CHAPTER IV

EFFECT OF SLAUGHTERING METHODS ON CHEMICAL CHANGES OF BROILER CHICKEN MEAT DURING REFRIGERATED STORAGE

4.1 INTRODUCTION

Chicken 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 (Liu et al., 2012). However, chicken meat is a perishable product which deteriorates quickly if it is improperly handle.

Processing of poultry involves slaughtering, packaging and storage. 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. Lipid oxidation, protein degradation and the loss of other valuable molecules are the consequence of meat spoilage process. There are three main mechanisms responsible for meat spoilage during refrigerated storage post mortem: (a) microbial spoilage, (b) lipid oxidation and (c) autolytic enzymatic spoilage (Dave & Ghaly, 2011).

Microbial spoilage by psychrotrophs are the major cause of spoilage in meat at chilling temperature. They belong to microbial genera of both Gram positive such as lactic acid bacteria and Gram negative bacteria such as *Pseudomonas* spp. and *Enterobacteriaceae* (Gill & Newton, 1978; Holzapfel, 1998). Species of *Pseudomonas* are particularly responsible for spoilage of meat stored at refrigerated temperature (Labadie, 1999; Jay et al., 2003; Ercolini et al., 2007). However, the microflora of vacuum packaged chill stored meat in most cases is characterised by psychrotrophic lactic acid bacteria (Dainty et al., 1992; Borch et al., 1996; Nychas et al., 1998).

Lipid oxidation (often measured with *TBARS test*) is an important factor after microbial spoilage that causes meat deterioration in both fresh and cooked meat products (Ladikos & Lougovois, 1990; Nam et al., 2002; Ahn et al., 2009). Lipid oxidation controls the formation of free radicals which forms aldehydes; a volatile compound responsible for the development of rancid flavours and change meat colour (Guillen & Guman, 1998; Ahn et al., 2009). The rate of lipid oxidation in refrigerated meat products is dependent on fat content, fatty acid composition, haem pigments and transition metal ion content particularly iron and copper (Addis, 1986; Du et al., 2000; Choe & Min, 2006; Min et al., 2008). TBARS values between 5 mg MDA/kg meat and above (Insausti et al., 2001) are considered as acceptable threshold for detecting fitness for human consumption.

Since blood component especially haemoglobin has been reported to be a powerful promoter of lipid oxidation (Alvarado et al., 2007), there is a need to evaluate the extent of lipid oxidation as affected by residual blood, haem pigments, iron and copper in broiler chicken slaughtered by two methods (HM and NHM) meat during refrigerated storage. Hence the objective of this study was to determine the effect of slaughtering methods (HM and NHM) on the chemical changes that occur during refrigerated storage of broiler chicken meat.

4.2 MATERIALS AND METHODS

Breast meat (200 g) from five carcasses of previously slaughtered birds form both HM and NHM were kept in polythene bags and stored at 4°C. At predetermined time intervals (day 1, 3, 5, 7 and 9) samples were taken for further analysis.

4.2.1 Determination of Mineral Contents

Mineral content namely iron (Fe^{2+}), calcium (Ca^{2+}), zinc (Zn^{2+}), magnesium (Mg^{2+}), copper (Cu^{2+}) and manganese ($Mn^{2+)}$ were determined using the wet method of minerals determination as described by Addeen et al. (2014). One day refrigerated breast meat (0.5 g) was digested in HNO_3/H_20_2 (2:1) using a Perkin Elmer microwave reaction system (Model Anton Paar Multiwave 3000) equipped with eight high-pressure quartz vessels. After digestion was completed, the mixture was transferred to a volumetric flask and the volume was made up to 100 ml with deionised water.

The reference elements standards were prepared as listed in Appendix 1 then measured by a Perkin-Elmer Analyst 800 atomic absorption spectroscopy. The mineral content was calculated as shown below and expressed as mg/kg wet sample.

$$\text{mg mineral/kg sample} = \frac{X \times TV \times 100}{\text{Sample weight} \times 1000}$$

Where X = concentration of the sample in mg/L

TV = total volume of sample in ml

4.2.2 Determination of haem iron content

Haem iron content of chicken meat was determined by the method of Cheng and Ockerman (2004) 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) allowed to stand for a few minutes. The absorbance of the clear filtrate was read at 640nm using Implen Nanophotometer P330 (Implen GmbH, München, Germany) against an acid acetone as blank. Haem iron content was calculated as follows;

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

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

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

4.2.3 Determination of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined according to the method of Benjakul and Bauer (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 3600xg, 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.

