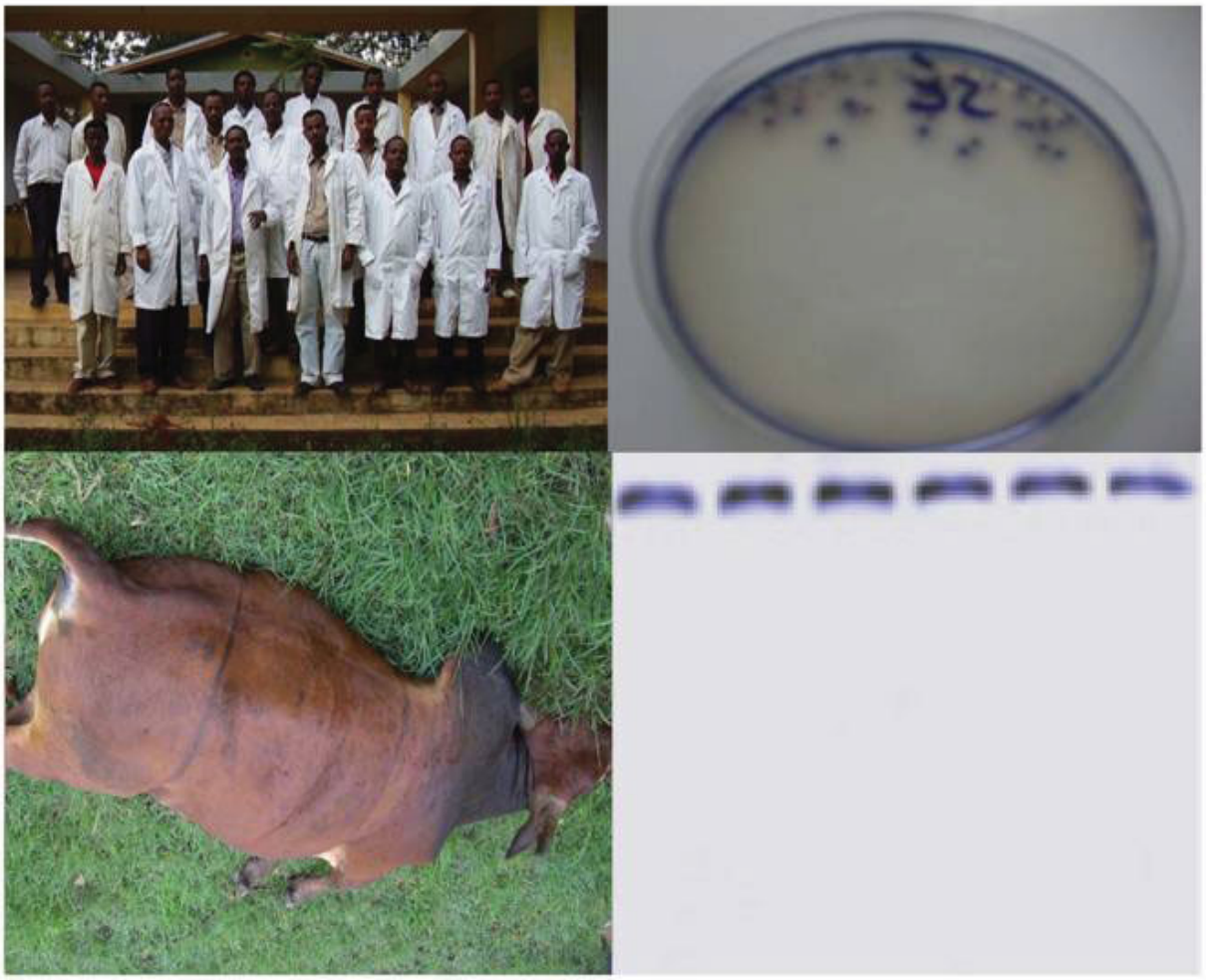
Department of Animal Sciences

Georg-August University of Goettingen

The Safety and Quality of Horro Beef Muscles



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The Safety and Quality of Horro Beef Muscles

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By

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Acronyms and Abbreviations

AAA	Addis Ababa Abattoir
AC	Aerobic Count
ACT	Amount of Connective Tissue
AHRI	Armauer Hansen Research Institute
ALERT	All African Leprosy, Tuberculosis, Rehabilitation & Training
API	Analytical Profile Index
BF	*Biceps femoris*
CC	Coliform Counts
CCP	Critical Control Point
CFU	Colony Forming Units
CL	Critical Limits
DMb	Deoxymyoglobin
EC	Enterobacteriaceae Count
HACCP	Hazard Analysis and Critical Control Point
IMS	Immunomagnetic Separation
IS	*Infraspinatus*
LL	*Longissimus lumborum*
MMb	Metmyoglobin
MRA	Metmyoglobin Reductase Activity
OMb	Oxymyoglobin
PBW	Peptone Buffered Water
PCA	Plate Count Agar
PVC	Polyvinyl Chloride
RF	*Rectus femoris*
RM	*Rhomboideus*
SM	*Semimembranosus*
Tn-T	Troponin-T
USDA	United States Department of Agriculture
WBSF	Warner-Bratzler Shear Force

Chapter 1:
General Introduction

General introduction

Safety and quality are the two vital components against which consumers judge beef during purchase and consumption. Broadly, beef safety includes examination and inspection of slaughter livestock before they are allowed to enter any slaughtering or packing facility (a*nte-mortem inspection)*, and conducting thorough examination of every carcass, including organs like the lymph nodes and other organs to identify any signs of disease or unwholesome condition (p*ostmortem inspection*). The inspection system continues throughout the entire processing segment of the establishment including careful examination of both uncooked and fully cooked products (product inspection). The presence of hazard is what makes beef unsafe and, when broadly defined, hazard includes any biological, physical and/or chemical factor which can lead to the condemnation of slaughter animal, derived carcass or its products.

Savell (1995) states that neither traditional meat inspection nor supposedly good manufacturing practices can reliably assure the attainment and maintenance of high safety standards for meat in respect of contamination with hazards. HACCP is a generic food safety term applied as systematic preventive tool to safeguarded beef consumers from health endangering hazards in the process from farm to fork. It allows the beef slaughter and processing centers to identify potential beef safety hazards that are reasonably likely to occur in the process or type of product being produced and establish points of control to prevent them from occurring. Application of HACCP is based on seven principles (Codex, 1997) and is currently seen as mandatory action if beef industry is to remain competitive.

The definition of beef quality, according to Savell (1993) includes its use in the manufacturing sector where freedom from defects, consistency, compliance to manufacturing specifications, meeting or exceeding the expectations of customers. Beef quality is also a generic term used to describe properties and perceptions of beef (Maltin *et al*., 2003). Beef quality governing main factors are

product-oriented, process oriented, user-oriented and quality control (Brunsø, 2005). It has also been defined as a composite of those characteristics that differentiate individual units of a product and have the significance in determining the degree of acceptability by buyer (Shewfelt, 1990). These levels of perception (Shewfelt, 1990) are external quality characteristics that can be perceived by the sense of sight and touch without ingesting the product while internal quality is characterized by senses of taste, smell, and texture and combine with visual appearance in determining acceptability.

Several factors may contribute for variability of beef quality including the characteristics of the live animal that can affect tenderness and flavour (Klastrup *et al*., 1984), the feeding regime, handling and transport systems (Huff and Parrish, 1993). Similarly, good slaughtering techniques are a necessity to ensure that the beef quality and safety is not compromised (Field, 1971). It is generally agreed that *postmortem* events are the main determinants of beef quality (Maltin *et al*., 2003) and it is likely that the *postmortem* properties of beef are to be determined at different levels ranging from the molecular to mechanical. Organoleptic parameters such as color, palatability, shelf stability and biophysical aspects like proteolytic activity of calpain enzymes, degradation of key myofibrillar proteins, autoxidation of myoglobin, the activity of myoglobin reductase describe the *postmortem* events of beef quality and are the main concerns.

