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Physico-chemical changes and microbiological quality of refrigerated broiler chicken meat slaughtered by two different methods

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Abstract

Slaughtering is the first step in meat processing. It involves killing an animal for the production of meat. Effectiveness of slaughter is determined by the amount of blood removed from the animal. This study aimed to compare the chemical changes and microbiological quality of broiler chicken meat slaughtered by Halal and Non-Halal slaughter methods during refrigerated storage. A total of sixty (60) broiler chickens were slaughtered by: i) Neck cutting (NC) - by severing the jugular veins, carotid arteries, trachea and the oesophagus according to the Islamic ritual method of slaughter and (ii) Neck poking (NP) - by poking the neck of the bird with a sharp object. Residual blood was quantified by measuring the haem iron content in the breast meat samples. Storage stability of chicken meat was evaluated by measuring the extent of lipid oxidation determined by thiobarbituric acid reactive substances (TBARS) and by assessing the microbiological quality of the meat. Haem iron content decreased significantly ($P<0.05$) during 9-day storage at 4°C. Haem iron content ranged between 1.31-2.55 mg/100g sample and 2.05-3.25 mg/100g sample in neck cut and neck poked chickens respectively. Slaughter method had no significant effect ($P>0.05$) on chicken meat lipid oxidation at 1, 3, and 9 day of storage at 4°C. However, at 5 and 7 day of storage, significant differences ($P<0.05$) were observed, with neck poked meat samples recording significantly higher levels of malondaldehyde (MDA) than that from neck cut samples. A significantly ($P<0.05$) higher total viable count (TVC) and lactic acid bacteria (LAB) count were observed in neck poked samples as compared to the neck cut samples throughout the storage time. The total viable count and LAB counts reached the highest value of 6.28 $\log_{10}$ CFU/g and 3.93 $\log_{10}$ CFU/g respectively after 9 d of refrigerated storage in neck poked meat samples as compared to 5.26 $\log_{10}$ CFU/g and 3.76 $\log_{10}$ CFU/g recorded in neck cut meat samples after 9 d of refrigerated storage respectively. This study showed that slaughter method had a positive effect on chemical changes and microbial quality of chicken meat during refrigerated storage.

Introduction

Poultry meat account for about 33% of the world meat consumption (FAOSTAT, 2010) and consumers' demand for high quality poultry meat is ever increasing. According to the Food and Agriculture Organization statistics, the average per capita consumption of poultry meat has quadrupled since the 1960s (11 kg in 2003 compared with 3 kg in 1963). This increase may be due to the fact that poultry meat is cheaper with good nutritional profile, easy to prepare and it is well suited for quick menus. Globally, chicken meat is regarded as the cheapest commercially produced meat in a global market and consumption is estimated to increase to about 34% by 2018 (Jung *et al*., 2011). Chicken meat is generally regarded as better than red meat because it is a white meat which contains less fat and cholesterol, easy to handle portions and has no religious restrictions unlike pork and beef (Liu *et al*., 2012). However, chicken meat is a perishable product which deteriorates quickly if it is improperly processed.

The first step in meat processing is the slaughtering process. Slaughtering is the process of killing an animal for the production of meat. A good slaughtering practise ensures maximum blood drainage but the method of slaughtering is dependent on the amount of blood bled. Alli *et al.* (2011) reported a maximum blood drainage which had a favourable effect on the keeping quality of chicken meat for birds slaughtered using the Halal method. Residual blood left in the carcass as a result of improper bleeding may decrease the shelf life and hence the quality of the meat product because haemoglobin which

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is an important component of blood is a powerful promoter of lipid oxidation (Alvarado *et al.*, 2007). The Halal slaughter method which involves severing the jugular veins, carotid arteries, trachea and the oesophagus is a method prescribed for the Muslims to slaughter animals by ensuring maximum blood removal because blood consumption is forbidden.

After slaughtering operation, the next problem processors face is how to maintain the quality of meat from spoilage when the meat product is not meant for immediate consumption. Quick refrigeration after slaughter is one of the ways to prolong the quality of meat. Refrigeration after slaughter is essential to retard microbial growth, lipid oxidation and spoilage. However, during extended refrigerated storage, chemical changes and microbial growth may occur in the meat product and the rate at which these changes occur is dependent on the state of the meat during slaughtering and processing.

