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ORIGINAL ARTICLE

Changes of microbial spoilage, lipid-protein oxidation and physicochemical properties during post mortem refrigerated storage of goat meat

Azad Behnan SABOW,[1,7] Awis Qurni SAZILI,[1,2,6] Zeiad Amjad AGHWAN,[5,8] Idrus ZULKIFLI,[1,2,6] Yong Meng GOH,[2,3] Mohd Zainal Abidin AB KADIR,[5,9] Khadijah NAKYINSIGE,[1,10] Ubedullah KAKA[4] and Kazeem Dauda ADEYEMI[1,11]

[1]*Department of Animal Science, Universiti Putra Malaysia,* [2]*Institute of Tropical Agriculture, Universiti Putra Malaysia,* [3]*Department of Veterinary, Universiti Putra Malaysia,* [4]*Department of Veterinary Clinical Studies, Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia,* [5]*Department of Electrical and Electronic Engineering, Faculty of Engineering, Universiti Putra Malaysia,* [6]*Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Selangor, Malaysia,* [7]*Department of Animal Resource, University of Salahaddin, Erbil,* [8]*Department of Animal Science, College of Agriculture, University of Mosul, Mosul, Iraq,* [9]*Centre for Electromagnetic and Lighting Protection Research (CELP),* [10]*Department of Food Science and Nutrition, Islamic University, Mbale, Uganda; and* [11]*Department of Animal Production, University of Ilorin, Ilorin, Nigeria*

ABSTRACT

Examined was the effect of post mortem refrigerated storage on microbial spoilage, lipid-protein oxidation and physicochemical traits of goat meat. Seven Boer bucks were slaughtered, eviscerated and aged for 24 h. The Longissimus lumborum (LL) and Semitendinosus (ST) muscles were excised and subjected to 13 days post mortem refrigerated storage. The pH, lipid and protein oxidation, tenderness, color and drip loss were determined in LL while microbiological analysis was performed on ST. Bacterial counts generally increased with increasing aging time and the limit for fresh meat was reached at day 14 post mortem. Significant differences were observed in malondialdehyde (MDA) content at day 7 of storage. The thiol concentration significantly reduced as aging time increased. The band intensities of myosin heavy chain (MHC) and troponin-T significantly decreased as storage progressed, while actin remained relatively stable. After 14 days of aging, tenderness showed significant improvement while muscle pH and drip loss reduced with increase in storage time. Samples aged for 14 days had higher lightness ($P < 0.05$) and lower ($P < 0.05$) yellowness and redness. Post mortem refrigerated storage influenced oxidative and microbial stability and physico-chemical properties of goat meat.

Key words: *goat meat, lipid–protein oxidation, microbial spoilage, physicochemical traits, refrigerated storage.*

INTRODUCTION

Goat meat (chevon) has been recognized as lean meat with positive dietary values and it is one of the main sources of red meat in human diets (Ilie *et al.* 2012). Because of its relatively low intramuscular fat and cholesterol levels when compared to similar cuts in mutton and beef, goat meat is preferred by many meat consumers (Adam *et al.* 2010). In spite of its low lipid content, goat meat has a high proportion of unsaturated fatty acids and is a source of conjugated linoleic acid (Pearce *et al.* 2010) which are good for human health and have anti-inflammatory, anti-thrombotic and anti-atherosclerotic benefits. This has led to the development and expansion of goat meat industries worldwide in order to meet consumers' demands (Dhanda *et al.* 2003).

Meat quality is influenced by ante and post mortem factors. The ante mortem factor comprises animal species, sex, age, muscle groups, gene regulation and nutritional status (Hou *et al.* 2014), while post mortem factors basically involve the refrigerated storage of meat, often termed aging (Atanassova *et al.* 2008). During aging, the conversion of muscle to meat occurs along with quantitative transformations in many metabolites (Choi *et al.* 2010).

Correspondence: Awis Qurni Sazili, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia. (Email: awis@upm.edu.my)
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Consequently, meat becomes spoiled and unfit for human consumption (Doulgeraki *et al*. 2012). It has been reported that bacteria levels between log6 to log7 colony forming units (cfu)/2 cm or /g during refrigerated storage are critical levels for meat spoilage (Insausti *et al*. 2001). In addition to microbial spoilage, lipid and protein oxidation are also regarded as the main cause of sensory, functional and nutritional quality deterioration in meat (Dai *et al*. 2014). Metabolic and other processes lead to formation of reactive oxidative species, including hydroxyl, superoxide, peroxide and nitric oxide radicals that are able to interact with proteins and lipids during meat maturation and storage (Falowo *et al*. 2014). Lipid oxidation changes lead to unpleasant taste and odor, protein degradation, color deteriorations, reduction in shelf life and the accumulation of toxic compounds, which could affect consumers' health (Falowo *et al*. 2014; Sabow *et al*. 2015). Insausti *et al*. (2001) reported that thiobarbituric acid reactive substances (TBARS) values equal to or higher than 5 mg malondialdehyde/kg meat comprise the threshold for identifying fitness for human consumption. The chemical transformations emerging during protein oxidation are in charge of several biological modifications that affect meat quality, such as protein solubility and protein fragmentation and aggregation (Nieto *et al*. 2013). Moreover, these chemical changes have been reported to negatively affect meat color and tenderness as well as reduce its water holding capacity (Delles & Xiong 2014).