There is no published information regarding states of quality of Horro beef in view of consumers' preferences and quality *per se* to guide market oriented production. On the other hand, beef animal production could be meaningful only when deemed to meet the need of the beef consumers. Therefore, assessment of quality in relation to the parts of the beef and consumers' need would benefit current Horro beef stakeholders and future improvement programs. Hence, this prototype study can be viewed as benchmarking effort of the safety and quality of

Horro beef muscles in the general beef improvement interest of Regional State of Oromia and Federal Democratic Republic of Ethiopia.

Before the start of sample collection for the thorough study of safety and quality of Horro beef, however, it was important to have prior knowledge of management aspects of the Horro cattle breed of the study area. Therefore, diagnostic survey was conducted for three months and the prevailing management aspects were analyzed for probable influence they may exert on the parameters (broadly, safety and quality) studied. Accordingly questionnaire was developed and pre-tested with elderly livestock farmers of the area and grass root level development agents from Oromia Bureau of Agriculture. Three districts (Horro, Bako, and Danno) were purposely chosen while household (HH) respondents from the districts chosen were randomly recruited. Initially 30 HH respondents were recruited from three sub-districts within each district but only 20 HH/ sub-district rearing at least two heads of cattle were actually participated in the interview.

The management aspects addressed in the survey mainly included feed type, sex and market age of the animal frequently sold for slaughter, grazing area, distance of watering point from the shelter, shelter type and ploughing history of the steer sold for slaughter. The questionnaire was open type so that comments from the respondents could be documented. Further, Horro beef slaughter stock sales point and to slaughter house delivery routes were identified. Bivariate analyses of the variables were conducted using Chi-square analysis and t-test. Based on the preliminary data results, criteria were set for sampling of experimental animals. Once the category of the experimental animals was decided, belonging steers were either purchased live and slaughtered or local butcheries were contacted for arrangement of desired muscle sample collection.

Moreover, it was realized that Horro slaughter stocks were available for sale at primary markets in the production area with several destiny of slaughter houses. Butchers might purchase the slaughter animal for informal slaughter in their local area or livestock traders might buy and re-sale at the secondary or terminal markets. Butchers buying the stock at the terminal market deliver the animals to slaughter house of their interest to get slaughtering service. Among destination centers for slaughter stock of Horro beef animals, Addis Ababa abattoir (AAA) and the supply chain was considered for assessment of the safety of beef derived from Horro cattle. The consideration of this supply chain and use of Horro beef was important because it addresses consumers not only in the State of Oromia but also include those in City Administration of Addis Ababa.

Chapter 2:

Application of HACCP to the supply and processing chain of Horro beef animals in Ethiopia[1]

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Application of HACCP to the supply and processing chain of Horro beef animals in Ethiopia

Abstract

Prototype studies with the objective of Hazard Analysis and Critical Control Point (HACCP) application in the Horro beef production to consumption chain were conducted in Ethiopia. The chain involved terminal market for sales of slaughter stocks, carcass dressing line in Addis Ababa abattoir (AAA) and butcheries concerned with retailing of the derived carcasses. At first, baseline microbial data to implicate hygienic level of the process chain were collected and then potential hazards identified. Enumeration involved aerobic plate (AC), Enterobacteriaceae (EC) and coliform (CC) counts. From each plate of enumeration, randomly picked 8-12 colony forming units (CFU) were subjected to Analytical Profile Index (API 20E) biochemical test strips for characterization of the hazard. At latter stages, additional samples were collected following similar procedures of the enumeration work for selective detection of specific hazards expected in slaughter stock and the derived carcass processing environment. Samples were selectively enriched and immunomagnetic separation (IMS) assay performed. Post-IMS cultures bead matrix were sub-cultured and final identification of confirmed hazards were carried out using Microbact GNB 24E (MB1074A, Oxoid), 12L (MB1128, Oxoid) and 24E (MB1132, Oxoid). The pure cultures of all categories of isolates were serotyped based on the reactions of somatic or cell wall (O) and flagellar (H) antigens.

Enumeration data indicated that process stages had significant ($p < 0.05$) effects on AC, EC and CC. The mean log CFU ranged from 3.567-4.128, 3.742-4.059, and 3.757-4.037 CFU/cm^2/g^2 respectively for AC, EC and CC. The incidences of the hazards varied between 10.4 and 22 % for Klebsiella pneumoniae and E. coli O157:H7, respectively. Hazards were distributed along all process stages studied. Following the principles of HACCP, critical control points and limits were

identified and monitoring and evaluation procedures suggested based on the results. It was recommended that stakeholders could help the goal of HACCP implementation achievable, especially the State of Oromia and the Federal Government of Ethiopia could formulate policy either for mandatory or voluntarily HACCP application in both domestic and export abattoirs. The practice of a full scale HACCP plan should prevent hazards which may endanger consumer health and also increase competence of the country's beef export potential in the global trade market.

Key words: Horro beef; Addis Ababa; Abattoir; Hazard; HACCP; Oromia; Ethiopia

2.1. Introduction

Beef is the most favored food consumed in Ethiopia often uncooked. The slaughter beef stocks are purchased from extensive or semi intensive-management systems and either trekked or trucked to slaughter points. Based on the recommendation of Solomon (1975), the official veterinarians visually assess *ante mortem* and *postmortem* inspection. Approved carcasses are stamped, immediately loaded onto trucks and dispatched to butcheries for retailing. Obviously, such gross inspection is not a strict way to assure safety of the carcass processed from production to consumption chain (Asseged et al., 2004). Under such unreliable inspection systems, spoilage of the beef and public health issues endangered by beef-borne hazards may become an issue and calls for increased attention.

Butchery men in Addis Ababa purchase Horro beef animals and submit them to the Addis Ababa abattoir (AAA). Purchased animals are tagged, transported and unloaded at the lairage of AAA for slaughter. After slaughter and dressing service at the AAA, the butchers receive the carcasses for retailing in their butcheries. Thus, substantial numbers of Addis Ababa city dwellers depend on the supply

and retailing of carcasses of Horro beef animals. However, the extent of exposed, sickened and/or deceased consumers and the potential cost of beef-borne illnesses in the country is not well documented. Neither level of safety nor epidemiological data following the supply and processing chain of Horro cattle slaughter stock were documented. The fact that beef is often consumed uncooked in Ethiopia may increase the risk posed by beef-borne pathogens on consumers' health. Also there is need for the country to improve the safety of carcasses, not only for domestic consumption but also to comply with international beef export trade standards.

Despite the demand for improved safety standards, the health risk posed by microbial hazards is ever increasing (Notermans et al., 1995). In most food processes the points where food becomes a microbiological hazard are few, and these points can be specifically controlled and monitored once identified (Kilsby and Pugh, 1981). As a remedy for the threat, Hazard Analysis and Critical Control Point (HACCP) is a most effective means for preventing microbial contamination of meat carcasses during slaughter (Bolton et al. 2002). HACCP is an optimal framework for building science-based process control (s) to prevent food safety hazards in food production systems (FSIS, 1996). It would be beneficial for each slaughter facility to develop its own baseline reference data on hazards or potential hazards and customize available generic HACCP plans to match site specific circumstances and processes (Vanne et al., 1996). Hence, an effective HACCP system must be based on accurate baseline data on the types and levels of potential contamination at each stage of production (Gill et al., 1996) as processing plants differ in terms of the range and levels of contamination present (Vanne et al., 1996).