Many researches in meat science have documented the effect of genotype, diet, sex, and rearing techniques on post mortem changes in meat during refrigerated storage (Anadon 2002; Berri *et al.*, 2005; Bianchi *et al*., 2006) but reports on post mortem changes in meat as a result of slaughtering method is still very limited. Therefore, the objective of this study was to determine the effect of slaughter on chemical changes and microbiological quality of broiler chicken meat during refrigerated storage.

Materials and Methods

Sample preparation and slaughter methods

Broiler chickens were obtained from a fresh market in Semarak, Nilai, Negeri Sembilan, Malaysia. A total of 60 broiler chickens approximately weighing 2 kg and of the same marketable age were used for this experiment. The birds were grouped into two groups of thirty birds per group based on the slaughter method used. The birds were slaughtered by: (i) neck cutting which was done by severing the jugular veins, carotid arteries, trachea and the oesophagus according to the Islamic ritual method (NC); (ii) neck poking which involved the use of a sharp object to poke the neck of the birds thereby creating a small hole for blood drainage (NP).

After slaughtering, the birds were left for about 3-5 minutes for effective blood drainage and to make sure the birds were dead before processing. Afterwards, the birds were immersed in hot water 60°C for two minutes to help in feathers scalding and the feathers were removed mechanically by using a feather picking machine. The birds were then eviscerated and internal organs removed. The birds were packed in styrofoam box with ice and immediately taken to the laboratory. The carcasses were allowed to cool for 6-8 h post-mortem in ice before deboning. After deboning, the breast meat *(Pectoralis major)* were kept in the refrigerator (4ºC) overnight and prepared for further analyses.

Drip loss

Drip loss was evaluated according to the method of Wang (2005). A 200 g fresh sample of breast meats were weighed prior refrigeration at 4ºC and reweighed after 24 h refrigeration. The difference in the initial weight and final weight was calculated in percentage as the drip loss during 24 h refrigerated storage.

$$\% \text{ Drip Loss} = \frac{W1-W2}{W1} \times 100$$

Where W1= weight of sample after deboning
W2 = weight of sample after 24 hours chilling

Colour determination

Meat colour was evaluated 24 hours post-mortem storage at 4ºC. The lightness *(L^*),* redness *(a^*)*, and yellowness *(b^*)* values of the chicken meat were measured using a Hunter Labscan colorimeter (Minolta CR-300, Minolta Corp., Ramsey, NJ). The colorimeter was calibrated using a standard white ceramic tile. Colour was evaluated on the breast meat in an area free of obvious colour defects, bruises, and blood spots as described by Dadgar *et al.* (2010).

Cook loss

Overnight thawed samples of breast fillets were individually packaged in plastic bags, sealed and cooked to an internal temperature of 75 ± 1ºC in an 80 ± 0.5ºC water bath (WNB7, Memmert GmbH) for 25-35 min. Samples were immediately cooled in water for 20 minutes, then weighed. Cook loss was calculated as the percentage weight lost during cooking (Dadgar *et al*., 2010).

$$\% \text{ Cook Loss} = \frac{C1- C2}{C1} \times 100$$

C1= sample weight after thawing
C2= sample weight after cooking

Ultimate pH measurement

Ultimate pH was determined on samples using the slurry method described by Dadgar *et al.* (2010) with modifications. Ultimate pH was measured by homogenizing 5 g of meat sample in 20 ml of deionised water using a homogenizer (Yellow line

DI 25 basic, Colonial Scientific, Richmond, VA) at 13600 rpm for 60 sec. the pH of the homogenate was determined using a pH meter (Mettler Toledo pH meter, Greifensee, Switzerland) calibrated at pH 4.0 and 7.0.