Although microbiological quality and shelf life of fresh meat during refrigerated storage have been the subject of many studies, most of them deal with beef (Marino *et al*. 2013), pork (Nieto *et al*. 2013), lamb (Santé-Lhoutellier *et al*. 2008), chicken (Delles *et al*. 2014) and rabbit (Nakyinsige *et al*. 2014) meat but not chevon. There is hardly any study relating lipid and protein oxidation to changes in physicochemical characteristics of goat meat. Therefore, the present study aimed to determine microbial spoilage, lipid and protein oxidation as well as some physicochemical traits of chevon during 14 days of refrigerated storage.

MATERIALS AND METHODS

Animal welfare

The experiment was undertaken following the animal ethics guidelines of the Research Policy of Universiti Putra Malaysia.

Animals and slaughtering procedure

A total of seven male crossbred Boer goats weighing 22.84 ± 1.66 kg and at about 7 months old were obtained from a commercial farm. The animals were slaughtered according to halal slaughter procedure as outlined in the Malaysian Standard MS1500: 2009 (Department of Standards Malaysia 2009). The slaughter procedure was conducted in a research abattoir at the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. The halal slaughter was performed by a licensed slaughterman. The head of each animal was pulled dorsally to stretch the neck in order to facilitate exsanguination. A transverse section was performed with a sharp knife. The neck cut severed skin, muscle, esophagus, trachea, carotid arteries, jugular veins and major nerves without decapitating the head.

Carcass sampling and storage

After evisceration and carcass dressing, the left Longissimus lumborum (LL) muscle from the sixth and eighth lumbar vertebrae was removed and divided into three parts. The first part was snap frozen in liquid nitrogen before being stored at −80°C and assigned for subsequent determination of pH (pre-rigor), myofibrillar fragmentation index (MFI), lipid and protein oxidation at day 0. The second part was dissected at three specific periods, that is, 1, 7 and 14 days, then properly labeled, vacuum packaged and stored in a 4°C chiller for drip loss determination (Honikel 1998). The last portion was directly stored at −80°C and assigned for subsequent determination of color and shear force at day 0. The Semitendinosus (ST) muscle was aseptically packed in stomacher bags and stored at 4°C for microbial enumeration. The remaining carcasses were then hung in the 4°C chiller and the subsequent sampling was carried out at specific periods (1, 7 and 14 days post mortem). Upon completion of each aging period, LL muscle chops of approximately 2.5 cm thick were dissected, labeled, vacuum packaged and stored at −80°C until subsequent analyses.

Microbiological analysis

At 0, 1, 7 and 14 days post mortem, a sample (5 g) of meat from ST muscle was drawn aseptically and transferred to a stomacher bag containing 45 mL of sterile 2.25% peptone water solution (Merk KGaA, Darmstadt, Germany). The sample was homogenized using a stomacher (Inter Science, Saint-Nom-la-Bretèche, France) for 2 min at room temperature. For microbial enumeration, 0.1 mL samples of serial dilutions (1:10 diluent and peptone water) of meat homogenates were spread on the surface of dry media. Ten-fold dilutions were spread on Petri dishes in duplicate for enumerations of total aerobic count (TAC) on Plate Count Agar (Merck KGaA, Darmstadt, Germany), lactic acid bacteria on Man, Rogosa and Sharpe agar (Merk KGaA, Darmstadt, Germany), *Enterobacteriaceae* on Violet Red Bile Glucose Agar (Merck KGaA, Darmstadt, Germany) and *Pseudomonas* spp. on Centrimide Agar (Merck KGaA,

Darmstadt, Germany). For all bacterial counts, plates were incubated at 32°C for 72 h, except for *Pseudomonas* spp. which was incubated at 25°C for 72 h (Bórnez *et al.* 2009). A colony counter (Stuart®, Burlington, VT, USA) was used for counting. The data (growth counts) were transformed to log10 values.

Muscle pH

Meat pH was determined using a portable pH meter (Mettler Toledo, AG 8603, Zurich, Switzerland) following the indirect method described by AMSA (2012). Approximately 0.5 g of each crushed muscle sample was homogenized (Wiggen Hauser® D-500, Berlin, Germany) for 30 s in 10 mL ice cold deionized water in the presence of 5 mmol/L sodium iodoacetate (Merck Schuchardt OHG, Hohenbrunn, Germany) to prevent further glycolysis. The pH of the resultant homogenates was measured using the electrode attached to the pH meter.

Determination of thiobarbituric acid reactive substances (TBARS)

The TBARS in the LL muscles was determined using QuantiChromTM TBARS Assay Kit (DTBA-100; BioAssay Systems, Hayward, CA, USA) following the manufacturer's description of the colorimetric protocol.

Determination of myofibrillar protein oxidation

Extraction of myofibrillar proteins

Myofibrillar proteins were isolated using an extraction buffer containing 150 mmol/L NaCl, 25 mmol/L KCl, 3 mmol/L MgCl2, 4 mmol/L ethylenediaminetetraacetic acid (EDTA) at pH 6.5 as described by Morzel *et al.* (2006). The total protein concentration of the sample was determined by the method of Bradford (1976) using Protein Assay Kit II 500-0002 (Bio-Rad, Hercules, CA, USA). Bovine serum albumen (BSA) was used to prepare protein standards.