However, no published reports on the specific hazard type and levels of contamination in Ethiopia are available following the supply and process chain of Horro beef to enable identification of severity or the risk category. Stages at which the microbial hazards colonize the beef carcasses also deserve

investigation as these would help to demarcate critical control points and set limits beyond which occurrence of the hazard would either risk public health, incur economic loss or both possibilities. Therefore, application of generic HACCP to the Horro beef production to consumption chain is a prototype study which can be implicated as a useful tool not only for production of safe beef but also to minimize consumers' health risks. Moreover, it would provide a baseline investigation beneficial to enable the country to compete for safe beef export markets as such experience could further be adopted by export abattoirs. Hence this study was conducted with objectives to identify microbial hazards, characterize associated risks, and set critical control points and critical limits of the hazard *per se* so as to enable application of HACCP as a tool to minimize risk to the public of disease(s) caused by the potential microbial hazards.

2.2. Materials and methods

2.2.1. Description of the supply chain and slaughter operation

The study was conducted following the supply chain of Horro beef slaughter stock to Addis Ababa abattoir (AAA). The AAA, located in Addis Ababa city (9.03^0 N 38.7^0 E), is a high throughput carcass processing center. The abattoir is mandated to provide slaughter services for retailer butchers based in Addis Ababa city for in city carcass retailing. An initial meeting was held with staff members of the slaughter division of the AAA to develop flow charts of the Horro slaughter stock supply chain, carcass dressing operation in the abattoir, and its distribution to develop plans for sampling and assessing hazards as well as to discuss issues deemed necessary.

The following points were made clear.

1. Species and identity of the stocks slaughtered in the AAA
2. Flow chain of the Horro cattle slaughter stock
3. Lairage and carcass dressing line flow steps

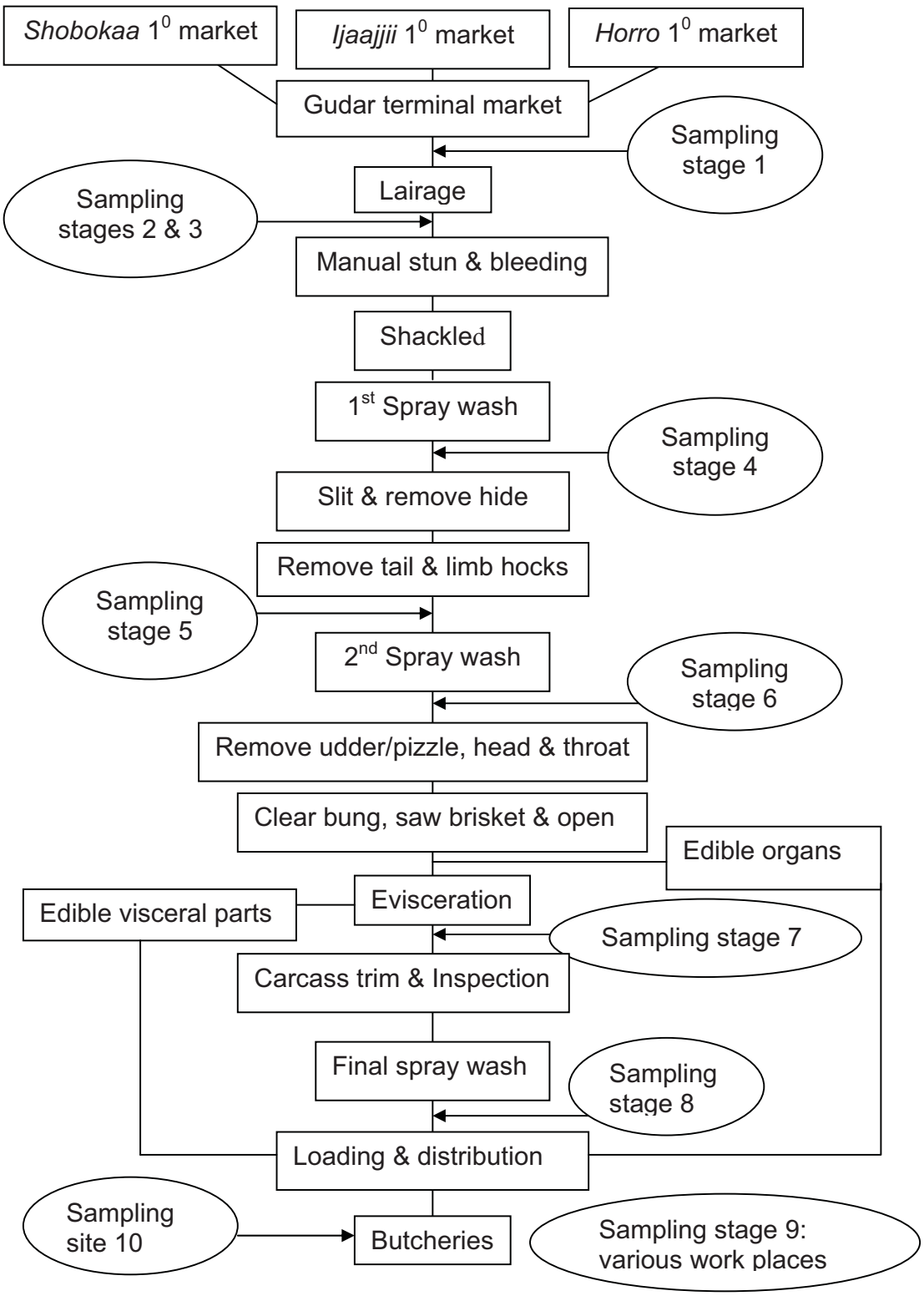

Figure 2.1. Flow diagram of Horro beef supply chain, carcass dressing and

distribution of Addis Ababa abattoir. Other components not shown in the Fig. 1 were considered to have no health significance to Horro beef consumers as they were removed from the carcass chain.

4. The number of personnel involved in slaughter operation and utensils used by the operators.
5. Horro slaughter cattle supply chain and dressing flow diagram was developed at the site and validated.

The substantial amount of beef stock consisted of the Horro cattle breed. While slaughter of cattle was a regular activity, that of sheep and goats was occasional. The throughput per day of beef slaughter ranges from 600-700 animals. The abattoir undertakes an independent flow chain of the carcasses for each species. The number of carcass operators range from 50-60 persons and slaughtering is conducted manually. Slaughter service is carried out on every day of the week. *Ante mortem* and carcass-by-carcass *postmortem* inspection is an integral part of the daily activity conducted jointly by staff veterinarians of the abattoir and statutorily mandated staff of the Veterinary Department of Federal State Ministry of Agriculture and Rural Development of Ethiopia. Fit carcasses and edible organs are finally washed and distributed at ambient temperatures to the butchers for retailing.

Butchers purchase from resellers at terminal markets and transfer the stock to the lairage of AAA. Animals from the lairage were entered to the abattoir and individually stunned by knife on a restraining floor. After bleeding and shackling, the carcasses were raised to the processing rail and spray washed with water (about 20 ^{0}C). At the first work stand, the carcasses were skinned, extremities like tail and hocks were removed. The carcasses were then moved to a 2nd stand for spray wash, operations on the udder/pizzle, head and throat. These trimmed extremities and the hide were removed from the carcass dressing line for purposes other than consumption. The carcasses were further moved to a 3rd

stand for carcass splitting and eviscerating operations, and then to a 4th stand for final trimming. Then the official inspectors examine fitness of the carcass and fit carcasses, viscera as well as edible organs were finally spray washed with lukewarm water (about 20 ^{0}C) and were loaded in trucks for distribution to butcheries.