Determination of haem iron content

Haem iron content of chicken meat was determined on 1, 3, 5, 7 and 9 days of refrigerated storage at 4°C. The method of Cheng and Ockerman (2004) was followed with slight modifications. On each day for analysis, 2 g of ground meat sample was thoroughly mixed with 9 ml of acid acetone (90% acetone, 8% deionised water and 2% HCl v/v/v) and allowed to stand for 1 h at room temperature. The mixture was filtered with a Whatman No. 1 filter paper (Whatman International, Ltd, Maidstone, England) and allowed to stand for a few minutes. The absorbance of the clear filtrate was read at 640 nm using Implen Nanophotometer P330 (Implen GmbH, München, Germany) against an acid acetone as blank. Haem iron content was calculated as follows;

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

$$\text{Where total haem pigment (ppm)} = A_{640} \times 680$$

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

Determination of thiobarbituric acid reactive substances (TBARS)

Thiobarbituric Acid Reactive Substance was determined according to the method of Benjakul and Baeur (2001) with modifications. Ground chicken meat (1 g) was mixed with 5 mL of a solution containing 0.375% of 2-thiobarbituric acid (TBA), 15% trichloroacetic acid (TCA) and 0.25N HCl. The mixture was incubated in water bath at 95°C for 15 min, followed by cooling with running water. The mixture was centrifuged at 3600 x g, 4°C for 20 min (Combi 514R, Hanil BioMed Inc., Korea). The supernatant was collected and the absorbance was read at 532 nm using Implen Nanophotometer P330 (Implen GmbH, München, Germany). TBARS value was calculated from the standard curve of malonaldehyde (0-2 ppm) by extrapolation and results were expressed as mg malonaldhyde/kg wet sample. The same procedure was repeated on 3, 5, 7 and 9 days of refrigerated storage at 4°C.

Microbiological analyses

Breast meat (200 g) from five carcasses of previously slaughtered birds from both NC and NP meat samples were kept in zipper bags and stored at 4°C. At predetermined days (day 1, 3, 5, 7 and 9); samples were taken for microbial analysis.

Total viable count

On each sampling day, 5 g of ground breast meat samples was added to 45 ml of sterile phosphate buffered saline solution. The mixture was homogenised in the stomacher (Stomacher® 400 Circular Seward). Appropriate dilutions were transferred to already prepared plate count agar (PCA) (Oxoid CM0361). Plates were incubated at 37°C for 24 hr to enumerate the viable plate counts.

Lactic acid bacteria count

Ground breast meat (5 g) sample was added to 45 ml of sterile phosphate buffered saline. The mixture was homogenised in the stomacher (Stomacher®400 Circular Seward). Appropriate dilutions were spread on de Man, Rogosa and Sharpe (MRS) agar (Oxoid CM0361) plates containing 0.8% calcium carbonate. Plates were incubated anaerobically in anaerobic jars with AneroGen TM (Oxoid) at 37°C for 48 h. The colonies obtained were tested for catalase activity by placing a drop of 4% hydrogen peroxide solution on the cells. Bubbles formation indicated the presence of catalase in the cells. Gram staining was done and the morphology of the bacteria was observed using a Nikon microscope (Nikon Eclipse 80i).

Data analysis

All data obtained were analysed using the Student's t-test of Minitab 16 and the level of significance was determined at $P \leq 0.05$.

Results and Discussion

Drip losses and colour measurement

A significant decrease ($P<0.05$) in drip loss was observed after 24 h refrigerated storage in NC broiler meat (0.43%) compared to NP (0.58%) meat samples (Table 1). However, no significant difference ($P>0.05$) was observed between cook loss of NC (16.64%) and NP (18.12%) samples. The ability of fresh meat to retain water is one of the most important quality attributes of raw meat products (Huff-lonergan and Lonergan, 2005). The water holding capacity is the ability of meat to hold water under stress condition and can be evaluated from the amount of drip and cook losses (Zayas, 1997). Higher drip and cook losses observed in NP meat samples is consistent with the results obtained by Addeen *et al*. (2014) and D'Agata *et al*. (2009) who observed that Islamic

Table 1. Drip losses, pH and colour (24 h post-mortem) of chicken meat obtained from NC and NP[a].

