammad, as recorded in the book of Hadith, the tradition. Islamic law is referred to as Shariah and has been interpreted by Muslim scholars over the years. The basic principles of the Islamic law remain definite and unaltered. However, their interpretation and application may change according to the time, place and circumstances. Apart from 2 basic sources of Islamic law, Quran and the Sunnah, 2 other sources of jurisprudence are used in determining the permissibility of food, when a contemporary situation is not explicitly covered by the first 2 basic sources. The first is Ijma′ meaning a consensus of legal opinion. The second is Qiyas, meaning reasoning by analogy. In the latter case, the process of Ijtihad, or exerting one-self fully to derive and answer to the problem, is used. Animal-derived food ingredients like emulsifiers, tallow and enzymes must be made from animals slaughtered by a Muslim to be Halal (Regenstein *et al.,* 2003).

1.2.1.2 Halal requirements

The classification of animals into halal or haram is clearly stated in the Quran. The permission to kill animals quoted in the religious texts. The meat of the birds that do not use their claws to hold down food, such as chickens, turkeys, ducks, geese, pigeons, doves, partridges, quails, sparrows, emus, and ostriches is permitted. There are special requirements for slaughtering the animal:

- An animal must be of a Halal species
- It must be slaughtered by an adult and sane (mentally competent) Muslim
- Allah's name must be invoked at the time of slaughter
- Slaughtering must be done by cutting the throat in a manner that induces rapid and complete bleeding, resulting in the quickest death. The generally accepted method is to cut at least 3 of the 4 passages (i.e., the carotids, jugular vein, trachea, and esophagus).Some Islamic scholars do accept machine slaughter, particularly of poultry. In recent years, however, the trend has gone back towards requiring hand slaughter of these animals (Regenstein *et al.,* 2003).

The meat of slaughtered animals is called "zabiha" (or "dhabiha") meat. Prophet Muhammad emphasized certain condition for the handling of animals. He said, "Verily Allah has prescribed proficiency in all things. Thus, if you kill, kill well; and if you perform dhabiha, perform it well. Let each one of you sharpen his blade and let him spare suffering to the animal he slays." (Khan, 1991).

Islam places great emphasis on gentle and humane treatment of animals, especially before and during slaughter. Islam encourages conditions include giving the animal proper rest and water, avoiding conditions that create stress, not sharpening the knife in front of the animals, using a very sharp knife to slit the throat, etc. Only after the blood is allowed to drain completely from the animal, the animal which has become lifeless can further be subjected to the dismemberment (cutting off of horns, ears, legs, and so on). Unlike kosher, soaking and salting of the carcass is not required for Halal; Halal meat is therefore treated like other commercial meat.

Fish and other creatures that live in water need not be ritually slaughtered. Similarly, there is no special method of killing the locust. The meat of

the animals that die of natural causes (diseases, for example, or being gored by other animals, being strangled, falling from a height, beating, or killed by wild beasts) is unlawful to be eaten, unless one saves such animals by slaughtering before they actually become lifeless. Fish that dies naturally and is floating on water or lying out of water is still Halal as long as it does not show any signs of decay or deterioration (Regenstein *et al.,* 2003).

1.2.1.3 Conditions and Method of Islamic slaughtering (Dhabh or Zabh)

Dhabh is a clearly defined method of killing an animal for the sole purpose of making its meat to fit for human consumption. The word dhabh in Arabic means purification or rendering something good or wholesome. Dhabh method is also called dhakaat in Arabic, which means purification or making something complete. The following conditions must be fulfilled for dhabh to meet the requirements of the shariah (jurisprudence).

The Slaughter Person

The person performing the act of dhabh must be of sound mind adult Muslim. The person can be of either sex. If a person lacks or loses the competence through intoxication or loss of mental abilities, he or she may not perform Halal slaughter. The meat of an animal killed by an idolater, a non-believer, or someone who has apostatized from Islam is not acceptable.

The Instrument

The knife used to perform dhabh must be extremely sharp to facilitate quick cutting of the skin and severing of blood vessels to enable the blood to flow immediately and quickly, in other words, to bring about an immediate and massive hemorrhage. Muhammad said: “Verily God has prescribed proficiency in all things. Thus, if you kill, kill well; and if you perform dhabh, perform it well. Let each of you sharpen his blade and let him spare suffering to the animal he slays” (Khan, 1991). The use of an instrument that kills the animal by cutting its skin but not severing the jugular vein is forbidden. It is also a tradition not to sharpen the knife in front of the animal about to be slaughtered (Raiz and chaudry, 2004).

The Cut

The incision should be made in the neck at some point just below the glottis and the base of the neck. Traditionally, camels used to be slayed by making an incision anywhere on the neck. This process is called nahr, which means spearing the hollow of the neck. With modern restraining methods and stunning techniques, this procedure might not be appropriate any longer. The trachea and the esophagus must be cut in addition to the jugular veins and the carotid arteries. The spinal cord must not be cut. The head is therefore not to be severed completely. It is interesting to note that the kosher kill is very similar to the traditional method of dhabh described, except that the invocation is not made on each animal.

The Invocation

Tasmiyyah or invocation means pronouncing the name of God by saying Bismillah (in the name of Allah) or Bismillah Allahu Akbar (in the name of God, God is Great) before cutting the neck. Opinions differ somewhat on the issue of invocation, according to three of the earliest jurists. According to Imam Malik, if the name of God is not mentioned over the animal before slaughtering, the meat of such animal is Haram or forbidden, whether one neglects to say Bismillah intentionally or unintentionally. According to the jurist Abu Hanifah, if one neglects to say Bismillah intentionally, the meat is haram; if the omission is unintentional, the meat is Halal. According to Imam Shafii, whether one neglects to say Bismillah intentionally or unintentionally before slaughtering, the meat is Halal so long as the person is competent to perform dhabh (Khan, 1991). It is also enough to state here that the above tradition does not prove that the pronouncing of God's name is not obligatory in performing dhabh. In fact, the tradition emphasizes that the pronouncing of God's name will be a widely known matter and will be considered an essential condition of dhabh (Khan, 1991).

1.2.1.4 Islamic law in relation to blood as food

According to the Quran verses, blood that pours forth is prohibited for consumption. It includes blood of permitted and non-permitted animals alike. Liquid blood is generally not offered for sale or consumed by Muslims or non-Muslims. There is general agreement among Muslim scholars that anything made from blood is unacceptable. Products like blood sausage and ingredients like blood albumin are

either Haram or questionable at best, and should be avoided for product formulations (Regenstein *et al*., 2003).

"He has forbidden you only the Maitah (dead animals), and blood, and the flesh of swine, and that which is slaughtered as a sacrifice for others than Allah..."

(Al-Baqara, 2:173)

"Forbidden to your (for food) are: Al-Maitah (the dead animals, cattle, beasts not slaughtered), blood, the flesh of swine, and that on which Allah's Name has not been mentioned while slaughtering,..."

(Al-Maeda, 5:3)

"Say (O Mohammad sas): "I find not in that which has been revealed to me anything forbidden to be eaten by one who wishes to eat it, unless it be Maitah (a dead animal) or blood poured forth (by slaughtering of the like), or the flesh of swine (pork)..."

(Al-An'am, 16:115)

Another important Islamic principle to note is that one cannot make Haram what Allah has made Halal. One question remains: Is there a limit on how much blood is permitted to be retained in the meat? Tafsir Ibn Kathir, in the commentary of verse 5:3, mentions the following hadith reported by Ahmad and Ibn Majah.

Narrated Ibn 'Umar (May Allah be pleased with him): Allah's Messenger (peace be upon him) said, "Two types of dead animals and two types of bloods have been made lawful for us, the two types of dead animals are locust and fish (seafood), while the two types of bloods are the liver and the spleen"

Thus it can be seen that two important organs from the cardiovascular system namely the liver where blood is processed, filtered and stored and the spleen where red blood cells are produced, stored and removed are Halal. It will be seen earlier that such organs contain far more blood cells per gram than muscle cells. Therefore, it can be argued from a Halal perspective that the residual amount of blood permissible in meat should not exceed that normally found in the liver or the spleen. Furthermore, red and white cells and platelets (components of blood) are produced in the bone marrow and in the absence of any references to the contrary bone marrow is considered Halal.

There are conflicting reports regarding the permissibility of cutting the spinal cord during Halal slaughter. It is unacceptable because carcass convulsions are required to 'squeeze all blood out of the meat' (Khan, 1991). In contrast, Gregory (2007) stated that a minority of Halal slaughterman severs the spinal cord, whilst a further minority also practiced decapitation.

1.2.2 Poultry slaughtering process

Poultry production and processing involve a series of interrelated steps designed to convert domestic birds into ready-to-cook whole carcasses, cut-up carcass parts, or various forms of deboned meat products (Alan, 2001). A good poultry slaughtering process must fulfill hygienic-sanitation requirement.

1.2.2.1 Stunning slaughtering process

The "stunning" has been used to render the bird unconscious prior to killing. Several methods have been developed to accomplish this goal. The most common and one of the simplest is electric shock. Electrical stunning (ES) system for poultry was initially developed to immobilize the bird long enough to allow physical manipulation of the bird for alignment in automated neck cutting equipment (killer) and to reduce carcass damage due to unconscious physical activity such as wing flapping and violent muscular contractions during bleeding (McNeal *et al*., 2003). The primary functions of stunning are to render the animal unconscious for easier handling during slaughter and to render the animal insensible to pain.

There are several alternative methods to ES including gas stunning using CO2, cervical dislocation, and even no stunning because ES is not required by law before slaughter of poultry. Gas (CO2) stunning is an alternative method used by the European Economic Commission because it can quickly render the bird unconscious (McNeal and Fletcher, 2003). After being stunned, general slaughter procedures are used to exsanguinate the broiler. Generally, this includes a bilateral or a unilateral neck cut to severe the carotid artery and jugular vein. Decapitation is an acceptable means of killing chickens as described by the American Veterinary Medical Association (1993). Decapitation after low-voltage ES can be an alternative to high-voltage stunning used in the EU, which can cause meat quality problems such as hemorrhaging. Decapitation can cause a higher pH at 24 h postmortem and has no effect on color, water-holding capacity, or tenderness when compared with other methods (McNeal and Fletcher, 2003). Studies have shown that decapitation can be used successfully as an alternative to conventional ES method based on ensuring an irreversible loss of consciousness while not negatively affecting carcass and meat quality (McNeal *et al.*, 2003; McNeal and Fletcher 2003).

1.2.2.2 Slaughtering process without stunning (Halal)

The OIE (2009), the EU, and the USA, permit slaughtering without stunning to allow Jews and Muslims to practice their religious beliefs. To improve animal welfare, some religious authorities will accept stunning either immediately before or immediately after the throat cut.

The traditional method of slaughtering in Islam is to slit the throat, cutting the carotid arteries, jugular veins, trachea, and the esophagus, without severing the head. It must be done by a Muslim of sound mind and health while pronouncing the name of God on each animal or bird. A common pronouncement is Bismillahi Allahu Akbar, which means, in the name of Allah, Allah is great. Slaughtering by hand is still preferred by all Muslims and quite widely followed in the Muslim countries and other countries where Muslims control the slaughterhouses (Sams, 2001). The birds must be completely lifeless before they enter the hot water bath. The conditions for defeathering, such as water temperature, chlorine level, etc. are the same for Halal processing as for regular poultry processing. However, in poultry

processing plants where both Halal and non-halal birds are processed, halal birds must be completely segregated during defeathering, chilling, eviscerating, processing, and storing. Further processing, such as marinating, breading, and application of batters or rubs, should also be done under the supervision of a qualified halal inspector by using thoroughly cleaned equipment. Non-meat ingredients such as spices, seasonings, and breadings must also be Halal approved (Regenstein and Chaudry, 2001). One main argument against pre-slaughter stunning according to Muslim factions is that the stunning process can hinder blood loss, as stunning supposedly changes the muscular, neurological and cardiovascular status of the animal (Anil *et al.,* 2004). Slaughtering chicken without stunning is much more difficult than slaughtering stunned chicken. The chicken has to be handled properly without hurting it. The chicken has to be taken out from the basket by holding the thighs and wings. Then the chicken is hold with one hand, whilst the other hand holds a knife to slaughter. Similar to the procedure of chicken slaughtering with stunning, in this procedure, the butcher must have spare knifes to ensure the sharpness and cleanliness. After slaughtering, the chicken is placed into a basket or specific container (box) which is easy to clean up. In this procedure, it is necessary to ensure that the blood flows perfectly (around 3-5 minutes) or until the chicken completely dies. Halal method can cause severe pain to the animal when a small knife is used, as numerous attempts are made to severe all the vessels in the neck (Grandin and Regenstein, 1994).

1.2.3 Effect of slaughtering methods on the quality of poultry meat

1.2.3.1 Factors affecting bleeding at the time of slaughtering

The major factors affecting bleeding include the blood vessels severed, size and patency of the sticking wound, orientation of the carcass (e.g. horizontal or vertical), cardiac arrest, vasodilatation or vasoconstriction in the capillary bed, tonic muscle contractions squeezing blood capillaries and vessels and clonic activity causing movement of blood towards the sticking wound (Gregory, 2005). Processing factors including time (to bleed) and dressing procedures (to allow blood to escape) also affect bleeding. Other factors such as health, live-weight, breed, sex, stress and hydration levels were reported to affect bleeding in sheep (Khalid, 2011).

Four theories have been put forward to explain the cause of the blood capillary rupture that leads to blood splash, but none of these have been proven. Firstly, it could be due to counteracting muscle contractions during stunning, causing localised tearing of the capillary bed (Gregory, 1998). However, haemorrhages can be induced electrically in tissues such as the brain, which does not possess any skeletal muscle (Hassin, 1933). If this applies more broadly, it is unlikely that the localised striated muscle contraction is the only cause of vessel rupture. Secondly, Shaw *et al.* (1971) suggested that one of the contributing factors may be arteriolar dilatation. This was based on the finding that, in rats, propranolol reduced and phentolamine increased the extent of blood splash. The effects of these drugs would not necessarily be limited to the arterial side of the circulation, as venous dilatation could also play a role (Vanhoutte *et al.*, 1981). Presumably, it is engorgement of the capillary bed which encourages rupture of vessels when placed under pressure. Thirdly, the blood vessels may be unduly fragile. Histological examination of blood splash in meat has shown that the vessels that burst are on the venous side of the capillary bed, which has less elastic walls than the arterial side, and so are weaker and more prone to damage. Blood splash is common in animals that have died from anticoagulant poisoning and these animals have developed capillary fragility (Fulton and Berman, 1964; Littin, 2004). It has been noted that lambs in a flock that had a high prevalence of blood splash had poor blood coagulation in terms of prothrombin time (Restall, 1981). It was suggested that the common link might be ingestion of excessive amounts of coumarins from pasture. The raised susceptibility to blood splash in unweaned lambs might be a low Vitamin K status because of their milk-based diet. Fourthly, during intense generalized muscle body contractions, such as those during electrical stunning, the venous and arterial systems experience severe external pressure. Squeezing of the veins results in large rises in venous pressure, which is transmitted to the capillary system at sites that can be some distance from the contractions. The venules in the capillary bed probably burst where they are weakest, or where venous pressure is particularly high. This referral of pressure, causing distant petechial haemorrhage, has been seen in other contexts when sudden intense pressures have been applied to veins (French and Callender, 1962). Engorgement of the venous circulation would presumably exaggerate this effect.

It is well recognised in the broiler processing industry that high frequency currents result in fewer birds with engorged wing veins and wing haemorrhages in comparison with low frequencies (50 or 60 Hz). This has recently been confirmed for turkeys (Wilkins and Wotton, 2002), and it was found that the effects of high frequency electrical stunning on breast meat were fall in pH and quality which were considered to be minor. High frequency electrical stunning resulted in fewer carcases with broken coracoid and furculum bones, and a lower prevalence of haemorrhages in the meat at these sites. Bleeding efficiency is greater with high frequencies, and the prevalence of cardiac arrest at stunning is lower (Mouchoniere *et al*., 1999).

Bloody pygostyles can be an unsightly blemish in poultry. McNeal *et al*. (2003) found that one way of reducing this problem was to decapitate the birds after stunning, instead of using the normal neck cutting method. Decapitation after electrical stunning also had the advantage that the body lost physical activity sooner after cutting. Evidently, disruption of neurotransmission through the spinal cord led to earlier termination of the convulsions. Gregory *et al*. (1999) found in a limited trial that when the prevalence of blood spots in breast muscle was high, it could be reduced by performing a ventral neck cut instead of the conventional dorsal cut. Stunning only the head results in breast muscle haemorrhages at the humeruscoracoid joint, whereas with whole body stunning, the haemorrhages tend to be in the middle of the muscle (Hillebrand *et al*., 1996).

Bleeding efficiency and bleeding rate at sticking are influenced by several factors. A cardiac arrest at the start of bleeding will slow the rate of blood loss, and in some situations it can result in less blood loss. This is not an inevitable consequence of inducing a cardiac arrest during electrical stunning (Gregory, 1998). The role of severing the vagus nerves at sticking on subsequent bleeding efficiency and residual blood in the carcass has not been examined, but it is known that vagal severance affects the distribution of blood flow in different organs and it reduces the blood pressure and cardiac output responses during haemorrhage (Schertel *et al*., 1991). This could have implications for different sticking methods.

Physiological factors are those that influence the distribution of total blood volume between peripheral vascular beds and central large vessels, whilst

mechanical factors influence the drainage of blood from the vessels at sticking. Delayed bleeding does not significantly affect blood retention or meat quality (Warriss, 1984).

1.2.3.2 Effect of slaughtering methods on bleeding and meat quality

The Islamic hanging method seems to be the best bleeding method among the three methods, compared with Islamic traditional slaughtering method and electric stunning method. Therefore, it is recommend to be used for slaughtering of the broiler chickens. According to Ali *et al.* (2011), the amount of blood collected after slaughtering the broiler chickens using the three different methods (Islamic traditional, Islamic hanging and electric stunning methods) varied. The electrically stunned group had the lowest weight of blood, whilst that slaughtered by the Islamic hanging method showed the highest weight of blood collected. Islamic hanging without stunning leads to more bleeding and this may be attributed to the effect of gravity and the rapid speed of blood flow in the blood vessels before clotting (Ali *et al.*, 2011).

Blood content in breast muscle from birds subjected to different slaughter treatments can be estimated by measuring the haemoglobin content in aqueous tissue extracts. There was no significant difference in haemoglobin content among treatments in which a bleeding step was used (Table 1). Mean haemoglobin content ranged from 8.37 to 8.72 mol/kg of tissue in the bled groups, which was 13 to 17% lower in extracts from chicken breast muscle of bled bird, compared with un-bled birds. This finding indicated that bleeding removed little blood from the breast muscle. Three to four capillaries surround each muscle fiber (Mathieu-Costello, 1993), which might explain why there is poor blood removal from breast muscle after bleeding. When the neck is cut to bleed, the blood pressure drops rapidly. Thus, there is not enough driving force to empty the numerous capillary beds in the muscle. Haemoglobin contents in the breast muscle of un-bled, stun and bled, electrocuted and bled, and decapitated and halal killed chickens were 0.36, 0.19, 0.22, 0.17, and 0.17 mg/g of soft tissue, respectively (Griffiths *et al.*, 1985).

Table 1 Haemoglobin content in chicken breast muscle with different slaughtering methods

Treatment	Haemoglobin in breast muscle (µmol/kg)
CO_2 slaughter, not bled	10.04 ± 1.70[a]
No stun	8.37 ± 1.80[b]
Electrical stun	8.72 ± 1.66[b]
CO_2 stun	8.53 ± 1.38[b]
Electrical stun and decapitation	8.40 ± 1.72[b]

Source: Alvarado *et al*. (2007)

Electrical stunning (ES) has been associated with a decrease in carcass blood loss (Papinaho and Fletcher, 1995a), although some exceptions have also been reported (Dickens and Lyon, 1993). Recent research suggests that electrical stunning only affects the rate of early blood loss and has little effect on the ultimate carcass blood loss (Papinaho and Fletcher, 1995b).

Meat derived from animals slaughtered without stunning showed higher pH values, lower drip loss and some petechial hemorrhages (DAgata *et al*., 2009). Meat derived from ritual slaughter had an unpleasant aspect due to some small red spots on the surface.

Breast meat tenderness, as measured by cooked meat shear values, has been shown to be affected by electrical stunning, although these effects seem to be minimal on final meat quality. Lee *et al*. (1979) reported that stunning decreased shear values by 30% on 24-h-old breasts. Stunning amperage has a greater deleterious effect on breast muscle characteristics than voltage. As amperage is increased, breast muscle hemorrhaging and the incidence of broken bones increases in broilers (Gregory and Wilkins, 1989b). Increased amperages were shown to increase in breast muscle hemorrhages in turkeys (Gregory and Wilkins, 1989a). Decapitation method caused a higher pH at 24 h postmortem and had no effect on color, water-holding capacity, or tenderness when compared with other methods (McNeal and Fletcher, 2003). Decapitation can be used successfully as an alternative to conventional ES

method based on ensuring an irreversible loss of consciousness without negative effect on carcass and meat quality (McNeal *et al*., 2003; McNeal and Fletcher 2003).

Blood spots continue to be a problem in the poultry processing sector. High frequency electrical stunning and gas stunning were reported to tackle this problem. High frequency electrical stunning in poultry usually results in a shorter-lasting stun (Hillebrand *et al*., 1996; Mouchoniere *et al*., 1999). The main commercial advantage with high frequencies is fewer blood spots and other haemorrhages in the carcass, and this could be linked to the reduced muscle tension during stunning. High frequency stunning can also lead to less blood retention in the viscera (Turcsa´n *et al*., 2003).

Stunning broilers with Ar or CO_2 decreased carcass defects and poor quality meat when compared with ES (Mojan Raj *et al*., 1992). Hirschler and Sams (1993) indicated that CO_2 stunning reduced the incidence of carcass defects, specifically, broken clavicles and hemorrhages in the breast, thigh, and shoulder when compared with ES. Also, this stunning method was shown to accelerate rigor development as indicated by a more rapid pH decline early PM and, therefore, can reduce the need for aging (Mojan Raj, 1994).

1.2.4 Lipid oxidation mediated by haemoglobin

1.2.4.1 Structure and function of haemoglobin

Haemoglobin is the major haem protein of red blood cells and is responsible for the transportation of oxygen to the tissues (Perutz, 1990). Haemoglobin consists of four polypeptide chains, two of α and β chains. The four chains are held together by covalent attractions. Each α chain is in contact with β chain (Figure 1). The haemoglobin from different fish species varies with respect to the difference in the amino acid sequences. Differences in the numbers and type of amino acids were reported in haemoglobin from different sources (Perutz, 1990; Jensen *et al*., 1998).

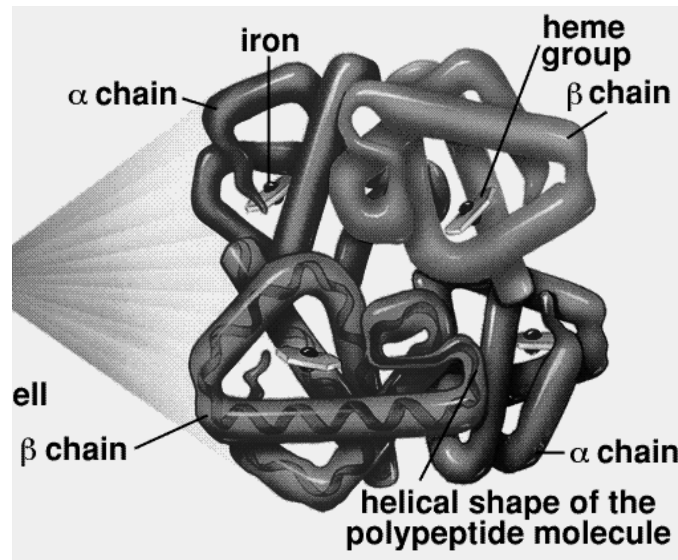


Figure 1. Haemoglobin structure displaying haem group with iron attached
Source: Mader (1997).

Capacity of haemoglobin to bind with oxygen depends on the presence of non-polypeptide units namely a haem group. The haem buried in the hydrophobic pocket of the haemoglobin consists of an organic part and an iron atom (Dickerson and Geis, 1986). The organic part, protoporphyrin, is made up of four pyrrole rings. The four pyrroles are linked by methene bridge to form tetrapyrrole ring. Four methyl, two vinyl and two propionate side chains are attached to tetrapyrrole ring. The iron atom in the haem binds to the four nitrogen in the center of the protoporphyrin ring. The iron can form two additional bonds, one on either side of the haem plane. These binding sites are termed the fifth and sixth coordination positions. At the fifth coordination position, the iron is directly bonded to histidine of globin called proximal histidine while the sixth coordination position is the oxygen binding site. The iron atom can be in the ferrous (2+) or the ferric (3+) oxidation state and the corresponding forms of haemoglobin are called ferrohaemoglobin and ferrihaemoglobin, respectively. Ferrihaemoglobin is also called as methaemoglobin, where water molecule occupies the sixth coordination site. Only ferrohaemoglobin, the 2+ oxidation state, can bind oxygen. Therefore, most haemoglobin molecules are found either with no oxygen (deoxyhaemoglobin, unliganded Hb) or with four oxygens (oxyhaemoglobin, liganded Hb) (Fermi *et al.*, 1984).

Oxygen binding property of haemoglobin is affected by pH. In the physiological range, a lowering of pH decreases the oxygen affinity of haemoglobin. Increasing concentration of CO_2 also lowers the oxygen affinity. These linkages between the binding of O_2 and concentration of H^+ and CO_2 are known as Bohr effect (Riggs, 1988). Haemoglobin of some fish expressed a large decrease in both oxygen affinity at low pH. This characteristic of haemoglobin is known as the Roots effect (Brittain, 1987). Typically, Bohr effect expresses its role when blood pH drops from about 7.4 to 6.5 (Stryer, 1988). A further decrease in blood pH is considered the Root effect (Manning *et al.,* 1998).