2.2.2. Sampling and hazard assessment plan

Based on the principles of HACCP (Codex, 1997), samples for hazard identification were collected at ten process stages from terminal market to butchery on five slaughter days per week for five consecutive weeks. Samples at the terminal markets of the stock production area were collected by randomly selecting the animals at the point of loading to the AAA (stage 1). Sampling locations were from brisket and flank area on animal hide surface (stage 1) (MSC, 2002). The sampling stages in the abattoir were randomly sampled substrates in the lairage (stage 2), animal hide surface in the lairage (stage 3), hide surface immediately after first spray wash (stage 4), carcass surface immediately before 2nd spray wash (stage 5), carcass surface immediately after second spray wash (stage 6), at evisceration from visceral contents (stage 7), from carcass surfaces after the final spray wash at the point of loading (stage 8), composite sample from stands, abattoir's walls, washing table after routine cleansing and operators' hands (stage 9) and in butcheries from retail cut tables (stage 10). Locations of samples taken from hide of the animal at all stages in the premises of the abattoir coincide with and that from carcasses underlay the sample locations taken from hide of live animal at the terminal market. Other samples taken other than from animal/carcass were randomly taken from the specified category.

On each day of sample collection, beef animals and derived carcasses were coded for sampling as one complete sampling unit. Following each coded animal/carcass unit a total of 250 microbial samples (n = 5 days x 5 weeks = 25

samples per sampling stage) were collected. Sponge samples at stages 1, 3, 4, 5, 6, and 8 were aseptically collected following the technique of MSC (2002) from two locations (brisket and flank region) on the surface of either the tagged slaughter animal or its carcass. A sterile sponge (polyurethane, 10 ×10 cm^2) was moistened by 15 ml of 0·1% (w/v) Peptone Buffered Water (PBW) (Catalog No. B8910, Difco) and swabbed 10 times against the fur of the animal or wiped 10 times horizontally and vertically on the carcass in a delineated area (sterile metal template) of 10x10 cm^2. The same side of the sponge was used for each stage's sampling location. After swabbing, each sponge sample was kept in a tightly fastened bag. For the substrate in the lairage and visceral contents (digesta) after evisceration (stages 2 and 7), 25 gm samples were collected, rinsed with PBW, macerated and each put into a labeled screw capped borosilicate bottle.

Sponge samples taken at work areas (stage 9) were randomly aseptically collected from operator's hands and knives, stands, abattoir's walls, and washing table after the final cleansing activity. Samples from the surfaces of each work area were taken from delineated (100 cm^2) area per the work areas described above. Operative's knife hands were sampled according to Bell and Hathaway (1996). The knife hands were placed into a sterile plastic bag containing 50 ml of sterile PBW (0.1%, w/v). The sampler gripped the bag around the operative's arm just above the wrist joint and then vigorously shook the relaxed hand inside the plastic bag for 2 s. On completion of the 'hand shake' procedure, the plastic bag was squeezed down over the knife hand to maximize dilution fluid recovery. Then the bag was removed and tightly secured. The approximate hand area sampled was determined by tracing an outline of the hand onto graph paper. The sample area was twice the area of hand outline to account for both sides of hands sampled. Samples from the operators' knife were collected by dragging a sterile sponge (polyurethane, 10 ×10 cm^2) moistened by 15 ml of 0·1% (w/v) PBW (drag swabs). The area of the knife was defined by tracing an outline of the knife onto graph paper. The sample area was twice the area of knife outline to account for both sides of knife sampled. Microbial contaminations identified for stage 9 were

expressed in terms of each unit area, pooled together as one composite sample per stage 9 and calculated to give homogenate dilution (1/10).

After collection, all the samples were immediately transported to in a cooler on ice packs the microbiology laboratory for analysis at the Armauer Hansen Research Institute (AHRI)/All African Leprosy, Tuberculosis, Rehabilitation and Training Center (ALERT).

2.2.3. Hazard enumeration

Once in the laboratory, sample homogenates (1:10, w/v) were prepared with appropriate volumes of 0·1% peptone buffered water (PBW) (Catalog No. B8910, Difco) by thoroughly mixing the bags of sponge samples for 20 seconds (MSC, 2002) and by macerating, followed by homogenizing the faecal and visceral samples for 30 min using Orbital incubator (INR200, Gallenkamp, England).

From the non-selective enrichment broths appropriate serial dilutions were made and incubated for 24 h at 37 ^{0}C. Broth culture (0.1 mL) was spread plated on Plate Count Agar (PCA) (BO0263, Oxoid) at 37 ^{0}C for 24 h for enumeration of aerobic counts (AC). Enterobacteriaceae (EC) and total coliforms (CC) were enumerated on MacConkey Agar (MACA) (CM0115, Oxoid) and Coliform Agar (CA) (87959 HiCrome, Fluka, GmbH) after incubation at 37 ^{0}C for 24 h. Plates with 25-250 CFU were selected and colony forming units (CFU) counted using colony counter (Cat No 3.081 002, Schütt count^{plus}, GmbH, Germany). Numbers of colonies per ml or g (N) were calculated (Maturin and Peeler, 1998) as follows:

$N = \Sigma C / [(1 * n_1) + (0.1 * n_2)] * (d)$

Where N = Number of colonies per ml or g of product

C = Sum of all colonies on all plates counted

n_1 = Number of plates in first dilution counted

n_2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

2.2.4. Hazard confirmatory tests

After counting of CFU, 8-12 colonies were picked randomly by sterile swab from MACA and CA plates and subjected to miniaturized and standard Analytical Profile Index (API 20E) biochemical test strips (BioMerieux, Marcy l'Etoile, France API) for characterization of the hazard.

The plastic test strips each holding twenty mini-test tubes were inoculated with a saline suspension of the isolated culture. A few tubes (CIT, VP and GEL) were completely filled and some tubes (ADH, LDC, ODC, H_2S, URE) were overlaid with mineral oil such that anaerobic reactions could be carried out. After incubation in a humidity chamber for 18-24 h at 37°C, the color reactions were read and the reactions were converted to a seven-digit code. The code was fed into the manufacturer's database (apiweb, bioMerieux) via computer and the computer determined identity of the hazard was recorded.

2.2.5. Selective detection of high risk posing hazards

Detection of *E. coli O157:H7, Listeria monocytogenes* and Salmonella species generally followed the methods of (FDA, 2002; Kofitsyo et al., 1995).

2.2.5.1. Selective enrichment

Sponge, fecal and tissue samples were taken from all supply and process stages. The samples at each stage were transferred into screw cap tubes each containing 225 mL of modified Tryptone Soya Broth containing novobiocine (mTSB + N) (BO0869E, Oxoid), University of Vermont Medium (UVM-I) (SR0142 & CM0863, Oxoid) and Buffered Peptone Water (BPW) (CM0509, Oxoid), for selective enrichment of *E. coli O157:H7*, *Listeria monocytogenes* and *Salmonella*

enterica, respectively. The samples were kept in cooler boxes and transported to the laboratory for analysis.

Once in the laboratory, all sample tubes of mTSB + N, UVM-I and BPW broth culture were incubated at 42 ^{0}C, 30 ^{0}C and 37 ^{0}C, respectively, for 24 h. Following incubation, 2.5 mL of each enriched broth sample were pooled into sterile tube to represent one composite sample per process stage for identification. Thereafter, the Immunomagnetic separation (IMS) technique was applied using Dynabeads® anti-*E. coli O157*, anti-Listeria and anti-Salmonella bead (Dynal) consisting of magnetic beads coated with *E. coli O157*, Listeria and Salmonella antibodies, as recommended by the manufacturer.