Parameters	NC	NP	Statistical Significance
Drip loss (%)	0.48±0.08	0.58±0.05	*
L*	53.77±3.60	52.07±1.98	*
a*	6.88±0.81	8.36±0.71	*
b*	18.13±5.02	19.20±3.74	N.S
Cooking loss (%)	16.64±1.27	18.12±1.54	N.S
pH	5.95±0.14	6.17±0.15	*

[a]Means ± standard deviation (n=30), NC = Neck cut by severing the jugular veins, carotid arteries, trachea and the oesophagus; NP = neck poking using a sharp object; * means significant at P<0.05, NS means not significant at P<0.05. All analysis was carried with 24h refrigerated samples

slaughtered animals (chickens and cattle) showed lower drip loss compared with those slaughtered by conventional, Non-Halal methods. Lawrie (1998) reported that a high drip loss is associated with the loss of valuable protein and flavour compound hence making the meat product of poor quality. Also, Cheng and Sun (2008) associated stress and different slaughtering methods as an important factor that influences drip loss. Therefore, the high drip and cook loss observed in NP meat samples may be attributed to stressful slaughtering resulting in the depletion of the glycogen reserve in the muscle.

The CIE system of colour profile as lightness *(L^*),* redness *(a^*)* and yellowness *(b^*)* of meat samples is shown in Table 1. The L^* (lightness) values of meat samples was significantly (P<0.05) higher in NC samples compared to NP samples. Similarly, significant (P<0.05) difference was observed in a^* (redness) values of both NC (6.88) and NP (8.36). However, no significant (P>0.05) difference was recorded for the yellowness *(b^*)* value of NC (18.13) and NP (19.20). Alvarado et al. (2004) and Bourbab and Idaomar (2012) reported a significant (P<0.05) lower meat colour L^* (lightness) and a^*(redness) values for perfectly bled and imperfectly bled broiler chickens respectively. Similar observation was noted in NC broiler chicken. In contrast a higher a^* value was observed for NP that could be as a result of residual blood left in the carcass of birds (Bourbab and Idaomar, 2012)

Ultimate pH (pHu) after 24 h refrigeration

The ultimate pH of chicken meat after 24 h post-mortem is shown in Table 1. After 24 h of refrigerated storage, pH of meat was significantly (P < 0.05) lower in NC compared to NP samples. High pH observed in meat samples indicates depletion of glycogen as a result of animal stress before or during slaughtering (Hambrecht *et al*., 2004). Stress during slaughtering process aids glycogen use up and reduction in the level of lactic acid by bringing the animal to early rigor mortis. Also the high pHu can also be attributed to the residual blood in the carcass as a result of imperfect bleeding (Bourbab and Idaomar, 2012). Also, a negative relationship was reported (Fletcher, 1995; Allen *et al.*, 1997; Barbut, 1998) between breast meat L^* value and breast pH. Hence, the high ultimate pH observed in NP samples after 24 h of refrigerated storage can be attributed to stressful slaughtering and imperfect bleeding.

Haem iron content

A significant (P<0.05) decrease in the haem iron content of breast meat was observed during 9 d storage at 4°C (Table 2). At 1, 3, 5 and 9 d of storage at 4°C, NC samples recorded a significant (P<0.05) lower haem content values compared to NP samples and, the haem iron content were 2.55, 2.17, 1.98, and 1.31 mg/100 g sample for the 1, 3, 5 and 9 days of storage respectively. A similar result was reported by Addeen *et al*. (2014) and Luciano *et al*. (2009) for chicken and lamb meat. Declines in haem iron content with increasing storage time were probably due to haem breakdown, causing the release of non-haem iron (Benjakul and Bauer, 2001). This released non haem-iron can stimulate lipid oxidation of muscle during extended storage (Tappel, 1995). It has also been reported that L^* and a^* values of breast and thigh meat decreased with storage time (Yang and Chen, 1993), hence this can explain the drop observed in the haem iron content as storage time increased. Furthermore, Purchas *et al.* (2003) reported that the drip losses from meat during storage contained significant amount of iron and particularly soluble haem iron. This decline in the haem iron content as storage time increases can also be attributed to the loss iron in meat as a form of

Table 2. Effect of slaughtering methods on haem iron content of broiler chicken meata

Storage time (days)	NC mg/100g	NP mg/100g	Statistical Significance
1	2.55±0.21	3.25±0.29	*
3	2.17±0.08	2.97±0.11	*
5	1.98±0.14	2.75±0.07	*
7	1.76±0.16	2.36±0.29	NS
9	1.31±0.26	2.05±0.20	*

[a]Means ± standard deviation (n=5), NC = Neck cutting; NP = Neck poking, * means significant at P<0.05, NS means not significant at P<0.05.