Determination of free thiol content

Protein thiols were quantified following Elman's method using 2,2-dithiobis(5-nitropyridine) (DTNP) (Winterbourn 1990) with slight modifications by Morzel *et al.* (2006). The free thiol concentration was measured using a spectrophotometer (Spectronic Instruments Scottsdale, AZ, USA) at 386 nm and was calculated using an absorption coefficient of 14 g/cm. The final results were expressed as nanomoles of free thiols per milligram of protein.

Sodium dodecyl sulfate polyacrylamide get electrophoresis (SDS-PAGE)

Myofibrillar proteins were incubated for 10 min at 90°C in a sample buffer containing 30% (v/v) glycerol, 5% (v/v) ß-mercaptoethanol, 2.3% (w/v) SDS, 62.5 mmol/L Tris–HCl (pH 6.8) and 0.05% (w/v) bromophenol blue at a ratio of 1:1 (v/v). One-dimensional SDS-PAGE was performed according to the method of Laemmli (1970) using polyacrylamide gels of 8 cm × 5.5 cm (length × width) and 0.8 mm thickness. Twelve percent resolving gels were prepared for actin and troponin-T, whereas 5% resolving gels were prepared for myosin heavy chain (MHC). The resolving gels were overlaid with 4% stacking gel solution and kept overnight at 4°C to allow complete polymerization. The protein load was adjusted to 30 μg per lane. Proteins were separated in running buffer containing 0.025 mol/L Tris base, 0.192 mol/L glycine, 0.1% SDS at pH 8.3 under constant voltage of 120 V and 400 mA for 90 min, following which the tracking dye reached the bottom of the gel. Protein bands were stained with 0.05% Coomassie blue staining solution for 60 min and destained with destaining solution for 30 min. The bands of myofibrillar proteins were visualized using GS-800 Calibrated Imaging Densitometer (Bio-Rad) (Fig. 1).

Western-blotting

The fractionated proteins that were initially separated from the samples based on their molecular weight through gel electrophoresis were transferred from the gel onto polyvinylidene difluoride (PVDF) membranes

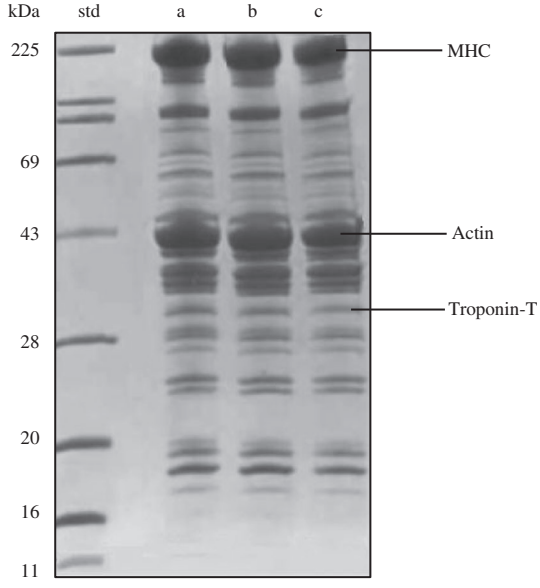

Figure 1 Representative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showing the myofibrillar protein bands of Longissimus lumborum muscle in goats during 14 days of refrigerated storage. Lanes a-c: aging for 0, 7 and 14 days, respectively. Equal amount of protein (30 μg) of each sample was loaded and electrophoresed on a separate 12% SDS-PAGE under 120 V of constant voltage for about 90 min. The gels were then stained with Coomassie blue staining for 60 min and destained with destaining solution for 45 min. The bands of myofibrillar proteins were visualized using GS-800 Calibrated Imaging Densitometer.

using Trans-Blot® SD semi-dry transfer system cell (Bio-Rad). MHC was transferred at constant amperage of 250 mA per gel, voltage limit of 25 V for 135 min, whereas actin and troponin-T were transferred at the same amperage and voltage for 45 min. After transfer, membranes were blocked for 3 h at room temperature in blocking solution (5% BSA in TBS-T buffer (100 mmol/L Tris-HCl; 150 mmol/L NaCl; 0.05% Tween 20). Blots were washed three times (10 min per wash) in phosphate-buffered saline + Tween 20 and then were incubated overnight at room temperature with the primary antibody which was diluted 1:500 in TBS-T containing 3% BSA. Monoclonal Anti-Myosin (Skeletal, Fast), produced in mouse; Cat #. M4276, Monoclonal Anti-Myosin (Skeletal, Slow), antibody produced in mouse; Cat # M842, Anti-actin antibody produced in rabbit; Cat # A2066 227 and monoclonal anti-troponin-T antibody produced in mouse; Cat # T6277 from Sigma-Aldrich (St Louis, MO, USA) were the primary antibodies used for MHC, actin and troponin-T, respectively. Subsequently, the membranes were incubated with secondary antibody (anti- mouse immunoglobulin G (IgG: whole molecule) peroxidase, antibody developed in rabbit; Cat # A9044 from Sigma-Aldrich) diluted 1:10 000 of in 3% BSA in TBS-T buffer for 90 min at room temperature. This was followed by three times washing with TBS-T buffer. The blocked membranes were detected using a DAB substrate kit Code: E885 DAB SUBSTRATE SYSTEM (aMReSCO®; Solon, OH, USA). MHC, actin and troponin-T band intensities were measured using GS-800 Calibrated Imaging Densitometer (Bio-Rad) followed by quantification of the bands intensity using Quantify One® software (Bio-Rad, USA). The concentrations of each protein were determined (Gadiyaram *et al.* 2008) using the following formula:

$$\text{Concentration (mg)} = \text{Relative intensity} \times \text{Area}$$

The proportion of protein in each band was calculated using the formula:

$$\text{Proportion (mg)} = \left[\text{Concentration of the protein} / \text{Concentration of all proteins in a band}\right] \times \text{Amount of protein loaded}$$