2.2.5.2. Immunomagnetic separation

Following the manufacturer's suggested protocol, a 1 mL aliquot of each enriched culture and 20 *µ*L of Dynabeads (Dynal) were combined in a 1.5 ml microfuge tube. The tubes were briefly vortexed and incubated for 20 min at room temperature with intermittent shaking. Tubes were placed into a magnetic particle concentrator and left undisturbed for 10 min to allow the magnetic beads to concentrate onto the side of the tubes. The supernatants were aspirated using sterile Pasteur pipettes, leaving the beads concentrated on the sides of the tubes. The concentrates were re-suspended in 1 mL PBST (0.15 *M* NaCl, 0.01 *M* Na2HPO4, pH 7.4, and 0.05% Tween 20) wash solution. Tubes were shaken to evenly distribute the beads in the wash solution and allowed to sit undisturbed for 10 before the supernatants were decanted. The washing step was repeated twice. After the third final washing process, all the pathogen-beads complexes were re-suspended in 100 *µ*L PBS-Tween 20. For further selective enrichment of BPW broth, 100 µL of re-suspended bead–Salmonella matrix was transferred onto Rappaport Vasilliadis soya peptone broth (CM 866, Oxoid) and incubated at 42 ^{0}C for 18–24 h.

2.2.5.3. Sub-cultivation

Post-IMS cultures bead matrix were sub-cultured onto their respective media. For confirmation of *E. coli*, 50 µL of the re-suspended samples were be inoculated onto sorbitol MacConkey agar (SMAC) (CM0813, Oxoid) supplemented with cefixime (SR0191, Oxoid) and potassium telluride (SR0030, Oxoid) using sterile cotton swab and incubated anaerobically at 37 ^{0}C for 24 h. Three to five non-sorbitol fermenting (NSF) colonies were picked and sub-cultured on Violet Red Bile Agar (VRBL) (CM107, Oxoid) containing 4-methylumbelliferyl B-D glucorinide (BR0071, Oxoid). The colonies grown on VRBL agar plate were incubated at 37 ^{0}C for 18-24 h and checked by UV light (4W/ 366 nm) for fluorescence activity in the dark.

For confirmation of Listeria and Salmonella species, 50 mL of each the Dynabeads–Listeria and Dynabeads–Salmonella complexes were streaked onto Palcam (SR150 and CM0877, Oxoid) and Oxford (SR140 and CM0856, Oxoid), and modified brilliant green agar (CM0329, Oxoid) and Xylose Lysine Deoxycholate (CM0469, Oxoid) agar plates, respectively. The cultures of Listeria and Salmonella were incubated at 30 ^{0}C for 36-48 h and 37 ^{0}C for 24 h, respectively. Three colonies of each culture were re-streaked onto TSA (CM131, Oxoid) and respectively incubated at 30 ^{0}C and 37 ^{0}C for 24 h.

2.2.5.4. Biochemical confirmation and serotyping

The non-fluorescent colonies (b-glucorinidase-negative colonies) of *E. coli* culture were analyzed with Wellcox® *E. coli O157:H7* rapid latex agglutination test (R30959601, Oxoid). The biochemical test of TSA positive Listeria cultures followed Gram stain and morphological confirmation. The TSA positive Salmonella cultures were inoculated onto urea broth (RO0338B, Oxoid), lysine decarboxylase broth (CM0308, Oxoid) and triple sugar iron (CM0277a) slants.

Final identification of confirmed *E. coli*, Listeria and Salmonella isolates were carried out using the Microbact GNB 24E (MB1074A, Oxoid), 12L (MB1128, Oxoid) and 24E (MB1132, Oxoid), respectively, according to the manufacturers' instructions. The pure cultures of all categories of isolates were serotyped based on the reactions of somatic or cell wall (O) and flagellar (H) antigens with a series of polyvalent and monovalent antisera according to the manufacturer's instructions.

2.2.6. Data analysis

The quantitative data sets of 25 obtained through the supply and process stages were transformed to log bacterial counts. In all calculations for sets of AC, log values of 1·8/100 cm^2 were assumed for samples in which counts were less than 60 CFU/100 cm^2. In all calculations involving sets of EC or CC counts, log values of 1.5/100 cm^2 were assumed for samples in which EC or CC were not detected at the level of 1 CFU/100 cm^2. Data was analyzed as of one way ANOVA (SAS, 2003) on the assumption that the log values were normally distributed (Kilsby and Pugh, 1981). Significant mean separation ($p < 0.5$) were done using Ryan–Einot–Gabriel–Welsch multiple range test (REGWQ) option of ANOVA procedure (SAS, 2003). Data of the identified hazards was reported as percentage and qualitatively for each process stage.

2.3. Results and Discussion

2.3.1. Enumeration and identification of the hazards

Bacterial hazards are a major concern in the production of food of animal origin (Notermans et al., 1994). The contamination of the flesh by such hazards is likely to provide a risk to human health and is considered undesirable. Therefore, identification of the hazard and its entry point following production to consumption

chain of the meat is essential. Fig. 2 illustrates studies conducted to address these issues and results obtained from enumeration of AC, EC and CC.

There were significant differences in the numbers of bacteria recovered at the different stages within the supply and processing chain of Horro beef. The mean log CFU/cm^2/g^2 AC varied between 3.567 and 4.128 for the samples of carcass at stages 8 and 2, respectively. Following similar distribution pattern of AC, the mean log CFU/cm^2/g^2 EC varied between 3.742 and 4.059 for the samples at stages 8 and 2, respectively. The distribution of CC gave mean log range of 3.757-4.037 CFU/cm^2/g^2 respectively at stages 10 and 2. The overall values of AC were in the range of values (3.5 to 10.7 log CFU/g^2) reported by Gashe and Mpuchane (2000) for beef products at butcheries.

The highest significant ($p < 0.05$) stages of contamination were animal hide surface and substrates in the lairage across all hazards enumerated. The highest log mean number of hazards enumerated at lairage stage may be linked to the disposal site of unfit carcasses, trims and drainage effluents via ditch accumulated over years in an open site located in about 100m from the lairage. In a similar study, the major contamination points during swine slaughter were identified as pig-related, such as faecal and pharyngeal, and environmental (Borch et al., 1996). The contamination levels at terminal market (stage 1) were comparable with levels of contamination at stages 4, 5 and 9 in the case of AC and EC, and to stage 4 and 7 in the case of CC. The log mean contamination levels of all contaminants at stage 4 indicate that the 1st spray wash activity of the abattoir had significantly reduced the log mean contaminants at stage 2 and 3 but not significantly reduced the contamination level relative to the stage 1.