4.2.4 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

4.2.4.1 Sample Preparation

Protein from chicken breast was extracted after 1 and 9 d storage at 4°C following the method of Zaman et al. (2012) with modifications. Approximately 1 g of muscle sample was homogenized cold (on ice) using homogenizer (Yellow line DI 25 basic, Colonial Scientific, Richmond, VA) in 10 ml of 0.25 M phosphate buffer (pH 7.5) with 0.1% triton X-100. Solution was then centrifuged at 6000×g for 30 min at 4° C (Combi 514R, Hanil BioMed Inc., Korea.) and supernatant was collected and preserved in 1ml aliquots at -80^{0}C.

4.2.4.2 Protein Quantification

Protein was quantified using the Bradford assay following the method of Bradford (1976). Bradford reagent (GE Healthcare, Uppsala, Sweden) 800 µl of was mixed with 200 µl of sample solution. Absorbance was measured at 595nm using Implen Nanophotometer P330 (Implen GmbH, München, Germany). Protein concentration was determined from the standard curve from serial dilutions of bovine serum albumin (BSA, BIO-RAD USA).

4.2.4.3 Preparation of separating gel solution (12.5%)

The separating gel solution was prepared as described by Laemmli (1970) as shown in Table 6. After the mixture has been completely dissolved, the gel mixture was immediately loaded into the casing with a pipette and filled to the line on the casing. It was allowed to polymerize for about 20-30 min at room temperature.

Table 6: Separating gel solution (12.5% Acrylamide)

Ingredients	Volume (ml)
H_2O	10.2
0.5 M Tris-HCl, pH 8.8	7.5
20% (w/v) SDS	0.15
Acrylamide/Bis-acrylamide (30%/0.8% w/v)	12.0
10% (w/v) ammonium persulfate (APS)	0.15
TEMED	0.02

4.2.4.4 Preparation of stacking gel solution (4%)

The stacking gel was also prepared as described by Laemmli (1970) as shown in Table 7. The stacking gel solution was carefully loaded on the top of the separating gel. After pouring the stacking gel, the comb was carefully inserted on the top of the casing, and left for about 10-15 min to solidify at room temperature.

Table 7: Stacking Gel Solution (4% Acrylamide)

Ingredients	Volume (ml)
H_2O	3.075
0.5 M Tris-HCl, pH 6.8	1.25
20% (w/v) SDS	0.67
Acrylamide/Bis-acrylamide (30%/0.8% w/v)	0.67
10% (w/v) ammonium persulfate (APS)	0.025
TEMED	0.005

4.2.4.5 Sample buffer Preparation

Stock sample buffer (0.06 M Tris-C1, pH 6.8, 2% SDS, 10% glycerol, 0.025% Bromphenol Blue) was prepared as shown in Table 8.

Table 8: Preparation of the sample buffer

Ingredients	Volume (ml)
H_2O	4.8
0.5 M Tris-HCl, pH 6.8	1.2
10% (w/v) SDS	2.0
Glycerol	1.0
0.5% Bromphenol Blue (w/v water)	0.5

4.2.4.6 Preparation of running buffer

Running buffer (1000 ml) was prepared by mixing 0.025 M Tris base, 0.192 M glycine, 0.1% (w/v) SDS with distilled water at pH 8.3.

SDS-PAGE was used to detect the rate of protein denaturation during refrigerated storage using the method described by Laemmli (1970) with 12.5% acrylamide separating gel and 4% acrylamide stacking gel. The gel was subjected to electrophoresis at 120 volts for 120 min using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). Protein bands were stained with Coomassie Brilliant Blue R-250 (Bio Basic Canada Inc.) overnight and destained with 40% methanol and 10% acetic acid. A standard marker (Bio Rad laboratories, Inc. Bio-Rad) was used as reference to estimate the molecular weight of the protein bands. Determination of molecular weight of protein bands obtained was calculated as listed in Appendix 2.

4.2.5 Data Analysis

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

4.3 RESULTS

4.3.1 Mineral Contents of Broiler Chicken Meat

Six mineral (Fe, Cu, Mg, Mn, Ca and Zn) of fresh breast meat sample were evaluated using the atomic absorption spectrophotometer (Table 9). The Fe, Ca and Mg of breast meat samples from HM were significantly ($P<0.05$) lower than NHM. Fe content of HM was 10.00mg/kg, Ca content was 120.30 mg/Kg and Mg content was 302.34 mg/Kg. The Fe, Ca and Mg content of NHM were 13.77mg/kg, 147.87 mg/Kg, and 317.10 mg/Kg respectively.

