

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

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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/100 g 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 4°C 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.

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Introduction

Eating is considered as a matter of worshipping God for Muslim. Islamic law prescribes a set of dietary rule, called “Halal” (legal, permitted by Allah) which lists the permitted food and prohibits 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 (Sams, 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 CO₂) 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 (Sams, 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

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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.

Materials and Methods

Chemicals

Bathophenanthroline disulphonic acid, sodium dodecyl sulphate (SDS), ammonium thiocyanate and β -mercaptoethanol (β ME) were purchased from Sigma (St. Louis, MO, USA). Trichloroacetic acid, anhydrous sodium sulphate, sodium nitrite, ferrous chloride, iron standard solution and standard 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).

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, 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 being bled for 3 min, the chicken was plucked in a rotary-drum picker for 30 sec 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.

Chemical analyses

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 rate 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.

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:

$$\text{Haem iron content (ppm)} = \text{Total pigment (ppm)} \times 0.0822$$

$$\text{where total pigment (ppm)} = A_{640} \times 680.$$

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

Determination of non-haem iron content

Non-haem iron content was measured as described by Schrickler *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, (400 µL) was used. The concentration of the non-haem iron was expressed as mg/100 g wet sample.

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 4000 g for 20 min (MIKRO20, Hettich Zentrifugan, 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 malonaldehyde/kg wet sample.

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 & 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.

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 11,000 rpm for 1 min. The mixtures were incubated at 85°C 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.

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 250°C and detector temperature of 270°C. The oven was programmed from 170 to 225°C at a rate of 1°C/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.

Analysis of volatile compounds

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

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 an 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 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 270°C.

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 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 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.

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.

Physical analysis

Determination of colour

Colour of chicken meat was determined by measuring L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values 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 ($L^* = 93.55$, $a^* = -0.84$, $b^* = 0.37$).

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

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

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.

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 35°C for 2 days. For psychrophilic bacteria count, the incubation was conducted at 4°C for 7 days.

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).

Results and Discussion

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 1. 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.*, 2010). 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.

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

Haem iron and non-haem iron contents

The changes of haem iron content in chicken meat during refrigerated storage are shown in Figure 1(A). At day 0, UN sample had higher haem iron content (3.41 mg/100 g sample) than did slaughtered samples ($P < 0.05$). This was in agreement with the highest Fe content in UN sample (Table 1). Haem iron contents of 2.41, 2.35 and 2.56 mg/100 g 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

Table 1. 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 ^a	12.47±1.26 ^a	14.21±0.35 ^a	18.10±1.05 ^b
Mg	870.48±2.54 ^a	959.62±1.43 ^b	930.79±1.84 ^b	965.41±2.65 ^b
Cu	0.41±0.07 ^a	1.03±0.34 ^a	0.38±0.02 ^a	4.35±0.64 ^b
Zn	55.98±1.87 ^b	47.10±0.52 ^b	24.80±0.67 ^a	16.16±1.18 ^a
Ca	136.85±1.93 ^a	390.26±0.82 ^b	108.99±1.22 ^a	129.26±1.05 ^a
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 ($p < 0.05$).

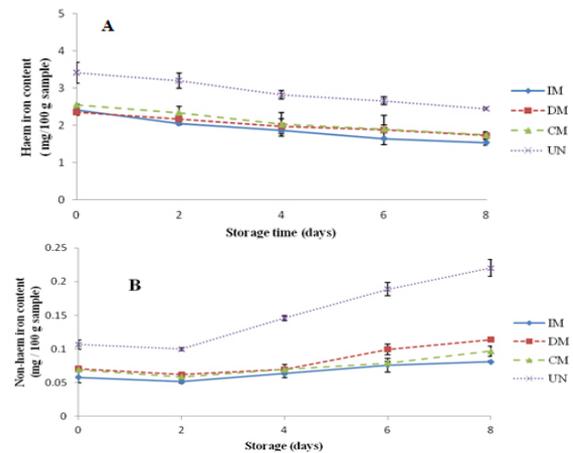


Figure 1. Haem iron (A) and non-haem iron contents (B) of chicken meat with different slaughtering methods and un-bled sample during 8 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Bar represent standard deviation (n = 3)

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/100 g 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 1(B). At day 0, the content of non-haem iron found in IM, DM, CM and UN samples were 0.05, 0.07, 0.07 and 0.11 mg/100 g sample, 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

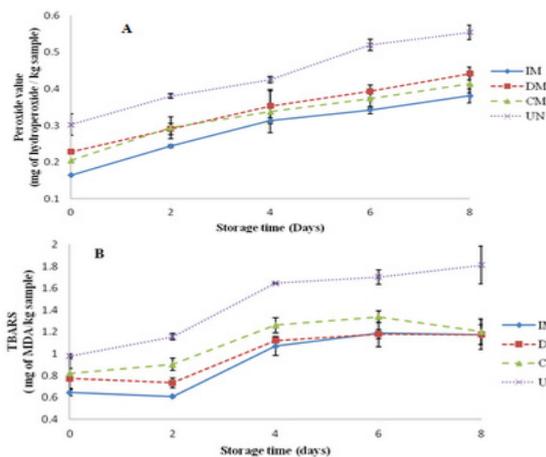


Figure 2. PV (A) and TBARS (B) of chicken meat with different slaughtering methods and un-bled sample during 8 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Bar represent the standard deviation ($n = 3$)

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.