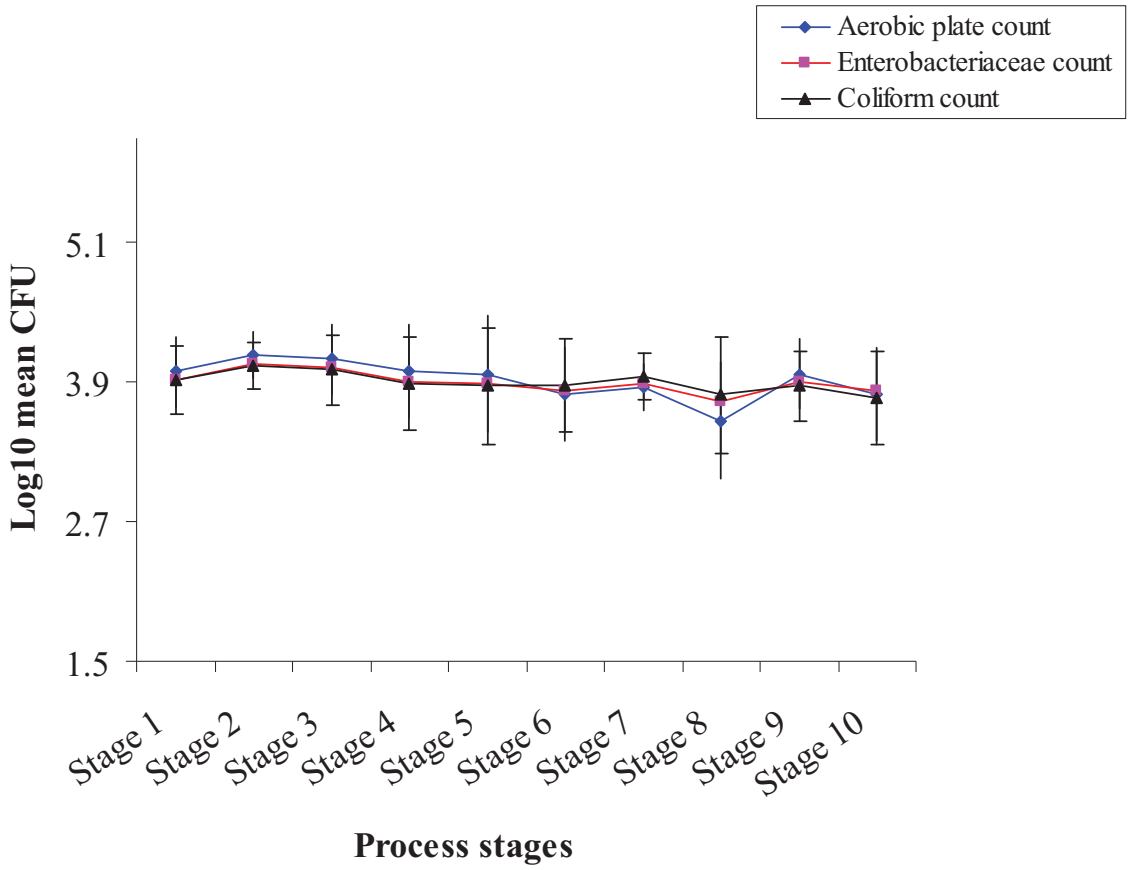

Figure 2.2. Log mean (CFU/cm^2/g^2 sample) of hazards at selected processing stages of Horro beef supply and slaughter chain. The process stages at which samples collected were animal hide surface at terminal market (stage 1), substrates in the lairage (stage 2), hide surface of animal in the lairage (stage 3), hide surface immediately after the 1st spray wash (stage 4), carcass surface immediately before 2nd spray wash (stage 5), carcass surface immediately after 2nd spray wash (stage 6), at evisceration (stage 7), carcass surface after the final spray wash at the point of loading (stage 8), from stands of carcass operation, abattoir's walls, viscera and other internal organs washing table after routine cleansing (stage 9) and in the butchery from retailing table (stage 10). Samples collected from animal hide surface or carcasses overlay each other and were from brisket and flank area.

The stage of hide pulling had been implicated as a major point of bacterial contamination of beef carcasses (Madden et al., 2004). A further significant reduction of contamination by AC and EC were observed after the second spray wash at stage 6. However, the 2nd spray wash didn't significantly affect the contamination level of CC at stages 4, 5 and 6. The log means count of AC, EC and CC significantly increased at stage 7 compared to stage 6 indicating the possibility for recontamination of the carcass by these hazards during evisceration.

The final spray wash before loading carcasses in trucks significantly reduced the log means to lowest level of the contaminants in the abattoir. In the butchery, the log means of AC and EC significantly increased compared with contamination levels at stage 8. The contaminants at the work area gave significantly higher log mean counts compared to stages 6, 7 and 8 in the case of AC, stages 6 and 8 in the case of EC, and stage 8 in the case CC within the abattoir. The increased level of contaminants at stage 9, regardless of its process stage in tier, is important because the contaminants could have access to carcass contact for recontamination at any stages in the abattoir. This explanation is in agreement with the observation of Barros et al. (2007) who stated without proper hygienic control, the environment in slaughterhouses and butcher shops could act as an important source of microbiological contamination. The overall distribution pattern of CC gave slightly lower than both AC and EC except at stages 6, 7 and 8 where it showed log mean higher than both AC and EC. The higher log means of both AC and EC relative to CC at stages 6, 7 and 8 may have been related to environment favorable to the formers. In a similar study, Madden et al., (2004) also reported higher mean of EC at washing stages before dispatch than at hide removal of beef carcass processing stages and explained it in terms of the probability of redistributing bacteria by the washing practice.

Kotula et al. (1975) reported that surface microbial populations of 1.0×10^5 cm^{-2} or more were able to cause health problems. On the other hand it had been

reported that microbe numbers of only 500 cells are able to cause illness in man (Robinson, 1981). The findings of the present work reported the log mean contaminants in the rage of the previous two studies' findings. Of course, the difficulty of making valid microbial comparisons between different slaughter procedures has been discussed by several authors (Madden et al., 2007; Bell and Hathaway, 1996). Therefore, comparisons of our results were made in the most general terms because of scarcity of literature reports on very similar work. Regardless of the numbers, attachment of bacteria to food product or product contact surfaces leads to mild poisoning to serious cases of food-borne infection or intoxication requiring hospitalization, serious hygienic problems, economic losses due to food spoilage, and complaints of low quality product (Brown, 2002, Mattila-Sandholm and Wirtanen, 1992).

Generally, the most unacceptable levels of contamination were caused by the filthy conditions in the lairage and the environment under which slaughter steers had been raised, as well as the working environment in the abattoir. The enumeration of AC, EC and CC indicates that there may be a way for beef-borne pathogens to enter the system at any point in the chain and that action should be taken to improve hygiene. For instance, using logistic regression, Ceylan et al. (2008) predicted positive relationship of EC with availability of pathogenic hazard *Listeria monocytogenes*. Hence, in order to assure the hygiene of the carcass supply to butchery, stakeholders must make efforts to control feces and filthy conditions so as not to spread the contaminants from hides, viscera and the working environment to the carcass all along the farm to fork chain. The sources of contamination identified in the present study are in agreement with the reports of Bell and Hathaway (1996) who stated the contact between the carcass and the hide, the fleece in the case of sheep and lambs, allows contamination with a mixture of microorganisms derived from the animal's pre-slaughter environment, including those of faecal, soil, water and feed origin.

The enumeration study showed that the overall effect of spray wash led to a quantitative over all decrease of log means (CFU/cm^2/g^2) of 0.561, 0.317 and 0.164 for hazards AC, EC and CC, respectively and implicated the terminal markets and, thereafter, from lairage to the final carcass retail in butchery shops generally had substantial effects on the load and type of bacteria from the slaughtered steers and derived carcasses (Fig 2). Further identification works illustrated important pathogenic hazards which may be considered qualitatively rather than quantitative (incidence) (Table 1) distribution throughout the process stages. Although the incidence reported for each pathogenic hazard gave a

Table 2.1

Types and distribution frequencies of identified hazards within the supply and processing chain of Horro beef

Hazard name	Number of examined samples	Number of positive samples	Incidence (%)	Biohazard level[1]
Citrobacter freundii	250	39	15.6	01
Citrobacter youngae	250	39	15.6	01
E. coli	250	55	22	01 and 02
E. coli O157:H7	250	37	14.8	02
Klebsiella pneumoniae	250	26	10.4	02
Kluyvera species	250	27	10.8	02
Listeria monocytogenes	250	35	14	02
Morganella morganii	250	43	17.2	02
Proteus mirabilis	250	36	14.4	02
Proteus vulgaris	250	36	14.4	02
Pseudomonas aeruginosa	250	34	13.6	02
Salmonella species	250	40	16	02
Serratia fonticola	250	32	12.8	01
Serratia liquefaciens	250	29	11.6	01
Serratia odorifera	250	31	12.4	01

[1]Biohazard level according to DEHS (2007) and Suchman and Laxon (2004): Level 01 means biological agents that are unlikely to cause disease in healthy individuals or animals (low individual and community risk) and level 02 indicates pathogens that can cause human or animal disease but, under normal circumstances.

range of 10.4 to 22% seemingly low, qualitative consideration of each hazard may most reflect their implications for carcass safety and the consequent risk they may pose on public health. Hence, both the enumeration and hazard findings (Table 1) confirm the importance of re-examining and possibly modifying individual and combined processes within the scope of HACCP.