Table 3. The TBARS values (mg MDA/kg sample) of broiler breast meat of both NC and NP during 9 days refrigerated storage at 4°C[a].

Storage time (days)	NC	NP	Statistical Significance
1	0.47±0.03	0.65±0.05	N.S
3	0.77±0.05	0.85±0.04	N.S
5	1.31±0.05	1.48±0.07	*
7	1.44±0.03	1.53±0.05	*
9	1.52±0.04	1.59±0.04	N.S

[a]Means ± standard deviation (n=5) NC = Neck cutting; NP = Neck poking, * means significant at P<0.05, NS means not significant at P<0.05.

drip losses in this present study.

Thiobarbituric acid reactive substances (TBARS)

The TBARS values were estimated from the standard curve of malonaldehyde (Table 3). An increase in TBARS values was observed in the breast meat during 9 d storage at 4°C. The result from this study is similar to findings of Chueachuaychoo *et al.* (2011), Addeen *et al.* (2014) and Nakyinsige *et al.* (2014) who all reported a significant increase (P<0.05) in TBARS value with increase in storage time in chicken, turkey and rabbit meat respectively. However, TBARS values obtained for both NC and NP did not reach the 5 mg MDA/kg meat and above acceptable point for detecting fitness for human consumption as reported by Insausti *et al.* (2001). Nakyinsige *et al.* (2014) attributed the extent of lipid oxidation to pre-slaughter stress and early post-mortem pH decline.

Total viable count

The total viable counts of NP samples during 9 d refrigerated storage at 4°C were consistently higher than those samples from NC (Figure 1). Similar observations were reported by Alli *et al.* (2011); the total viable count of poultry slaughtered by three methods increased with storage time. They reported that the Halal slaughtered chicken recorded lower total viable counts during 6 h (3.82 $\log_{10}$CFU/g) and 96 h (8.71 $\log_{10}$ CFU/g) of refrigerated storage at 4°C compared to electrically stunned chickens (3.88 $\log_{10}$CFU/g and 8.79 $\log_{10}$CFU/g). In addition, Alvarado *et al.* (2007) reported a lower total viable count for broiler chicken slaughtered by unilateral neck cutting without stunning at day 0 (3.05 $\log_{10}$CFU/g) and day 5 (3.67 $\log_{10}$CFU/g) of refrigerated storage at 4°C compared to CO_2 stunned bled birds (3.05 $\log_{10}$CFU/g and 3.72 $\log_{10}$CFU/g) and CO_2 stunned un-bled birds (3.85 $\log_{10}$CFU/g and 5.05 $\log_{10}$CFU/g). The method of slaughter used in this study was also without stunning, but the birds slaughtered by the NP were done by poking the neck without neck cutting. A negative relationship exists between blood loss as a result of slaughter method and microbial count (Nakyinsige *et al.*, 2014). Blood is an excellent medium for bacterial growth due to its high nutritive value which serves as substrate for most bacteria (Alvarado *et al.*, 2007; Alli *et al.*, 2011; Addeen *et al.*, 2014; Nakyinsige *et al.*, 2014). The high microbial counts in NP meat samples observed during refrigerated storage may be caused by lower blood loss during slaughter. Bacteria count between 10^7 and 10^9 CFU/cm^2 during refrigerated storage is used as the cut-off point for determining fitness for

