

REGULAR ARTICLE

# Slaughtering method affects lipid oxidation, volatile profile and overall quality of chicken patties during storage

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## ABSTRACT

Quality of cooked chicken patties obtained from various slaughtering methods including Halal method (HM), decapitation method (DM), conventional neck cut method (CM) and un-bled sample (UN) was monitored during 12 days of refrigerated storage. Cooked patties from HM 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 hexanal and octanal contents detected by solid phase micro extraction–gas chromatography–mass spectrometry (SPME–GC–MS). After 12 days of storage, cooked patties from HM chicken had the lowest  $a^*$  but the highest  $L^*$  value and exhibited the highest hardness in comparison with other samples. For sensory property, the highest likeness score was observed for cooked patties from HM chicken ( $P < 0.05$ ). Additionally, mesophilic bacteria count (MBC) and psychrophilic bacteria count (PBC) were lowest in cooked patties from HM chicken ( $P < 0.05$ ). Thus, amongst different slaughtering methods tested, Halal slaughtering method was found to be more effective in maintaining the quality of cooked chicken patties during the refrigerated storage.

**Keywords:** Halal slaughtering; Haemoglobin; Lipid oxidation; Quality; Chicken meat

## INTRODUCTION

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 methods applied, 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 (D'Agata 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).

Since last decade, popularity of the processed products from the chicken have gained momentum due to their high nutritional quality. These products are available at comparatively low cost 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. (2014) reported that chicken meat obtained from Halal slaughtering method contained the lowest haem and non-haem iron amount and had the lowest lipid oxidation during refrigerated storage of 8 days. Those chicken meats can yield the products e.g. patties with

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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. Therefore, this study aimed to elucidate the impact of Halal slaughtering method on quality of cooked patties, in comparison with other methods during the extended refrigerated storage.

## MATERIALS AND METHODS

### 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).

### 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 four groups, three representing groups slaughtered by three different slaughtering methods. Those included 1) Halal method, 2) Decapitation method and 3) Conventional neck cut method. Fourth group represented un-bled sample which were used as control. The samples thus obtained were referred to as “HM”, “DM”, “CM” and “UN”, respectively. Samples were blinded, which means they were coded and codes were noted down. All the slaughtering methods were carried out according to the regulation mentioned by “Bureau of Livestock Standards and Certification”, Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand. For all slaughtering methods, broilers were bled for 3 min, and then the chicken was plucked in a rotary-drum picker for 30s and eviscerated. Breast muscle was dissected from the carcasses. Haem iron contents of UN, HM, DM and CM determined as per the method of Aneesa et al. (2014) were 3.55, 2.28, 2.49 and 2.56 mg/100g, respectively. UN, HM, DM and CM samples had non-haem iron contents of 0.107, 0.051, 0.061 and 0.064 mg/100 g, respectively.

Breast was minced in meat mincer (Panasonic, Japan) with a hole diameter of 5 mm. The minced breast muscle was mixed with sodium chloride (1% w/w) and 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 different analyses during 12 days of refrigerated storage. Lipid oxidation and microbial counts were evaluated on day 0, 3, 6, 9 and 12. Volatile compounds, colour and texture profile analysis were conducted on day 0 and 12, and sensory evaluation was done on day 0 and 6.

### Chemical analyses

#### *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 malonaldehyde/kg patties.

#### *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 were 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 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.

#### *Determination of volatile compounds by solid phase micro extraction–gas chromatography–mass spectrometry (SPME–GC–MS)*

Volatile compounds were determined by SPME–GC–MS (Iglesias and Medina, 2008) by following steps.

### 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.

### 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.

### 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 in term of abundance of each identified compound.

### Physical analyses

#### Colour ( $L^*$ , $a^*$ , $b^*$ , $\Delta E^*$ )

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,  $\Delta E^*$ , was calculated by the following equation (Berns, 2000):

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

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

### 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).

### 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 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 psychrophilic bacterial count, a pour plate method was used and the incubation was conducted at 4°C for 7 days.