2.3.2. Critical control points and limits

Considering the risk likely posed by the identified hazards on public health (i.e. the severity of the hazard), the hazard were categorized either under biohazard level one or two. It was defined (DEHS, 2007; Suchman and Laxon, 2004) that level one and two biohazards respectively represent biological agents that are unlikely to cause disease in healthy individuals or animals (low individual and community risk) and pathogens that can cause human or animal disease under normal circumstances. Among the identified biohazard level 2, the highest incidence was for *Morganella morganii* (17.2%) followed by Salmonella species (16%). The hazard *Klebsiella pneumoniae* (10.4 %) showed the least percentage incidence. Within the limits of isolation procedures followed, the incidence reported herein was in comparison with reports of Gashe and Mpuchane (2000). However, our report on incidence of *Listeria monocytogenes* was lower compared to incidence reported by Ceylan et al. (2008). Based on the guideline for listing, inclusion or deletion of the potential hazards (Notermans et al., 1994), hazards of biosafety level 02 would be considered for critical control point (CCP) and critical limit establishments.

The FSIS (1996) final rule stated that the risk of foodborne illness associated with food-borne pathogens is largely avoidable and can be minimized by proper implementation of HACCP. With this recommendation in mind, we followed the HACCP model Codex (1997) for the present study to describe possible ways of HACCP implementation in AAA so as to reduce or eliminate public health risk. The Codex (1997) HACCP model consisted of four steps of decision tree and

seven principles to enable preventive measures against risks likely to endanger consumer health. We conducted the analysis of hazard (principle 1) based on the flow diagram of the Horro beef supply and process chain developed at the onset of the study (Fig.1). The figure indicates that the stock purchase commenced either at primary markets in the production area and/or at terminal markets and that the process completed with retail sales of the carcass at the butchery for uncooked and/or cooked beef consumption. The type and intensity of the identified hazards following the chain were as indicated in Table 1, and the lists of potential hazards and the stages at which the hazards colonize the carcass were given in Table 2.

The identification of the CCPs across the process stages (principle 2) were done in consultation with the experts of slaughter department of the AAA and guided with decision tree of Codex (1997) as indicated in Table 2. When the stage was not important as CCP, it was either determined as CCP2 or assigned not important for critical control stage. Establishment of critical limits for preventative measures associated with each identified CCP (principle 3) was based on the biohazard level definition (DEHS, 2007; Suchman and Laxon, 2004) attached to the hazards identified. For hazards defined as biohazard level 2, the concept of zero tolerance was used because infectious organisms such as Salmonella can cause health disorders even when present in low numbers and they should not be deleted from the list of potential hazards (Notermans et al., 1994). Also the stages at which the hazards were identified were considered to determine the critical limits. If the stage at which the hazard identified was not set as CCP, setting limits to the hazards were not considered important.

For hazards identified at stage 1, 2, and 3 of the supply and process chain, both the terminal market and lairage may not be considered as CCP because improvement of husbandry practices and keeping better level of lairage hygiene

Table 2.2

Hazard distribution along process stages and suggested CCP of the hazards identified.

Step	Hazard	Q1 [a]	Q2 [b]	Q3 [c]	Q4 [d]	CCP: ccp1 or ccp2 [e]
Stage 1	*Proteus mirabilis*	No	No	-	-	Not ccp
	Proteus vulgaris	No	No	-	-	Not ccp
	Serratia fonticola	No	No	-	-	Not ccp
Stage 2	*E. coli O157:H7*	Yes	No	Yes	Yes	Not ccp
	Listeria monocytogenes	Yes	No	Yes	Yes	Not ccp
	Morganella morganii	Yes	No	Yes	Yes	Not ccp
	Pseudomonas aeruginosa	Yes	No	Yes	Yes	Not ccp
	Proteus mirabilis	Yes	No	Yes	Yes	Not ccp
	Proteus vulgaris	Yes	No	Yes	Yes	Not ccp
Stage 3	*Morganella morganii*	Yes	No	Yes	Yes	Not ccp
	Proteus mirabilis	Yes	No	Yes	Yes	Not ccp
	Proteus vulgaris	Yes	No	Yes	Yes	Not ccp
	Pseudomonas aeruginosa	Yes	No	Yes	Yes	Not ccp
Stage 4	*E. coli O157:H7*	Yes	No	Yes	No	ccp2
	Listeria monocytogenes	Yes	No	Yes	No	ccp2
	Morganella morganii	Yes	No	Yes	No	ccp2
	Pseudomonas aeruginosa	Yes	No	Yes	No	ccp2
Stage 5	*Morganella morganii*	Yes	No	Yes	Yes	Not ccp
Stage 6	Kluyvera species	Yes	No	Yes	No	ccp2
	Salmonella species	Yes	No	Yes	No	ccp2
Stage 7	*Klebsiella pneumoniae*	Yes	Yes	-	-	ccp1
	Morganella morganii	Yes	Yes	-	-	ccp1
	Pseudomonas aeruginosa	Yes	Yes	-	-	ccp1
	Salmonella species	Yes	Yes	-	-	ccp1
Stage 8	Kluyvera species	Yes	Yes	-	-	ccp1
	Salmonella species	Yes	Yes	-	-	ccp1
Stage 9	*Morganella morganii*	Yes	No	Yes	Yes	Not ccp
Stage 10	*E. coli O157:H7*	Yes	Yes	-	-	ccp1
	Morganella morganii	Yes	Yes	-	-	ccp1
	Salmonella species	Yes	Yes	-	-	ccp1

[a] Question 1: Do preventive control measure exist for the identified hazard? (Yes/No)

[b] Question 2: Is the step specifically designed to eliminate or reduce the likely occurrence of a hazard to an acceptable level? (Yes/No)

[c] Q3: Could contamination with identified hazard (s) occur in excess of acceptable level (s) or could this increase to unacceptable levels? (Yes/No)

[d] Q4: Will a subsequent step eliminate the identified hazard (s) or reduce likely occurrence to acceptable level (s)? (Yes/No)

[e] Critical control point (CCP): Type of control point assigned to the process stage are either CCP1 (those must be designated critical control points, if the hazardous contamination can be wholly prevented or eliminated) or CCP2 (if the contamination by the hazard can be only minimized or substantially reduced).

would suffice to reduce the pathogenic hazards. For instance, Oosterom and Notremans (1983) reported that the observance of strict hygiene in pig-fattening units brought immediate benefits in the reduction and elimination of Salmonella and other pathogens. After the first spray wash (stage 4), hazards were identified indicating the effect of the wash was not enough to eliminate available hazards. The stage also leads to hide removal and is where the chance of direct hazard contamination from hide surface or cross contamination from operators' hands/knives while removing the hide to carcass could be possible. Similarly, stage 6 is after the second spray wash that leads to carcass trimming operation and open up, where not only the carcass external surface but also internal surface as well could be cross contaminated. While operations at both stages 4 and 6 indicate possible chances for cross contamination or redistribution of the hazards, and that, though, not crucially important for elimination of the hazards, they could be manipulated to reduce the hazards. Therefore, designation of the stages as CCP2 would help to attract monitoring attention and temperature manipulation at the first and second washings so to reduce the hazards. Regarding temperature manipulation, Gill et al. (1996) reported that wash water temperatures of 85 ^{0}C or higher are necessary if a decontamination effect is to be obtained. Experiments of washing carcasses for 20 s with water at 85 ^{0}C had been reported to yield a 2 log CFU/cm^2 reduction in *E. coli* (Gill et al., 1996) and *Salmonella typhimurium* numbers (Van Netten et al., 1995). The decontamination effects of such washing could be enhanced by the inclusion of additional wash components. For example, lactic acid treatment of beef carcasses, prior to chilling, has been shown to reduce bacterial levels up to 3 log cycles (Castillo et al. 2001). Similarly, the addition of chlorine or trisodium phosphate to the wash

water significantly decreases aerobic and enterobacteriaceae counts on poultry carcasses (Whyte et al., 2001).