1.2.4.2 Lipid oxidation mediated by haemoglobin

Haemoglobin can be a source of activated oxygen due to haemoglobin autoxidation, and haem or iron can be released from the protein to promote lipid oxidation. Haemoglobin can be present in many different forms (Everse and Hsia, 1997). Both oxidized and the reduced forms can be pro-oxidative. Several mechanisms of the pro-oxidative power of haemoglobin were proposed (Everse and Hsia, 1997). Oxyhaemoglobin (Fe^{2+}-O_2) can autoxidize to methaemoglobin (Fe^{3+}) releasing its oxygen as a superoxide anion radical ($O_2^{\bullet-}$). This radical can further dismutate to hydrogen peroxide, which can activate the methaemoglobin to form hypervalent ferryl haemoglobin (Fe^{4+}=O). Although this species is only transient in nature and has a short half-life, it is capable of peroxidizing lipids and is thought to be the main species responsible for haemoglobin-induced lipid oxidation in muscle products (Alayash, 1999). This ferryl species has a protein radical form and exerts its action by abstracting an electron from the lipid substrate leaving a free radical substrate, species, which can cause further oxidation. The superoxide released on autoxidation can also lead to the formation of species ($HOO^\bullet$ and $HO^\bullet$) that are pro-oxidative, $HO^\bullet$ more than $HOO^\bullet$. In a second mechanism, which is poorly understood, the protein may act as a pseudo-lipoxygenase (Everse and Hsia, 1997).

Blood contains a high amount of haem protein (HP) which can induce lipid oxidation metHP reacts with peroxides to stimulate the formation of compounds capable of initiating and propagating lipid oxidation (Everse and Hsia, 1997). After being slaughtered, the iron atom in the haem ring of the haem proteins is primarily in

the ferrous (+2) state. Conversion of ferrous haem protein to met (+3) haem protein (metHP) is a process known as autoxidation. Autoxidation appears to be a critical step enhancing lipid oxidation. Additionally, haem or iron can be released from the Hb during post-mortem handling and storage, thereby promoting lipid oxidation (Richards and Hultin, 2002).

Hemoglobin and myoglobin are important factors determining poultry meat quality. Bruises, hemorrhages, and poor bleeding efficiency can negatively affect color of the meat and skin and shorten shelf-life (Griffiths and Nairn, 1984). Residual blood in the carcass is often associated with a meaty flavor and result in decreased shelf-life. Generally, myoglobin is relatively unimportant in connection with broiler breast meat quality. Nishida and Nishida (1985) and Kranen *et al.* (1999) reported that hemoglobin was the only detectable heme pigment found in chicken breast muscle. Therefore, haem protein in the breast muscle of broilers is hemoglobin, and excessive hemorrhaging of blood into broiler breast muscle caused by different stunning and slaughter techniques can increase the hemoglobin content. This increased hemoglobin content in the muscle can decrease shelf-life and can cause increased oxidation (Alvarado *et al.*, 2007).

Bleeding decreases lipid oxidation in intact but not in minced mackerel dark muscle, compared with controls that are not bled (Richards and Hultin, 2002). This finding is attributed to increased rupture of blood vessels in the intact tissue from un-bled fish. Maqsood *et al.* (2011) studied the effect of bleeding on chemical changes of Asian seabass slices during iced storage. The initiation and propagation of lipid oxidation were more pronounced in the un-bled samples, compared with the bled samples. Bleeding of fish decreased the Hb content. Consequently, lipid oxidation in the bled samples could be retarded as evidenced by the lower PV and TBARS in the bled samples (Maqsood *et al.*, 2011).

Slaughtering is a stressful period, and blood vessel rupture in chicken filets could be variable due to the stunning regimen used. When tissue extracts are prepared for hemoglobin analysis, the tissue is disintegrated so that hemoglobin from ruptured and un-ruptured blood vessels will be measured equally. This procedure may explain why ES decreased lipid oxidation more effectively than other stunning

methods yet haemoglobin content was not lower in samples from ES-treated birds (Alvarado *et al*., 2007).

1.2.5 Composition and quality of poultry meat

Quality is the most important criterion from the point of view of the consumer (Levie, 1970). There are two major aspects of meat quality, "nutrition quality" which is objective and "eating quality" as perceived by the consumer which is highly subjective (Bender, 1992).

1.2.5.1 Nutrition quality

Meat from poultry contains several important classes of nutrients and is low in calories. The fat contains essential fatty acid; the proteins which are a good source of essential amino acid (Van Heerden *et al*., 2002; Wattanachant *et al*., 2004) and also excellent source of the water-soluble vitamins and mineral such as iron and zinc (Van Heerden *et al*., 2002; Boccia *et al*., 2005).

Chicken contains about 16.44-23.31% protein, 0.37-7.20% fat, 0.19-6.52% ash, and 72.8-80.82% moisture content (Al-Najdawi and Abdullah, 2002; Wattanachant *et al*., 2004; Chuaynukool *et al*., 2007). Chemical composition of poultry meat has been shown to be related to species, breed, muscle type, sex, age, and method of carcasses processing (Wattanachant *et al*., 2004; Boccia *et al*., 2005; Chuaynukool *et al*., 2007). Ding *et al*. (1999) showed significantly different fat contents between broiler and local chicken. Abeni and Bergoglio (2001) reported the significant difference in ash content of breast muscle from 3 stains chicken broiler. In general, carcasses in young birds have a higher proportion of moisture than the old one, while fat content increases with age (Mountney and Parkhurst, 1995; Moran, 1995). Wattanachant *et al*. (2004) found that indigenous chicken muscle contained higher protein content but lower fat and ash contents compared to broiler muscles ($P<0.001$). Ang and Huang (1983) found that hand deboned meat (HDM) and mechanically deboned meat (MDM) have similar protein contents. Muscle types of different chicken breeds are also different in chemical composition (Chuaynukool *et al*., 2007) as shown in Table 2.

Table 2 Chemical composition of *Pectoralis m.* and *Biceps femoris m.* from different breeds of Thai chicken

Chemical composition	Broiler		Thai indigenous chicken		Spent hen	
	Pectoralis muscle	Biceps femoris muscle	Pectoralis muscle	Biceps femoris muscle	Pectoralis muscle	Biceps femoris muscle
Protein (%)	21.02	16.98	23.05	20.34	20.34	16.44
Fat (%)	1.33	0.51	2.88	1.14	1.64	1.28
Ash (%)	1.44	1.43	0.60	0.75	0.19	1.29
Moisture (%)	76.62	77.30	74.39	80.82	74.83	79.42

Source: Chuaynukool *et al.* (2007)

Amino acids represent over 90% of the crude protein in the body of poultry (Hunton, 1995). Poultry, like mammals, store excess energy as fat in adipose tissues (Rose, 1997). Adipose tissue contains 60-80% lipid, of which 99% is triacylglycerol, whereas white muscle may contain 1% lipid, only half of which is triacylglycerol (Katz *et al.*, 1966). The fatty acid composition of triacylglycerol tends to consist mainly of oleic, palmitic, linoleic, stearic and palmitoleic acid in decreasing amounts with very small contentsof C20 and C22 PUFA (Enser, 1999).

1.2.5.2 Eating quality

Eating quality of meat include color (appearance), flavor, texture, and juiciness. These characteristic of meat are influenced by various intrinsic and extrinsic factors, such as muscle structure and its chemical composition, antemortem stress, as well as postmortem handling and storage condition (Xiong et al., 1999; Fletcher, 1999).

1.2.5.2.1 Color

Color is an important attribute for customer satisfaction (Sam and Alvarado, 2004). It is important for both the consumer's initial selection of raw meat and for the consumer's final evaluation of the cooked product (Fletcher, 1999). The

characteristic color of meat is a function of two factors: the meat pigment and the light-scattering properties (Varnam and Sutherland, 1995).

Meat pigments

Myoglobin content has been shown to be primarily related to species, muscle type, sex, and age of the animal (Fletcher, 1999; Young and West, 2001). The relationship of animal species, muscle type, and animal age on meat myoglobin content and visual color was reported by Miller (1994). White meat from 8-week-old poultry had the lowest myoglobin content (0.01 mg/g meat), compared to 26-week-old male poultry white meat (0.1 mg/g meat), young turkey white meat (0.12 mg/g meat), 8-week poultry dark meat (0.4 mg/g), 26-week male poultry dark meat (1.5 mg/g), 24-week male turkey dark meat (1.5 mg/g) and compared to 5-month-old pork (0.30 mg/g), young lamp (2.50 mg/g), dark meat fish species (5.3-24.4 mg/g), white meat fish species (0.30-1.0 mg/g), and 3-year-old beef (16-20 mg/g).

1.2.5.2.2 Flavor

Flavor is one of the main eating quality attributes together with appearance, and texture (Farmer, 1999). It plays an important sensory aspect of the overall acceptability of meat product (Shahidi, 1994). Factors which might affect flavor, include the production factors of age, sex, genotype, diet and stocking density, the method of slaughter, the post slaughter time, evisceration, time and temperature of chilling and storage, and finally, cooking method (Farmer, 1999). Lipid also plays an important role in the overall flavor of meat which is distinct and species dependent (Mottram and Edwards, 1983). Lipid oxidation is well known as the cause of rancidity development, but it can also contribute to desirable food flavor (Farmer, 1999). Susceptibility of meat to lipid oxidation depend on several factors such as: (1) the unsaturated fatty acid content (Jensen *et al.*, 1997a, b; Woods and Church, 1999); (2) the fatty acid composition of lipid fractions (Dawson *et al.*, 1990; Nawar, 2000; Jensen *et al.*, 1997a,b); (3) the balance between antioxidant/pro-oxidant factors (Rhee *et al.*, 1996; Botsoglou *et al.*, 2003); and (4) processing methods (Dawson *et al.*, 1990; Ang and Huang, 1993).

Fat in poultry are more susceptible to rancidity since they are richer in unsaturated fatty acid than other meats (Jensen *et al.*, 1997a, b; Pikul *et al.*, 1984).

Wilson *et al*. (1976) reported that oxidative changes, as measured by the thiobarbituric acid (TBA) numbers, occurred most readily in turkey meat, followed by chicken, pork, beef and mutton. Within the same species of poultry, dark meat was found to generate higher TBA number than white meat (Botsoglou *et al*., 2003). Both triacylglyceride (TG) and phospholipid (PL) will undergo oxidation; however, several studies have shown that the PL fraction contributes to more lipid oxidation in meat than the TG (Alasnier *et al*., 2000), which may be due to a higher degree of polyunsaturation (Alasnier *et al*., 2000).

Pikul *et al*. (1984) pointed out that fat from chicken breast meat contained two times more malondialdehyde than fat from leg meat, due to a larger PL fraction in fat from breast meat. However, the TBA numbers were higher in leg meat because the breast meat contained much less total fat. Ang (1988) similarly reported that when meat was cooked, the initial TBA number were highest in leg meat, followed by liver, breast, and skin. After 5-day storage at 4°C, however, liver had highest TBA numbers, followed by leg meat, breast meat, and skin. Skin PL contains only about one half of polyunsaturated fatty acid with 20- and 22-carbon atoms, compared to leg and breast tissues (Pikul *et al*., 1985).

1.2.5.2.3 Texture

The most important quality attribute of chicken meat is texture (Smith and Fletcher, 1988; chrystall, 1994). Tenderness is a quality attribute uniquely important to meat texture. Tenderness of meat may be simply defined as the ease of teeth to cut meat fibers during mastication. In general terms, the degree of tenderness is directly related to quality, rated very high on the palatability scale (Levis, 1970). For intact or non-comminuted meat, tenderness or toughness is determined by two groups of components: the connective tissue and the muscle fibers (Xiong *et al*., 1999), and other factors such as post-mortem carcass aging temperature and time (Dunn *et al*., 2000; Wattanachant, 2003).

The role of connective tissue on meat tenderness was elucidated (Brooks and Savell, 2004). Since epimysium is normally removed when cooked meat is consumed, only the intramuscular connective tissue (IMCT), perimysium and endomysium, present a realistic toughness problem to meat and meat product (Xiong

et al., 1999). The amount, composition, and arrangements of IMCT directly affect the texture of meat (Wattanachant *et al*., 2004). Total amount of connective tissue from different muscles of the same animal are different (Liu *et al*., 1996; Wattanachant *et al*., 2004). The specific influence of intramuscular connective tissue depends on their thickness, the amount of collagen present, as well as the density and type of cross linkages between collagen fibrils (Xiong *et al*., 1999; Wattanachant *et al*., 2004). A high correlation between the thickness of perimysium and shear value have been reported by Liu *et al*. (1996) and Wattanachant *et al*. (2004). Wattanachant *et al*. (2004) reported that perimysium thicknesses were 14.2 µm for *Biceps femoris* muscle and 7.10 µm for *Pectoralis* muscle of indigenous chicken muscle, which were thicker than those of broiler muscle, which were 9.93 µm for *Biceps femoris* muscle and 3.87 µm for *Pectoralis* muscle. Perimysium thickness of the muscles correlated well with the magnitude of the shear value of the muscleas previously reported by Wattanachant *et al*. (2004); Thai indigenous *Biceps femoris* (5.20 kg), broiler *Biceps femoris* (2.89 kg), Thai indigenous *Pectoralis* (1.78 kg), and broiler *Pectoralis* (1.20 kg).

Muscle fibers or muscle cell have a threadlike appearance and surrounded by sacrolemma, which is a cell membrane overlayed with endomysial connective tissue (Lluch *et al*., 2001). The size of the muscle fiber bundles can determine the texture of the muscle (Lawrie, 1991). However, there is an indirect correlation between muscle fiber diameter and tenderness. Ozawa *et al*. (2000) found that the white muscle fiber content (larger diameter and narrow Z-lines) was negatively correlated ($p<0.05$) with shear value, while at the same time red muscle fiber content (wild Z-lines) was positively correlated ($p<0.05$) to this value. The average of the sarcomere length of raw muscle from Thai indigenous chicken, spent hen and broiler were significant different ($p<0.05$) in range of 1.55-1.62 µm for *Pectoralis* muscle and 1.53-1.64 µm for *Biceps femoris* muscle (Chuaynukool *et al*., 2007). Sarcomere length is used as a measure of muscle contraction and is highly correlated with tenderness of pre-rigor and rigor meat (Lyon and Buhr, 1999). Lower resistance to shearing and greater tenderization is associated with longer and shorter sarcomeres.

1.2.6 Pathogen and spoilage bacteria in poultry meat

Meat is recognized as one of the most perishable foods. This is due to its chemical composition that favours microbial growth to unacceptable levels. Growth of microorganisms contributes significantly to meat deterioration or spoilage. When large numbers of microorganisms are present, raw meat, becomes unappealing and unsuitable for human consumption (Gram *et al*., 2002; Fung, 2010). The initial microbial load of meat depends on the physiological status of the animal at slaughter, the spread of contamination into slaughterhouses and during processing, while temperature and other conditions of storage during distribution can also influence the rate of spoilage (Nychas *et al*., 2008). Intrinsic factors of meat such as pH and water activity are not inhibitory to growth of microorganisms owning to their neutral and high initial values, respectively (Mataragas *et al*., 2006). Poultry meat and products are highly perishable foods, which are susceptible to organoleptic changes i.e. off-odors, off-flavors and slime. Those alterations are a result of microbial growth (Lambert *et al*., 1991; Jackson *et al*., 1997). Depending on the degree of processing, their spoilage varies between 4 and 10 days under refrigeration (Marenzi, 1986). The microbiological quality and safety of commercially processed poultry products are major areas of concern for producers, consumers, and public health officials worldwide. Products excessively contaminated with microorganisms are undesirable from the standpoint of public health, storage quality, and general aesthetics (Cunningham, 1982; Mead, 1989). The psychrotrophic bacterial flora on freshly dressed broiler chicken carcasses are heterogeneous *Flavobacteria* spp., *Shewanella putrefaciens, Acinetobacter* spp., *Corynebacteria* spp., *Moraxella* spp. and *Pseudomonas fluorescent*. They are commonly found in aerobically stored, chilled poultry meats (Barnes and Impey, 1968; Lahellec and Colin, 1979). Barnes and Impey (1968) reported that the microorganisms on the poultry skin at the time of spoilage were predominantly *Pseudomonas* spp., with *Acinetobacter* spp. and *S. putrefaciens* being present in lower numbers. During slaughter, the intestinal content will inevitably contaminate the broiler carcasses (Berrang and Dickens, 2000; Stern and Robach, 2003). This may be associated with the contamination of foodborne pathogens such as *Salmonella* spp. and *Campylobacter* spp. During processing of

poultry, cross-contamination and subsequent increase in bacterial load of the final product might occur (Luber, 2009). *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp., which all cause diseases in man, can be found on poultry carcasses and in poultry processing plants (Jorgensen *et al*., 2002; Miettinen *et al*., 2002). Psychrotrophic bacteria, generally *Pseudomonas* spp., have been identified as the predominant microorganism responsible for spoilage of aerobically-stored meat products (Gill and Newton, 1978; Ikeme *et al*., 1982; Pooni and Mead, 1984)

Campylobacter jejuni is a zoonotic enteric pathogen causing human gastroenteritis. It has reservoirs in a wide variety of animal species, including domestic animals. The illness is transmitted primarily through foods of animal origin. *C. jejuni* grows between 30 °C and 45 °C with an optimal range of 42 °C to 45 °C, and a pH range of 5.8 to 8.0 (Kelana and Griffiths, 2003). The approximate body temperature of the chicken (from41 °C to42 °C) and the environment of the chicken gut makes the chicken become an optimum host for *C. Jejuni* (Nachamkin, 1995). The role of *C. jejuni* as a foodborne pathogen is associated with its ability to survive in food during storage and handling. Temperature is a key factor in the survival of *C. jejuni* survived longer at 4 °C than at room temperature in the different media tested (Mihaljevic *et al*., 2007). Studies with chicken meat revealed that the colony-forming units (CFUs) of *C. jejuni* decreased in ground chicken meat and on chicken skin during refrigerated storage at 4 °C during one week by 0.81-log CFU/g and during frozen storage at -20 °C by 0.57 - 1.57-log CFU/g within two weeks (Bhaduri and Cottrell, 2004).

Salmonella is one of the most common pathogen associated with foodborne illness. Salmonellosis is the illness caused by this bacterium (CDC, 2005). Red meat and poultry are good sources of *Salmonella*. Generally meat or poultry meat are the most vulnerable foods for the growth of *Salmonella*, particularly *Salmonella enteritidis* (AMI, 2004). Poultry products are important vehicles for *Salmonella* transmission to humans and have been incriminated in numerous *Salmonella* outbreaks (Baeumler *et al*., 2000; Davies and Breslin, 2003).

Poultry can become contaminated with *Listeria* spp., either environmentally or from healthy carrier birds during production in the farm (Skovgaard and Morgen, 1988). In poultry abattoir and processing plant, improper

cleaning and disinfecting of environment and equipments and also mishandling of the products may lead to *Listeria* contamination of poultry carcasses and the final products (Loura *et al.*, 2005; Uyttendaele *et al.*, 1997). Meat and meat products have frequently been contaminated with *L. monocytogenes* and may serve as vehicle of other pathogenic organisms. The frequent occurrence of *L. monocytogenes* in meat may pose a potential risk for consumers, particularly for immune compromised people. Outbreaks and sporadic cases of listeriosis are predominately associated with ready-to-eat foods (Pradhan *et al.*, 2009; WHO/FAO, 2004). An important factor in foodborne listeriosis is that the pathogen can grow to significant numbers at refrigeration temperatures when given sufficient time (ICMSF, 1996; MacGowan *et al.*, 1994; WHO/FAO, 2004).

1.3 Objectives of study

1. To study the effect of slaughtering methods on quality and storage stability of raw and cooked broiler chicken meat.
2. To investigate the effect of haemoglobin from broiler chicken on pro-oxidative activity and lipid oxidation in chicken meat model system.
3. To investigate the effect of whole chicken blood from broiler chicken on growth of pathogen and spoilage bacteria.

CHAPTER 2

Effect of Islamic slaughtering on chemical compositions and post-mortem quality changes of broiler chicken meat

2.1 Abstract

Halal or Islamic slaughtering process is implemented for 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. This study aimed to compare chemical compositions and post-mortem quality of broiler chicken breast meat obtained from different slaughtering methods. Chicken breast meat from Islamic slaughtering method, decapitation method, conventional neck cut method and un-bled sample contained haem iron contents of 2.41, 2.35, 2.56 and 3.41 mg/100g sample with Fe content of 10.09, 12.47, 14.21 and 18.10 mg/kg, respectively. Similar haem and non-haem iron contents were found amongst bled samples. During the storage at 4ºC for 8 days, chicken meat from Islamic slaughtering method showed the lower peroxide value and thiobarbituric acid reactive substances within the first four days of storage, compared with others ($P<0.05$). There were no differences in protein patterns of chicken meat obtained from different slaughtering methods. PUFA content of chicken meat from Islamic slaughtering method was higher than that of samples bled with other methods after 8 days of storage. Higher mesophilic bacteria count total viable count and psychrophilic bacterial count were observed in un-bled sample, as compared to the slaughtered samples ($P<0.05$). Un-bled samples had the higher a*, ΔE*and ΔC* values than bled counterparts, and L*- and a*- values decreased after 8 days of storage. Islamic slaughtering method could lower Fe or haem in the muscle, thereby lowering lipid oxidation in post-mortem chicken meat.

2.2 Introduction

Eating is considered as a matter of worshiping God for Muslim. Islamic law prescribes a set of dietary rule, called "Halal" (legal, permitted by Allah) which lists the permitted food and prohibit the consumption of meat not obtained according to Islamic rules, covering livestock handling before and during slaughter (Regenstein *et al*., 2003; Bonne and Verbeke, 2008). Halal is also refers to the aspects of reliable, food quality, wholesome, hygiene and safety. Muslims must make an effort to obtain halal food of good quality. For non-Muslim consumers, halal foods are often perceived as specially selected and processed to achieve the halal standards of quality (Sam and Alvarado, 2001).

Poultry production and processing involve a series of interrelated steps for conversion of domestic birds into ready-to-cook carcasses with several forms (Alan, 2001). However, poultry meat is perishable if it is not handled properly. Amount of blood retained in meat in one of the most important factors affecting the quality changes, contamination and deterioration (Ali *et al*., 2011). Blood is considered to be an excellent medium for the growth of bacteria. The amount of blood bled by the animal depends on the slaughtering method (Ali *et al*., 2011). Blood components, especially haemoglobin, are powerful promoters of lipid oxidation and may decrease the shelf-life of meat and fish products (Alvarado *et al*., 2007; Maqsood *et al.,* 2011).

Several slaughtering methods have been used in the world. Those include the Halal method, hanging method and stunning (electrical and CO_2) method. The Islamic method is a traditional method of slaughtering for halal foods. Stunning prior to slaughtering is not permitted. Harsher electrical stunning results in higher incidence of hemorrhaging and broken bones (Sam and Alvarado, 2001). The chicken meat can be haram (forbidden to be eaten by Islamic law) when the slaughtering is not appropriate or does not follow the Islamic guidance (Bonne and Verbeke, 2008). Halal slaughtering must be executed by a throat cut in order to bring the animal to a quick death without suffering, by reaction of carotid arteries, jugular veins, trachea and esophagus and the absence of previous stunning, allowing a rapid and complete bleeding (Grandin and Regenstain, 1994). In Thailand, slaughtering process for

chicken can be varied, depending on the belief or practice. However, Islamic or Halal method has been believed to render the considerable bleeding, and it may be beneficial for shelf-life extension or quality maintenance of chicken meat. However, a little information about the effect of slaughtering methods on quality of post-mortem broiler chicken meat, regarding chemical compositions, lipid oxidation and microbial growth has been reported. Therefore, the objective of this study was to evaluate the impact of different slaughtering methods, especially Islamic slaughtering method, on chemical composition and post-mortem quality changes of chicken meat.

2.3 Materials and methods

2.3.1 Chemicals

Bathophenanthrolinedisulphonic acid, sodium dodecyl sulphate (SDS), ammonium thiocyanate and β-mercaptoethanol (βME) were purchased from Sigma (St. Louis. MO, USA). Trichloroacetic acid, anhydrous sodium sulphate, sodium nitrite, ferrous chloride, iron standard solutionandstandard plate count agar (PCA) were obtained from Merck (Darmstadt, Germany). Disodium hydrogen phosphate, sodium dihydrogen phosphate, 2-thiobarbituric acid, cumenehydroperoxide, 1,1,3,3-tetramethoxypropane, acrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED) and bis-acrylamide were procured from Fluka (Buchs, Switzerland). Methanol, acetone and chloroform were obtained from Lab-Scan (Bangkok, Thailand)

2.3.2 Preparation and slaughtering of broilers

Thirty-two broilers were obtained from a poultry farm in Songkhla, Thailand. Broilers with the age of six weeks and the body weight of approximately 2 kg were divided into three groups. Three slaughtering methods were used. Those included 1) Islamic method; cutting carotid arteries, jugular veins, trachea and esophagus, 2) decapitation method; cutting carotid arteries, jugular veins, trachea esophagus including spinal cord and 3) conventional neck cut method; cutting carotid arteries and jugular veins. Un-bled sample was used as the control. The samples obtained were reference to as "IM", "DM", "CM" and "UN", respectively.

After being bled for 3 min, the chicken was plucked in a rotary-drum picker for 30sec and eviscerated. Breast muscle was dissected from the carcasses. The sample (150-200 g) was packaged in a polyethylene bag, kept at 4°C and taken for analyses at day 0, 2, 4, 6 and 8.

2.3.3 Chemical analyses

2.3.3.1 Determination of minerals

Iron (Fe^{2+}), copper (Cu^{2+}), manganese (Mn^{2+}), magnesium (Mg^{2+}), zinc (Zn^{2+}) and calcium (Ca^{2+}) contents were determined using the inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Model 4300 DV, Perkin Elmer, Shelton, CT, USA) according to the method of AOAC (2000). Sample (4 g) was mixed well with 4 mL of 70% nitric acid. The mixture was heated on the hot plate until digestion was completed. The digested sample was transferred to a volumetric flask and the volume was made up to 10 mL with deionised water. The solution was then subjected to analysis. Flow rates of argon to plasma, auxiliary and nebuliser were maintained at 15, 0.2, and 0.8 L/min, respectively. Sample flow rate was set at 1.5 mL/min. The content of mineral was calculated and expressed as mg/kg wet sample.

2.3.3.2 Determination of haem iron content

Haem iron content of chicken meat was determined as described by Cheng and Ockerman (2004) with a slight modification. Ground sample (2 g) was mixed with 9 mL of acid acetone (90 % acetone, 8 % deionised water and 2 % HCl v/v/w). The mixture was macerated with a glass rod and allowed to stand for 1 h at room temperature. The mixture was filtered with a Whatman No.42 filter paper (Whatman International, Ltd, Maidstone, England) and the absorbance of the filtrate was read at 640 nm against an acid acetone used as blank. Haem iron content was calculated as follows:

Haem iron content (ppm) = Total pigment (ppm) x 0.0822

where total pigment (ppm) = A_{640} x 680.

The haem iron content was expressed as mg/100g wet sample.