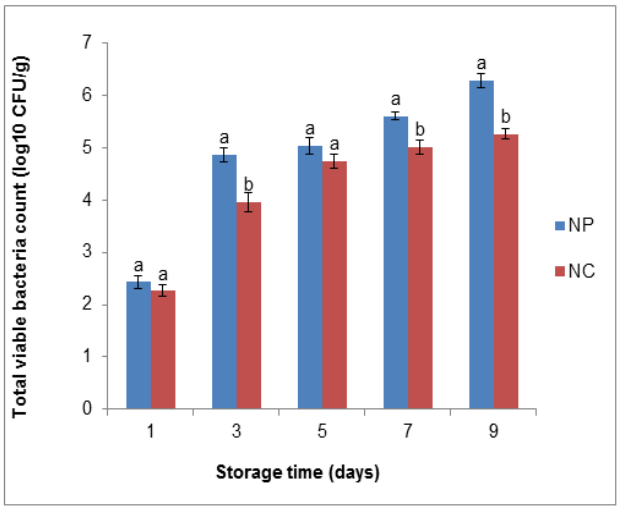

Figure 1. Total viable count of NC and NP samples during 9 d of storage at 4°C. NC= Neck cutting method, NP= Neck poking method. ab means with different letters differ significantly at P<0.05.

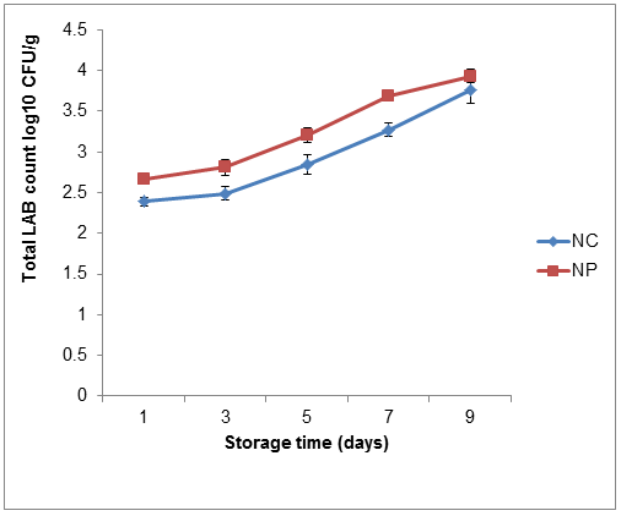

Figure 2. LAB count (log_{10} CFU/g) of NC and NP meat samples during 9 d of refrigerated storage at 4°C

human consumption (Insausti *et al.*, 2001; Jeremiah, 2001).

The microbial count of both NC and NP were within the cut-off point limit and, therefore may be acceptable for human consumption. Also, meat ultimate pH (>6.0) significantly affect the growth of spoilage bacteria (Lawrie and Ledward, 2006). Therefore, the high ultimate pH (6.17) in the NP meat may be one of the reasons responsible for high total viable count.

Lactic acid bacteria count

Lactic acid bacteria behave as facultative anaerobes and are capable of growing under high CO_2 concentration. The LAB count in this present study continuously increased throughout the 9 d of storage at 4°C with the NP samples recording higher LAB counts compared to the NC samples (Figure 2). Similar result was reported by Jouki and Khazaei (2011) who also observed an increase in LAB counts of camel meat during 18 d of storage at 4°C.

However, recently Sabow *et al.* (2015) reported that slaughter methods did not significantly affect LAB counts in goat meat (chevon) during 7 d of storage at 4°C. Although, Halal slaughter (without stunning) recorded lower LAB counts throughout 7 d storage compared to anaesthesia slaughter (anaesthesia with halothane before exsanguination). They attributed the higher level of LAB growth in anaesthesia slaughter group to a faster pH decline caused by minimal anaesthesia that caused aging to start earlier in the goat meat. Nortje and Shaw (1989) suggested that spoilage ensues in meat products when the lactic acid bacteria count reaches 7 log CFU/g. However, the LAB counts in NP and NC did not reach 7 log CFU/g during the 9 d of refrigerated storage.

Conclusion

These results indicate that methods of slaughtering affect the chemical and microbiological changes in chicken meat during refrigerated storage. However, chickens slaughtered by neck cutting following the Halal method of slaughter had the least lipid oxidation, haem iron content and total bacteria count during the 9 d refrigerated storage period used in this study. Hence, to ensure a better shelf life for chicken meat products during refrigerated storage, neck cutting in accordance with the Islamic ritual method of slaughter is the most favourable to ensure maximum blood drainage and better quality meat.

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