However, Zn and Mn content of HM was significantly ($P<0.05$) higher than NHM. The Zn and Mn content of HM were 7.20 and 8.58 mg/Kg, respectively. The Zn content of NHM was 0.80 mg/Kg and Mn content was 1.48 mg/Kg. No significant ($P>0.05$) difference was observed in Cu content for both HM (0.76 mg/Kg) and NHM (1.48 mg/Kg).

Table 9: Effect of slaughtering methods on mineral contents of broiler chicken meat[a]

Mineral Contents (mg/kg)	HM	NHM	Statistical Significance
Zn	7.20±0.71	0.80±0.43	*
Mg	302.34±2.49	317.10±1.46	*
Fe	10.00±1.64	13.77±2.76	*
Cu	0.76±0.39	1.48±0.61	N.S
Ca	120.30±2.41	147.87±2.70	*
Mn	8.58±2.74	1.48±1.90	*

[a]HM = Halal method; NHM = Non-Halal method, n = 5, * means significant at P<0.05, NS means not significant at P<0.05.

4.3.2 Haem Iron Content of Broiler chicken meat during Storage

A significant (P<0.05) decrease in the haem iron content of breast meat was observed during 9 d storage at 4°C (Table 10). At 1, 3, 5 and 9 d of storage at 4°C, HM samples recorded a significant (P<0.05) lower haem content values compared to NHM samples and, the haem iron content were 2.55, 2.17, 1.98, and 1.31 mg/100 g sample, respectively. The haem iron content of NHM samples was higher during 9 d storage at 4°C and ranged between 2.05-3.25 mg/100g sample. However, on day 7 of storage at 4°C, no significant difference (P>0.05) was observed in the haem iron content values for both HM and NHM samples although HM recorded a lower value (1.76 mg/100 g sample) compared to NHM (2.36 mg/100 g sample).

Table 10: Effect of slaughtering methods on haem iron content of broiler chicken meat[a]

Storage time (days)	HM mg/100g	NHM 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]HM = Halal method; NHM = Non-Halal method, n = 5, * means significant at P<0.05, NS means not significant at P<0.05.

4.3.3 Thiobarbituric Acid Reactive Substance (TBARS)

Extent of lipid oxidation in broiler chicken breast meat during 9 d of storage at 4°C was evaluated by measuring the TBARS values (Table 11). The TBARS values were estimated from the standard curve of malonaldehyde (Figure 4). An increase in TBARS values were observed in the breast meat during 9 d storage at 4°C. Slaughter methods had no significant (P>0.05) effect on broiler chicken meat lipid oxidation on 1, 3 and 9 d of storage although the TBARS values increases with storage time. The TBARS values of HM on 1, 3 and 9 d of storage were 0.47, 0.77 and 1.52 MDA/kg sample, respectively. Similarly, NHM recorded TBARS values of 0.65, 0.85 and 1.59 MDA/kg sample respectively on day 1, 3, and 9 of storage at 4°C.

However, on day 5 and 7 of storage at 4°C, significant (P<0.05) differences were observed in the TBARS values. NHM showed significantly (P<0.05) higher values of

1.48 and 1.53 MDA/kg sample compared to 1.31 and 1.44 MDA/kg sample recorded for HM.

Figure 4: Standard curve of malonaldehyde (0-2ppm)