2.3.3.3 Determination of non-haem iron content

Non-haem iron content was measured as described by Schricker *et al.* (1982) with a slight modification. Ground sample (1 g) was placed in a screw cap test tube, and 50 µL of 0.39% (w/v) sodium nitrite was added. A mixture (4 mL) of 40% trichloroacetic acid and 6 N HCl (ratio of 1:1 [v/v], prepared freshly) were added. The tightly capped tubes were placed in an incubator shaker at 65°C (Memmert, D-91126, Schwabach, Germany) for 22 h, and then cooled down at room temperature (25–30°C) for 2 h. The supernatant (400 µL) was mixed with 2 mL of the non-haem iron colour reagent (prepared freshly). After vortexing and standing for 10 min, the absorbance was measured at 540 nm. The colour reagent was prepared by mixing a 1:20:20 ratio (w/v/v) of: (1) bathophenanthrolinedisulfonic acid (0.162 g, dissolved in 100 mL of double-deionised water with 2 mL thioglycolic acid); (2) double-deionised water; and (3) saturated sodium acetate solution.

The non-haem iron content was calculated from an iron standard curve. The iron standard solution, ranging from 0 to 2 ppm, was used. The concentration of the non-haem iron was expressed as mg/100 g wet sample.

2.3.3.4 Determination of thiobarbituric acid reactive substances (TBARS)

TBARS were determined according to the method of Benjakul and Bauer (2001) with some modification. Ground chicken meat (1 g) was mixed with 5 mL of a solution containing 0.375% TBA, 15% TCA and 0.25N HCl. The mixture was heated in boiling water for 10 min, followed by cooling with the running water. The mixture was centrifuged at 4000g for 20 min (MIKRO20, HettichZentrifugan, Germany). The supernatant was collected and the absorbance was read at 532 nm using a UV-160 spectrophotometer. TBARS value was calculated from the standard curve of malonaldehyde (0-2 ppm) and expressed as mg malonaldhyde/kg wet sample.

2.3.3.5 Determination of peroxide value (PV)

PV was determined as per the method of Richards and Hultin (2002) with a slight modification. Ground sample (1 g) was mixed with 11 mL of

chloroform/methanol (2:1, v/v). The mixture was homogenised at a speed of 13,500 rpm for 2 min using an UltraTurrax T25 homogenizer (Janke and Kunkel, Staufen, Germany). The homogenate was then filtered using Whatman no.1 filter paper. Two millilitres of 0.5% NaCl were then added to 7 mL of the filtrate. The mixture was vortexed at a moderate speed for 30 sec using a Vortex-Genie2 mixer 4 (Bohemia, NY, USA) and then centrifuged at 3000 g for 3 min using a RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA) to separate the sample into two phases. Two millilitres of cold chloroform/methanol (2:1) was added to 3 mL of the lower phase. Twenty-five microlitres of 30% ammonium thiocyanate and 25 µL of 20 mM iron (II) chloride were added to the mixture. The reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at 500 nm. A standard curve was prepared using cumenehydroperoxide at the concentration range of 0.5–2 ppm.

2.3.3.6 Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out as per the Laemmli method (Laemmli, 1970). Sample (3 g) was homogenised in 5% (w/v) SDS (27 mL) at a speed of at 11,000 rpm for 1 min. The mixtures were incubated at 85ºC for 1 h and then centrifuged at 6,000 g for 10 min. The solution was mixed with the sample buffer containing 1.5 M βME at a ratio of 1:1 (v/v). The samples (25 µg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). Protein bands were stained with 0.125% Coomassie Brilliant Blue R-250 and destained in 25% methanol and 10% acetic acid. Standard markers including myosin from rabbit muscle (200 kDa), β-galactosidase from *Escherichia coli* (116 kDa), phosphorylase b from rabbit muscle (97 kDa), bovine serum albumin (66 kDa), glutamic dehydrogenase from bovine liver (55 kDa), glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle (36 kDa), and carbonic anhydrase from bovine erythrocytes (29 kDa) were used for MW estimation.

2.3.3.7 Analysis of fatty acid profile

Firstly, lipids were extracted from chicken meat as per the Bligh and Dyer method (Bligh and Dyer 1959). Fatty acid methyl esters (FAMEs) were then prepared according to the method of AOAC (2000). The prepared methyl esters were injected to the gas chromatography (Shimadzu, Kyoto, Japan) equipped with the flame ionisation detector (FID) at a split ratio of 1:20. A fused silica capillary column (30 m x 0.25 mm), coated with bonded polyglycol liquid phase, was used. The analytical conditions were: injection port temperature of 250ºC and detector temperature of 270ºC. The oven was programmed from 170 to 225ºC at a rate of 1ºC/min (no initial or final hold). Retention times of FAME standards were used to identify chromatographic peaks of the samples. Fatty acid content was calculated, based on the peak area ratio and expressed as g fatty acid/100 g lipid.

2.3.3.8 Analysis of volatile compounds

Volatile compounds were determined by solid phase micro extraction–gas chromatography–mass spectrometry (SPME–GC–MS) (Iglesias and Medina 2008).

2.3.3.8.1 Extraction of volatile compounds. Three grams of sample was homogenised at a speed of 13,500 rpm for 2 min with 8 mL of saturated NaCl in an ultra-pure water. The mixture was centrifuged at 2000 g for 10 min at 4ºC. The supernatant (6 mL) was heated at 60ºC with an equilibrium time of 10 h in a 20 mL headspace vial. Finally, the SPME fibre (50/30 μm DVB/Carboxen™/PDMS StableFlex™) (Supelco, Belle-fonte, PA, USA) was exposed to the head space of the vial containing the sample extract and the volatile compounds were allowed to absorb in the SPME fibre for 1 h. The volatile compounds were then desorbed in the GC injector port for 15 min at 270ºC.

2.3.3.8.2 GC-MS analysis. Analysis was performed in a HP 5890 series II gas chromatography coupled with HP 5972 mass selective detectors, equipped with a splitless injector, and coupled with a quadrupole mass detector (Hewlett Packard, Atlanta, GA, USA). The compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m 0.25 mm ID,

with film thickness of 0.25 lm). The GC oven temperature programme was 35ºC for 3 min, followed by an increase of 3ºC/min to 70ºC, then an increase of 10ºC/min to 200ºC, and finally an increase of 15ºC/min to a final temperature of 250ºC, and hold for 10 min. Helium was employed as a carrier gas, with a constant flow of 1.0 mL/min. The injector was operated in the splitless mode and its temperature was set at 270ºC. The transfer line temperature was maintained at 265ºC. The quadrupole mass spectrometer was operated in the electron ionisation (EI) mode and the source temperature was set at 250ºC. Initially, a full scan mode data was acquired to determine the appropriate masses for the later acquisition in selected ion monitoring (SIM) mode, under the following conditions: mass range: 25–500 amu and scan rate: 0.220 sec/scan. All analyses were performed with ionisation energy of 70 eV, a filament emission current of 150 µA, and an electron multiplier voltage of 500 V.

2.3.3.8.3 Identification of volatile compounds. Identification of the compounds was done by consulting ChemStation Library Search (Wiley 275.L). Identification of compounds was performed, based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search (Wiley 275.L). Quantification limits were calculated to a signal-to noise (S/N) ratio of 10. Repeatability was evaluated by analysing 3 replicates of each sample. The identified volatile compounds related with lipid oxidation were presented in the form of normalised area under peak of each identified compound.

2.3.4 Physical analysis

2.3.4.1 Determination of colour

Colour of chicken meat was determined by measuring L* (lightness), a*(redness/greenness) and b* (yellowness/blueness) value using a colourimeter (JP7100F, Juki Corp, Tokyo, Japan). The colourimeter was standardised by black and white tile. Colour differences, ΔE*, was calculated by the following equation (Berns 2000).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where, ΔL^*, Δa^* and Δb^* represent the difference in the colour parameters between the sample and the white standard ($L^*= 93.55$, $a^* = -0.84$, $b^*=0.37$).

$$\Delta C^* = C^*_{sample} - C^*_{standard}$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

2.3.4.2 Determination of drip loss

Drip loss of chicken meat was determined by calculating a percentage of weight loss, relative to the initial weight as described by Woelfer *et al*. (2002). At the designated storage time, samples were taken immediately from the containers, gently blotted dry and weighed. Drip loss was then calculated.

2.3.5 Microbiological analysis

Mesophilic bacteria count was analysed following Bacteria Analytical Manual (BAM, 2001) using a pour plate method. Ground sample (25 g) was placed in a stomacher bag containing 225 mL of 0.15 M phosphate buffer saline (PBS) (pH 7.2). After mixing for 1 min in a Stomacher blender (M400, Seward, West Sussex, UK), the appropriate dilutions were prepared. Mesophilic bacteria count was determined by a pour plate method. One mL of appropriate dilution of homogenate was transferred on plate count agar and incubated at 35ºC for 2 days. For psychrophilic bacteria count, the incubation was conducted at 4ºC for 7 days.

2.3.6 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie 1980) Analysis was performed using the Statistical Package for Social Science package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

2.4 Results and discussion

2.4.1 Effect of slaughtering methods on mineral composition of chicken meat

Mineral contents of chicken meat with different slaughtering methods including Islamic slaughtering method, decapitation method, conventional method and un-bled sample at day 0 are shown in Table 3. Mg was found as the most predominant mineral in chicken meat, regardless of slaughtering methods used. Amongst all samples, IM sample showed the lowest Mg content ($P<0.05$). Ca constituted as the second abundant mineral, followed by Zn. Zn was found at the highest content in IM sample ($P<0.05$). UN sample had the highest Fe content (18.10 mg/kg sample) ($P<0.05$), whilst IM sample showed the lowest Fe content (10.09 mg/kg sample) ($P<0.05$). Cu content was lower in slaughtered samples, compared with un-bled sample. It was noted that Mn was found only in chicken meat with IM sample. Fe and Cu could act as pro-oxidants in the chicken meat during storage. Transition metal ions, particularly Cu and Fe, are known to be major catalysts of oxidation (Thanonkaew *et al.* 2006). The results suggested that bleeding during slaughtering process could remove blood, but the rate of removal might be varied. Fe content was lowest in IM sample ($P<0.05$), indicating the most effective removal of blood from chicken. Throat cut is implemented for Islamic method, in which the major vein was cut, facilitating the bleeding (Gregory *et al.* 2012). For UN sample, broiler died during transportation due to the stress. This meat contained a large amount of blood retained and could be the major source of pro-oxidants, especially Fe.

Table 3 Mineral content of chicken meat with different slaughtering methods and un-bled sample

Mineral contents (mg/kg)	IM	DM	CM	UN
Fe	10.09±1.43^{b}	12.47±1.26^{b}	14.21±0.35^{b}	18.10±1.05^{a}
Mg	870.48±2.54^{b}	959.62±1.43^{a}	930.79±1.84^{a}	965.41±2.65^{a}
Cu	0.41±0.07^{b}	1.03±0.34^{b}	0.38±0.02^{b}	4.35±0.64^{a}
Zn	55.98±1.87^{a}	47.10±0.52^{a}	24.80±0.67^{b}	16.16±1.18^{b}
Ca	136.85±1.93^{b}	390.26±0.82^{a}	108.99±1.22^{b}	129.26±1.05^{b}
Mn	2.72±0.64	ND	ND	ND

ND: Not detectable or below detection limit.IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Values are mean ± SD (n=3). Different lowercase letters within the same row denote the significant difference (P<0.05).

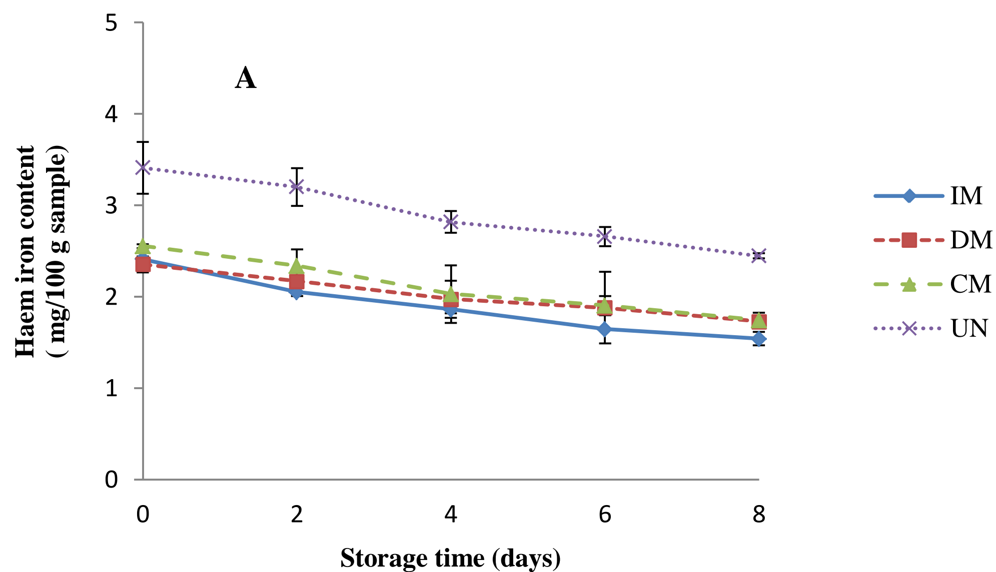
2.4.2 Effect of slaughtering methods on chemical changes of chicken meat during refrigerated storage

2.4.2.1 Haem iron and non-haem iron contents

The changes of haem iron content in chicken meat during refrigerated storage are shown in Figure 2(A). At day 0, UN sample had higher haem iron content (3.41 mg/100g sample) than did slaughtered samples (P<0.05). This was in agreement with the highest Fe content in UN sample (Table 3). Haem iron contents of 2.41, 2.35 and 2.56 mg/100g sample were found for IM, DM and CM samples, respectively. This reconfirmed that the blood was effectively removed from the muscle via bleeding. Blood contains a high amount of haemoglobin, a major haem protein found in blood. Haemoglobin is made up of four polypeptide chains with each chain containing one haem group; each haem consists of an iron atom coordinated inside the porphyrin ring (Richard *et al.,* 2007). During the refrigerated storage, the gradual decreases in haem iron contents of all samples were observed up to 8 days (P<0.05). Decreases in haem iron content with increasing storage time were probably due to haem breakdown, resulting in the release of non-haem iron (Benjakul and Bauer, 2001). The released iron can stimulate lipid oxidation of muscle during the extended storage (Tappel, 1995). After storage for 8 days, IM sample showed the lower haem iron content (1.54 mg/100g sample) than did samples with other slaughtering methods (P<0.05). Hemoglobin content in the chicken meat of Halal or Islamic method was lowest, compared with that found in other slaughtering methods (Griffiths *et al.*, 1985).

The changes in non-haem iron content in chicken meat are depicted in Figure 2(B). At day 0, the content of non-haem iron found in IM, DM, CM and UN samples were 0.058, 0.071, 0.069 and 0.107 mg/100g meat, respectively. Amongst all samples, UN sample had the highest non-haem iron content (P<0.05). For UN sample,

the marked increase in non-haem iron content was found after the first 2 days of storage ($P<0.05$). For slaughtered samples, non-haem iron content increased after 4 days of storage, regardless of slaughtering methods used. These results suggested that the disruption of porphyrin ring probably occurred during storage, leading to the release of free iron named “non-haem iron”. Damage of porphyrin ring during storage was suggested as the cause of breakdown of haem molecules and the release of iron (Gomez-Basauri and Regenstein, 1992). Non-haem iron has been known to function as the potent catalyst of lipid oxidation in muscle foods (Kanner, 1994). Additionally, Decker and Hultin (1990a, 1990b) reported that the deterioration of subcellular organelles, e.g. mitochondria, and the release of cytochrome c, could be responsible for the increase in soluble hemin. The increase in non-haem iron content with increasing storage period was coincidental with the decrease in haem iron content (Figure 1A). The results suggested that blood retained in muscle more likely underwent destruction, leading to the release of prooxidative iron.



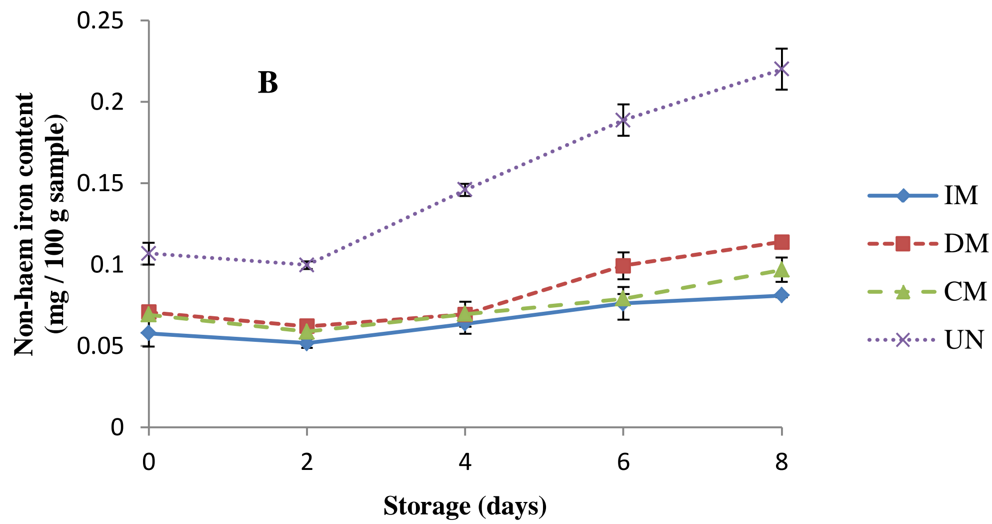


Figure 2 Haem iron (A) and non-haem iron contents (B) of chicken meat with different slaughtering methods and un-bled sample during 8 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Bar represent standard deviation (n=3)

2.4.2.2 Lipid oxidation products

Lipid oxidation products in chicken meat during 8 days of refrigerated storage were monitored by measuring PV and TBARS value (Figure 3). The continuous increase in PV was noticeable in all samples during the refrigerated storage ($P<0.05$). The result indicated that lipid oxidation took place continuously in the chicken meat, in which hydroperoxide was formed as evidenced by the increase in PV. When comparing PV of all samples, PV of UN sample was higher than those of slaughtered samples during 8 days of storage ($P<0.05$). It was noted that IM sample showed the lowest PV ($P<0.05$) throughout the storage, indicating the lower lipid oxidation taken place in the muscle. This was in accordance with the lowest Fe content as well as non-haem iron content in this sample (Table 3, Figure 2B). The higher PV in UN sample indicated that lipid oxidation was more pronounced, more likely associated with the higher blood retained in the meat.

TBARS values of chicken meat increased, especially during 2-4 days of storage ($P<0.05$). Higher TBARS was observed for UN sample, compared with slaughtered sample throughout the storage ($P<0.05$). After 4 days of storage, no marked changes were noticeable in all samples, whilst PV still increased (Figure 3A). The loss of low molecular weight oxidation products during the advancement of oxidation might lead to the constant TBARS values. Higher blood retained with coincidentally higher Fe and non-haem iron contents might induce the lipid oxidation to a higher extent. Haemoglobin in muscle was reported to accelerate lipid oxidation (Richard and Hultin, 2002). Apart from haemoglobin, the blood also contain large amount of white blood cells, which can also generate superoxide, hydrogen peroxide and hydroxyl radical which are known to enhance the lipid oxidation (Gabig and Babior 1981). Therefore, Islamic slaughtering method could lower lipid oxidation in chicken meat to some degree during the extended refrigerated storage.

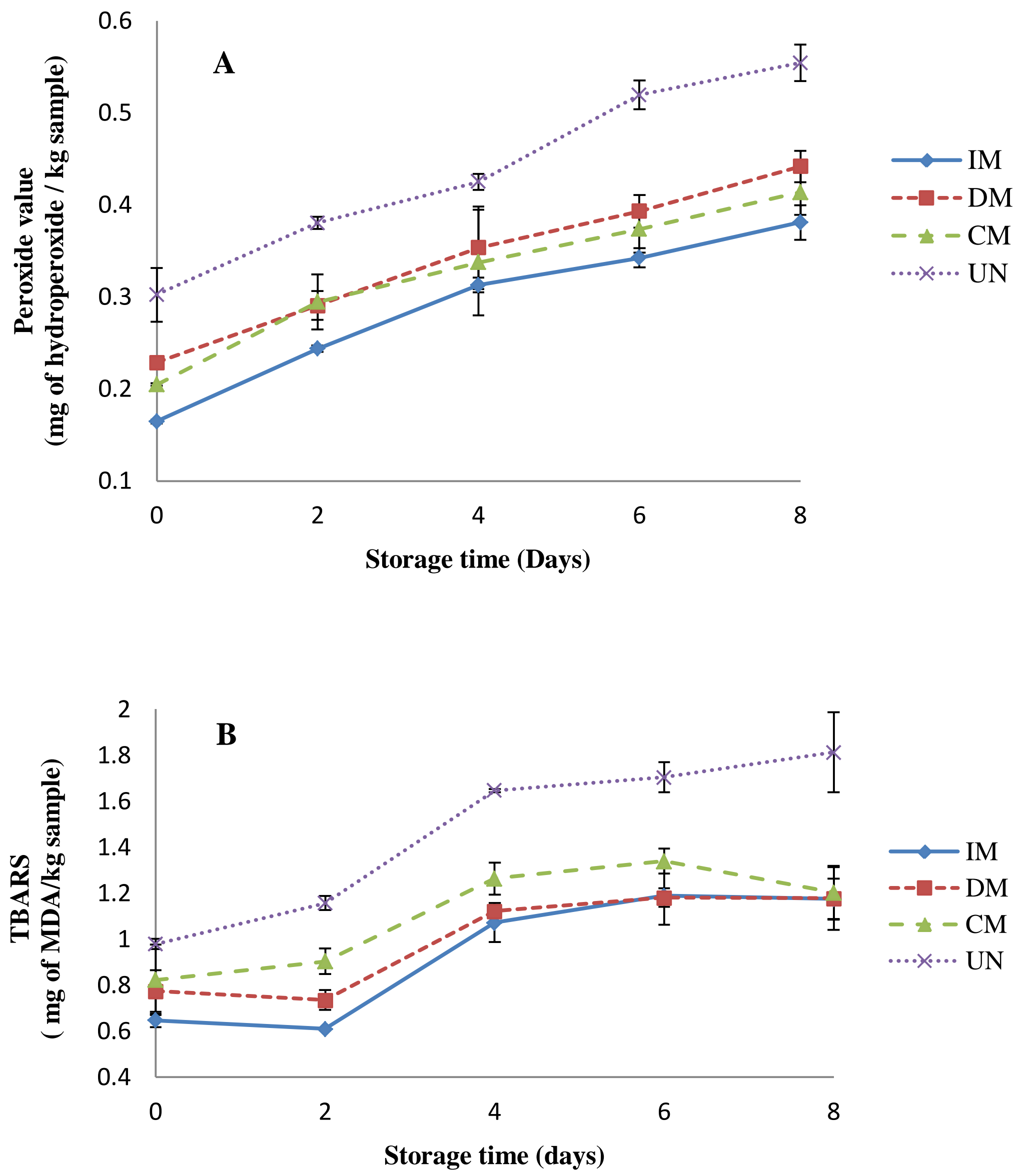


Figure 3 PV (A) and TBARS (B) of chicken meat with different slaughtering methods and un-bled sample during 8 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Bar represent the standard deviation (n=3)

2.4.2.3 Fatty acid profile

Fatty acid profiles of chicken meat with different slaughtering methods including Islamic slaughtering method, decapitation method, conventional method and un-bled sample after 8 days of refrigerated storage in comparison with day 0 are shown in Table 4. Chicken lipid (day 0) contained oleic acid (C 18:1 n-9) as the dominant fatty acid, followed by palmitic acid (C 16:0) and linoleic acid (C 18:2 n-6). It consisted of EPA and DHA at very low contents. Monounsaturated fatty acids constituted as the major components in chicken lipids. PUFA constituted amount 21.50 g/100 g lipid. These unsaturated fatty acids underwent oxidation during the extended storage. This was evidenced by the decrease in PUFA at day 8 of storage. Amongst all slaughtered samples, IM sample had the highest PUFA content after 8 days of storage. This was in agreement with the lowest PV and TBARS in this sample (Figure 2). The decrease PUFA was coincidental with the increase in SFA content. When PUFA underwent oxidation, the proportion of SFA, which was more stable to oxidation, became increased. Furthermore, some triglycerides might be hydrolysed, releasing free fatty acids, which were more susceptible to oxidation, compared with esterified forms. During storage, triglycerides and phospholipids underwent hydrolysis into free fatty acid, which were prone to oxidation (Thiansilakul *et al.* 2010). Thus, slaughtering process had the impact on oxidation to some degree and determined fatty acid profile of chicken meat after refrigerated storage.