### 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 ( $5 \times 10^5$  CFU/g) (ICMSF, 1986), The samples therefore were considered safe to be tasted by the sensory panelist. The samples were evaluated by 30 untrained 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). Samples from each group were coded with 3-digit random codes and were offered to the panelist in a completely randomized order. Panelists were seated in separate booths of the sensory laboratory approved by ISI standards. All panelists were asked to evaluate for appearance, odor, taste, texture and overall likeness. Sensory evaluation was conducted in single session on day 0 and 6 of storage with each panelist evaluating 3 samples for each treatment.

### Statistical analysis

The experiments were run in triplicate with three different lots or batches of samples for every method of slaughtering. Data obtained 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).

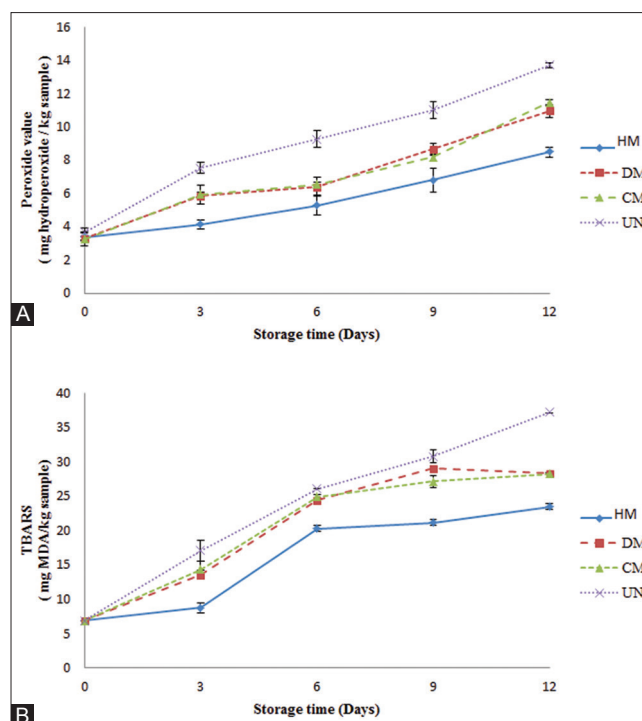
## RESULTS AND DISCUSSION

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

#### Lipid oxidation

Lipid oxidation in cooked chicken patties during refrigerated storage of 12 days as monitored by PV and TBARS value is shown in Fig. 1. The increases in PV of all samples were observed with increasing storage time ( $P < 0.05$ ). However, the rate of increase was different. When comparing PV of all samples, PV of HM 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 ( $P < 0.05$ ). Without bleeding, blood in the UN sample could serve as the source of haem and non-haem irons, known as pro-oxidants. UN chicken meat used for patties preparation had the highest haem and non-haem contents. This result was in agreement with our previous work (Addeen et al., 2014). When broilers were slaughtered, the bleeding occurred. As a result, the blood was less retained as indicated by lower haem iron content. However, bleeding efficacy could be varied, depending on the methods used. The result suggested that patties prepared from HM sample, containing the lowest haem and non-haem iron contents, was less susceptible to lipid oxidation.

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



**Fig 1.** Changes in PV (A), and TBARS (B) of cooked patties from chicken with different slaughtering methods during 12 days of refrigerated storage. HM: Halal method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled. Bar represent the standard deviation (n=3).

haem proteins, could take place. Those pro-oxidants could induce the lipid oxidation as indicated by the increase in PV. Due to the lowest PV in HM sample, the lowest content of pro-oxidants in this sample might be associated with the lowest oxidation. HM chicken meat contained the lower haem iron and non-haem iron content than chicken from other slaughtering methods (Addeen et al., 2014). Heating might cause disruption of porphyrin ring of the haemoglobin and myoglobin resulting in 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, 1996). For HM 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 the highest TBARS value than slaughtered

samples throughout the storage ( $P < 0.05$ ). Blood containing haemoglobin could serve as the source of pro-oxidants, which induced lipid oxidation in cooked patties (Richard et al., 2007). Blood is also known to carry a good amount of white blood cells having potential to generate superoxide, hydrogen peroxide and hydroxyl radicals. Therefore, chicken from Halal slaughtering method yielded the cooked patties with the decreased susceptibility to lipid oxidation, as evidenced by the lowest TBARS value.