Determination of physicochemical meat characteristics

Shear force

Shear force values were determined following the procedure previously described by Sazili *et al.* (2005). Briefly, the frozen samples of LL muscle were thawed overnight at 4°C and cooked in a heated water bath to an internal temperature of 78°C monitored using a digital thermocouple thermometer (HI 145–00 thermometer, HANNA® Instruments, Woonsocket, RI, USA). The cooked samples were then kept overnight at 4°C. From each cooled sample, at least three replicate blocks measuring 1 cm × 1 cm × 2 cm (height × width × length) were cut parallel to the muscle fiber orientation and sheared with a Volodkevich bite set attached to the TA.HD plus® texture analyzer (Stable Micro System, Surrey, UK). The equipment was calibrated at 5 kg for weight, 10 mm return distance for height and the blade speed was set at 10 mm/s. Shear force values were reported as the average peak positive force of all block values of each individual sample and expressed in kilogram (kg).

Drip loss

Drip loss of LL muscle was determined by calculating a percentage of weight loss, relative to the initial weight as described by Honikel (1998). At the designated storage time, samples were taken immediately from the polyethylene plastic bags, gently blotted dry and weighed. Drip loss was then calculated.

Myofibril fragmentation index

The myofibril fragmentation index (MFI) was measured according to the method of Hopkins *et al.* (2000) with slight modification as described by Nakyinsige *et al.* (2014). The MFI is the value of absorbance of the myofibrillar suspension after being diluted to a final protein concentration of 0.5 ± 0.05 mg/mL, measured at 540 nm with a spectronic®20 GENESYS™ spectrophotometer (Spectronic Instruments, Scottsdale, AZ, USA) and multiplied by 150 (Hopkins *et al.* 2000). All samples collected at 0, 1, 7 and 14 days from meat matured at 4°C, after myofibrillar extraction and measuring myofibrillar fragmentation index, were examined and photomicrographied using an image analyzer microscope attached with charge-coupled device (CCD) camera (Olympus, BX. 51. TF, Tokyo, Japan).

Color

Meat color was determined by measuring L* (lightness), a* (redness) and b* (yellowness) values using a colorimeter Color Flex spectrophotometer (Hunter Lab Reston, Reston, VA, USA). The colorimeter was calibrated against black and white reference tiles prior to use and the color values of samples (approximately 15 mm of thickness) determined after a 30 min blooming period (AMSA 2012) using illuminant D65 as the light source. Hue angle and chroma were calculated using the formula ($\tan^{-1}(b^*/a^*)$) and $\sqrt{(a^2 + b^2)}$, respectively.

Statistical analysis

All values are reported as the means ± SE. Variables were analyzed as repeated measures using the General Linear Model (GLM) procedure of Statistical Analysis System package (SAS) Version 9.1.3 software (SAS Institute Inc., Cary, NC, USA). Duncan's test was used to separate means with a *P*-value of 0.05.

RESULTS AND DISCUSSION

Microbiological changes

The microbiological quality of meat is affected by various factors such as the physiological condition of the animal at slaughter time, the spread of contamination during slaughter, temperature and storage conditions (Koutsoumanis & Sofos 2004). Furthermore, Eze and Ivuoma (2012) showed that refrigerated storage affected the growth of microorganisms in meat and meat products. Table 1 shows the growth of microbial spoilage in goat meat during 14 days post mortem. Generally, bacterial counts increased ($P < 0.05$) with increase in aging time. Total aerobic counts were the highest, followed by *Pseudomonas* spp., *Enterobacteriaceae* and lactic acid bacteria. In goat meat, total aerobic counts reached about 6.017 log10 cfu/g during refrigerated storage at 4°C. *Pseudomonas* spp. is a Gram-negative psychotropic bacteria dominated at refrigeration temperatures and regarded as a major spoilage microorganism in red meat (Jouki & Khazaei 2011). In the current study, *Pseudomonas* spp. reached 3.866 log10 cfu/g on day 14 of storage and its count was higher than lactic acid bacteria and *Enterobacteriaceae*. *Pseudomonas* spp. develop faster and have higher tendency toward oxygen than other microorganisms (Jay 2000). The levels of all microbes detected throughout the 14 day storage in the current study are acceptable as reported by Insausti *et al.* (2001). According to these authors, spoilage takes place when the levels of total viable count and/or *Enterobacteriaceae* count reach log7–8 cfu/g. Likewise, Nortjé and Shaw (1989) observed that spoilage occurs when the lactic acid bacteria count reaches log7 cfu/g.