Table 4 Fatty acid profile of chicken meat with different slaughtering methods and un-bled sample after 8 days of at day 0 and 8 of refrigerated storage

Fatty acids (g/100 g lipid)	Day 0	Day 8			
		IM	DM	CM	UN
C12:0	0.07±0.18	0.07±0.01	0.09±0.01	0.07±0.01	0.16±0.08
C14:0	0.44±0.14	0.43±0.04	0.44±0.02	0.42±0.02	0.61±0.13
C15:0	0.07±0.61	0.06±0.05	0.06±0.01	0.05±0.49	0.07±0.01
C16:0	22.15±0.43	23.68±1.36	23.96±1.89	22.69±1.06	23.46±1.06
C17:0	0.10±0.18	0.07±0.16	0.08±0.01	0.08±0.01	0.12±0.01
C18:0	5.27±0.53	6.28±0.62	6.04±0.10	6.19±0.58	6.94±0.58
C20:0	0.06±0.23	0.05±0.24	0.05±0.01	0.37±0.06	0.06±0.01
C22:0	0.02±0.18	ND	ND	0.09±0.01	ND
C24:0	0.21±0.19	0.26±0.13	0.22±0.01	0.21±0.07	0.29±0.03
C14:1	0.08±0.02	0.11±0.01	0.09±0.01	0.07±0.01	0.08±0.01
C15:1	1.12±0.26	1.46±0.14	1.20±0.18	0.04±0.01	0.02±0.01
C16:1	0.41±0.14	0.34±0.13	0.36±0.06	0.41±0.07	0.41±0.04
C16:1 *n-7*	3.61±1.61	4.50±0.45	4.16±0.08	3.80±0.52	3.27±0.32
C17:1	0.07±0.01	0.05±0.02	0.05±0.01	0.05±0.01	0.08±0.01
C18:1 *n-9 t*	0.09±0.91	0.18±0.01	0.12±0.05	0.11±0.01	0.09±0.02
C18:1 *n-11t*	0.06±0.14	ND	0.08±0.01	0.07±0.01	0.08±0.04
C18:1 *n-9*	40.25±0.36	38.09±1.80	40.45±1.22	43.23±0.52	40.86±4.08
C18:1 *n-7*	1.97±0.12	2.44±0.24	2.36±0.13	2.08±0.23	1.96±0.19
C20:1 *n-9*	0.37±0.17	0.39±0.04	0.38±0.13	0.36±0.06	0.30±0.03
C22:1 *n-9*	0.05±0.01	0.04±0.01	0.05±0.01	0.05±0.01	0.03±0.01
C18:2 *n-6*	17.43±0.91	14.15±1.45	13.49±0.42	13.98±0.45	14.87±0.48
C18:2*t9t12*	0.05±0.21	0.08±0.01	0.06±0.01	0.05±0.01	0.05±0.01
C20:2 n-6	0.41±0.11	0.55±0.04	0.50±0.15	0.39±0.03	0.31±0.03
C22:2	ND	0.04±0.01	ND	ND	ND
C18:3 *n-3*	0.55±0.08	0.44±0.03	0.46±0.15	0.45±0.05	0.25±0.06
C18:3 *n-6*	0.13±0.02	0.14±0.01	0.13±0.08	0.14±0.01	0.11±0.01
C20:3 *n-6*	0.43±0.12	0.74±0.03	0.70±0.01	0.03±0.01	0.01±0.04
C20:3 *n-3*	0.02±0.01	ND	0.04±0.01	ND	0.03±0.01
C20:4 *n-6*	2.14±0.21	2.08±0.15	1.76±0.12	1.67±0.73	0.66±0.16
C20:4 *n-3*	0.02±0.01	ND	0.03±0.01	ND	0.02±0.01
C18:4 *n-3*	ND	0.10±0.05	ND	0.05±0.01	ND
C20:5 *n-3* (EPA)	0.09±0.01	0.13±0.08	0.12±0.01	0.03±0.01	0.11±0.01
C22:6 *n-3* (DHA)	0.23±0.02	0.28±0.02	0.27±0.07	0.20±0.37	0.32±0.04
Saturated fatty acid (SFA)	28.38±1.83	30.89±1.58	30.95±1.59	30.18±1.11	31.71±0.67
Monounsaturated fatty acid (MUFA)	48.06±1.80	47.60±1.76	50.26±2.52	50.26±0.12	47.17±0.74
Polyunsaturated fatty acid (PUFA)	21.50±1.15	18.75±0.37	17.55±0.25	17.00±0.80	16.74±0.49

ND: non-detectable IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Values are mean ± SD (n=3).

2.4.2.4 Protein Patterns

Protein patterns of chicken meat with different slaughtering methods and UN sample at day 0 and day 8 of storage is shown in Figure 4. All samples contained myosin heavy chain (MHC) and actin as the major proteins. Additionally, all samples also consisted of tropomyosin and troponin T. In general, there were no differences in protein patterns between all samples with different slaughtering methods at day 0. After 8 days of storage, protein with MW of 205 kDa was generated, plausibly caused by the degradation during refrigerated storage. It was noted that slight degradation of MHC and tropomyosins was noticed in UN sample after 8 days of storage, whereas no change in protein pattern was found in all slaughtered samples. The degradation of MHC and tropomyosins in un-bled sample at the end of storage was probably attributed to endogenous and microbial proteinases (Masniyom *et al*., 2004). Moreover, protein oxidation can also lead to protein degradation (Park *et al.,* 2007). This could result in the changes in textural property of chicken meat after the extended storage.

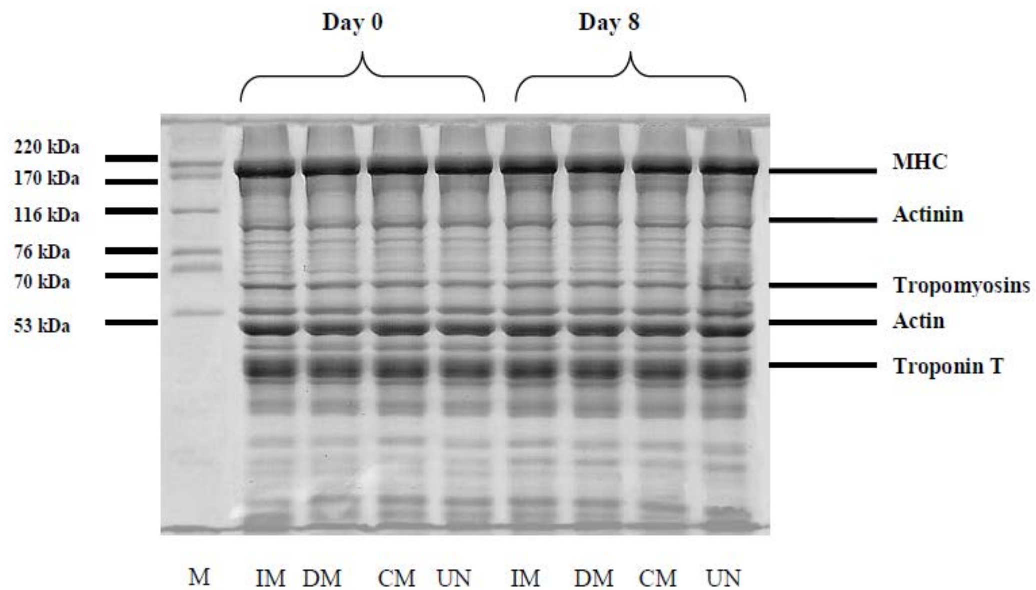


Figure 4 Protein pattern of chicken meat with different slaughtering methods and un-bled sample at day 0 and day 8 of refrigerated storage. M: Molecular-weight markers; IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled.

2.4.2.5 Volatile Compounds

Selected volatile compounds in chicken meat at day 0 and meat with different slaughtering methods after 8 days of refrigerated storage are presented in Table 5. Chicken meat contained PUFAs, which are prone to oxidation. The oxidation of PUFAs is known to produce volatile aldehydic compounds including hexanal, heptanal, octanal, etc. (Yasuhara and Shibamoto, 1995). Aldehydes are the most prominent volatiles produced during lipid oxidation and have been used successfully to follow lipid oxidation in a number of foods, including muscle foods (Ross and Smith, 2006). Amongst all the aldehydic compounds, heptanal, octanal, haxanal and nonanal were the major aldehydes in chicken meat. Tetradecanal, 1-Octanal and 2-decenal were also detected in chicken meat. After 8 days of storage, all aldehydes increased. Amongst all sample, UN samples showed the highest abundance. Aldehydes have been used as indicators of lipid oxidation because they possess low

threshold values and are the major contributors to the development of off-flavour and odor (Boyd *et al.*, 1992; Ross and Smith 2006). The results indicated that lipid oxidation and a greater decomposition of hydroperoxide formed were more pronounced in the UN samples. This was in agreement with the highest prooxidant content in this sample. Autoxidation of polyunsaturated fatty acids in chicken leads to formation of aldehydes, which can produce off-odours, thereby limiting the shelf-life of chicken. Rancidity developed from the autoxidation of lipid leads to unacceptability by the consumers (Iglesias and Media, 2008). Nonanal constituted as the most abundant aldehyde in chicken meat. For hexanal, which has been reported to affect the off-odor caused by lipid oxidation, IM sample had the lowest abundance, compared with other samples. Alcohol was detected in all samples. The alcohol was the secondary product produced by the decomposition of hydroperoxide (Girand and Durance 2000). It was noted that UN sample contained higher abundance of 1-Octen-3-ol than slaughtered sample. Furan are formed by the decomposition of 12-hydroperoxide of linolenate (18:3n-3), the 14-hydroperoxide of eicosapentaenoate (20:5n-3) and the 16-hydroperoxide of docosahexaenoate (22:6n-3). Those compounds can undergo β-cleavage to produce a conjugated diene radical, which can react with oxygen to produce a vinyl hydroperoxide. Cleavage of the vinyl hydroperoxide by loss of a hydroxyl radical leads to the formation of alkoxyl radical, that undergoes cyclisation and produces furan (Maqsood and Benjakul 2011). The abundance of 2-pentylfuran found in the UN sample was higher, compared with slaughtered samples. In general, the lower amount of secondary oxidation products, including aldehydes, alcohols and furan in the slaughtered samples was in accordance with the lower PV and TBARS (Fig. 3). Thus, the bleeding was found to be effective in retarding the formation of secondary lipid oxidation products in chicken meat during refrigerated storage and slaughtering methods affected the volatiles in chicken meat to same degree.

Table 5 Volatile compounds in chicken meat with different slaughtering methods and un-bled sample after 8 day of refrigerated storage

Compounds (Abundance x 10^{6})	Day 0*	Day 8			
		IM	DM	CM	UN
Hexanal	89±0.04	239±0.12	274±0.35	280±0.58	489±1.35
Heptanal	51±1.34	387±1.65	328±1.39	334±1.48	797±1.63
2-pentylfuran	188±0.23	267±0.58	290±0.49	249±0.36	1150±0.32
Octanal	38±1.03	1348±1.52	1348±1.73	1453±1.65	2238±1.96
Nonanal	219±1.56	1763±0.98	1763±1.53	1704±1.04	3219±1.94
1-Octen-3-ol	87±0.86	169±0.52	151±0.38	127±0.43	252±0.59
Benzaldehyde	198±1.05	215±1.07	227±1.12	218±1.29	298±1.05
Nonenal	43±0.48	187±0.63	176±0.92	187±0.48	243±1.32
1-Octanal	287±1.76	396±1.29	361±1.45	459±1.93	787±1.64
2-Decenal	142±1.28	376±1.18	347±1.16	391±1.20	942±1.28
Tetradecanal	104±1.15	226±1.26	229±0.95	208±1.17	391±1.10
Phenol	434±1.93	633±1.87	605±1.14	670±0.93	935±1.18
Isopropyl myristate	105±0.98	164±0.63	198±1.29	147±1.19	289±0.91
Hexadecanoic acid	168±0.75	83±1.17	159±1.14	104±0.97	212±1.15

ND: Non detectable IM: Islamic method; DM: Decapitation method;
CM: Conventional neck cut method and UN: Un-bled. Values are mean ± SD (n=3).
*IM obtained at day 0 was used.

2.4.3 Effect of slaughtering methods on physical changes of chicken meat during refrigerated storage.

2.4.3.1 Colour

Colour of chicken meat with different slaughtering methods and UN sample at day 0 and 8 of storage is shown in Table 6. There was no differences in L*-value between all samples at day 0 ($P>0.05$). However, UN sample had higher a* and ΔE* values ($P<0.05$). Higher a* value could be the result of the blood retained in the meat. After 8 days of storage, the lightness value (L*) and redness (a*) of all broiler chicken breast meat decreased ($P<0.05$). The oxidation of haemoglobin and myoglobin to form met-hemoglobin or met-myoglobin resulted in discolouration of chicken meat, particularly un-bled sample during storage (Alvarado *et al.*, 2007).

Boulianne and King (1998) stated that a* value was positively correlated with total pigment, myoglobin, and iron concentration of chicken meat and the change in a* value was correlated well with myoglobin content. Myoglobin might undergo oxidation during chilled storage to form Met-myoglobin, resulting in more brownish in colour. It was noted that slaughtering methods had the impact on chicken meat colour differently. IM and DM samples had the lower a*-value, but higher b*-value after 8 days of storage, compared with other samples (P<0.05). Therefore, the appropriate slaughtering method could reduce the discolouration of chicken meat during the storage, thereby maintaining the quality and acceptability of chicken meat.

Table 6 *L*,a*,b* ΔE*and ΔC** values of chicken meat with different slaughtering methods and un-bled sample at day 0 and day 8 of refrigerated storage

Storage time (days)	Sample	L*	a*	b*	ΔE*	ΔC*
0	IM	48.55±2.13aA	0.85±0.81bA	11.33±1.45abA	46.35±1.93abA	10.46±1.43bA
	DM	49.20±0.51aA	1.01±0.68bA	10.33±2.35bA	45.49±0.87bA	9.50±2.27bA
	CM	49.49±1.74aA	1.93±0.35bA	10.77±1.03abA	45.36±1.85bA	10.03±1.01bA
	UN	46.57±4.58aA	7.01±0.90aA	12.57±1.36aA	49.17±4.19aA	13.49±0.91aA
8	IM	46.54±1.72aB	0.58±0.45cB	11.76±0.57aA	48.16±1.69bA	10.86±0.56aA
	DM	47.69±0.79aB	0.48±0.24cB	10.95±0.60abA	47.08±0.75bA	10.05±0.61abA
	CM	47.28±1.53aB	0.62±0.19bB	9.74±1.11cA	47.22±1.54bA	8.84±1.10cA
	UN	42.42±1.54bB	2.29±1.43aB	10.49±0.51bcB	52.24±1.41aA	9.90±0.61bB

Values are mean ± SD (n=6). Different lowercase letters within the same storage time in the same column denote the significant difference (P<0.05). Different uppercase letters within the same sample in the same column denotes the significant difference (P<0.05).IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled.

2.4.3.2 Drip Loss and Cooking Loss

Drip loss and cooking loss of chicken meat with different slaughtering methods and UN sample at day 0 and day 8 of refrigerated storage are shown in Table 7. Drip loss was measured to obtain an overall assessment of water binding properties of muscle. With increasing storage time, drip loss of all samples increased (P<0.05). During storage, pH of meat decreased slightly. pH was decreased from pH 7.7 -7.4 to pH 6.4-6.2 after 8 days of storage (data not shown). The acids formed were likely contributed to the decrease in pH of which meat probably induced the denaturation of proteins, thereby decreasing water holding capacity. This was evidenced by the increased drip loss. At day 0, IM sample showed a lower drip loss value, compared with other samples (P<0.05). This result was in agreement with DAgata *et al.* (2009) who reported that meat derived from animals with halal slaughtering showed the lower drip loss, compared with that from conventional slaughtering method. Nevertheless, there was no difference in drip loss between samples stored at day 0 and 8 (P>0.05).

Cooking loss is another parameter to evaluate water holding capacity after heating and an increase in cooking loss was related to low water holding capacity of protein due to their denaturation (Zayas, 1997). During heating, water within the muscle located in the narrow channels between the filaments was released as meat shrinks (Bertola *et al*., 1994). This resulted in cooking loss when being heated. The result showed that cooking loss of IM sample was lower than that of samples from other slaughtering methods (P<0.05). Cooking loss generally increased after 8 days of storage (P<0.05). During the extended storage, lipid oxidation took place especially for UN sample. Lipid oxidation products have been known to induce protein cross-linking in the muscle (Bertola *et al*., 1994). As a result, the denaturation of proteins was enhanced with concomitant decrease in water holding capacity after cooking. This was obvious in UN sample, in which the highest oxidation occurred. The result suggested that slaughtering method had the impact on water holding capacity of chicken meat to some degree, after slaughtering and the extended storage.

Table 7 Drip loss and cooking loss of chicken meat with different slaughtering methods and un-bled sample at day 0 and 8 of refrigerated storage

Storage time (days)	Sample	Drip loss (%)	Cooking loss (%)
0	IM	3.55±0.10bA	18.92±2.44bB
	DM	4.25±0.30aA	19.02±1.40bB
	CM	4.60±0.57aA	20.00±0.63bB
	UN	4.64±0.36aA	23.47±1.71aB
8	IM	4.11±0.52bA	21.38±2.39bA
	DM	4.97±0.55abA	21.72±2.03bA
	CM	4.87±0.48abA	21.60±0.70bA
	UN	5.82±1.71aA	26.87±0.68aA

Values are mean ± SD (n=6). Different lowercase letters within the same storage time in the same column denote the significant difference (P<0.05). Different uppercase litters within the same sample in the same column denotes the significant difference (P<0.05). IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled.

2.4.4 Effect of slaughtering methods on microbiological changes of chicken meat during refrigerated storage

Mesophilic bacteria count (MBC) and psychrophilic bacteria count (PBC) of chicken meat with different from each slaughtering methods during refrigerated storage are depicted in Figure 5. MBC and PBC of all sample increased with increasing storage time (P<0.05). MBC and PBC of UN sample were higher than those of slaughtered samples throughout the storage (P<0.05), suggesting that bleeding was effective in retarding the growth of bacteria in chicken meat. Bleeding could lower MBC in chicken meat, regardless of slaughtering methods used. There were no differences in MBC and PBC between chicken meat from three slaughtering methods (P>0.05). However, blood could be retained in the bled sample to some extent and this would serve as the nutrient for microbial growth. After slaughtering, the blood pressure drops rapidly and it does not have enough driving force to empty the numerous capillary in muscle (Alvarado *et al*., 2007). The blood enriched with nutrients for microbial growth could induce the enumeration of bacteria, which were

contaminated from skin, viscera or environment during handing, slaughtering and dressing. *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, Enterobacteriaceae, *Escherichia coli*, *Campylobacter* spp., and *C. perfringens* were found as the dominant bacteria in chicken meat (Jorgensen *et al*., 2002; Miettinen *et al*., 2002). The bacteria continued to grow as far as there was available nutrients. For the meat with low residual blood, the available nutrients were depleted rapidly (Ali *et al*., 2011). High bacterial growth shortened the shelf-life of chicken meat and led to the enhanced deterioration of the product. Thus, the blood present in the UN sample provided a suitable substrate for the growth of microorganisms as evidenced by higher MBC and PBC throughout the storage of 8 days. MBC of UN sample exceeded 10^7cfu/g, the upper limit for the chicken to be safe for consumption (ICMSF, 1986), at day 6. When storage time increased, psychrophilic bacteria became dominated as indicated by higher count for all samples, whilst the increasing rate of mesophilic bacteria was lowered. Thus, bleeding played a role in microbial load of chicken meat to some extent.

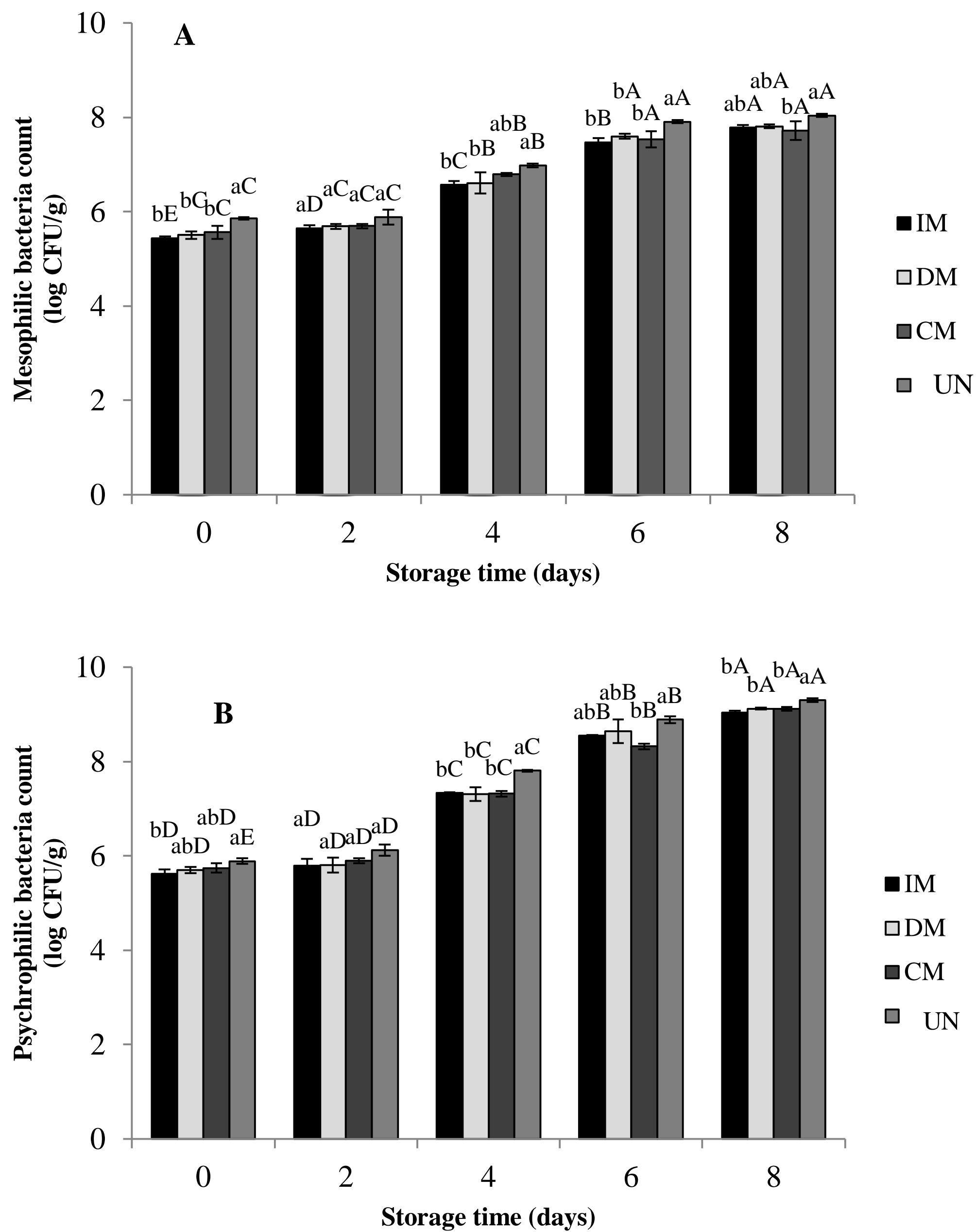


Figure 5 Mesophilic bacteria count (A) and psychrophilic bacteria counts (B) of chicken meat with different slaughtering methods and un-bled sample during 8 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Different lowercase letters on the bars within the same storage time indicate the significant differences (P<0.05). Different uppercase letters on the bars within the same sample indicate the significant differences (P<0.05).

2.5 Conclusion

Chicken meat from Islamic slaughtering methods showed the lower haem and non-haem iron contents with coincidental lowerer lipid oxidation than meat obtained from other slaughtering methods. The oxidation of lipids induced by iron from haem, along with microbial growth, was plausibly the main cause of deterioration and losses in quality of chicken meat. Thus, Islamic slaughtering method yielded chicken meat with the better quality and oxidative stability during post-harvest storage.

CHAPTER 3
Impact of Halal slaughtering method on the quality changes of cooked chicken patties during refrigerated storage

3.1 Abstract

Quality of cooked patties from chicken obtained from various slaughtering methods including Islamic method (IM), decapitation method (DM), conventional neck cut method (CM) and un-bled sample (UN) was monitored during 12 days of refrigerated storage. During the storage, cooked patties from IM chicken showed the lowest peroxide value (PV) and thiobarbituric acid reactive substances value (TBARS), compared with others ($P<0.05$). This was coincidental with the lowest abundance of the hexanal and octanal, the major secondary lipid oxidation products. After 12 days of storage, cooked patties from IM chicken had the lower a*, b*, ΔE* values, but the higher L* value, compared with those prepared by other slaughtering methods. Cooked patties from IM chicken also exhibited the higher hardness and cohesiveness, in comparison with other samples. For sensory property, the higher likeness score of all sensory attributes was observed for cooked patties from IM chicken at day 6 of storage ($P<0.05$). Additionally, mesophilic bacteria count (MBC) and psychrophilic bacteria count (PBC) were lower in cooked patties from IM chicken ($P<0.05$), compared with other samples. Thus, Islamic slaughtering method showed the superior preventive effect toward lipid oxidation and microbial growth to other methods, and could maintain the quality of cooked chicken patties during the refrigerated storage, effectively.

3.2 Introduction

Animal meat obtained from Islamic slaughtering method must be prepared following Islamic law prescribes a set of dietary rule, called “Halal” (legal, permitted by Allah) which lists the permitted food. The consumption of meat not obtained according to Islamic rules, covering livestock handling before and during slaughter, is prohibited (Regenstein *et al.*, 2003; Bonne and Verbeke, 2008). Halal refers to the aspects of reliable food quality, wholesome, hygiene and safety

(Regenstein *et al*., 2003). Muslims are supposed to make an effort to obtain halal food of good quality. For non-Muslim consumers, halal foods are often perceived as specially selected and processed to achieve the halal standards of quality (Riaz and Chaudry, 2004).

Poultry production and processing involve a series of interrelated steps, converting domestic birds into different forms such as ready-to-cook whole carcasses, cut-up carcass parts, or deboned meat product (Alan, 2001). However, poultry meat is perishable if it is not handled properly. This is in relation with microbial growth, which directly determines the safety and the shelf-life of chicken meat. One of the most important factors affecting the level of contamination and enhance the deterioration is the amount of blood left within the carcass after bleeding (Ali *et al.*, 2011). Depending on the degree of processing or slaughtering, the spoilage of chicken meat varies between 4 and 10 days under refrigeration (Marenzi, 1986). Blood is considered to be an excellent medium for the growth of bacteria due to its high nutritive value. The amount of blood bled by the animal depends on the slaughtering method (DAgata *et al.,* 2009). Furthermore, blood components, especially haemoglobin, are powerful promoters of lipid oxidation and may decrease the shelf-life of meat products (Alvarado *et al.,* 2007).

In the last decade, chicken-based meat products have become increasingly popular worldwide due to their high nutritional quality with low cost and are available as either fresh or precooked chicken, mainly packaged and stored under refrigerated condition (Barbut, 2002). In Thailand poultry slaughtering can be varied, depending on the belief or practice. However, halal slaughtering method has been believed to render the complete bleeding, and it may be beneficial for shelf-life extension or quality maintenance of chicken meat. Recently, Addeen *et al.* (2013) reported that chicken meat obtained from Islamic slaughtering method contained the lowest haem and non-haem iron amount and had the lower lipid oxidation during refrigerated storage of 8 days. Those chicken meat can yield the products e.g. patties with higher oxidation stability or longer shelf-life, compared with chicken meat obtained from other slaughtering methods. However, no information on the quality of cooked patties from chicken obtained by different slaughtering methods has been reported. This study aimed to elucidate the impact of Islamic slaughtering method on

quality of cooked patties, in comparison with other methods during the extended refrigerated storage.

3.3 Materials and Methods

3.3.1 Chemicals

Trichloroacetic acid, ferrous chloride and standard plate count agar (PCA) were obtained from Merck (Darmstadt, Germany). 2-thiobarbituric acid and 1,1,3,3-tetramethoxypropane (Malonaldehyde) were purchased from Sigma (St. Louis. MO, USA). Cumene hydroperoxide was procured from Fluka (Buchs, Switzerland). Methanol, chloroform, hydrochloric and ammonium thiocyanate were obtained from Lab-Scan (Bangkok, Thailand).

3.3.2 Sample preparation and storage condition

Thirty-two broilers with the age of six weeks and body weight of approximately 2 kg were obtained from a poultry farm in Songkhla, Thailand. The broilers were divided into three groups having three different slaughtering methods. Those included 1) Islamic method, 2) Decapitation method and 3) Conventional neck cut method. Un-bled sample was used as the control. The samples obtained were referred to as "IM", "DM", "CM" and "UN", respectively.