### Volatile compounds as analyzed by solid phase micro extraction–gas chromatography–mass spectrometry (SPME–GC–MS)

Volatile compounds in cooked chicken patties after 12 days of refrigerated storage are displayed in Table 1. Chicken muscle contained high amount of polyunsaturated fatty acids (PUFAs) (Addeen et al., 2014). PUFAs oxidation has been known to produce several volatile compounds including hexanal, heptanal, octanal, etc (Yasuhara and Shibamoto, 1995). Moreover, volatiles including aldehyde (hexanal, heptanal, octanal, 2-heptanal, nonanal, 2-octenal, nonenal and tetradecanal), alcohol (1-octen-3-ol) and other volatile substances (tetradecane) are generated at 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 highest abundance of all volatile compounds. It was postulated that the highest lipid oxidation and greater decomposition of hydroperoxide occurred in UN sample, compared with slaughtered samples. Aldehydes are the known to be the most prominent volatile compounds produced during lipid oxidation and are associated with off-odour (McGill et al., 1974). This observation was similar to previous studies (Du et al. 2001). Cooked sample had the increased the amount of volatiles, especially hexanal and pentanal; this could be due to the higher oxidation, compared with raw meat (Bagorogoza et al., 2001). Aldehydes are important with respect to off-odour formation because they possess low threshold values which

make their contribution to the off-odour very prominent (Ross and Smith, 2006).

Alcohols and ketones are considered to be secondary lipid oxidation products formed by the decomposition of hydroperoxide (Maqsood and Benjakul, 2011). Desirable mushroom-like odour besides green and plant-like aromas is imparted by 1-octen-3-ol (Josephson et al., 1986). In general, the lower amount of secondary oxidation products, including aldehyde and alcohol, in HM sample was in accordance with the lower PV and TBARS (Fig. 1). For DM and CM samples, the latter showed the higher abundance, compound than the former, except heptanal, 2-heptenal and 1-octen-3-ol. Thus, the Halal slaughtering method was found to be effective in retarding the formation of secondary lipid oxidation products in cooked chicken patties.

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

#### Colour of chicken patties as affected by different slaughtering methods

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 2. 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 oxidized haem iron) is formed, leading to the tan colour (Gokalp et al., 2004). At day 0, the cooked chicken patties from UN sample had higher  $a^*$ ,  $b^*$ ,  $\Delta E^*$  but lower  $L^*$  value than those from the slaughtered samples ( $P < 0.05$ ). Boulianna and King (1998) showed a strong positive correlation between total pigment concentration and  $a^*$  value.

After 12 days of storage  $a^*$ ,  $b^*$  and  $\Delta E^*$  values in cooked chicken patties decreased ( $P < 0.05$ ). This coincided with

**Table 1: Volatile compounds in cooked patties from chicken with different slaughtering methods after 12 day of refrigerated storage**