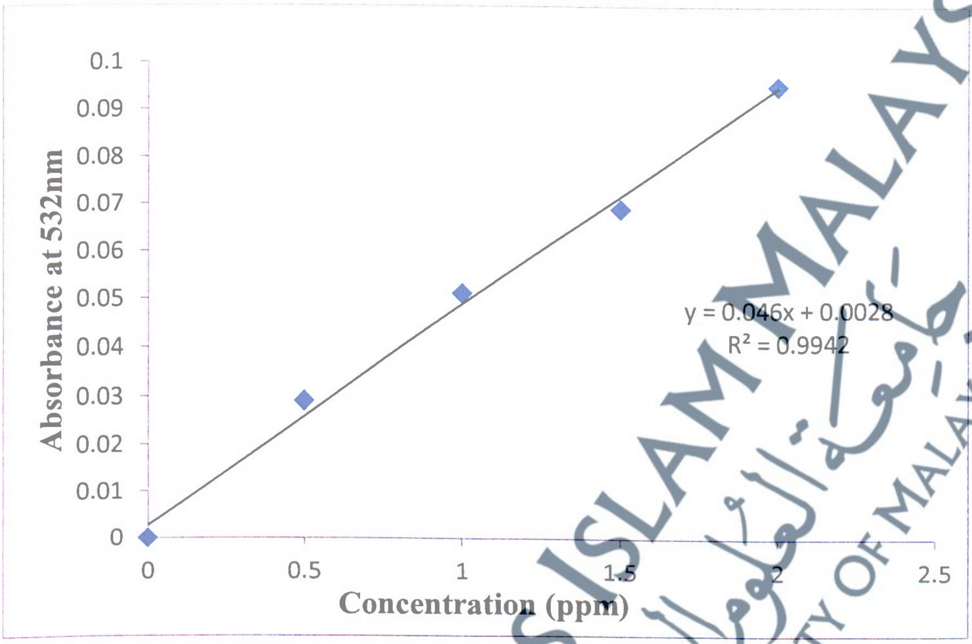


Table 11: The TBARS values (mg MDA/kg sample) of broiler breast meat of both HM and NHM during 9 days refrigerated storage at 4°C[a].

Storage time (days)	HM	NHM	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] HM = Halal method; NHM = Non-Halal method, n = 5, * means significant at P<0.05, NS means not significant at P<0.05.

4.3.4 Protein Degradation during 4°C storage

The changes in breast meat muscle proteins were extrapolated from the standard curve of bovine serum albumin (BSA) (Figure 5). The protein concentration in broiler chicken meat slaughtered by HM and NHM method ranged between 1.28-1.52mg/ml (Table 12). At day 1 of storage at 4°C the protein concentration in both HM and NHM were 1.52 mg/ml and 1.44 mg/ml respectively. However, at day 9 of storage at 4°C, a significant decrease in the protein concentration was observed in both HM (1.39 mg/ml) and NHM (1.28 mg/ml).

Protein degradation occurred in the broiler chicken breast meat during storage as indicated in the protein patterns of HM and NHM (Figure 6). Similar protein patterns were observed during 1 day refrigeration at 4°C for both HM and NHM. Six protein bands were obtained during 1 day refrigerated storage at 4°C and the molecular weight of the band ranged between 44KDa-146.8KDa. However, at day 9 of storage at 4°C, additional bands were recorded for HM (29.1KDa) and NHM (7.7KDa and 29.1KDa respectively).

Figure 5: A standard curve of Bovine Serum Albumin (BSA)