After bleeding for 3 min, the chicken was plucked in a rotary-drum picker for 30 s and eviscerated. Breast muscle was dissected from the carcasses, followed by mincing. The chicken breast was minced using a food processor at high speed for 3 min (MK- Panasonic, 5087M, Selangor Darul Ehsan, Malaysia). The minced breast muscle was mixed with sodium chloride (1% w/w) and was formed into patties (30 g with approximately 1 cm thickness and diameter of 5 cm). The patties were heated by steaming until the core temperature reached 80°C (Naveena *et al.,* 2008). After cooling for 10 min at room temperature, the patties were packaged in polyethylene bag and kept at 4°C. Samples were taken for analyses at day 0, 3, 6, 9 and 12.

3.3.3 Chemical analyses

3.3.3.1 Determination of thiobarbituric acid reactive substances (TBARS)

TBARS value was determined according to the method of Benjakul and Bauer (2001) with some modifications. Ground sample (1 g) was mixed with 5 mL of a solution containing 0.375% TBA, 15% TCA and 0.25 N HCl. The mixture was heated in boiling water for 10 min, followed by cooling with the running water. The mixture was centrifuged at 4000g for 20 min (MIKRO20, Hettich Zentrifugan, Germany). The supernatant was collected and the absorbance was read at 532 nm using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). TBARS value was calculated from the standard curve of malonaldehyde (0-2 ppm) and expressed as mg malonaldhyde/kg patties.

3.3.3.2 Determination of peroxide value (PV)

PV was determined as per the method of Richards and Hultin (2002) with a slight modification. Ground sample (1 g) was mixed with 11 mL of chloroform/methanol (2:1, v/v). The mixture was homogenised at a speed of 13,500 rpm for 2 min using an UltraTurrax T25 homogeniser (Janke and Kunkel, Staufen, Germany). The homogenate was then filtered using Whatman no.1 filter paper (Whatman International, Ltd, Maidstone, England). Two millilitres of 0.5% NaCl was then added to 7 mL of the filtrate. The mixture was vortexed at a moderate speed for 30 s using a Vortex-Genie2 mixer 4 (Bohemia, NY, USA) and then centrifuged at 3000g for 3 min using a RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA) to separate the sample into two phases. Two millilitres of cold chloroform/methanol (2:1) was added to 3 mL of the lower phase. Twenty-five microlitres of 30% ammonium thiocyanate and 25 μL of 20 mM iron (II) chloride were added to the mixture (Shantha and Decker, 1994). The reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at 500 nm. A standard curve was prepared using cumene hydroperoxide at the concentration range of 0.5–2 ppm. PV was expressed as mg hydroperoxide/kg patties.

3.3.3.3 Determination of volatile compounds

Volatile compounds were determined by solid phase micro extraction–gas chromatography–mass spectrometry (SPME–GC–MS) (Iglesias and Medina, 2008).

3.3.3.3.1 Extraction of volatile compounds. Three grams of sample were homogenised at a speed of 13,500 rpm for 2 min with 8 mL of saturated NaCl in ultra-pure water. The mixture was centrifuged at 2000 g for 10 min at 4°C. The supernatant (6 mL) was heated at 60°C with an equilibrium time of 10 h in a 20 mL headspace vial. Finally, the SPME fibre (50/30 µm DVB/Carboxen™/PDMS StableFlex™) (Supelco, Belle-fonte, PA, USA) was exposed to the head space of the vial containing the sample extract. The volatile compounds were allowed to absorb in the SPME fibre for 1 h. The volatile compounds were then desorbed in the GC injector port for 15 min at 270°C.

3.3.3.3.2 GC-MS analysis. GC-MS analysis was performed in a HP 5890 series II gas chromatography coupled with HP 5972 mass selective detectors, equipped with a splitless injector, and coupled with a quadrupole mass detector (Hewlett Packard, Atlanta, GA, USA). The compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m 0.25 mm ID, with film thickness of 0.25 µm). The GC oven temperature programme was 35°C for 3 min, followed by an increase of 3°C /min to 70°C, then an increase of 10°C /min to 200°C, and finally an increase of 15°C /min to a final temperature of 250°C, and hold for 10 min. Helium was employed as a carrier gas, with a constant flow of 1.0 mL/min. The injector was operated in the splitless mode and its temperature was set at 270°C. The transfer line temperature was maintained at 265°C. The quadrupole mass spectrometer was operated in the electron ionisation (EI) mode and the source temperature was set at 250°C. Initially, a full scan mode data was acquired to determine the appropriate masses for the later acquisition in selected ion monitoring (SIM) mode, under the following conditions: mass range: 25–500 amu and scan rate: 0.220 s/scan. All analyses were performed with ionisation energy of 70 eV, a filament emission current of 150 µA, and an electron multiplier voltage of 500 V.

3.3.3.3.3 Identification of volatile compounds. Identification of the compounds was done by consulting ChemStation Library Search (Wiley 275.L). Identification of compounds was performed, based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search (Wiley 275.L). Quantification limits were calculated to a signal-to noise (S/N) ratio of 10. Repeatability was evaluated by analysing 3 replicates of each sample. The identified volatile compounds related with lipid oxidation were presented in the form of normalised area under peak of each identified compound.

3.3.4 Physical analyses

3.3.4.1 Colour (L*, a*, b*, ΔE*)

Colour of cooked chicken patties was determined by measuring L* (lightness), a*(redness/greenness) and b* (yellowness/blueness) value using a colourimeter (JP7100F, Juki Corp, Tokyo, Japan). The colourimeter was standardised by black and white tile. Colour differences, ΔE*, was calculated by the following equation (Berns, 2000):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where, ΔL*, Δa* and Δb* represent the difference in the colour parameters between the sample and the white standard (L*= 93.55, a* = -0.84, b*=0.37)

3.3.4.2 Texture profile analysis (TPA)

TPA was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England) with cylindrical aluminum probe (50 mm diameter). The samples was cut into cylinders (30 mm height×20 mm diameter) and placed on the instrument's base. The tests were performed with two compression cycles. TPA textural parameters were measured at room temperature with the following testing conditions: crosshead speed of 5.0 mm/s, 50% strain, surface sensing force of 99.0 g and threshold of 30.0 g. The time interval between the first and the second compressions was 1 s. The Texture Expert version 1.0 software (Stable Micro Systems, Surrey, England) was used to collect and process the data. Hardness,

springiness, cohesiveness, gumminess and chewiness were calculated from the force–time curves generated for each sample (Bourne, 1978).

3.3.5 Microbiological analysis

Mesophilic bacteria count was analysed following Bacteria Analytical Manual (BAM, 2001) using pour a plate method. Ground sample (25 g) was placed in a stomacher bag containing 225 ml of phosphate buffer saline (PBS) 0.15M (pH 7.2). After mixing for 1 min in a Stomacher blender (M400, Seward, West Sussex, UK), the appropriate dilutions were prepared. One ml of appropriate dilution of homogenate was transferred on plate count agar and incubated at 35°C for 2 days. For psychophilic bacteria count, a pour plate method was used and the incubation was conducted at 4°C for 7 days.

3.3.6 Sensory analysis

Cooked patties obtained from chicken with three different slaughtering methods and un-bled sample were subjected to sensory analysis at day 0 and 6 of storage. The microbial load in all samples tested did not exceed the microbiological limit (5×10^5 CFU/g) (ICMSF, 1986). Before testing, the samples were wrapped with aluminum foil and steamed for 3 min. The samples were evaluated by 30 panelists from the Department of Food Technology with the age of 25-35, using the 9-point hedonic scale, where 9 = like extremely; 7= like moderately; 5 = neither like or not dislike; 3 = dislike moderately; 1 = dislike extremely (Meilgaard *et al.*, 2006). All panelists were asked to evaluate for appearance, odor, taste, texture and overall likeness.

3.3.7 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie, 1980). Analysis was performed using the Statistical Package for Social Science package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3.4 Results and Discussion

3.4.1 Chemical changes of cooked patties from chicken with different slaughtering methods during refrigerated storage

3.4.1.1 Lipid oxidation

Lipid oxidation in cooked chicken patties during refrigerated storage of 12 days as monitored by PV and TBARS value is shown in Figure 6. The increases in PV of all samples were observed with increasing storage time (P<0.05). However, the rate of increased was different. When comparing PV of all samples, PV of IM sample was lower than those obtained from other slaughtering methods as well as UN sample during the 12 days of storage (P<0.05). UN sample showed the higher PV, compared with the slaughtered samples (P<0.05). During cooking, proteins underwent denaturation and the loss of antioxidative enzyme, e.g. catalase and glutathione peroxidase might occur. Simultaneously, the release of catalytically-active iron from metallo-proteins, especially haem proteins, could take place. Those prooxidants could induce the lipid oxidation as indicated by the increase in PV. Due to the lower PV in IM sample, the lower content of prooxidants in this sample might be associated with the lower oxidation. Addeen *et al*. (2013) reported that IM chicken meat contained the lower haem iron and non-haem iron content than chicken from other slaughtering methods. When heat was applied, the disruption of porphyrin ring probably occurred, leading to the release of free iron named “non-haem iron”. Damage of porphyrin with the release of iron is known to induce lipid oxidation in muscle (Bertola *et al.,* 1994).

TBARS values of all cooked chicken patties increased as the storage time increased (P<0.05). The increase in TBARS might be caused by the formation of decomposition products during the advancement of oxidation (Nawar, 2000). For IM sample, no change in TBARS was noticeable within the first 3 days of storage (P> 0.05). The sharp increase was found during 3-6 days. Thereafter, slight increase in TBARS was observed up to day 12. Similar result was obtained for DM and CM samples. Although PV increased continuously, TBARS remained unchanged, particularly during 9-12. This might be due to the loss of volatile lipid oxidation products to a higher rate. Overall, UN sample had higher TBARS value than

slaughtered samples throughout the storage (P<0.05). Blood containing haemoglobin could serve as the source of prooxidants, which induced lipid oxidation in cooked patties (Richard *et al*., 2007). Blood also contain a large amount of white blood cells, which can also generate superoxide, hydrogen peroxide and hydroxyl radical (Gabig and Babior, 1981). Therefore, chicken from Islamic slaughtering method yielded the cooked patties with the decreased susceptibility to lipid oxidation, as evidenced by the lower TBARS value.

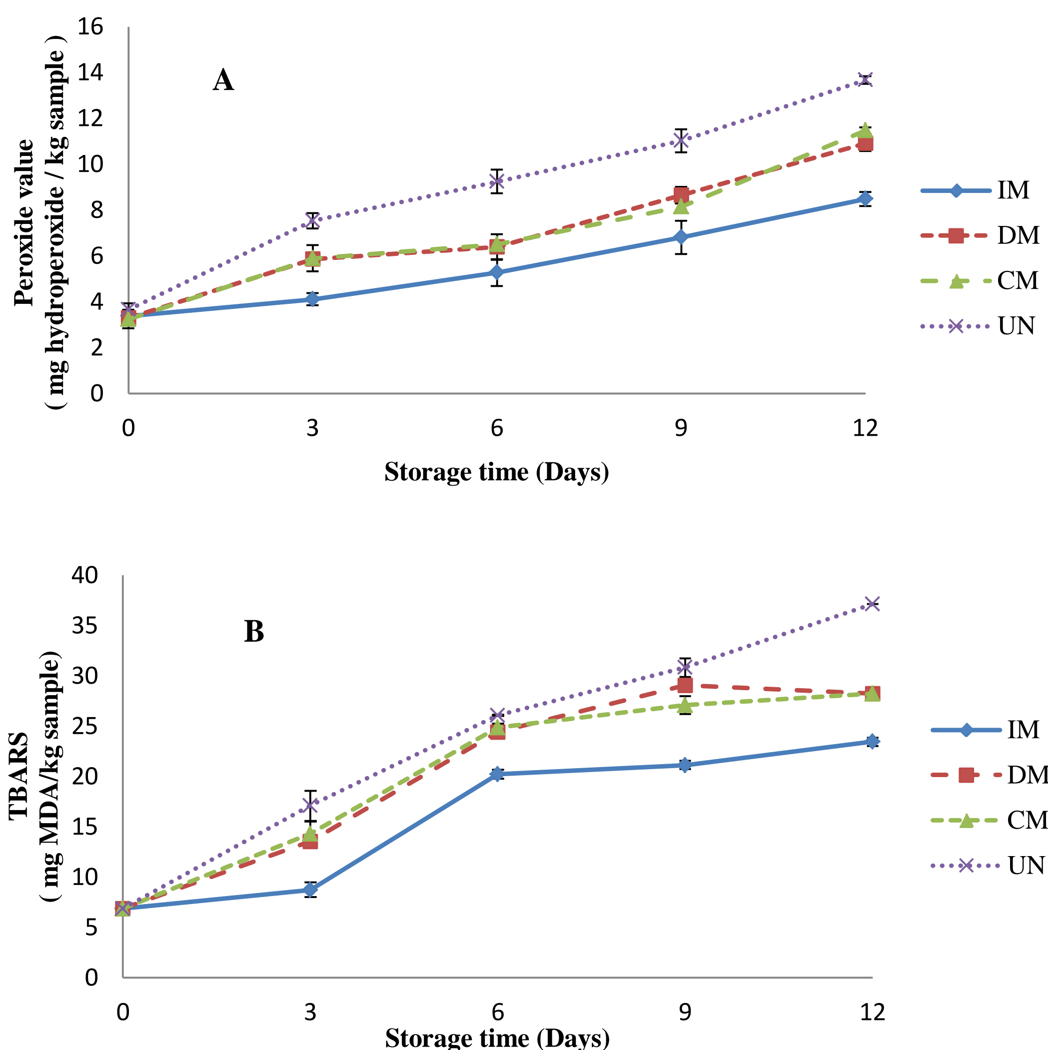


Figure 6 Changes in PV (A) and TBARS (B) of cooked patties from chicken with different slaughtering methods during 12 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Bar represent the standard deviation (n=3)

3.4.1.2 Volatile compounds

Volatile compounds in cooked chicken patties after 12 days of refrigerated storage are displayed in Table 8. Chicken muscle contained high amount of polyunsaturated fatty acids (PUFAs) (Addeen *et al.,* 2013). Oxidation of PUFAs known to produce volatile compounds including hexanal, heptanal, octanal, etc (Yasuhara and Shibamoto, 1995). Several volatiles including aldehyde (hexanal, heptanal, octanal, 2-heptanal, nonanal, 2-octenal nonenal and tetradecanal), alcohol (1-octen-3-ol) and other volatile substances (tetradecane) were formed to a higher extent after 12 day of storage. It was noted that hexanal and octanal were the major oxidation products in the chicken meat. Amongst all samples, UN sample contained higher abundance of all volatile compounds. It was postulated that higher lipid oxidation and greater decomposition of hydroperoxide occured in UN sample, compared with slaughtered samples. Aldehydes are the most prominent volatiles produced during lipid oxidation and are associated with off-odour (McGill *et al.,* 1974). This observation was similar to previous studies (St. Angelo *et al.,* 1988; Larick *et al.,* 1992; Du *et al.,* 2001). Cooked sample had the increased the amount of volatiles, especially haxanal and pentanal; this could be due to the higher oxidation, compared with raw meat (Bagorogoza *et al.,* 2001). Aldehydes have been used as indicators of lipid oxidation because they possess low threshold values and are the major contributors to the development of off-flavour and odour (Boyd *et al.,* 1992; Ross and Smith, 2006). Alcohols and ketones are the secondary products produced by the decomposition of hydroperoxide (Maqsood and Benjakul, 2011). 1-octen-3-ol imparts a desirable mushroom-like odour besides green and plant-like aromas (Josephson *et al*., 1986). In general, the lower amount of secondary oxidation products, including aldehyde and alcohol, in IM sample was in accordance with the lower PV and TBARS (Figure 6). For DM and CM samples, the latter showed the higher abundance, compound with the former, except heptanal, 2-heptenal and 1-octen-3-ol. Thus, the Islamic slaughtering method was found to be effective in retarding the formation of secondary lipid oxidation products in cooked chicken patties.

Table 8 Volatile compounds in cooked patties from chicken with different slaughtering methods after 12 day of refrigerated storage

Compounds (Abundance x10^7)	Day 0*	Day 12			
		IM	DM	CM	UN
Hexanal	485±0.97	702±0.92	748±0.98	785±1.15	967±1.54
Heptanal	90±1.19	109±1.16	199±1.93	112±0.91	273±0.87
Octanal	237±0.93	405±1.45	442±1.15	477±1.18	596±1.29
2-Heptenal	ND	19±0.38	29±1.28	18±1.10	49±0.92
Nonanal	236±1.17	334±1.53	412±1.76	517±1.28	582±1.64
2-Octenal	54±1.20	137±0.52	181±0.48	214±1.64	254±1.97
1-Octen-3-ol	136±1.93	167±1.18	196±1.05	186±1.32	209±1.59
Tetradecane	ND	29±0.63	75±0.86	77±0.65	113±0.94
Nonenal	ND	50±1.76	69±1.56	80±1.43	107±1.83
Tetradecanal	14±1.04	38±1.05	46±1.03	50±0.62	91±0.94

ND: not detectable IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Values are mean ± SD (n=3).
*IM obtained at day 0 was used.

3.4.2 Physical changes of cooked patties from chicken with different slaughtering methods during refrigerated storage

3.4.2.1 Colour

Colour of cooked patties from chicken with different slaughtering methods and UN sample at 0 and day 12 of refrigerated storage is shown in Table 9. Colour is an important factor for consumer acceptance of cooked meat products (Aksu *et al.*, 2005). Poultry meats contain varying amounts of pigments including haemoglobin, myoglobin, and cytochrome c. During cooking of meats, hemichrome (denatured globin and oxidised haem iron) is formed, leading to the tan colour. Haemoglobin and myoglobin are almost completely denatured between 80 and 85 ºC (Lawrie, 1955). At day 0, the cooked chicken patties from UN sample had higher a*, b* ΔE* but lower L* value than those from the slaughtered samples ($P<0.05$). Boulianne and King (1998) showed a strong positive correlation between total pigment concentration and a* value. After 12 days of storage a*, b*and ΔE* value in

cooked chicken patties decreased (P<0.05). This coincided with the increase in L* value (P>0.05), except for patties from UN samples, where no change in L*-value was observed (P>0.05). The whitening was plausibly due to an increased destruction of pigment with the extended storage time. The coincidental decreases in a* and b* values might be due to the loss of chroma, resulting from the changes in haem pigments. However, the cooked patties from IM chicken showed the lower a*, b* ΔE* and higher L*value throughout the storage, compared with others. The result suggested that Islamic method could exhibit the most effective removal of blood from chicken. As a result, the changes in colour mediated by the blood or pigments retained in chicken meat could be lowered.

Table 9 *L**, *a**, *b** and *ΔE** values of cooked patties from chicken with different slaughtering methods at day 0 and day 12 of refrigerated storage

Storage time(days)	Samples	*L**	*a**	*b**	*ΔE**
0	IM	83.31±0.22aA	0.53±0.02dA	14.65±0.22cA	17.63±0.44cA
	DM	80.65±0.23cA	0.82±0.35cA	15.62±0.32bA	20.05±0.26bA
	CM	82.82±0.66bA	1.40±0.36bA	15.93±0.62bA	19.03±0.30bA
	UN	77.40±1.41dA	2.37±0.21aA	18.22±0.51aA	24.29±1.33aA
12	IM	85.34±0.30aA	0.34±0.02dB	12.48±0.34cB	14.68±0.25cA
	DM	84.31±0.29bA	0.74±0.20cB	17.50±0.28aB	19.52±0.16bB
	CM	83.38±0.83cA	1.13±0.30bB	15.48±0.60bB	18.33±1.12bA
	UN	77.67±0.81dA	2.16±0.16aB	17.52±0.33aB	23.58±0.83aA

Values are mean ± SD (n=3). Different lowercase letters within the same storage time in the same column denote the significant difference (P<0.05). Different uppercase letters within the same treatment in the same column denotes the significant difference (P<0.05). IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled

3.4.2.2 Textural properties

Textural properties of cooked patties from chicken with different slaughtering methods and UN sample at day 0 and day 12 of refrigerated storage are shown in Table 10. At day 0, patties from IM and DM sample had the higher hardness and gumminess, compared with other samples (P<0.05). Nevertheless, there was no

difference in springiness and cohesiveness between all sample (P>0.05). The denaturation of myofibrillar proteins, the shrinkage of intramuscular collagen, as well as the shrinkage and dehydration of the actomyosin were observed in the range of 80-100 ºC (Bailey and Light, 1989). This more likely resulted in the formation of hard texture of cooked patties. Hardness, springiness and chewiness of all samples decreased after 12 days of storage (P<0.05). However, there was no change in cohesiveness after storage. The results indicated that softening of texture occurred after 12 days of storage. No changes in gumminess were found in patties from IM and CM sample (P>0.05). The changes in textural properties might be associated with the chemical and microbial deterioration. Lipid and protein oxidation are closely associated with deteriorative processes occurring in meat (Mercier *et al.,* 2004). Protein oxidation can negatively affect the sensory quality of fresh meat and meat products in terms of texture, tenderness and colour (Rowe *et al.,* 2004). Spoilage bacteria might decompose the proteins in patties, resulting in the textural weakening. Nevertheless, at the end of storage (12 days), patties from IM and CM samples had the similar textural properties except for chewiness, in which patties from IM sample showed the higher value, compared with those from CM sample (P<0.05).

Table 10 Texture properties of cooked patties from chicken with different slaughtering methods at day 0 and day 12 of refrigerated storage

Storage time (days)	Samples	Hardness (N)	Springiness (cm)	Cohesiveness (ratio)	Gumminess (N)	Chewiness (N cm)
0	IM	11.87±1.32aA	1.18±0.41abA	0.67±0.04aB	7.28±1.62abA	7.66±0.84aA
	DM	11.64±1.42aA	0.94±0.05bA	0.67±0.02aA	7.92±0.76aA	7.33±0.48abA
	CM	10.45±0.89bA	1.13±0.37abA	0.64±0.05aA	6.57±1.34bcA	6.75±0.91bcA
	UN	9.58±0.88bA	1.42±0.55aA	0.65±0.02aA	6.23±0.68cA	6.43±0.46cA
12	IM	10.14±1.29aB	0.80±0.13aB	0.73±0.04aA	6.83±1.26aA	6.07±1.37aB
	DM	9.87±0.65abB	0.74±0.25aB	0.67±0.08bA	6.74±0.57aB	5.60±0.47abB
	CM	9.43±0.97abB	0.82±0.03aB	0.68±0.02abA	6.20±0.83aA	5.25±0.58bB
	UN	8.20±1.84bB	0.75±0.23aB	0.69±0.06abA	5.27±0.64bB	4.30±0.66cB

Values are mean ± SD (n=3). Different lowercase letters within the same storage time in the same column denote the significant difference (P<0.05). Different uppercase letters within the same treatment in the same column denotes the significant difference (P<0.05). IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled

3.4.3 Microbiological changes of cooked patties from chicken with different slaughtering methods during refrigerated storage.

Mesophilic bacteria count (MBC) and psychrophilic bacteria count (PBC) of cooked patties from chicken slaughtered by different methods and UN sample during refrigerated storage are depicted in Figure 7. MBC and PBS of all cooked chicken patties increased when the storage time increased (P<0.05). At day 0, patties from IM and DM showed the lower MBC and PBC than those from CM and UN samples (P<0.05). Patties from UN sample showed the highest MBC. This might be due to the enumeration of bacteria during transportation of this sample. At the same storage time, patties from IM sample had the lower MBC and PBC, compared with others (P<0.05). The swift cutting of vessels of the neck used for Islamic method disconnects the flow of blood to the nerve of the brain responsible for pain. While dying, the animal struggles, writhers, shakes and kicks, not due to pain, but due to the contraction and relaxation of the muscles deficient in blood and due to the flow of

blood out of the body (DAgata *et al.,* 2009). The purpose is to drain out most of the blood which would serve as a good culture medium for microorganisms. The spinal cord must not be cut because the nerve fibres to the heart could be damaged during the process, causing cardiac arrest and stagnating the blood in the blood vessels. Blood is a good media of bacteria. Therefore the Islamic way of slaughtering is more hygienic as most of the blood containing bacteria that are the cause of several diseases are eliminated. The blood enriched with nutrients for microbial growth could induce the enumeration of bacteria, which were contaminated from skin, viscera or environment during handle of the broiler chickens. *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, Enterobacteriaceae, *Escherichia coli*, *Campylobacter* spp., and *C. perfringens* were found as the dominant bacteria in chicken meat (Jorgensen *et al*., 2002; Miettinen *et al.,* 2002). The bacteria continued to grow as far as there was available nutrients, whilst for those with low residual blood, the bacterial growth rate was depleted (Ali *et al.,* 2011). Mesophilic bacteria count of patties from UN sample exceeded 5×10^5 cfu/g, the limit for the chicken and chicken product to be safe for consumption (ICMSF, 1986), at day 12.

For PBC, it was not detected at day 0. During 3-12 days of storage, particularly 9-12 days, psychrophilic bacteria became dominant and the inhibition of mesophilic bacteria occurred at the low temperature was presumed. Psychrotrophic bacteria, generally *Pseudomonas* spp., have been identified as the predominant microorganism responsible for spoilage of aerobically-stored meat products (Pooni and Mead, 1984). At all storage times, patties from chicken slaughtering by Islamic method showed the lower PBS than others. Thus, IM chicken rendered cooked patties with the lowest microbial load as indicated by lower MBC and PBC.