| Compounds (Abundance $\times 10^7$ ) | Day 0 Control  | Day 12         |                |                |                |
|--------------------------------------|----------------|----------------|----------------|----------------|----------------|
|                                      |                | HM             | DM             | CM             | UN             |
| Hexanal                              | 485 $\pm$ 0.97 | 702 $\pm$ 0.92 | 748 $\pm$ 0.98 | 785 $\pm$ 1.15 | 967 $\pm$ 1.54 |
| Heptanal                             | 90 $\pm$ 1.19  | 109 $\pm$ 1.16 | 199 $\pm$ 1.93 | 112 $\pm$ 0.91 | 273 $\pm$ 0.87 |
| Octanal                              | 237 $\pm$ 0.93 | 405 $\pm$ 1.45 | 442 $\pm$ 1.15 | 477 $\pm$ 1.18 | 596 $\pm$ 1.29 |
| 2-Heptenal                           | ND             | 19 $\pm$ 0.38  | 29 $\pm$ 1.28  | 18 $\pm$ 1.10  | 49 $\pm$ 0.92  |
| Nonanal                              | 236 $\pm$ 1.17 | 334 $\pm$ 1.53 | 412 $\pm$ 1.76 | 517 $\pm$ 1.28 | 582 $\pm$ 1.64 |
| 2-Octenal                            | 54 $\pm$ 1.20  | 137 $\pm$ 0.52 | 181 $\pm$ 0.48 | 214 $\pm$ 1.64 | 254 $\pm$ 1.97 |
| 1-Octen-3-ol                         | 136 $\pm$ 1.93 | 167 $\pm$ 1.18 | 196 $\pm$ 1.05 | 186 $\pm$ 1.32 | 209 $\pm$ 1.59 |
| Tetradecane                          | ND             | 29 $\pm$ 0.63  | 75 $\pm$ 0.86  | 77 $\pm$ 0.65  | 113 $\pm$ 0.94 |
| Nonenal                              | ND             | 50 $\pm$ 1.76  | 69 $\pm$ 1.56  | 80 $\pm$ 1.43  | 107 $\pm$ 1.83 |
| Tetradecanal                         | 14 $\pm$ 1.04  | 38 $\pm$ 1.05  | 46 $\pm$ 1.03  | 50 $\pm$ 0.62  | 91 $\pm$ 0.94  |

ND: Not detectable HM: Halal method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled, values are mean $\pm$ SD (n=3)

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 HM chicken showed the lower  $a^*$ ,  $b^*$ ,  $\Delta E^*$  and higher  $L^*$  value throughout the storage, compared with others. The result suggested that halal slaughtering 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.

**Textural properties of chicken patties as affected by different slaughtering methods**

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 3. At day 0, patties from HM 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 ( $P>0.05$ ). Bailey and Light (1989) have reported that when the different meat proteins are heated in the range of 80-100°C, denaturation of myofibrillar proteins, the shrinkage

of intramuscular collagen, as well as the shrinkage and dehydration of the actomyosin occur. This more likely resulted in the formation of hard texture of cooked patties. In the presence of blood, especially for UN sample, haem protein as well as plasma proteins in blood might undergo coagulation along with muscle proteins. This might result in the impeded interaction of muscle proteins during cooking, thereby lowering the hardness 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 chewiness after storage. During storage of 12 days, the degradation of proteins might be induced by psychrophilic microorganisms, regardless of slaughtering processes used. The cleavages of proteins were mostly associated with the textural softening, as shown by the decreases in hardness, springiness and chewiness of cooked patties. The changes in textural properties might be associated with the chemical and microbial deterioration. Lipid and protein oxidation are known to have deteriorative effect in meat (Mercier et al. 2004). Texture, tenderness and colour of fresh meat and meat products are adversely affected by protein oxidation (Rowe et al., 2004). After 12 days of storage, no differences in hardness, springiness and gumminess were observed amongst all samples with different slaughtering methods ( $P>0.05$ ). Nevertheless, at the end of storage (12 days), patties from HM and CM samples had the similar textural

**Table 2:  $L^*$ ,  $a^*$ ,  $b^*$  and  $\Delta E^*$  values of cooked patties from chicken with different slaughtering methods at day 0 and day 12 of refrigerated storage**

| Storage time (days) | Samples | $L^*$        | $a^*$       | $b^*$        | $\Delta E^*$ |
|---------------------|---------|--------------|-------------|--------------|--------------|
| 0                   | HM      | 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                  | HM      | 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 ( $p<0.05$ ), HM: Halal method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled

**Table 3: 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                   | HM      | 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                  | HM      | 10.14±1.29aB | 0.80±0.13aB      | 0.73±0.04aA          | 6.83±1.26aA   | 6.07±1.37aB      |
|                     | DM      | 9.87±0.65bB  | 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      |

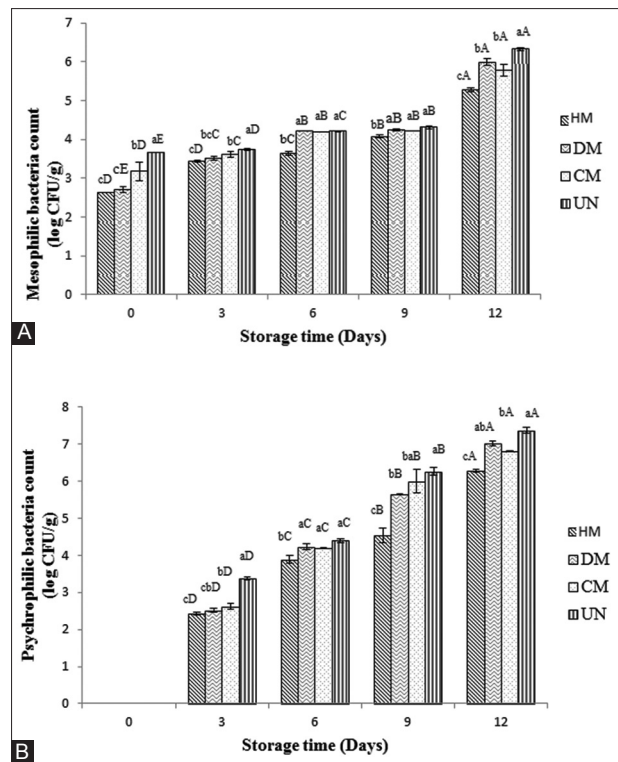
Values are mean±SD (n=3), different lowercase letters within the same storage time in the same column denote the significant difference ( $p<0.05$ ), different uppercase letters within the same treatment in the same column denotes the significant difference ( $p<0.05$ ), HM: Halal method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bled

properties except for chewiness, in which patties from HM sample showed the higher value, compared with those from CM sample ( $P<0.05$ ). Thus slaughtering methods showed the impact on the textural properties of cooked patties to some degree.

### 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 Fig. 2. MBC and PBC of all cooked chicken patties increased when the storage time increased ( $P<0.05$ ). At day 0, patties from HM 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. Unbled chicken meat contained the higher mesophilic bacterial count than the slaughtered samples (Addeen et al., 2014). Although cooking was able to partially kill the microorganisms, some heat tolerant microbes were survived. With higher initial load, the microbes were more retained as found in the patties from UN sample, even after cooking. At the same storage time, patties from HM sample had the lower MBC and PBC, compared with others ( $P<0.05$ ). The swift cutting of blood vessels of the neck applied in Halal slaughtering will stop the flow of blood to the nerve of the brain responsible for pain. Whilst slaughtering, 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 (D'Agata et al., 2009). The purpose of halal slaughtering is to drain out most of the blood which would serve as a good culture medium for microorganisms. In halal slaughtering method, the spinal cord must not be severed because the nerve fibres supplying to the heart could be damaged during the process, causing cardiac arrest and not allowing the blood to flow out from the blood vessels.