Muscle pH values

The ultimate pH of skeletal muscle is an influential factor in meat quality (Mortimer *et al.* 2014). Table 2 illustrates the impact of aging on the pH of LL muscle during refrigerated storage at 4°C. In general, pH values reduced significantly ($P < 0.05$) with storage time. The pH of LL muscle at days 1, 3, 7 and 14 was not significantly ($P > 0.05$) different but was significantly lower than that of day 0. The pH reduction is due to the conversion of muscle glycogen to lactic acid during the post mortem period (Scheffler *et al.* 2013). Typically, when an animal is exsanguinated and suffering from hypoxia, the muscle fibers resort to anaerobic glycolysis to maintain metabolic activities, serving as the only source of energy for the post mortem muscles. Thus, glycogen stores are depleted as they are converted to lactic acid which leads to the pH decline (Vieira & Fernández 2014). Values of pH measured in this study were in line with the findings of Hou *et al.* (2014) who reported that pH values in beef samples reduced significantly after 24 h of post mortem period with no significant changes during the next 20 days of aging. Furthermore, Abdullah and Qudsieh (2009) also established that pH was not affected by aging for 1 or 7 days. The ultimate pH (pHu) observed in the current study was higher than the pH (5.5-5.8) for normal meat. However, the pHu value observed in the current study is consistent with the ranges of pHu (> 6.0) observed in different breeds of goats (Husain *et al.* 2000; Guerra *et al.* 2011).The high pHu observed in goat meat has been attributed to the high susceptibility of goats' pre-slaughter stress which was linked to genotype effect on animal behavior (Hopkins & Fogarty 1998), implying that the excitable nature of goats predisposes them to yielding high pH meat.

Lipid-protein oxidation

Oxidation of lipid and protein of meat is the major non-microbial cause of quality deterioration during processing (Falowo *et al.* 2014). In fact, lipids and proteins are susceptible to oxidative damages because of the fast depletion of endogenous antioxidants after slaughter (Xiao *et al.* 2013), during refrigeration and frozen storage. Table 3 shows results for lipid and protein oxidation in goat meat during refrigerated storage at 4°C. In general, lipid oxidation values increased ($P < 0.05$) with storage time, and are in line with other studies in lambs

Table 1 Effect of aging on microbial spoilage of Semitendinosus muscle in goats

Parameter	Post mortem aging periods			
	0 day	1 day	7 days	14 days
Total aerobic count (log10 cfu/g)	2.894¶ ± 0.105	3.368§ ± 0.093	4.260‡ ± 0.144	6.017† ± 0.126
Lactic acid bacteria (log10 cfu /g)	0.719¶ ± 0.045	1.355§ ± 0.032	2.091‡ ± 0.050	3.443† ± 0.150
Enterobacteriaceae (log10 cfu /g)	1.558¶ ± 0.082	2.021§ ± 0.056	3.178‡ ± 0.047	3.830† ± 0.367
Pseudomonas spp. (log10 cfu /g)	0.378¶ ± 0.040	1.223§ ± 0.038	2.218‡ ± 0.098	3.866† ± 0.090

†, ‡, §, ¶ Least square means with different superscripts in the same row indicate significant differences in the aging period at $P < 0.05$.

Table 2 Effect of aging on the pH of Longissimus lumborum muscle in goats

Parameter	Post mortem aging periods				
	0 day	1 day	7 days	14 days	0 day
pH (unit)	6.827† ± 0.037	6.336‡ ± 0.084	6.203‡ ± 0.091	6.190‡ ± 0.103	6.194‡ ± 0.088

†, ‡ Least square means with different superscripts in the same row indicate significant differences in the aging period at $P < 0.05$.

Table 3 Effect of aging on lipid and protein oxidation of Longissimus lumborum muscle in goats

Parameter	Post mortem aging periods			
	0 day	1 day	7 days	14 days
Lipid oxidation (mg malondialdehyde/kg meat)	0.478§ ± 0.025	0.565§ ± 0.018	1.171‡ ± 0.029	2.090† ± 0.049
Free thiol content (nmol/mg protein)	41.727† ± 1.164	40.028† ± 0.736	34.581‡ ± 0.595	27.699§ ± 0.928

†, ‡, § Least square means with different superscripts in the same row indicate significant differences in the aging period at $P < 0.05$.

(Petron *et al.* 2007), goats (Sabow *et al.* 2015) and beef (Franco *et al.* 2009). No significant difference was observed in lipid oxidation of LL muscle at 0 and 1 day post mortem. However, the levels of lipid oxidation on days 7 and 14 were significantly ($P < 0.05$) higher than that of day 0 and 1. Lipids are chemically unstable. Thus, they are vulnerable to oxidation, particularly during post mortem and storage (Falowo *et al.* 2014). Lipid oxidation is a consequence of oxy- and/or lipid free radical generation and leads to the formation of toxic compounds such as MDA and cholesterol oxidation products (Morrissey *et al.* 1998). Lipid oxidation is involved in the deterioration of flavor, formation of rancid odors, discoloration and production of potentially toxic compounds which can affect consumers' health (Falowo *et al.* 2014). The threshold value of TBARS (5 mg MDA/kg) for detecting off-odors and off-taste (Insausti *et al.* 2001) was not reached in the current study.