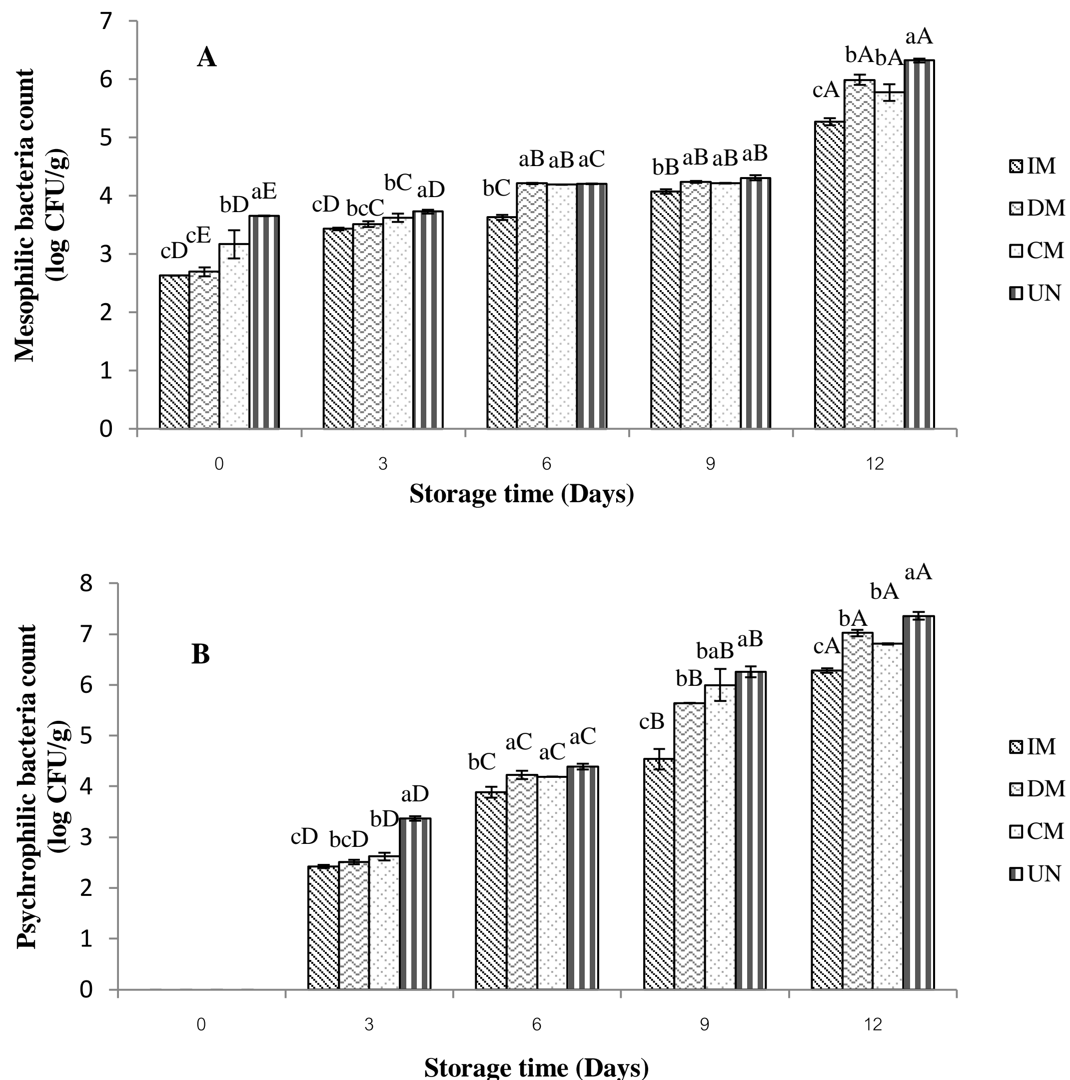


Figure 7 Mesophilic bacteria counts (A) and psychrophilic bacteria counts (B) of cooked patties from chicken with different slaughtering methods during 12 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Different lowercase letters on the bars within the same storage time indicate the significant differences (P<0.05). Different uppercase letters on the bars within the same treatment indicate the significant differences (P<0.05).

3.4.4 Sensory properties

Appearance, odor, texture, taste and overall likeness scores of all cooked chicken patties at day 0 and 6 of storage are depicted in Table 11. The scores of all sensory attribute were different, dependent on chicken used for patties

preparation. Islamic slaughtering method yielded patties with higher score for all attributes, except for taste ($P<0.05$). Patties from UN sample exhibited the lower scores for all attributes tested. This was related with the lower lipid oxidation in patties prepared from IM chicken, in which rancidity was developed to the lower extent. Lipid oxidation has been considered as the primary cause of flavor deterioration and the development of oxidized flavors in cooked stored meat (St. Angelo *et al.*, 1987; Shahidi, 1994; Thiansilakul *et al.*, 2010). In particular, the secondary products of lipid oxidation have been known to relate with warmed-over flavour (WOF) in cooked and chill stored meats (St. Angelo *et al.*, 1988). Cooked meat and meat products reheated after a short period of refrigerated storage develop a distinctive off-flavour referred to as WOF (Tims and Watts, 1958). WOF development is largely attributed to the autoxidation of polyunsaturated fatty acids, mainly in the phospholipids. Iron, in different forms, is an important catalyst in the reactions (Pearson *et al.*, 1977; Gray and Pearson, 1987). For odour likeness, patties from IM chicken showed no decrease in the score, whilst patties from DM and CM sample had the decrease in score. For overall likeness seen, patties from IM sample showed the higher score than other samples and no change in score was noticeable after 6 day of storage. The higher score of patties from IM sample was related with the higher hardness (Table 10) and the higher odour likeness score was in agreement with the lower PV and volatile compounds (Table 8)

Thus, cooked patties from chicken with Islamic slaughtering method showed the superior sensory property to those prepared from chicken obtained from other slaughtering methods.

Table 11 Hedonic scores of cooked chicken patties as affecting by slaughtering method at day 0 and day 6 of refrigerated storage

Attributes	Storage time (Day)	IM	DM	CM	UN
Appearance	0	7.90±0.70aA	6.76±0.77bA	6.81±0.60bA	5.29±1.65cA
	6	7.55±0.51aA	6.35±1.04bA	6.40±0.50bA	4.95±1.28cA
Odor	0	7.67±0.66aA	6.62±1.02bA	6.90±1.04bA	5.38±1.28cA
	6	7.40±0.50aA	5.70±1.30bB	5.65±0.88bB	4.60±1.43cA
Taste	0	7.43±0.51aA	6.76±1.18abA	6.76±0.94abA	6.10±1.37bA
	6	7.40±0.50aA	6.25±0.72bB	6.28±0.62bA	5.55±1.28cA
Texture	0	7.62±0.67aA	6.24±1.45bcA	6.67±1.02bA	5.76±1.76cA
	6	7.50±0.51aA	6.00±1.12bA	6.00±0.79bB	5.65±1.18bA
Overall	0	7.76±0.7aA	6.48±1.17bA	6.90±0.77bA	5.67±1.49cA
	6	7.68±0.65aA	6.15±0.81bA	6.48±0.57bB	5.20±1.19cA

Value are mean ± SD (n=30). Different lowercase letters in the same row denote the significant difference ($P<0.05$). Different uppercase letters within the same attribute in the same column denotes the significant difference ($P<0.05$). IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled

3.5 Conclusion

Cooked patties from Islamic slaughtering method showed the lower oxidation than those prepared from chicken with other slaughtering methods. Oxidation of lipid mediated by haem along with microbial growth was the main cause of deterioration and losses in quality of cooked patties. Thus, Islamic slaughtering method could be an effective practice to retard lipid oxidation and growth of microorganism during refrigerated storage, in which shelf-life could be extended.

CHAPTER 4

Haemoglobin-mediated lipid oxidation in washed chicken mince

4.1 Abstract

Impart of oxyhaemoglobin (OxyHb) and methaemoglobin (MetHb) on lipid oxidation and color of washed chicken mince during 8 days of refrigerated storage was investigated. Washed chicken mince added with oxyHb and metHb contained the higher peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS), compared with the control sample (P<0.05). Oxy-form was more pro-oxidative than met-form as evidenced by the higher PV and TBARS (P<0.05). Volatile lipid oxidation compounds detected by gas chromatography-mass spectrometry (GC-MS) were also formed at higher rate in the washed chicken mince added with oxyHb, compared with the control and that added with MetHb. At day 0 of storage washed chicken mince added with oxyHb showed the higher a*-value but lower L* and b* value when compared with the control sample. The decrease in a* value was more pronounced in the washed chicken mince containing oxyHb at day 8 of refrigerated storage. Thus, lipid oxidation and color of chicken mince was more likely governed by the form of haemoglobin.

4.2 Introduction

Lipid oxidation is one of the major deteriorations causing the quality losses in meat and meat products. This also leads to the shortened shelf-life (Contini *et al.,* 2014). Lipid oxidation rate of meat is dependent on the phospholipid composition as well as the amount of polyunsaturated fatty acids (PUFA) (Calkins and Hodgen, 2007). Raw chicken contains high amounts of oleic, linoleic and linolenic acids (Miranda *et al.,* 2012). As PUFA level increases, autoxidation and formation of volatile compounds increase (Elmore *et al.,* 1999). Lipid oxidation is generally induced by proxidants including haem and iron, etc (Thiansilakul *et al.,* 2010)

Haemoglobin (Hb) has been known to be an effective catalyst of lipid oxidation. Additionally, Hb can be a source of activated oxygen associated with its

autoxidation, and haem or iron can be released from the protein to promote lipid oxidation (Richard and Hultin, 2002). Hb has a quaternary structure characteristic of many multi-subunit globular protein. Hb is made up of four polypeptide chains and each chain contains one haem group. A haem group consists of an iron held in a heterocyclic ring, known as a porpyrin (Maqsood *et al.,* 2012). The iron atom in the haem ring of haem protein is primarily in the ferrous (+2) state. Conversion of ferrous haem protein to met (+3) counterpart is a process known as autoxidation (Richard and Dettmann, 2003). Autoxidation appears to be a critical step of haem in stimulating lipid oxidation. Methaemoglobin (MetHb) reacts with peroxides and induce the formation of compounds capable of initiating and propagating lipid oxidation (Everse and Hsia, 1997).

Hb was found to be the extractable pigment in white muscles of broiler chicken (Kranen *et al.,* 1999). Broiler chicken meat, containing substantial amount of Hb in the muscle, are considered to be highly prone to discolouration and lipid oxidation. Since slaughtering methods have been found to affect the blood retained in the carcass of meat. The oxidation of chicken meat can be influenced by slaughtering methods associated with the remaining Hb. However, a little information has been reported on relative efficacy of Hb to promote lipid oxidation in chicken meat. A better understanding of lipid oxidation initiated by haemoglobin would provide basic knowledge, leading to the development of preventive methods to inhibit lipid oxidation and improve overall quality of chicken meat, especially via the appropriate slaughtering. The objective of this investigation was to compare the pro-oxidative activity of broiler chicken Hb, both oxy- and met-forms, in washed chicken mince stored under the refrigerated condition.

4.3 Materials and Methods

4.3.1 Chemicals

Ferrous chloride was obtained from Merck (Darmstadt, Germany). 2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane (Malonaldehyde), streptomycin sulphate and sodium heparin were purchased from Sigma (St. Louis. MO, USA). Cumene hydroperoxide was procured from Fluka (Buchs, Switzerland). Methanol,

chloroform, hydrochloric and ammonium thiocyanate were obtained from Lab-Scan (Bangkok, Thailand). All chemicals used were of analytical grade.

4.3.2 Preparation of haemolysate

Firstly, chicken blood was collected from broiler. Blood was collected in a centrifuge tube rinsed with 150 mM NaCl solution containing sodium heparin (30 U/mL) with a blood to heparin ratio of 4:1. Haemolysate from collected blood was prepared according the method of Richards and Hultin (2000). Four volumes of cold 1 mM Tris buffer (pH 8.0) containing 1.7% NaCl were added to heparinized blood. Thereafter, the mixture was centrifuged at 700g for 10 min at 4°C using an RC-5B plus centrifuge (Avanti J-E, Beckman, Fullerton, CA). Plasma was then removed. Red blood cells were washed by suspending three times in 10 volumes of the above buffer and centrifuging at 700g. Cells was lyzed in 3 volumes of 1 mM Tris buffer (pH 8.0) for 1 h. One-tenth volume of 1 M NaCl was then added to aid in stromal removal before centrifugation at 28,000g for 15 min at 4°C. The supernatant termed "haemolysate" was stored at -40°C and was thawed before use.

Different elements including Iron (Fe^{2+}), copper (Cu^{2+}), manganese (Mn^{2+}), magnesium (Mg^{2+}), zinc (Zn^{2+}) and calcium (Ca^{2+}) were determined using the inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Model 4300 DV, Perkin Elmer, Shelton, CT, USA) according to the method of AOAC (1995). Haemolysate (3 mL) was mixed well with 4 mL of 50% HCl. The mixture was heated on the hot plate until digestion was completed. The digested sample was transferred to a volumetric flask and the volume was made up to 10 mL with deionized water. The solution was then subjected to analysis. Flow rates of argon to plasma, auxiliary and nebulizer were maintained at 15, 0.2, and 0.8 L/min, respectively. Sample flow rate was set at 1.5 mL/min. The concentration of element was calculated and expressed as mg/L sample.

4.3.3 Preparation of oxy- and met-haemoglobin

OxyHb and metHb were prepared according to the method of Tang et al. (2004) with some modifications. To obtain oxy-form, 1.5 mg of sodium dithionite was added to an aliquot of haemolysate solutions (3 mL; 0.1 mM haem). MetHb was

prepared by adding 1.5 mg of potassium ferricyanide to 3 mL of haemolysate solution (0.1 mM haem). The sodium dithionite and potassium ferricyanide were removed by dialysis of the sample against 10 volumes of cold 50 mM phosphate buffer, pH 7.0. The dialysis buffer was changed 20 times. The protein concentrations of the dialysates, representing oxyHb and metHb, were adjusted to 0.2 mg protein/mL. All samples were analyzed.

4.3.3.1 Absorption spectra

The absorption spectra of oxyHb and metHb solutions were taken using a spectrophotometer. The spectra were recorded from 350 to 750 nm at a scanning rate of 1000 nm/min using 50 mM phosphate buffer, pH 7.0, as blank.

4.3.3.2 Proportion of haemoglobin forms

The proportions of the two haemoglobin form; oxyHb and metHb, were calculated by a modified Krzywicki's equation (Tang *et al.,* 2004) as follows:

$$[OxyHb] = 0.722R_1 - 1.432R_2 - 1.659R_3 + 2.599$$

$$[MetHb] = -0.159R_1 - 0.085R_2 + 1.262R_3 - 0.520$$

$$[DeoHb] = 0.543R_1 + 1.594R_2 + 0.552R_3 - 1.329$$

Where $R_1 = A_{582}/A_{525}$, $R_2 = A_{557}/A_{525}$ and $R_3 = A_{503}/A_{525}$

4.3.4 Preparation of washed chicken mince

Washed chicken mince was prepared according to the method of Maqsood and Benjakul (2011). Broiler chicken with the age of six weeks and body weight of approximately 2 kg were obtained from a poultry farm in Songkhla, Thailand. After bleeding for 3 min, the chicken was plucked in a rotary-drum picker for 30 s and eviscerated. Breast muscle was dissected from the carcasses, followed by mincing. The mince was washed twice in iced distilled water using a mince-to-water ratio of 1:3 (w/w) by stirring continuously with a plastic rod for 2 min. The mixture was allowed to stand for 15 min, followed by dewatering on a fiberglass screen. The mince was then mixed with 50 mM sodium phosphate buffer (pH 7.0) at a mince/buffer ratio of 1:3 and homogenized using an Ultra-Turrex T25 homogenizer (Janke and Kunkel, Staufen, Germany) for 1 min. It was allowed to stand for 15 min

and then centrifuged at 15000 × g for 20 min at 4 °C. The resulting pellet was referred to as “washed mince”.

4.3.5 Effect of haemoglobin with different forms on lipid oxidation of washed chicken mince.

Washed mince (100 g) was added with 5 mL of haemoglobin with different forms to obtain the final concentration of 8 µmole of Hb/kg washed mince (Alvarado *et al.,* 2007). For the control samples, the haemoglobin was replaced by the same volume of distilled water. Streptomycin sulphate was added in the washed mince to obtain a final concentration of 200 ppm in order to inhibit the microbial growth. The sample was mixed manually. Washed mince (100 g) containing haemoglobin with different forms was packed in the polyethylene bags (14 x 8cm^2). The samples were stored in 4°C and randomly taken for analyses at day 0, 2, 4, 6 and 8. Volatile compounds were analyzed at day 0 and 8 day of storage.

4.3.6 Chemical analyses

4.3.6.1 Determination of peroxide value (PV)

PV was determined as per the method of Richards and Hultin (2002) with a slight modification. Ground sample (1 g) was mixed with 11 mL of chloroform/methanol (2:1, v/v). The mixture was homogenized at a speed of 13,500 rpm for 2 min using an UltraTurrax T25 homogeniser (Janke and Kunkel, Staufen, Germany). The homogenate was then filtered using Whatman no.1 filter paper (Whatman International, Ltd, Maidstone, England). Two milliliters of 0.5% NaCl was then added to 7 mL of the filtrate. The mixture was vortexed at a moderate speed for 30 s using a Vortex-Genie2 mixer 4 (Bohemia, NY, USA) and then centrifuged at 3000 g for 3 min using a RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA) to separate the sample into two phases. Two milliliters of cold chloroform/methanol (2:1) was added to 3 mL of the lower phase. Twenty-five microlitres of 30% ammonium thiocyanate and 25 µL of 20 mM iron (II) chloride were added to the mixture (Shantha and Decker, 1994). The reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at

500 nm. A standard curve was prepared using cumene hydroperoxide at the concentration range of 0.5–2 ppm. PV was expressed as mg hydroperoxide/kg.

4.3.6.2 Determination of thiobarbituric acid reactive substances (TBARS)

TBARS value was determined according to the method of Benjakul and Bauer (2001) with some modifications. Ground sample (1 g) was mixed with 5 mL of a solution containing 0.375% TBA, 15% TCA and 0.25N HCl. The mixture was heated in boiling water for 10 min, followed by cooling with the running water. The mixture was centrifuged at 4000g for 20 min (MIKRO20, Hettich Zentrifugan, Germany). The supernatant was collected and the absorbance was read at 532 nm using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). TBARS value was calculated from the standard curve of malonaldehyde (0-2 ppm) and expressed as mg malonaldhyde/kg.

4.3.6.3 Determination of volatile compounds

Volatile compounds were determined by solid phase micro extraction–gas chromatography–mass spectrometry (SPME–GC–MS) (Iglesias and Medina, 2008).

4.3.6.3.1 Extraction of volatile compounds. Three grams of sample were homogenized at a speed of 13,500 rpm for 2 min with 8 mL of saturated NaCl in ultra-pure water. The mixture was centrifuged at 2000 g for 10 min at 4°C. The supernatant (6 mL) was heated at 60°C with an equilibrium time of 10 h in a 20 mL headspace vial. Finally, the SPME fiber (50/30 μm DVB/Carboxen™/PDMS StableFlex™) (Supelco, Belle-fonte, PA, USA) was exposed to the head space of the vial containing the sample extract. The volatile compounds were allowed to absorb in the SPME fiber for 1 h. The volatile compounds were then desorbed in the GC injector port for 15 min at 270°C.

4.3.6.3.2 GC-MS analysis. GC-MS analysis was performed in a HP 5890 series II gas chromatography coupled with HP 5972 mass selective detectors, equipped with a splitless injector, and coupled with a quadrupole mass detector (Hewlett Packard, Atlanta, GA, USA). The compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m 0.25 mm ID,

with film thickness of 0.25 μm). The GC oven temperature programme was 35°C for 3 min, followed by an increase of 3°C /min to 70°C, then an increase of 10°C /min to 200°C, and finally an increase of 15°C /min to a final temperature of 250°C, and hold for 10 min. Helium was employed as a carrier gas with a constant flow of 1.0 ml/min. The injector was operated in the splitless mode and its temperature was set at 270°C. The transfer line temperature was maintained at 265°C. The quadrupole mass spectrometer was operated in the electron ionisation (EI) mode and the source temperature was set at 250°C. Initially, a full scan mode data was acquired to determine the appropriate masses for the later acquisition in selected ion monitoring (SIM) mode, under the following conditions: mass range: 25–500 amu and scan rate: 0.220 s/scan. All analyses were performed with ionisation energy of 70 eV, a filament emission current of 150 μA, and an electron multiplier voltage of 500 V.

4.3.6.3.3 Identification of volatile compounds. Identification of the compounds was done by consulting ChemStation Library Search (Wiley 275.L), based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search (Wiley 275.L). Quantification limits were calculated to a signal-to noise (S/N) ratio of 10. Repeatability was evaluated by analyzing 3 replicates of each sample. The identified volatile compounds related with lipid oxidation were presented in the form of normalized area under peak of each identified compound.

4.3.6.4 Color (L*, a*, b*, ΔE*)

Color was determined by measuring L* (lightness), a*(redness/greenness) and b* (yellowness/blueness) value using a colorimeter (JP7100F, Juki Corp, Tokyo, Japan). The colorimeter was standardised by black and white tile. Color difference, ΔE*, was calculated by the following equation (Berns, 2000):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where, ΔL*, Δa* and Δb* represent the difference in the color parameters between the sample and the white standard (L*= 93.55, a* = -0.84, b*=0.37)

4.3.5 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie, 1980). Analysis was performed using the Statistical Package for Social Science package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

4.4 Results and discussion

4.4.1 Composition and characterizations of haemolysate

4.4.1.1 Elements in haemolysate

Element compositions of chicken haemolysate are shown in Table 12. Fe was found as the most predominant element in chicken haemolysate. Ca constituted as the second abundant element, followed by Mg and Zn. Nevertheless, Cu and Mn were found at low concentrations. The high content of Fe (58.20 mg/l) was attributed to the presence of haemoglobin (Hb). Hb is the major haem protein of red blood cells and is responsible for the transportation of oxygen to the tissues (Perutz, 1990). Hb is made up of four polypeptide chains, where each chain consists of one haem group. A haem group contains an iron (Fe) ion held in a heterocyclic ring, known as a porphyrin (Maqsood *et al.,* 2012). Fe, Ca, Mg and Zn are minerals in the blood (Orden *et al.,* 1998). Fe and Ca could act as pro-oxidants in the chicken meat during storage. Transition metal ions, particularly Cu and Fe, are known to be the major catalysts of lipid oxidation (Thanonkaew *et al*., 2006).

Table 12 Element content of chicken blood haemolysate

Elements	Content (mg/L)
Fe	58.20±1.82
Cu	<0.001
Zn	1.45±0.63
Mn	<0.001
Ca	39.15±1.28
Mg	9.05±1.04

Values are mean ± SD (n=3).

4.4.1.2 The absorption spectra of Hb with different forms

The absorption spectra of oxyHb and metHb solutions from chicken blood haemolysate are depicted in Figure 8. The intense peaks in the blue region (350–450 nm) corresponding to the Soret bands, were found at 415 and 406 nm for oxyHb and metHb solutions, respectively. The Soret bands of oxyMb and metMb from meat were noticeable at 416 and 410 nm, respectively (Swatland, 1989). A Soret band results mainly from the interaction of the haem moiety with apomyoglobin. Hence it can be used to monitor the unfolding of hemoproteins (Chen and Chow, 2001). In the region of 450–700 nm, the peaks were found at wavelengths of 538 and 575 nm for the oxyHb solution and 500 and 628 nm for the metHb solution. The maximum wavelengths at 503, 557 and 582 nm representing metHb, deoxyHb and oxyHb, respectively. Those wavelengths have been used for determining the relative proportions of the Hb redox forms in aqueous solution (Tang *et al.,* 2004). The oxyHb solution had the highest proportions of oxyHb (87.24%), followed by metHb (9.08%) and deoxyHb (3.01%). For the metHb solution, metHb was dominant (86.99%); deoxyHb (6.41%) and oxyHb (6.43%) were present at lower levels. MetHb and oxyHb more likely underwent reduction and oxidation, respectively, during dialysis. This led to some changes in the target forms.

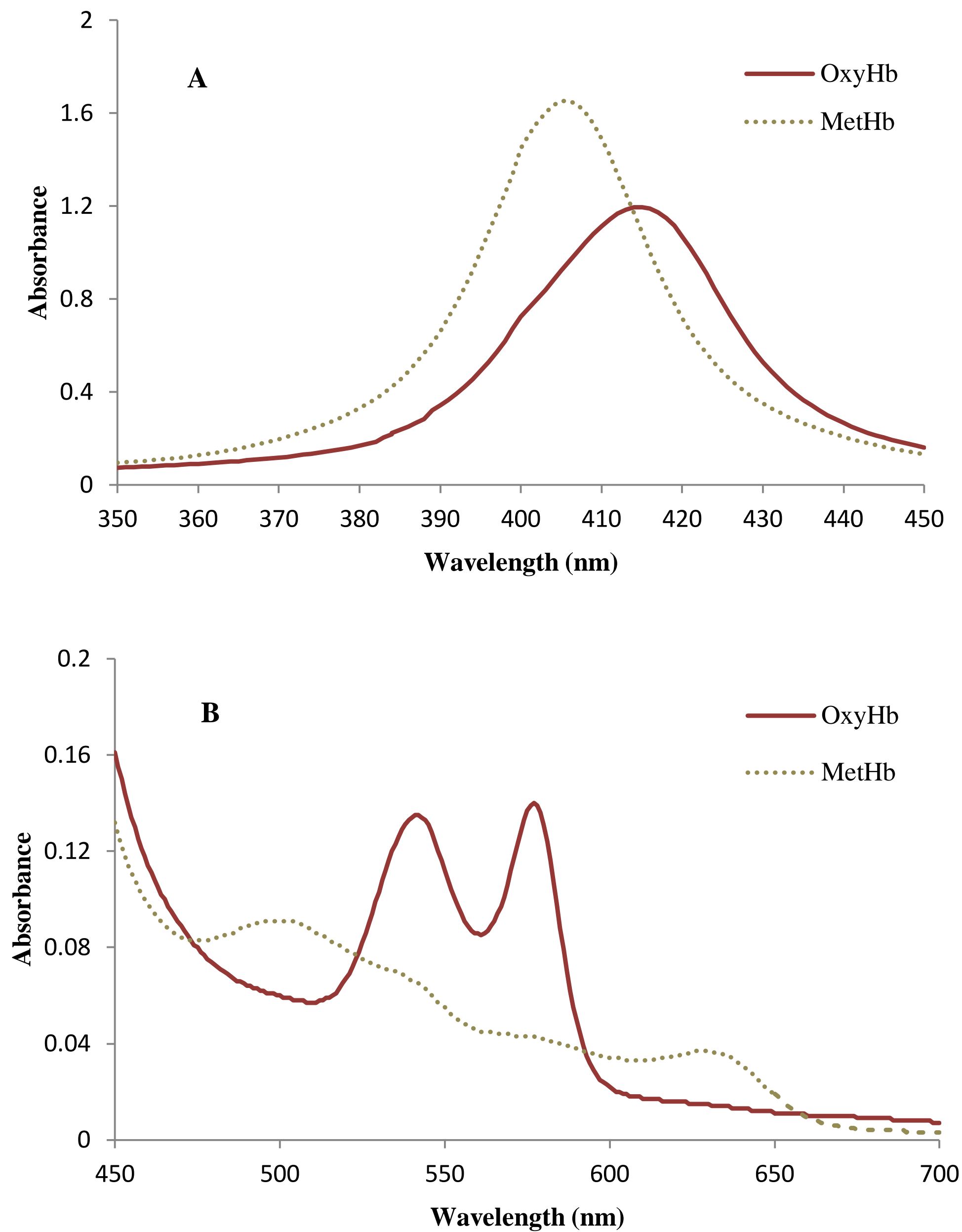


Figure 8 The absorption spectra in the region of 350-450 nm (A), 450-700 nm (B), of OxyMb and MetHb from chicken blood.