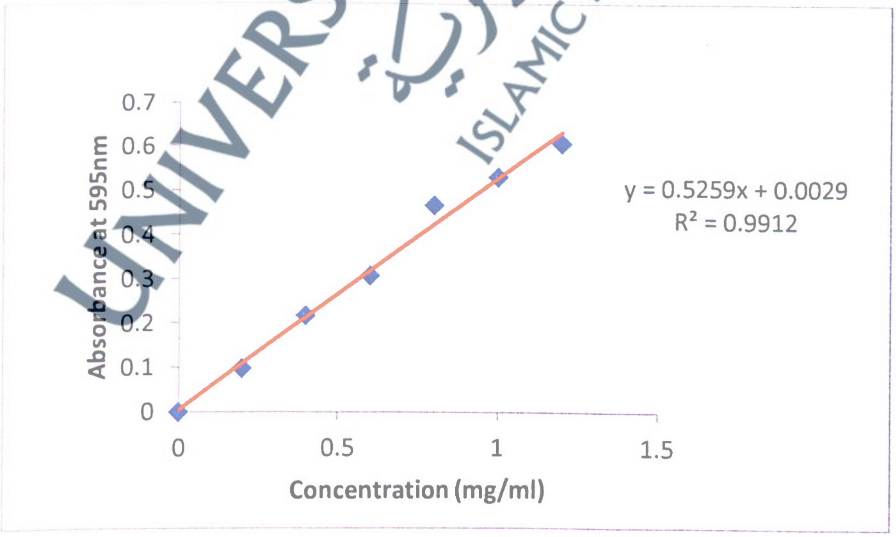
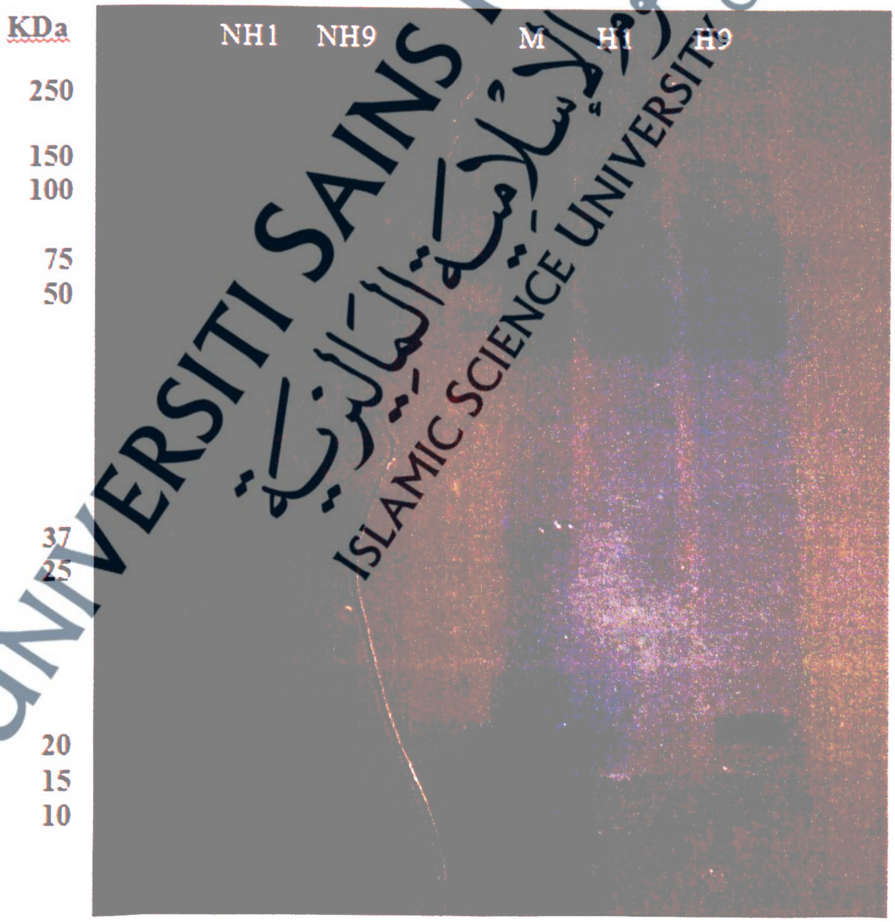


Table 12: Protein concentration of chicken meat solubilized in phosphate buffer[a]

Sample ID	Protein Concentration (mg/ml)
NH1	1.44±0.11
NH9	1.28±0.06
H1	1.52±0.08
H9	1.39±0.09

[a] NH1= Non-Halal sample after 24 h refrigeration at 4°C, NH9= Non-Halal sample after 9 days of refrigerated storage at 4°C, H1=Halal sample after 24 h refrigeration at 4°C, H9= Halal sample after 9 days of refrigerated storage, n = 5

Figure 6: SDS-PAGE patterns of broiler breast meat in 12.5% acrylamide separating gel and 4% acrylamide stacking gel at day 1 and day 9 of storage at 4°C. NH1= Non-Halal sample at day 1; NH9= Non-Halal sample at day 9; M= Molecular weight marker; H1= Halal sample at day 1; H9= Halal sample at day 9.



4.4 DISCUSSION

Iron is an important element of blood production and a large percentage of iron is found in the red blood cell of the blood called haemoglobin. Dietary iron present in meat exists in two forms as haem and non-haem iron. Haem iron is derived from the haemoglobin which is an important blood component. Haemoglobin is composed of four polypeptide chains with each chain containing one haem group; each haem consists of an iron atom coordinated inside the porphyrin ring (Richard et al., 2007). A strong positive relationship has been reported to exist between residual blood, haemoglobin content and iron content (Addeen et al., 2014; Nakyinsige et al., 2014). Oellingrath et al. (1990) reported that the haemoglobin content in meat is dependent on the extent of bleeding of the carcass.