Blood is a considered to be a rich medium which can support bacterial growth. Therefore, the halal way of slaughtering is considered to be more hygienic as most of the blood containing bacteria that can cause of several diseases are eliminated. Moreover, the nutrient rich blood if removed can reduce rate of multiplication and growth of bacteria which might contaminate 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 (Miettinen et al., 2002). Bacteria continued to grow as



**Fig 2.** Mesophilic bacteria counts (A) and psychrophilic bacteria counts (B) of cooked patties from chicken with different slaughtering methods during 12 days of refrigerated storage. HM: Halal 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 ( $p<0.05$ ). Bar represent the standard deviation ( $n=3$ ).

far as there were 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  $5 \times 10^5$  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. At all storage times, patties from chicken slaughtering by Halal method showed the lower PBS than others. Thus, HM chicken rendered cooked patties with the lowest microbial load as indicated by lower MBC and PBC.

### Sensorial properties of chicken patties as affected by different slaughtering methods

Appearance, odor, texture, taste and overall likeness scores of all cooked chicken patties at day 0 and 6 of storage are

**Table 4: Likeness scores of cooked patties from chicken with different slaughtering methods at day 0 and day 6 of refrigerated storage**

| Attributes | Storage time (Day) | Slaughtering methods |              |              |             |
|------------|--------------------|----------------------|--------------|--------------|-------------|
|            |                    | HM                   | DM           | CM           | UN          |
| Apperance  | 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±1.02bB  | 6.90±1.04bB  | 5.38±1.28cA |
|            | 6                  | 7.40±0.50aA          | 5.70±1.30bA  | 5.65±0.88bA  | 4.60±1.43cA |
| Taste      | 0                  | 7.43±0.51aA          | 6.76±1.18abB | 6.76±0.94abA | 6.10±1.37bA |
|            | 6                  | 7.40±0.50aA          | 6.25±0.72bA  | 6.28±0.62bA  | 5.55±1.28cA |
| Texture    | 0                  | 7.62±0.67aA          | 6.24±1.45bcA | 6.67±1.02bB  | 5.76±1.76cA |
|            | 6                  | 7.50±0.51aA          | 6.00±1.12bA  | 6.00±0.79bA  | 5.65±1.18bA |
| Overall    | 0                  | 7.76±0.7aA           | 6.48±1.17bA  | 6.90±0.77bB  | 5.67±1.49cA |
|            | 6                  | 7.68±0.65aA          | 6.15±0.81bA  | 6.48±0.57bA  | 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), HM: Halal method; DM: Decapitation method; CM: Conventional neck cut method and UN: Un-bleed

depicted in Table 4. The scores of all sensory attribute were different, dependent on chicken used for patties preparation. Halal slaughtering method yielded patties with highest score for all attributes, except for taste (P<0.05). Patties from UN sample exhibited the lowest scores for all attributes tested. This was related with the lower lipid oxidation in patties prepared from HM 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 (Thiansilakul et al., 2010). In particular, the secondary products of lipid oxidation have been known to relate with warmed-over flavor (WOF) in cooked and chill stored meats (St. Angelo et. al., 1988).

WOF is a distinct flavor which is known to occur in the meat which are reheated after a short period of refrigerated storage (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 (Gray and Pearson, 1987). For odour likeness, patties from HM 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 HM sample showed the higher score than other samples and no change in score was noticeable after 6 day of storage. The highest score of patties from HM sample was related with the highest hardness (Table 3) and the highest odour likeness score was in agreement with the lowest PV and volatile (Table 1). Since the patties samples stored for 12 days had the mesophilic bacterial counts close or above the limit ( $5 \times 10^5$  cfu/g), it was considered as unsafe for sensory testing. Thus, cooked patties from chicken with Halal slaughtering method showed the superior sensory property to those prepared from chicken obtained from other slaughtering methods.

## CONCLUSION

Cooked patties from Halal 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, Halal 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.

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### Author's contribution

Addeen, A. conducted the experiments and wrote the first draft of manuscript. Benjakul, S. designed the study, supervised the graduate student performing the experiments and revised the manuscript. Prodpran, T. participated in the data evaluation, design of experiment and revision of manuscript. All authors approved the final draft of the manuscript.

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