4.4.2 Changes in washed chicken mince added with Hb having different forms during refrigerated storage

4.4.2.1 Lipid oxidation

PV and TBARS of washed chicken mince with and without addition of oxyHb and metHb during 8 days of refrigerated storage are shown in Figure 9 (A) and Figure 9 (B), respectively. During storage, the increases in both PV and TBARS were noticeable in all samples. However, the rate of increase varied. Washed chicken mince without haem proteins (the control) showed no change in PV within the first 2 days. Slight increase in PV was found in the control during 2-4 day of storage. Thereafter, PV remained constant up to 8 days of storage ($P<0.05$). For the sample added with metHb, the highest increase in PV was found, compared with that containing oxyHb throughout the storage. The results showed that both oxy- and met-forms were capable of promoting lipid peroxide formation in washed mince. This indicated the pro-oxidative activity of haemoglobin in washed mince. The sample added with metHb showed the higher PV than that containing oxyHb throughout 8 days of refrigerated storage ($P<0.05$).

TBARS values of 1 mg MDA/kg in poultry may be associated with rancid off odors by consumers (Nam and Ahn, 2003). TBARS values slightly increased within the first 4 days of storage in all samples ($P<0.05$). At day 0, the sample added with Hb with both forms showed the higher TBARS value, compared with the control ($P<0.05$). In the presence of metHb or oxyHb, the rates of increases in TBARS were higher than that found in the control, suggesting the effective pro-oxidative activity of haem proteins in washed mince. When comparing between oxyHb and metHb, it was found that the sample added with oxyHb exhibited the higher TBARS ($P<0.05$). The higher TBARS value was found in sample added with oxyHb, while the lower PV value was noticeable. The result indicated that hydro peroxide in the sample added with oxyHb were more likely decomposed to a higher extent with the coincidental increase in the secondary oxidation product as indicated by higher TBARS value. A slight increase in TBARS value of the control was found with increasing storage time ($P<0.05$). Thus, the added Hb resulted in the higher lipid oxidation in the wash mince. Rapid autoxidation of oxyHb to metHb was closely

associated with their pronounced pro-oxidative activity in washed mince. When autoxidation takes place, MetHb and the superoxide anion radical ($O_2^{\bullet -}$) were formed. Additionally, $O_2^{\bullet -}$ dismutates to H_2O_2, which subsequently reacts with metHb to form the hypervalent ferrylhaemoglobin radical known to initiate lipid oxidation (Kanner and Harel, 1985). Lipid oxidation in washed mince containing oxyHb was associated with superoxide radicals generated during autoxidation (Richards and Dettmann, 2003). Superoxide radicals can be converted to H_2O_2 that facilitate the formation of ferrylHb catalyst (Kanner and Harel, 1985) and increase the reactivity of dissociated hemin (Robinson *et al.,* 2009). OxyHb, on the other hand, is gradually oxidized to metHb, which is more prone to hemin loss. Maqsood *et al.* (2012) reported that the reduced Hbs (oxyHb and deoxyHb) are known to be more pro-oxidative than metHb for a number of reasons. (i) Reduced Hbs can undergo autoxidation. Superoxide anion radical formed from reduced Hb (oxyHb) autoxidation can dismutate to form hydrogen peroxide. Hydrogen peroxide can activate the formation of metHb along with superoxide anion radical generated from oxyHb autoxidation (Misra and Fridovich, 1972). Reduced Hbs but not metHbs can act as Fenton reagents, producing the hydroxyl radical (Puppo and Halliwell, 1988). Alkoxyl radicals are formed in reduced Hb systems exposed to tert-butyl hydroperoxide, while haemichromes are formed in metHb systems (Thornalley *et al.,* 1983). Thus, different forms of Hbs had varying redox properties and susceptibility towards autoxidation, thereby promoting lipid oxidation differently.

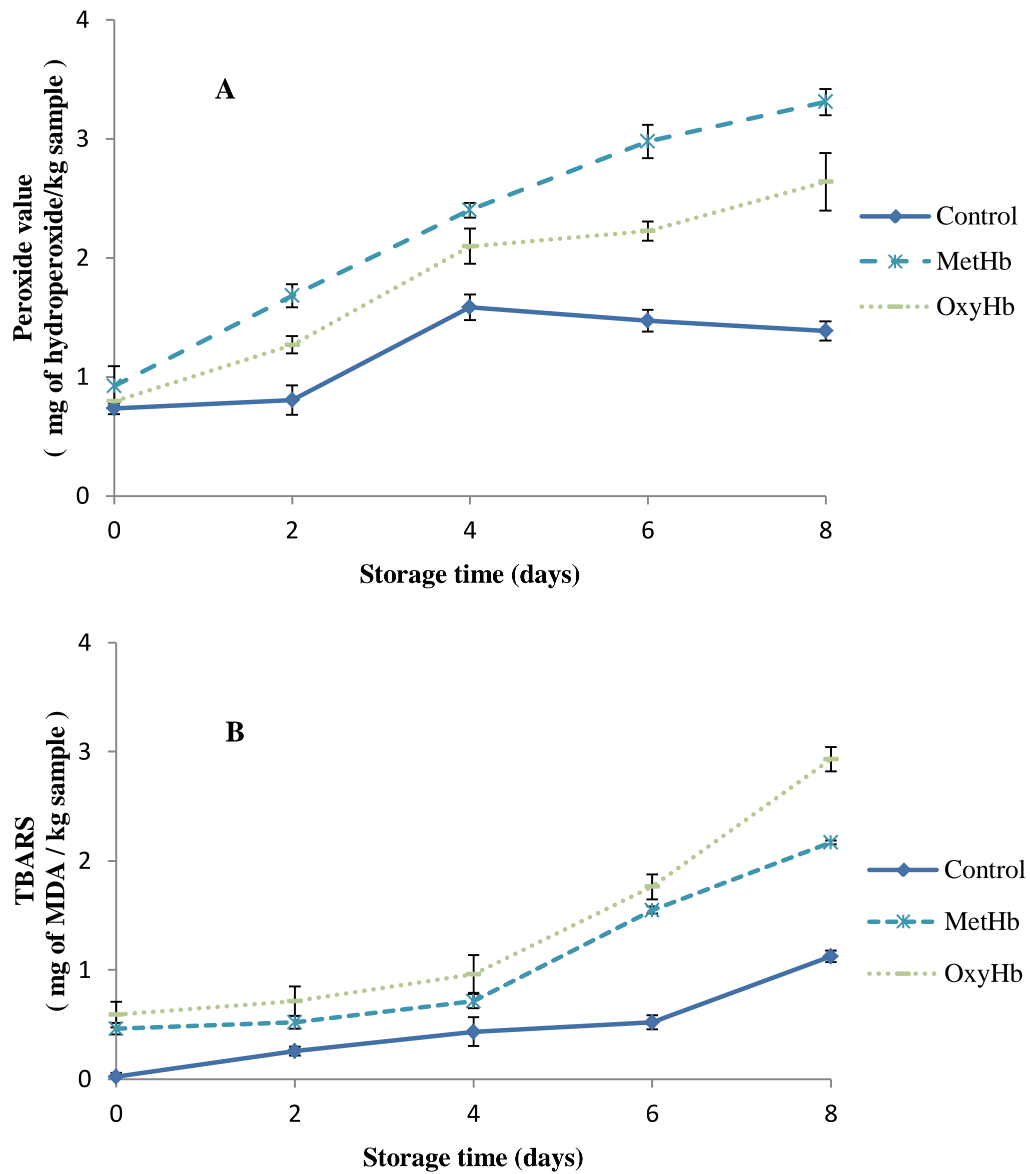


Figure 9 Changes in PV (A), and TBARS (B) of washed chicken mince without and with haemoglobin having different forms during 8 days of refrigerated storage. Bar represent the standard deviation (n=3)

4.4.2.2 Volatile compounds

Volatile compounds in washed chicken mince without and with metHb or oxyHb after 8 day of refrigerated storage are displayed in Table 13. Chicken muscle contained high amount of polyunsaturated fatty acids (PUFAs) (Almeida *et al.,* 2013). Chicken muscle contains high amounts of oleic, linoleic and linolenic unsaturated fatty acids (Wood and Church, 1999). Several volatiles including aldehyde (hexanal, heptanal, octanal, nonanal), alcohol (1-hexanol, 1-octen-3-ol, 1-dodecanol, 1-Octanol), ketone (cyclohexanone) and other volatile substances were formed to a higher extent after 8 day of storage. Aldehydes are the most prominent volatiles produced during lipid oxidation and have been used successfully to follow lipid oxidation in a number of foods, including muscle foods (Ross and Smith, 2006). For aldehydic compounds, hexanal, heptanal, octanal, and nonanal were found in washed chicken mince. Oxidation of PUFAs known to produce volatile compounds including hexanal, heptanal, octanal, nonanal etc (Yasuhara and Shibamoto, 1995). Pentanal, hexanal and nonanal are aldehydes formed during lipid peroxidation in poultry (Mielnik *et al.,* 2003). The control sample contained the lower amounts of volatile lipid oxidation products, when compared with the sample added with metHb or oxyHb ($P<0.05$). The results suggested that lipid oxidation and decomposition of hydroperoxides to the secondary volatile oxidation products were more pronounced in the samples incorporated with metHb or oxyHb, compared with the control sample. At days 8 of storage, hexanal, nonanal, 1-hexanal and 1-octanal were detected in the content. The higher formation of hexanal, heptanal, octanal and nonanal were observed in washed mince incorporated with oxyHb than that containing metHb ($P<0.05$). Higher formation of volatile lipid oxidation products in oxyHb added sample correlated well with the higher formation of TBARS value (Figure 9). The formation of aldehydes and other volatile oxidation product can produce off-odours (Boyd *et al*., 1992). Aldehydes have been used as the indicators of lipid oxidation because they possess low threshold value and are the major contributors to the development of off-flavour and odour (Boyd *et al.,* 1992; Ross and Smith, 2006). Heptanal and hexanal contributes to the rancidity in chicken (Schroll*, et al,* 1988).

Oleic acid is the most abundant fatty acid in meat and its degradation by oxidation produces octanal and nonanal (Garcia-Llatas *et al.,* 2006).

For alcoholic compounds, 1-hexanol, 1-octen-3-ol, 1-dodecanol and 1-octanol were higher in the sample added oxyHb and metHb, compared with the control sample. Alcohols are known as the secondary products produced by the decomposition of hydroperoxide (Girand and Durance, 2000). 1-hexanol contributes to a desirable flowery-like odour (Wang *et al.,* 2010). 1-octen-3-ol imparts a desirable mushroom-like odour, apart from green and plant-like aromas (Josephson *et al.,* 1986). Thus Hb, either Met-Hb or oxy-Hb, were able to catalyze the lipid oxidation in washed chicken mince intensively, but their pro-oxidative activity varied with form of Hb. The result also reconfirmed a higher impact of oxyHb than metHb on lipid oxidation.

Table 13 Effect of haemoglobin with different forms on volatile compounds in washed chicken mince at day 0 and 8 of refrigerated storage.

Compounds Abundance × 10^6	Day 0*	Day 8		
		Control	OxyHb	MetHb
1-Octen-3-ol	135±1.52	141±1.04	926±0.87	505±0.54
1-Dodecanol	183±0.97	182±0.64	272±1.24	215±1.76
Hexadecanoic	129±1.76	72±1.15	542±1.36	139±0.93
Hexanal	ND	94±0.91	1995±1.76	1245±1.32
Heptanal	ND	ND	520±0.66	359±0.58
Cyclohexanone	ND	ND	194±1.18	243±1.46
Octanal	ND	ND	427±0.93	264±0.94
Nonanal	ND	85±1.27	441±1.09	355±0.26
1-Hexanol	ND	260±0.83	402±0.84	376±1.80
1-Octanol	ND	69±1.43	381±1.26	342±1.04
Isopropyl myristate	ND	ND	198±0.37	459±0.49

ND: not detectable. Values are mean ± SD (n=3).

*Control sample obtained at day 0 was used.

4.4.2.3 Color

Color of washed chicken mince with and without addition of oxyHb and metHb at day 0 and 8 of refrigerated storage is shown in Table 14. At day 0, chicken without haemoglobin (control) had a higher L* value but lower ΔE* than those added with oxyHb or metHb ($P<0.05$). The a* value was higher for the sample added with oxy-Hb, ($P<0.05$). However, these were no differences in b*-value among all samples ($P>0.05$). The result indicated the efficiency of washing process in removal of water soluble pigments from chicken mince. After 8 days of refrigerated storage, the decreases in L* and a* value of all samples were noticeable ($P<0.05$). This coincided with the increase in ΔE* value ($P<0.05$), whereas no change in b* value was observed except for the sample with oxyHb ($P<0.05$). Control sample showed the higher L* value, compared with other samples ($P<0.05$). The sample with oxyHb had the higher a* value ($P<0.05$). Decreases in a* value in sample with oxyHb was more likely due to the formation of metHb, resulting from the oxidation of oxyHb. The result indicated the susceptibility of Hb to autoxidation with increasing storage time. The b* value of all sample remained unchanged after 8 days, but the increase in b* value of sample added with oxy-Hb immersed ($P<0.05$). There, the remaining blood or Hb in the chicken meat could induce the changes in color. Also, form of was an important factor determine the color of chicken meat.

Table 14 Effect of haemoglobin with different forms on the *L**, *a**, *b** *and ΔE** values in washed chicken mince at day 0 and day 8 of refrigerated storage

Storage time (days)	Samples	*L**	*a**	*b**	*ΔE**
0	Control	72.54±0.93aA	1.18±0.02cA	15.25±0.86aA	25.79±0.26bB
	Oxy-	69.03±1.42bA	1.65±0.12aA	14.95±0.28aB	28.64±1.36aB
	Met-	69.40±0.48bA	1.35±0.02bA	15.35±0.96aA	28.53±0.15aB
8	Control	68.35±0.19aB	0.45±0.07cB	15.16±0.28bA	29.25±0.27cA
	Oxy-	57.25±1.31bB	1.30±0.07aB	17.52±0.34aA	40.22±1.14bA
	Met-	53.38±0.83cB	0.98±0.77bB	14.82±0.73bA	42.73±1.01aA

Values are mean ± SD (n=6). Different lowercase letters within the same storage time in the same column denote the significant difference ($P<0.05$). Different uppercase letters within the same sample in the same column denotes the significant difference ($P<0.05$).

4.4 Conclusion

Haemoglobin in washed mince played an essential role in lipid oxidation during the extended refrigerated storage. A stronger prooxidative activity of oxyHb was found, compared with metHb. Therefore, the removed of blood via appropriate slaughtering method as were as washing process could be an effective means to lower off odour and off-color mediated by haem proteins.

CHAPTER 5

Effects of chicken blood on growth of pathogenic and spoilage bacteria in minced chicken during refrigerated storage

5.1 Abstract

Microorganisms are typically introduced to poultry meat through cross-contamination during processing, visible matters, including residual blood can further affect levels of contamination and deterioration of the meat. Effects of chicken blood and slaughtering methods on microorganism growth were investigated during 8 days of storage at refrigeration temperature. Broth with chicken blood added at a level of 5 µmol/g showed higher growth of *Listeria monocytogenes*, *Salmonella* Typhimurium, *Campylobacter jejuni* and *Pseudomonas aeruginosa*, compared with the no blood-added broth ($P<0.05$). Counts of these four strains were higher in the minced chicken with blood added at 10 µmol/g than those in the minced chicken with lower blood concentrations (2.5, 5, and 7.5 µmol/g) ($P<0.05$). Mesophilic and psychrophilic bacteria counts in the blood-added minced chicken increased from approximately 4.04 to 9.49 and from 3.54 to 9.84 log CFU/g, respectively, after 8 days of storage at 4°C. *Campylobacter jejuni* in the minced chicken from the Islamic slaughtering method showed higher growth than those from the conventional neck cut method. Our data suggest that chicken blood can promote bacterial growth, and that slaughtering methods can affect the microbial loads in the minced chicken during storage, which is likely mediated by the blood retained in the meat. A slaughtering method in which complete bleeding is performed or residual blood content is reduced as much as possible can be of effective approach for controlling some major pathogenic and spoilage bacteria normally present in chicken meat during; this can then lower the risk of pathogen contamination and extend the shelf-life of chicken meat.

5.2 Introduction

Poultry meat is generally perceived as cheap and wholesome for consumption. Additionally, there are no religious constraints on the consumption,

unlike pork, lamb or beef. These characteristics and other factors have made poultry meat by far the most popular food products worldwide with rapidly increase in consumption (FAO, 2012). Poultry products are highly perishable food; it usually deteriorates within a week of slaughtering (Marenzi, 1986). Due to its intrinsic factors, i.e., high pH, high protein and moisture contents, poultry meat typically facilities growth of pathogenic and spoilage organisms (Mataragas *et al*., 2006; Anang *et al*., 2007). Psychrotrophic microorganisms that survive processing operations may multiply during refrigerated storage and cause spoilage of poultry meat. High numbers of microorganism in raw meat can transform products to unsuitable and unappealing for consumption (Doulgeraki *et al*., 2012). Contamination of poultry meat with foodborne pathogens has been of a major public health concern. Several bacterial pathogens have been primarily associated with poultry-borne human illnesses, e.g., *Campylobacter*, *Salmonella*, and *Listeria monocytogenes*. *Salmonella* spp., while *Listeria monocytogenes* and *Campylobacter* spp. can be often found in poultry carcasses and in poultry processing plants (Jorgensen *et al*., 2002; Miettinen *et al*., 2002). The European Food Safety Authority (EFSA) reported that occurrence of *Campylobacter* continued to be high in broiler meat and *Campylobacter* was the most common cause of foodborne illnesses with 220, 209 confirmed human cases (EFSA, 2013). Poultry meat was also found to account for the most deaths (19%) between 1998 and 2008; many of those were caused by *Salmonella* and *Listeria* infections linked to contaminated poultry meat consumption (Painter *et al*., 2013).

While both pathogenic and spoilage organisms can be typically introduced to poultry meat through cross-contamination during processing operations (Fries and Graw, 1999), one potential factor that can affect the level of contamination and enhance the extent of deterioration is the amount of blood left within the carcass after bleeding. Blood is considered to be not only an excellent medium for the growth of bacteria due to its high nutritive value (Ali *et al*., 2011), but also a major defect that can cause undesirable short shelf-life (Griffiths and Nairn, 1984). The amount of blood bled from and retained in the animals can be dependent on the slaughtering method used. Halal or Islamic slaughtering is done by a throat cut in order to bring the animal to a quick death without suffering. This way also leads to complete bleeding while cutting carotid arteries, jugular veins, trachea and esophagus (Grandin and

Regenstain, 1994). This manner of the Halal slaughtering may allow for the presence of less amount of residual blood in the breast muscles of broilers, thus lowering the nutrient contents for supporting growth of microorganisms which can reduce pathogen contamination and prolong shelf-life of the poultry meat. The objective of this study was to investigate the impact of chicken blood as well as slaughtering methods (i.e., Halal and non-Halal methods) on microbiological quality of minced chicken during the refrigerated storage.

5.3 Materials and methods

5.3.1 Chemicals and media

Sodium heparin was purchased from Sigma (St. Louis, MO, USA). Sodium chloride was procured from Ajax Finechem (New South Wales, Australia). Brain Heart Infusion Agar (BHI), Pseudomonas Isolation Agar Base (PIA) and Xylose Lysine Deoxycholate agar (XLD) were purchased from Difco Laboratories (Detroit, MI, USA). Blood free Campylobacter selective agar base (mCCDA) was purchased from Oxoid (Basingstoke, Hampshire, England). Agar Listeria Ottaviani Agosti (ALOA) was purchased from AES laboratories (Marcy l'Etoile, France). Nutrient Broth (NB) and Plate Count Agar (PCA) were purchased from HiMedia (Mumbai, India).

5.3.2 Bacterial strains

Three pathogenic bacterial strains (*Listeria monocytogenes* DMST 1327, *Salmonella enterica* serovar Typhimurium DMST 562, *Campylobacter jejuni* PSU 03151) and one spoilage bacterial strain (*Pseudomonas aeruginosa* TISTR 781) were used in this study. These strains were obtained from the Department of Medicine Science, Ministry of Public Health, Nonthaburi, Thailand (DMST), Thailand Institute of Scientific and Technological Research (TISTR) and the Department of Microbiology, Faculty of Science, Prince of Songkla University (PSU). Prior to experiments, the bacterial strains were sub-cultured twice. An isolated colony of each strain was inoculated into 10 mL of Nutrient Broth (NB), followed by incubation at 37°C for 18 h for *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Salmonella*

enterica serovar Typhimurium, and at 42°C for 42 h for *Campylobacter jejuni.* Cultures (1 mL of each strain) were then separately transferred to another tube containing 10 mL of NB, and grown at the same corresponding temperatures and for another 18 h or 42 h. Overnight cultures from the second passage, representing approximately 10^{10} CFU/mL were used in each challenge experiment detailed below.

5.3.3 Effect of chicken blood on the growth of selected pathogenic and spoilage bacteria in medium broth

A broiler (age of 6 weeks with approx. 2 kg body weight) was obtained from a poultry farm in Songkhla, Thailand. Bleeding was performed prior to scalding and defeathering (picking). Chicken blood was collected by an aseptic technique using a sterilized syringe, and sterilized heparin was used as an anticoagulant. Blood was withdrawn into a sterilized syringe (20 mL) and transferred into a sterilized centrifuge tube, previously rinsed with 150 mM NaCl solution, containing 5 mL of sodium heparin (30 U/mL), representing a blood/heparin ratio of 4:1 (v/v). Approximately 40 mL of chicken blood was obtained from one broiler which was sufficient for further studies. Chicken blood mixed with heparin was kept at 4°C until use. A medium broth containing chicken blood (“blood broth”) was prepared by adding prepared blood into BHI broth. Blood broths containing different final blood levels in BHI (1, 2.5, and 5 µmol/mL) were prepared, following the procedures of haemoglobin quantification described by Richards and Hultin (2000), based on the standard curves using bovine haemoglobin standard (Sigma, St. Louis, Mo). Each blood broth was inoculated with 1 mL (10^4 CFU/mL) of the overnight cultures of pathogenic or spoilage bacteria prepared as detailed above. The blood broths inoculated with *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Salmonella* Typhimurium were incubated at 37°C and those inoculated with *Campylobacter jejuni* were incubated at 42°C under microaerophilic conditions (approximately 5% O_2, 10% CO_2 and 85% N_2). Controls, culture-inoculated broths without additional chicken blood, were included in the study. Bacterial growth was monitored every 6 h for 48 h. One milliliter of each blood broth was transferred into a test tube containing 9 mL of 0.85% normal saline solution (NSS), followed by a preparation of 10-fold

serial dilutions. Appropriate dilutions were used for microbiological analyses as detailed below.

5.3.4 Effect of chicken blood on the growth of microorganisms in non-Halal minced chicken

A broiler (age of 6 weeks with approx. 2 kg body weight) was obtained from a poultry farm in Songkhla, Thailand. After bleeding for 3 min by a normal neck cut, a broiler was plucked in a rotary-drum picker for 30 s and eviscerated. Breast muscles were dissected from the carcasses. The chicken breast was cut into wide strips of approximately 0.5 x 0.5 cm^2 using a sterilized knife. Chicken strips were minced using a food processor at high speed for 3 min (MK- Panasonic, 5087M, Selangor Darul Ehsan, Malaysia). Prepared chicken blood, as detailed above, was added to minced chicken (25 g) and mixed throughout in a sterile polyethylene bag to obtain different blood concentrations (2.5, 5, 7.5, and 10 µmol/g). Overnight cultures (1 mL) of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* were separately added into a sterile polyethylene bag containing blood added-minced chicken (25 g) with varied blood concentrations to achieve the final inoculum level of approximately 10^4 CFU/g. Controls, culture-inoculated minced chicken without additional chicken blood, were included in this study. Samples were stored at 4°C, and the growth of microorganisms was monitored every 2 days up to 8 days.

5.3.5 Effect of slaughtering methods on the growth of selected pathogenic and spoilage bacteria in minced chicken

Two broilers (age of 6 weeks with approx. 2 kg body weight) were obtained from a poultry farm in Songkhla, Thailand, and divided into two groups based on the slaughtering methods; (i) Islamic method and (ii) conventional neck cut method. After bleeding for 3 min, following each slaughtering method, broilers were plucked in a rotary-drum picker for 30 s and eviscerated. Breast muscles were dissected from the carcasses using a sterilized knife, followed by mincing using a food processor as detailed above. Cultures (1 mL) of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* were

separately added into a sterile polyethylene bag containing minced chicken (25 g) obtained from each slaughtering method to achieve the final inoculum level of approximately 10^4 CFU/g. Samples were stored at 4°C, and the growth of microorganisms was monitored every 2 days up to 8 days.

5.3.6 Microbiological analysis

Microbiological analysis was performed following the methods of Hasegawa (1987) and Cousin *et al*. (1992) with some modifications. Twenty five grams of minced chicken from each study were transferred into a stomacher bag containing 225 mL of 0.85% saline solution. Sample mixtures were homogenized by using a stomacher (400 Circulator, Seward, West Sussex, UK) for 1 min at 230 rpm. Aliquot of the homogenates (1 mL) was used to prepare 10-fold serial dilutions in 0.85% saline solution. Appropriate dilutions were used for microbial enumeration as detailed below.

5.3.7 Determination of mesophilic and psychrophilic bacteria counts

Mesophilic and psychrophilic bacteria counts were determined using the pour plate method according to Hasegawa (1987) and Cousin *et al*. (1992) with some modifications. One milliliter of appropriate dilution was transferred onto a sterile petri dish, and 15 mL of waterbath-equilibrated (45°C) plate count agar (PCA, 1.5% agarose) was poured and mixed with the diluted sample on the petri dish. Plates were incubated at 37°C for 2 days for mesophilic bacteria counts, and at 4°C for 7 days for psychrophilic bacteria counts.