Lipid oxidation products

Lipid oxidation products in chicken meat during 8 days of refrigerated storage were monitored by measuring PV and TBARS value (Figure 2). 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 PV content as well as non-haem iron content in this sample (Table 1, Figure 1B). The higher PV in

Table 2. Fatty acid profile of chicken meat with different slaughtering methods and un-bled sample after 8 days 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-11 t	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 n-7	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
Polysaturated 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 ($n = 3$).

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 2A). 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 Huntin, 2002). Apart from haemoglobin, the blood also contains 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.

Fatty acid profile

Fatty acid profiles of chicken meat with different slaughtering methods including Islamic slaughtering method, decapitation method, conventional method

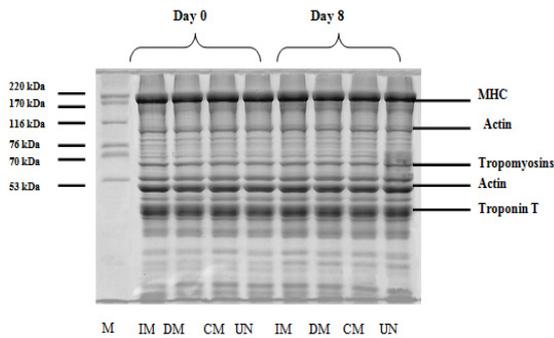


Figure 3. Protein pattern of chicken meat with different slaughtering methods and un-bled sample at day 0 and day 8 of refrigerated storage. M: Molecular-weight markers; IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled.

and un-bled sample after 8 days of refrigerated storage in comparison with day 0 are shown in Table 2. 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 acids, 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.

Protein patterns

Protein patterns of chicken meat with different slaughtering methods and UN sample at day 0 and day 8 of storage are shown in Figure 3. 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

Table 3. 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
Isopropylmyristate	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).

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.

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 3. 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, hexanal 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 decomposition of hydroperoxide formed were more pronounced in the UN samples. This was in agreement with the highest

Table 4. 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)	Samples	L*	a*	b*	ΔE*	ΔC*
0	IM	48.55±2.13aA	0.85±0.81baA	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±0.35cA	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±0.45bB	11.76±0.57aB	48.16±1.69bA	10.86±0.56aB
	DM	47.69±0.79aB	0.48±0.24bB	10.95±0.60abA	47.08±0.75bA	10.05±0.61abA
	CM	47.28±1.53aB	0.62±0.19cB	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.

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 is 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 alkoxy 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.

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

Colour

Colour of chicken meat with different slaughtering

Table 5. 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)	Samples	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 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.

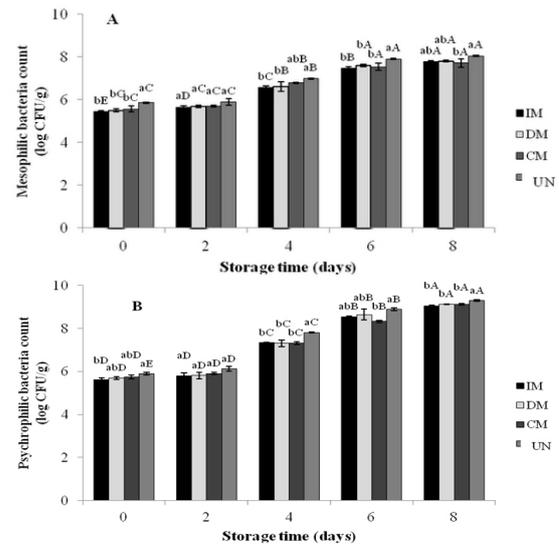


Figure 4. Mesophilic bacteria count (A) and psychrophilic bacteria counts (B) of chicken meat with different slaughtering methods and un-bled sample during 8 days of refrigerated storage. IM: Islamic method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Different lowercase 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).

methods and UN sample at day 0 and 8 of storage is shown in Table 4. There were 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 (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.

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 5. 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 ($P < 0.05$). At day 0, IM sample showed a lower drip loss value, compared with other samples ($P < 0.05$). This result was in agreement with D'Agata *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.

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 slaughtering methods during refrigerated storage are depicted in Figure 4. 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 handling, 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 10^7 cfu/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.

Conclusions

Chicken meat from Islamic slaughtering methods showed the lower haem and non-haem iron contents with coincidental lower 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.

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