Protein oxidation is a significant issue in meat quality assessment because muscle tissues involve high amounts of proteins playing a significant role in meat quality (Falowo *et al.* 2014). Meat protein oxidation is often evaluated by measuring the amount of protein thiols (the sulfhydryl group (SH) of a cysteine residue) and detection of protein disulfide cross-link formation by SDS-PAGE (Nieto *et al.* 2013). Protein oxidation measured by free thiol content was significantly ($P < 0.05$) influenced by aging time (Table 3). Based on quantification of protein thiol groups, the thiol concentration reduced from 41.727 to 27.699 nmol per mg protein throughout the post mortem storage. The protein thiols significantly reduce as protein oxidation increases in refrigerated storage. This finding is in line with previous studies on chill storage of meat (Zakrys-Waliwander *et al.* 2012; Nieto *et al.* 2013). Post mortem transformations in the muscle reduce the power of the antioxidant defense system (Baron & Andersen 2002). Accordingly, this increases the level of protein oxidation under the action of free radicals (Falowo *et al.* 2014). Oxidative reactions including the side chains of amino acids resulting in the formation of thiol groups (Lund *et al.* 2011). This conversion can also lead to loss of catalytic activity and increase the vulnerability to protein aggregation or degradation and loss of solubility (Rowe *et al.* 2004). The formation of disulfide bridges leads to the oxidization of thiol groups of cysteine (Sun *et al.* 2011). Sun *et al.* (2011) reported that thiols are only oxidized to a creation degree which indicates that all containing cycteine residues in the muscle tissue protein cannot show similar reactivity and some of the thiol groups are hidden in the protein and are protected from oxidation.

Degradation of myofibrillar proteins

The proteolytic pattern of myofibrillar proteins of LL in goats observed during 14 days of refrigerated storage using SDS-PAGE and Western blotting is represented in Figures 1 and 2, respectively. The SDS-PAGE patterns showed a decrease of bands corresponding to MHC as post mortem days increased. The actin band was relatively more stable. The bands of troponin-T also reduced with increase in aging period. The reduction in band intensity is a sign of protein oxidation in LL muscle during refrigerated storage at 4°C. The results of this study are in line with findings of Xue *et al.* (2012) which showed that increased bovine myofibrillar protein oxidation promoted the degradation of MHC but with insignificant effect on the degradation of actin. According to Marino *et al.* (2013), the intensity of troponin-T and band size along with the MHC highly decreased during postmortem.

The intensities of MHC, troponin-T and actin were determined by measuring the densitometric analysis of concentrations of each detected band. Table 4

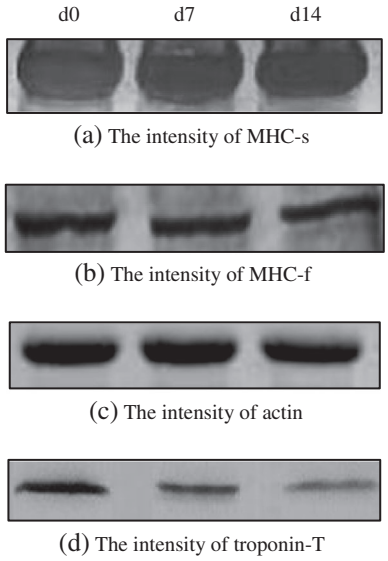

Figure 2 Western blotting analysis using anti-myosin heavy chain (MHC), anti-actin and anti-troponin-T antibodies following the developed protocol for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)goat Longissimus lumborum muscle during 14 days of refrigerated storage.

Table 4 Concentrations (mg) of selected myofibrillar proteins, including myosin heavy chain slow, myosin heavy chain fast, actin and troponin-T from goat Longissimus lumborum muscle

Parameter	Post mortem aging periods		
	0 day	7 days	14 days
Myosin heavy chain slow	12.348† ± 0.603	10.012‡ ± 0.809	8.883§ ± 0.883
Myosin heavy chain fast	2.723† ± 0.113	1.967‡ ± 0.074	1.123§ ± 0.084
Actin	6.191† ± 0.478	5.846† ± 0.325	5.576† ± 0.192
Troponin-T	0.845† ± 0.042	0.616‡ ± 0.035	0.464§ ± 0.034

†, ‡, § Least square means with different superscripts in the same row indicate significant differences in the aging period at $P < 0.05$.

shows that the concentrations of MHC and troponin-T highly ($P < 0.05$) decreased as aging period increased, while that of actin was stable during the 14 days storage at 4°C.

Myosin heavy chain protein constitutes the major part of thick filament in sarcomeres. It also contains 35% of the total skeletal muscle protein (Lefaucheur 2010). Furthermore, it is a substrate for μ-calpain (Lametsch *et al.* 2004). Electrophoretic analysis studies by Sun *et al.* (2011) and Addeen *et al.* (2014) observed that the protein band corresponding to MHC was the most sensitive protein to oxidation, whereas lower molecular weight proteins seemed to oxidize later. Ooizumi and Xiong (2004) showed that the initial oxidation of chicken myofibrils led to changes in myosin, especially intermolecular cross-linking of MHC and modifications of thiol groups at the myosin adenosine triphosphatase active site.