A significantly ($P<0.05$) low Fe content in HM meat samples is indicative of effective blood drainage in the carcass of the chicken since blood contains iron. High Fe content recorded for NHM meat shows low blood bleed out in the chicken. The result from this study is consistent with that obtained by Addeen et al. (2014) where low iron content was also reported for Islamic slaughtered birds compared to un-bled, conventional and decapitation methods of slaughter which recorded higher iron content values. Transition metals especially Fe and Cu have been reported to be a major promoter of lipid oxidation (Thanonkaew et al., 2006). In this study, it was observed that HM meat samples has significantly ($P<0.05$) lower Fe and Cu values compared to NHM.

A significant ($P<0.05$) decrease in the haem iron content was observed as storage time increased. 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 & 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 & Chen 1993), hence this can explain the drop observed in the haem iron content has 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 could also explain the decline in the haem iron content as storage time increases in this present study. A low haem iron content and Fe content in HM meat samples confirm effective blood removal in the carcass of broiler chicken meat.

Lipid oxidation is among the most critical quality parameters because it does not only interacts with protein oxidation (Hall, 1987) that causes meat discoloration (Guillen-Sans & Guman-Chozas, 1998), but also primarily responsible for producing many off-odour and rancid flavour in the meat during extended storage (Vercellotti et al., 1992; Min et al., 2005). Inefficient and improper bleeding may cause more blood (haemoglobin) to be retained in the breast meat. This retained haemoglobin could cause increased oxidation causing rancidity and reduced shelf life.

The 2-thiobarbituric acid reactive substances (TBARS) assay is most commonly used to determined lipid oxidation in meat which determines malondialdehyde (MDA) content (Broncano et al., 2009). The result obtained from this study shows that TBARS values increased with storage time in both HM and NHM (Table 11). At 1, 3 and 9 d post mortem, TBARS values of both HM and NHM were not significantly different (P>0.05) but values obtained for HM was lower compared to NHM. However a significant difference (P<0.05) in the TBARS values was observed on day

5 and 7 of refrigerated storage with HSM having the lower value of TBARS compared to NHM. The result from this study is similar to findings of Nakyinsige et al. (2014), Addeen et al. (2014), Chueachuaychoo et al. (2011), and Ziprin (2001) who also reported a significant increase ($P<0.05$) in TBARS value with increase in storage time in chicken, turkey and rabbit meat. However, TBARS values obtained for both HM and NHM did not reach the 5 mg MDA/kg acceptable threshold considered safe 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. Richard and Hultin (2002) also observed that effective bleeding significantly reduced rancidity in mackerel fish stored at 2^0C. Therefore, the high TBARS values recorded for NHM meat samples may be due to high a* (redder) and pH value (Table 4) and high Fe values (Table 9). HSM meat samples with lower TBARS value indicate that effective bleeding is essential to reduce the degree of lipid oxidation in meat.

Protein degradation involves the enzymatic breakdown of complex protein of meat tissues into simpler ones resulting in softening of meat. The enzymes can either be endogenous or microbial proteases (Masniyom et al., 2004). SDS-PAGE is a reliable method widely used to qualitatively analyse protein degradation during post-mortem storage by determination of molecular weight (Penny & Ferguson-Pryce, 1979). Protein bands from this study were found to be dispersed between higher (146.8 KDa) and lower (7.7 KDa) molecular weight (Figure 6). The protein pattern of breast chicken meat from both HM and NHM during day 1 of storage at 4^oC showed similar patterns with no observable changes. This result is consistent with the findings of Addeen et al., (2014) who also reported no observable changes in the protein patterns of chicken slaughtered by conventional neck cutting, decapitation, Islamic ritual method and un-bled method during day 0 of refrigerated storage at 4°C. However, at day 9 of refrigerated storage at 4°C, new bands of 29.1 KDa (HM) and 7.7 KDa (NHM) were produced. Protein degradation during extended storage time could be a consequence of spoilage microflora metabolism or due to endogenous enzymes (Signorini et al., 2007; Addeen et al., 2014). The discovery of a band with molecular weight of 7.7 KDa on day 9 of refrigerated storage at 4°C in NHM may be responsible for the tissue softening observed in the NHM meat.

4.5 CONCLUSION

Results obtained from this study showed that method of slaughter affected the chemical changes in meat during refrigerated storage at 4°C to some extent. HM meat showed better keeping quality with low iron content, haem content, lipid oxidation and lesser extent of tissue softening. In contrast, high values of these parameters during refrigerated storage as recorded in NHM meat indicate poor keeping quality.