5.3.8 Enumeration of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni*

Aliquots of appropriate dilutions (100 µl each) were spread plated onto (i) Pseudomonas Isolation Agar for *Pseudomonas aeruginosa* counts; (ii) Agar Listeria Ottaviani Agosti (ALOA) for *Listeria monocytogenes* counts; (iii) Xylose Lysine Deoxycholate (XLD) agar for *Salmonella* counts. Plates were incubated at 37°C 24 h. *Pseudomonas aeruginosa* colonies were green to blue-green with pigment that diffused into the medium (Arnaut-Rollier *et al*., 1999). *Listeria monocytogenes*

produced colonies with blue–green surrounding halo on ALOA (Willis *et al.,* 2006). *Salmonella* colonies were pink with or without black centers on XLD agar. *Campylobacter jejuni* was determined on Blood free Campylobacter selective agar base (mCCDA) (Nógrády *et al.,* 2008). Spread plate was prepared by spreading 100 µl of appropriate dilutions onto mCCDA, followed by incubation at 42°C for 48 h in a microaerophillic environment as detailed above. Colonies with grayish-white or creamy grey in color and moist in appearance indicated the presence of *Campylobacter jejuni* (Osiriphun *et al*., 2012).

5.3.9 Statistical Analysis

Each study was performed in three independent replicates with three different sets of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie 1980). Analysis was performed using the Statistical Package for Social Science package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

5.4 Results and discussion

5.4.1 Effect of chicken blood on the growth of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* in medium broth

Effect of chicken blood added into BHI broth ("blood broth") at different concentrations on the growth of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* is illustrated in Figure 10. Counts of each of the four tested strains in the blood broth increased with increasing incubation times ($P<0.05$). Broth at 5 µmol/mL yielded increased numbers of each strain throughout 48 h of the incubation period, from 3.43 to 8.87 log CFU/g for *Pseudomonas aeruginosa*, from 4.29 to 8.85 log CFU/g for *Listeria monocytogenes*, from 3.40 to 10.56 log CFU/g for *Salmonella* Typhimurium, and from 3.90 to 9.92 log CFU/g for *Campylobacter jejuni*. Overall, growth of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* in the blood broth at 2.5 and 5 µmol/mL increased rapidly

within 12 h of the incubation period. Counts of pathogenic strains tested, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni*, were higher in the blood broth at 2.5 and 5 µmol/mL, from 6–42 h of incubation as compared with those in the blood broth at 1 µmol/mL and the controls. Counts of the spoilage organism *Pseudomonas aeruginosa* in the blood broth at 2.5 and 5 µmol/mL were evidently higher from 0–42 h of incubation as compared with those in the blood broth at 1 µmol/mL and the controls. Results indicated that additional chicken blood in the medium broth could allow for more growth during the exponential phase of the microorganisms. Chicken blood is comprised of a variety of substrates, including glucose, lipid, amino acid, albumin, globulin, fibrinogen, hormone, vitamins and antibody (Perry et al., 1986); these substrates could be of additional nutrients to promote growth of bacteria, including *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni*. Higher growth rates of the pathogenic microorganisms affected by additional chicken blood can facilitate the bacterial cells to reach high numbers in a shorter time, thus achieving their infective doses more rapidly and more likely to cause foodborne illnesses among individuals.

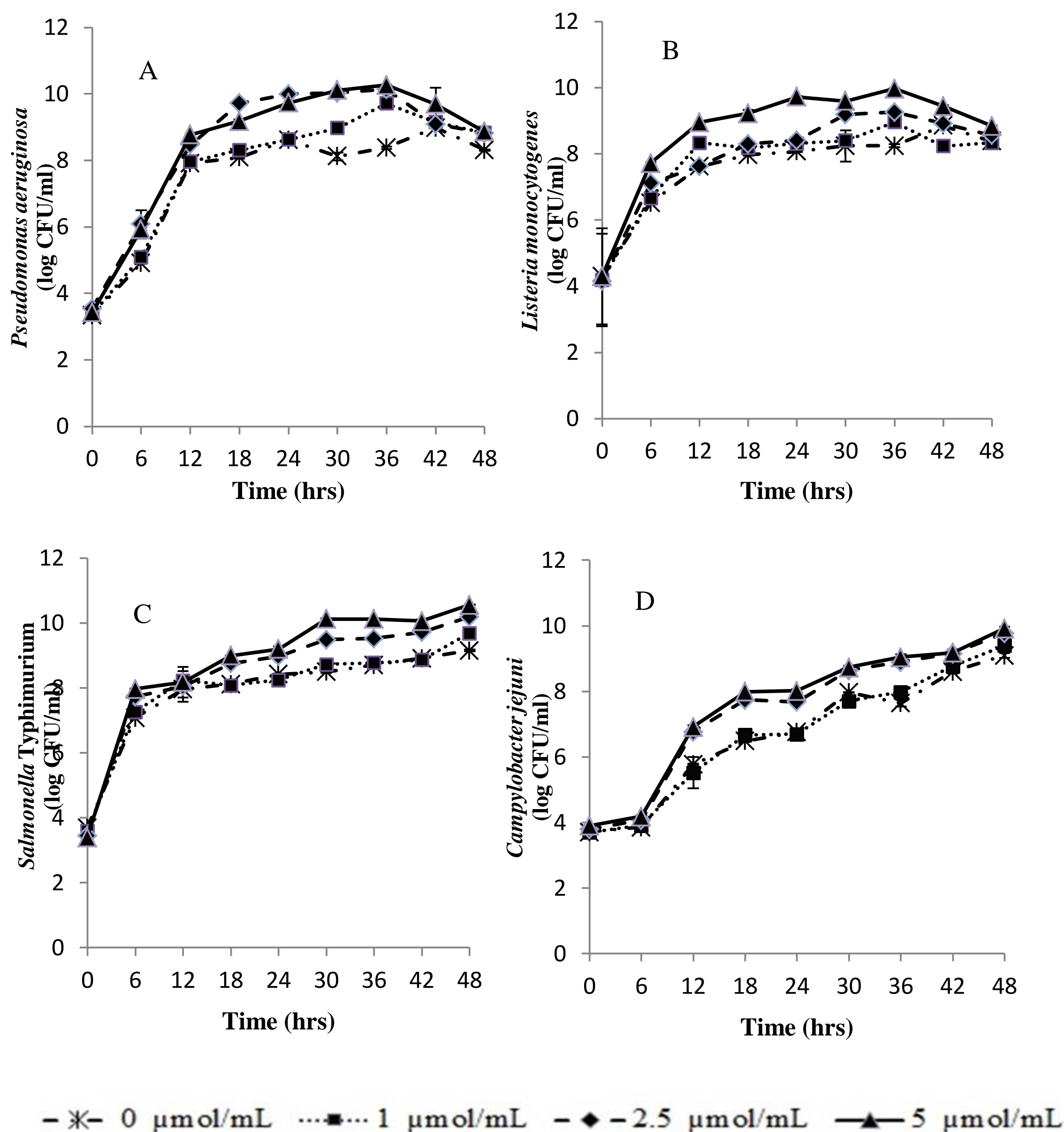


Figure 10 Effect of blood concentrations on the growth of *Pseudomonas aeruginosa* (A), *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Campylobacter jejuni* (D) in BHI broth.

5.4.2 Effect of chicken blood on the growth of microorganisms in non-Halal minced chicken

Mesophilic bacteria count (MBC) and psychrophilic bacteria count (PBC) of minced chicken with and without chicken blood added at different levels during 8 days of refrigerated storage are shown in Figure 11. MBC and PBC of minced chicken increased with increasing storage time, regardless of the blood concentrations present in the minced chicken ($P<0.05$). MBC and PBC of minced chicken with the high blood concentrations (7.5 and 10 µmol/g) increased at a higher rate, compared with other lower concentrations (2.5 and 5 µmol/g) and the controls (no blood added), after 2 days of storage. Especially, at day 4 of storage, MBC of minced chicken with 7.5 and 10 µmol blood/g exceeded 10^7 CFU/g, the upper limit for the chicken to be safe for consumption (ICMSF 1986). Spoilage of chicken generally occurs when the mesophilic bacteria count reaches 10^7–10^8 CFU/g (Linton *et al*., 2004), which is often after 4–10 days, depending on slaughtering techniques, the types and numbers of bacteria initially present and their growth rates (Upmann *et al.,* 2000). The presence of some residual blood in chicken muscles can be a good nutrient source for supporting the growth of microorganisms, including pathogenic and spoilage bacteria which could have been contaminated from skin, viscera, the processing operations or the environments during slaughtering.

Our data showed that PBC at days 0–2 was lower than MBC, and that PBC rapidly increased after 2 days of storage, suggesting that psychrophilic bacteria gradually grew during the refrigerated storage and became the dominant bacteria with increasing storage time. The previous study has shown that bacterial genera recovered from carcasses undergone processing and from carcasses stored at refrigeration temperatures included *Aeromonas*, *Chromobacterium*, and *Pseudomonas* (Hinton *et al*., 2004). In addition, *Pseudomonas* spp. became the predominant bacterial genera recovered from refrigerated carcasses after 7 days of storage at 4°C (Hinton *et al*., 2004). However, the initial microbial load of the meat also depends on the physiological status of the animal at slaughter, the spread of contamination into slaughterhouses and during processing, while temperature and other conditions of

storage during distribution can also influence the rate of spoilage (Nychas *et al.*, 2008).

Pathogenic and spoilage bacteria counts of the minced chicken with and without blood added at different concentrations during 8 days of refrigerated storage are shown in Figure 12. Overall, minced chicken with different blood concentrations showed higher counts of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* as compared to the controls (minced chicken with no blood added). Counts of the four strains were significantly higher in the minced chicken with blood added at a level of 10 µmol/g, compared with those in the minced chicken with lower blood concentrations and the controls ($P<0.05$). For *Listeria monocytogenes* and *Campylobacter jejuni*, there were no marked differences between the minced chicken with blood added at 7.5 and 10 µmol/g. Overall, minced chicken without blood had lower counts of *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, and *Campylobacter jejuni* at the end of storage (8 days) ($P<0.05$), except for *Listeria monocytogenes*. Minced chicken with blood added at different concentrations could allow for rapid growth of *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, and *Campylobacter jejuni* during 8 days and *Listeria monocytogenes* during 4 days of storage. Chicken meat is considered a food that supports growth of pathogens and spoilage organisms, numbers of these organisms could reach higher levels in the presence of some residual blood. This can be of a particular concern as growth of these pathogens and spoilage organism may be highly promoted, representing much higher counts than those in the blood-free chicken meat or chicken meat with minimized residual blood content while kept refrigerated for up to 4–8 days. Especially, some pathogens have the ability to survive and grow at refrigeration conditions (e.g., *Listeria monocytogenes*) and freezing temperatures. Foster and Mead (1976) reported almost 100% survival of five *Salmonella* serovars, including *Salmonella* Typhimurium in minced chicken breast during 100 days at -20°C.

The increase in the bacterial counts was affected by the accessible nutrients available for supporting growth of the bacteria. Our data showed that additional chicken blood had the direct effect on microbial growth for both pathogenic and spoilage bacteria in the minced chicken. It is therefore important to minimize

residual blood content in chicken meat during the slaughtering process. Appropriate washing should be necessarily emphasized. Poultry carcasses are regularly washed with water during slaughtering to remove not only some microbial contamination acquired during defeathering and evisceration, but also visible matter such as soil and possibly residual blood. Washing has been shown to reduce the numbers of pathogen contamination in chicken carcasses. For example, washing by multiple sprays from bleeding through chilling could result in the reduction of *Salmonella* prevalence on carcasses by 50–90% (Bolder, 2007). Rosenquist *et al*. (2006) showed that chicken carcass wash prior to the chilling operation could reduce *Campylobacter* about 0.83 and 0.97 log CFU/g.

Higher counts of pathogens and spoilage organism during storage could represent sufficient infective doses which are likely to increase the incidence of foodborne illnesses associated with consumption of contaminated chicken meat, as well as the likelihood of chicken meat becoming deteriorated more quickly. Results are in agreement with a study by Alvarado *et al*., (2007) that showed that blood components were powerful promoters of microbial growth and could decrease the shelf-life of the meat products. Occurrence of microorganisms in raw meat, especially in high numbers, can cause further changes, yielding the products that are unappealing and unsuitable for human consumption (Doulgeraki *et al*., 2012).

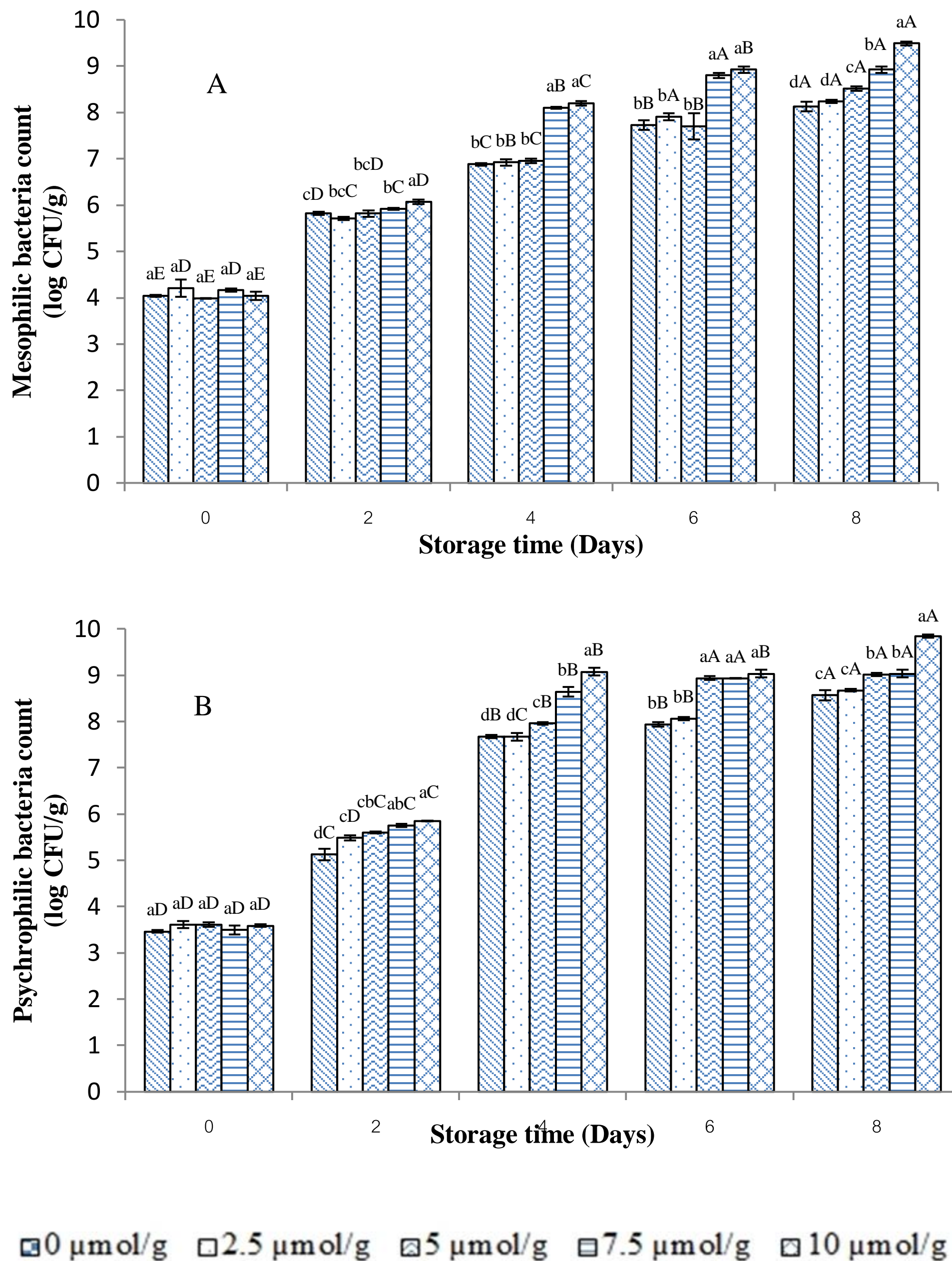


Figure 11 Effect of blood concentrations on the growth of mesophilic (A) and psychrophilic (B) bacteria counts in minced chicken during 8 days of refrigerated storage. Different lowercase letters on the bars within the same storage time indicate the significant differences (P<0.05). Different uppercase letters on the bars within the same treatment indicate the significant differences (P<0.05).

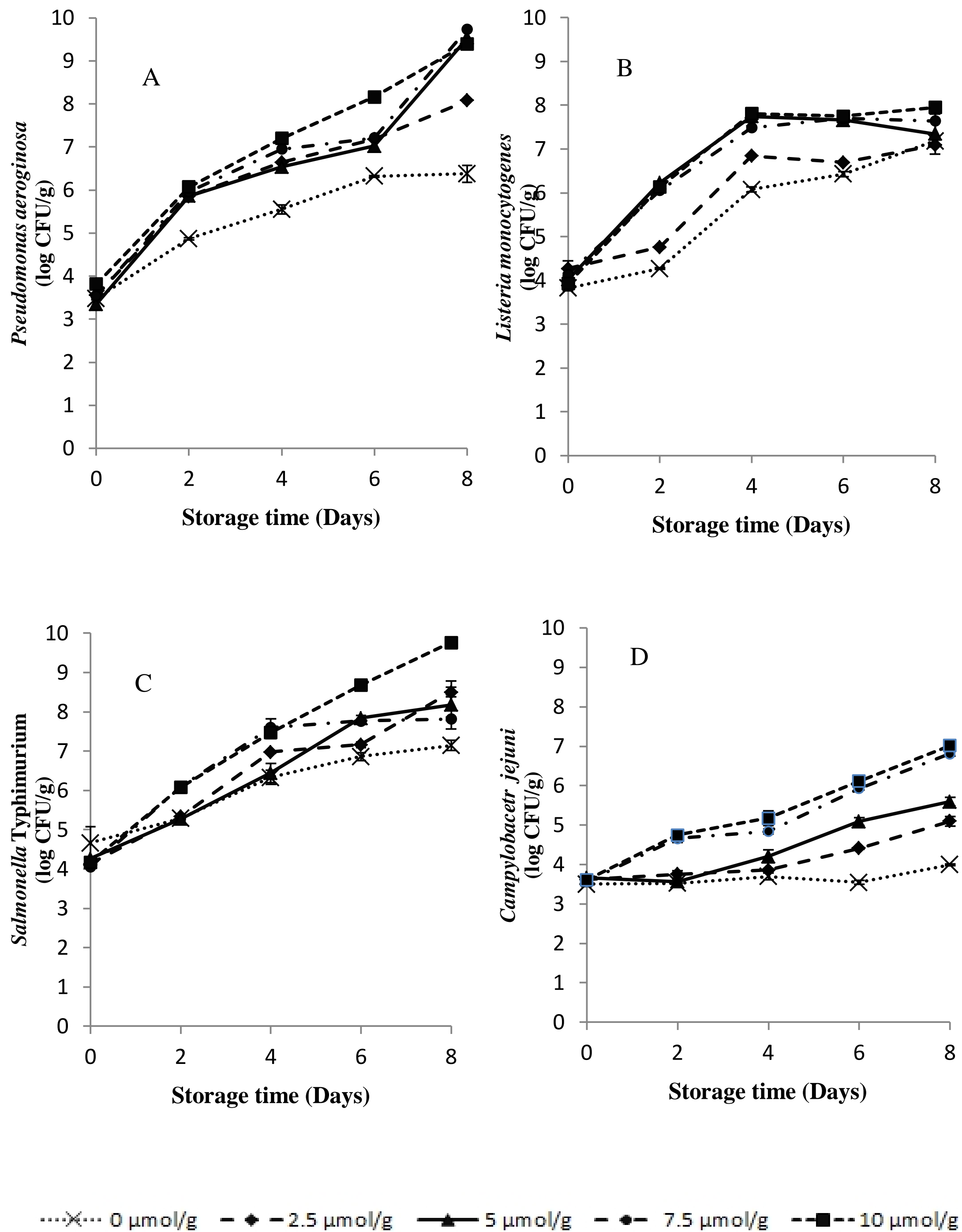


Figure 12 Effect of blood concentrations on the growth of *Pseudomonas aeruginosa* (A), *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Campylobacetr jejuni* (D) in minced chicken during 8 days of refrigerated storage.

5.4.3 Effect of slaughtering methods on the growth of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* in minced chicken

The microbial growth in minced chicken prepared from the Islamic slaughtering method (IM) and conventional neck cut method (CM) during refrigerated storage is shown in Figure 13. *Listeria monocytogenes, Salmonella* Typhimurium and *Campylobacter jejuni* constituted as the natural pathogen of chicken meat (Nierop *et al.*, 2005). Of these strains, only *Campylobacter jejuni* in the IM-minced chicken had higher growth than those found in the CM-minced chicken throughout 8 days of storage. Three other strains, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Salmonella* Typhimurium showed no difference in the counts in minced chicken from both slaughtering methods throughout 8 days of storage. The lower residual blood content in the IM-chicken might be correlated to the lower amount of nutrients available. The bacterial counts can be affected by the amount of residual blood left in the tissues of the broilers. Overall, the residual blood present in the CM-minced chicken provided a suitable substrate for the growth of pathogens. *Pseudomonas aeruginosa* counts in both CM- and IM-minced chicken increased rapidly from 3.54 to 9.74 and 3.35 to 9.53 log CFU/g, respectively. *Pseudomonas* spp., have been identified as the predominant microorganism responsible for spoilage of aerobically-stored meat products (Pooni and Mead, 1984). *Pseudomonas* spp. is the primary spoilage bacteria and their predominance in spoiled chicken may be attributed to their generation time being shorter than other spoilage and pathogenic bacteria (Mc-Meekin, 1975). *Pseudomonas* spp. are proteolytic and lipolytic bacteria ubiquitous and have a variety of strategies for utilizing diverse compounds, including various hydrocarbons, as carbon and energy sources (Mellor *et al.*, 2011). For *Listeria monocytogenes*, similar counts were observed between the IM- and CM-minced chicken during 8 days of storage. For the conventional neck cut method, if complete bleeding is not performed as in the Islamic slaughtering method, extra blood could be retained in the tissues and will likely to provide nutritious substrates for supporting growth of pathogenic and spoilage organisms.

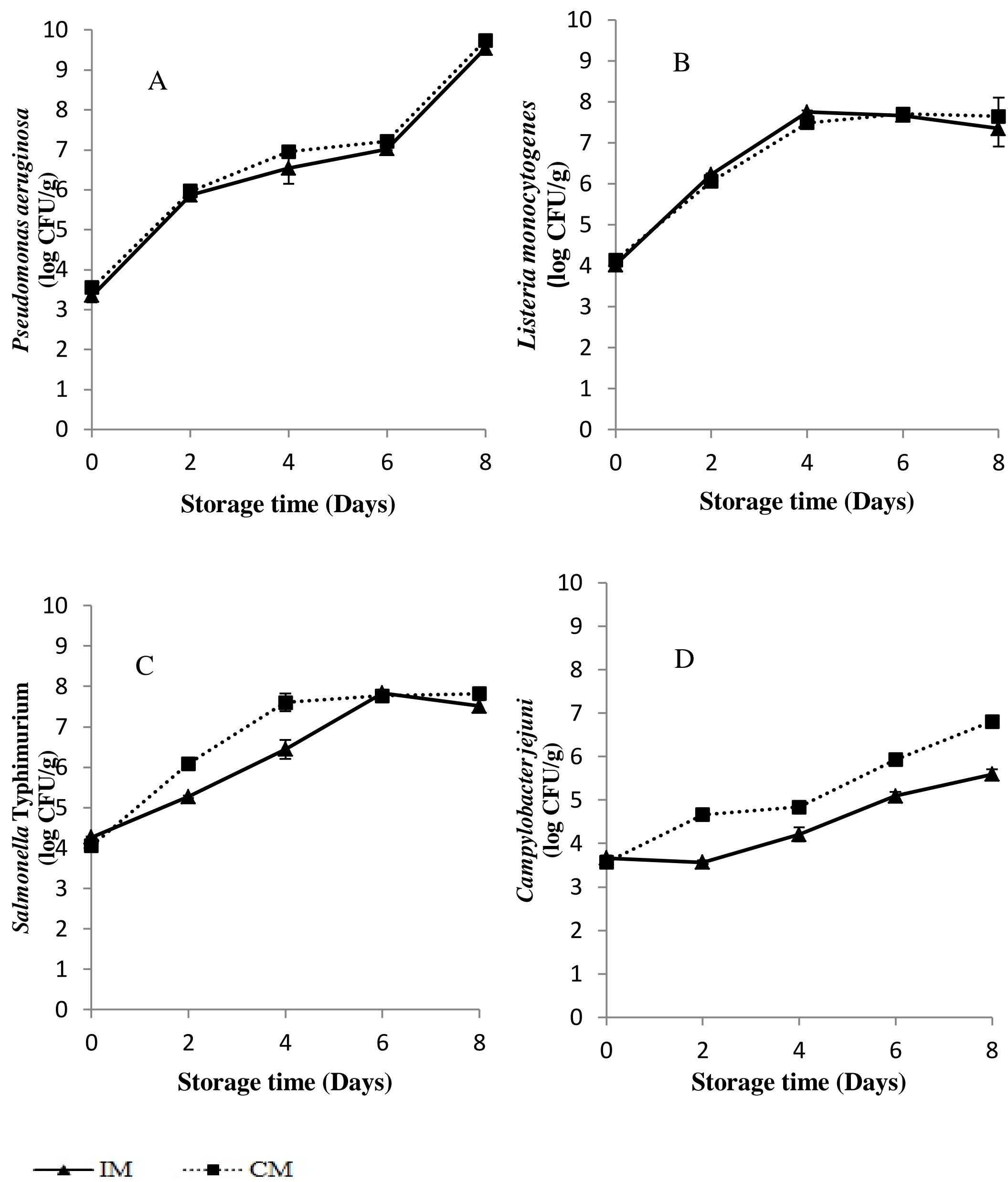


Figure 13 Effect of slaughtering methods on growth of *Pseudomonas aeruginosa* (A), *Listeria monocytogenes* (B), *Salmonella* Typhimurium (C) and *Campylobacetr jejuni* (D) in mined chicken during 8 days of refrigerated storage. IM, Islamic method; CM, conventional neck cut method.

5.5 Conclusion

Minced chicken with chicken blood added showed high growth of both pathogenic and spoilage bacteria (*Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni*). From this study, Islamic slaughtering method could lead to lower growth of the major foodborne pathogen *Campylobacter jejuni* in minced chicken. Thus, the slaughtering method, in which residual blood content is reduced as much as possible or complete bleeding is performed, can be of an effective approach for controlling some major pathogenic and spoilage bacteria often present in chicken meat. In addition, the washing step focusing on minimizing the residual blood content after bleeding needs to be emphasized to retard microbial growth in order to (i) prevent pathogenic bacteria to reach their infective doses more rapidly and (ii) delay deterioration of chicken meat caused by spoilage organism.

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