It has been reported that actin and actin-bundling proteins are significant in muscle contraction (Xue *et al.* 2012). The present research showed that actin remained unaffected during 14 days post mortem at 4°C. This oxidative stability of actin could be related to unavailability of oxidation sites, which might be hidden by the interaction of actin with myosin chains in myofibrillar suspensions (Xue *et al.* 2012). Gil *et al.* (2006) indicated that actin is degraded very little or not at all during meat aging at 0–5°C, even after 56 days.

Troponin-T is the tropomyosin-binding component of the troponin complex, regulating the calcium-dependent skeletal muscle contraction (Xue *et al.* 2012). In the present study, the Western blotting indicated that the degradation rate of troponin-T highly increased during post mortem at 4°C (Table 4). Similarly, studies in beef cattle (Martinaud *et al.* 1997) and lambs (Santé-Lhoutellier *et al.* 2008) documented that the degradation of troponin-T significantly increased during the post mortem aging periods. Moreover, Xue *et al.* (2012) demonstrated that the degradation rate of troponin-T decreased significantly in the oxidative modified group compared to the proteins that were not exposed to oxidation at the same time, indicating a good correlation between the two events (Xue *et al.* 2012).

Physicochemical meat characteristics

Shear force values

Tenderness is the most significant component of meat quality that influences consumers' eating satisfaction (Hildrum *et al.* 2009). Aging influences meat tenderness due to complex transformations in muscle metabolism (Marino *et al.* 2013). Table 5 shows the impact of aging on shear force values of LL muscle. The shear force values reduced significantly from day 1 to 14. Similar findings were found in other studies where post mortem aging increased meat tenderness (Marino *et al.* 2013; Lomiwes *et al.* 2014; Sabow *et al.* 2015). During post mortem, the

Table 5 Effect of aging on shear force values, myofibrillar fragmentation index (MFI), water holding capacity and color characteristics in goat Longissimus lumborum muscle

Parameter	Post mortem aging periods			
	0 day	1 day	7 days	14 days
Shear force (kg)	1.548† ± 0.029	1.417‡ ± 0.027	1.299§ ± 0.2756	1.008¶ ± 0.022
Myofibrillar fragmentation index	91.436¶ ± 0.466	106.920§ ± 0.987	123.247‡ ± 0.469	134.115† ± 1.851
Drip loss (%)	-	1.413§ ± 0.082	3.586‡ ± 0.184	5.437† ± 0.282
Lightness (L*)	29.853‡ ± 0.864	30.998‡ ± 0.818	32.681† ± 0.827	33.404† ± 0.756
Redness (a*)	14.755† ± 0.236	13.963† ± 0.334	12.704‡ ± 0.311	11.163‡ ± 0.291
Yellowness (b*)	13.300† ± 0.196	12.835† ± 0.337	10.729‡ ± 0.257	10.362‡ ± 0.380
Chroma (C*)	10.184§ ± 0.188	11.233‡ ± 0.276	11.263‡ ± 0.218	12.868† ± 0.233
Hue angle (H)	33.213¶ ± 0.398	35.957§ ± 0.699	38.938‡ ± 0.729	37.315† ± 0.764

†, ‡, §, ¶ Least square means with different superscripts in the same row indicate significant differences in the aging period at $P < 0.05$.
Hue = $[\tan^{-1}(b^*/a^*)]$; Chroma = $\sqrt{(a^2 + b^2)}$

accumulation of Ca^{2+} into the muscle sarcoplasm leads to the activation of μ-calpain which in turn destabilizes the intactmyofibrillar structure by degrading myofibrillar proteins involving titin, filamin, troponin-T and desmin (Lomiwes *et al*. 2014). Harris *et al*. (2001) observed that the 30 kDa fragment emerging from the parent troponin-T is a proper indicator of meat aging and tenderization because of its close relationship with meat tenderness, while Santé-Lhoutellier *et al*. (2008) described it as a potential proteolysis index. Troponin-T is present in the I-band areas of the intact myofibril, undergoing remarkable breakage during muscle aging. Thus, its disruption along with other I-band proteins titin and nebulin can contribute to myofibril fragmentation and tenderness (Taylor *et al*. 1995). Myofibrillar protein cross-linking in the form of binding and entrapping relating to the physical structures with increase in aging time could affect meat tenderness (Huff-Lonergan & Lonergan 2005). Moreover, Kang *et al*. (2011) showed that MHC isoforms are highly correlated with meat tenderness trait. This shows that MHC oxidation is a significant factor in post mortem meat tenderness.

Drip loss values

Meat has the potential to keep inherent water. This ability is defined as water-holding capacity (WHC), which is essential for both the industry and consumer (Modzelewska-Kapituła *et al*. 2015). Table 5 shows the drip loss of LL muscle during 14 days refrigerated storage at 4°C. The level of drip loss generally increased ($P < 0.05$) as aging time increased. As was shown in previous studies on meat originating from different animal species, aging decreased the WHC of meat (Jouki & Khazaei 2011; Farouk *et al*. 2012; Sabow *et al*. 2015). During aging, increase in protein oxidation may be responsible for the decreases in WHC. Similar findings have been reported in previous studies (Traore *et al*. 2012) where the ability of fresh muscle to maintain endogenous water was compromised when myofibrils were subjected to oxidizing agents. Lonergan *et al*. (2001) observed a positive correlation between drip loss and storage time. They demonstrated that the oxidative processes taking place in both lipid and protein fractions during storage might influence the ability of the protein to bind water, and this might result in a poor WHC. Furthermore, Bertram *et al*. (2007) reported that the loss of the ability of myofibrillar proteins to retain water took place upon their oxidation. Additionally, Estévez *et al*. (2011) found that a mechanism might reside in the loss of amino groups from acid side chains. This would reasonably result in the modification of the electronic arrangement and the isoelectric point of myofibrillar proteins. Moreover, based on previous studies, MHC oxidation is highly correlated with different aspects of meat quality, including drip loss and juiciness traits (Kang *et al*. 2011). On the other hand, WHC can affect or be affected by bacterial spoilage in meat (Olsson *et al*. 2007). Olsson *et al*. (2007) examined the effect of spoilage bacteria on the WHC of fish muscle during chilled storage. They observed that the absence of spoilage bacteria caused muscles to be able to hold water until 15 days post mortem. However, the presence of spoilage bacteria decreased the WHC from 15 day onwards. The proteolytic enzymes, likely originating from bacteria, play a vital role in post mortem degradation of collagen and myofibrillar proteins during the process of aging. As a consequence, they influence the WHC (Olsson *et al*. 2007).

Myofibril fragmentation index

The increase in MFI from day 0 to day 14 (Table 5) may be due to the breaking of myofibrillar proteins into segments at, or near, the Z-disk during post mortem storage (Shin *et al*. 2008). Hou *et al*. (2014) and Hopkins *et al*. (2004) observed that aging time significantly increased the MFI of the muscle in cattle and sheep respectively. The extent of fragmentation of myofibrils when exposed to homogenization is a sign of the degradation of muscle myofibrillar proteins under post mortem conditions (Li *et al*. 2012). According to Taylor *et al*. (1995), the MFI is a reliable indicator of the extent of proteolysis showing the degradation of key structural proteins, specially the rupture of the I-band of the sarcomere and breakage of myofibril linkages. Moreover, Marino *et al*. (2013) found that troponin-T degradation during aging time is related to structural changes such as MFI and troponin-T degradation along with the main increase of MFI during aging. They observed that this could be the consequence of a more intensive proteolysis.

Meat color characteristics

A customer's first appraisal of red meat quality is based upon its color, linking color to both perceived and actual values (Holman *et al*. 2015). As shown in Table 5, post mortem storage had significant effect on meat color. Initial color (L*, a* and b*) of the LL muscle at day 0 and 1 was not significantly different. The L*, a* and b* values of LL muscle on days 7 and 14 did not differ ($P > 0.05$) but were significantly different from that of day 0 and 1. The increased in L* value as storage progressed is in line with the report of Hou *et al*. (2014) and Li *et al*. (2012), which showed that aging improved muscle lightness. In the present study, redness of the meat decreased as storage progressed. This observation is in line with the finding of Lindahl (2011). Redness is the most significant color parameter for assessment of meat oxidation (Traore *et al*. 2012). This can be related to the oxidation of myoglobin (deoxymyoglobin or oxymyoglobin) to metmyoglobin because of decreased metmyoglobin reducing activity (MRA) that led to metmyoglobin accumulation in the meat (Xue *et al*. 2012). This change reduces the redness and makes the meat unpleasant for consumers (Filgueras *et al*. 2010). Zakrys *et al*. (2008) observed

that changes in a* values are related to lipid oxidation and are strongly correlated with the TBARS values. Moreover, Seydim *et al.* (2006) stated that reduction in redness is due to myoglobin oxidation, especially when meat pH is above 6. At high pH values, mitochondrial enzyme systems do not shut down and are able to use available oxygen. Zakrys *et al.* (2008) showed that instrumental a* value had a negative correlation, while L* value had a positive correlation with storage days post mortem, suggesting that samples became less red and lighter over the duration of storage. The difference in color during aging times was also evident from other color factors as shown by chroma (C*) and hue (H°). Oliete *et al.* (2006) observed that lengthening of the storage time of meat led to rises in C* and H° units.

Conclusion

This study indicated that protein oxidation took place during refrigerated storage of goat meat as evidenced through loss of thiol groups and degradation of MHC and troponin-T. The study also proposes lipid-protein oxidation as potential deteriorative changes in some physical characteristics in goat meat in addition to the commonly reported microbial spoilage. Short refrigerated storage (7 days) at 4°C can be recommended to improve goat meat tenderness without adverse effects on meat shelf life as indicated by oxidative stability and microbiological quality. However, longer aging times (14 days) may achieve further improvements in tenderness but meat shelf life is reduced significantly as evidenced through oxidation process (lipid and protein) which negatively influences color stability, flavor and protein stability and functionality.

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AUTHORS' CONTRIBUTION

AQS is the project leader who is also the main supervisor to ABS whom together with GYM contributed to the idea, design and execution of the study. ABS, NK, ZAA and AQS performed the microbiological analysis and lipid-protein oxidation measurement, while ABS, ZI, KDA and AQS determined the physicochemical traits of meat analysis. KDA, UK and MZAAK assisted in all animal procedures for the experiment. GYM and ABS were responsible for the statistical analysis. All authors contributed equally to the write-up of the final manuscript.

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