Stages five and nine were chosen for sampling to give signal whether the extremities removed during dressing operation and slaughter processing environment were important for redistribution or facilitate recontamination of the hazards. It must be clear, however, that neither the hide, the limbs and tails removed immediately before stage five would proceed in slaughter processing line, nor the stands, floors and walls at that stages could have direct contact with carcass, minimizing the possibility of their cross contamination. Moreover, to control the hazard identified at stage five the second spray wash and good hygiene practice could be considered appropriate. Hence, setting stages five and nine either as CCP1 or CCP2 seems unwise. In a similar study, Borch et al. (1996) stated that consideration of various working areas (like stage 9) for CCP might not be useful because endemic bacteria can be controlled by proper cleaning and disinfection because the organisms are useful only as indicators for the success of good manufacturing practice rules.

Stages 7 and 8 represent the final slaughter line stages of the AAA's current practice for both product categories: carcass and edible internal organs (kidney, tongue and viscera). Hazards like Salmonella species had been identified from both viscera and carcass surface at point of loading (after final wash). Taking the uncooked beef consumption behavior in Ethiopia, these slaughter line end products are ready for consumption as there were no further carcass process stages in the abattoir for possible control. This means that if the carcass or internal organs could be consumed, the hazards would be getting chance of direct in take to possibly risk consume health. Further, the hazards identified at those stages were widely reported to cause health risk. Therefore, designating both stages as CCP was essential. Also, considering the biohazard level definition of the hazards (Table 2) identified at stages 7 and 8, we suggest the critical limit for tolerance of these hazards needs to be non-existence. Similar

recommendations had been stated in the review of Hulebak and Schlosser (2002) on CCP of Salmonella species identified at evisceration step of beef slaughter. Once the carcass would be out of the abattoir, it would be distributed for retailing in the butchery with no control process step in between abattoir and butchery. The study also confirmed the presence of hazards on retailing table in the butchery. Therefore, hazards identified in the butchery can have direct intake access to the consumer especially where uncooked meat consumption in the restaurants connected to the butchery is common practice. Hence, butcheries must also be assigned CCP.

The requirements for monitoring of the CCP and establishing corrective actions to be taken when monitoring indicates a deviation from an established critical limit were dealt based on the general principles of HACCP implementation and based on the discussions held with the experts of AAA as there were no established functional HAACP team in the AAA in particular or in the country in general. It is clear that the AAA doesn't practice carcass chilling and also the carcass packing vehicle and butchery were furnished with carcass cooling units in the course of carcass distribution and retailing. Carcass chilling, however, has been implicated important to reduce levels of bacterial contamination of sheep carcass (Yalcin et al., 2004) and pork (Gill and Bryant, 1992), and that it can also be used as CCP because it prevents the proliferation of bacteria on warm carcass surfaces. Significant decrease of hazards may be possible in relation to specific hazard category to survive change of temperature from the carcass surface to chilling temperature because hazard properties such as sensitivity to temperature could be used to facilitate application of HACCP (Notermans et al., 1994). Interventions to suppress pathogen numbers in the gut, such as the inclusion of sodium chlorate in preslaughter feed which has been reported to reduce the levels of *Salmonella typhimurium* (Anderson et al., 2001) could also be useful. Therefore, for possible corrective action to achieve the critical limit of the hazards, future intervention should include chilling practice before loading beef carcass for retail distribution to achieve the limits suggested at stages 7 and

8. Within the given constraints to retail a chill hanged carcass in the butchery, consumption of uncooked beef could lead to a stage where hazards may pose direct health risk to consumers. Therefore, alternative strategies which focus on the identification of minor, commercially viable changes or enhancements to current elements of the carcass butchering practices are more likely to be adopted by the butchers. Meanwhile, cooking of the retailed carcass cuts, especially before consumption by immune-compromised segments of the consumers, could help to avert the likely risk posed by the hazards.

Hence the monitoring and corrective actions to be considered and implemented by the department of slaughter operation of the AAA should focus on achievement of the limits by enhancing the suggested process modifications. The verification step should not be restricted to the control criteria at specific CCPs. It should also include an analysis of any consumer complaints that occur after consumption of the product.

3.4. Implication

Available inspection guidelines and work procedures of the AAA were reviewed, expanded and incorporated into the HACCP monitoring and documentation process. The State of Oromia and the Federal Government of Ethiopia should also formulate either mandatory or voluntary HACCP application policy in each domestic and export abattoirs. The practice of full scale HACCP plan should prevent hazards which may endanger consumer health and also increase competence of the country's beef export potential in the global trade market.

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Chapter 3:

Assessment of the sensory quality and shelf stability of selected Horro beef muscles in Ethiopia[1]

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Assessment of the sensory quality and shelf stability of selected Horro beef muscles in Ethiopia

Abstract

*The objective of this work was to assess sensory quality and retail life of Horro beef muscles in Ethiopia. Six muscles: M. rhomboideus (RM), M. infraspinatus (IS), M. longissimus lumborum (LL), M. semimembranosus (SM), M. biceps femoris (BF) and M. rectus femoris (RF) were considered. Sensory quality of the muscles was rated by a 9- member trained panel for palatability, tenderness, juiciness, amount of connective tissue (ACT), lean color and surface discoloration attributes and measured by Warner-Bratzler Shear Force (WBSF). Retail life of the six muscles were evaluated for CIE 1976 L*a*b* color values across six days. Significant differences ($p < 0.05$) between muscles for all sensory attributes and WBSF were found. Significant L*a*b* color values of muscles and USDA quality grades by retail life were found ($p < 0.05$). Significant correlations ($p < 0.05$ or $p < 0.01$) of key parameters were also identified.*

Key words: Horro beef, meat quality, shelf stability, Ethiopian consumer preference

3. 1. Introduction

Horro cattle are the most common indigenous breed used for meat in the Oromia region of Ethiopia (McDowell, 2002). The slaughter stocks from the breed are sold to local or central markets where formal slaughter is practiced. After slaughter and dressing, butcheries hang unchilled carcasses by hind legs on hooks and retail in a room commonly painted white under incandescent light. Carcasses may be displayed for five to six days after slaughter. The cuts can be sold uncooked and/or roasted in the butchers' own premises or sold for home consumption.

Fiber orientation in intact muscles can affect palatability, particularly tenderness of beef (Meade, Johnson, & West, 1992). However, Horro beef butchers fabricate the carcass without regard to muscle type or fiber orientation in an attempt to compromise quality. Kukowski, Maddock, Wulf, Fausti, & Taylor (2005) noted such a traditional cutting system, where beef chuck was sold as low-priced roasts and steaks consisting of a number of different muscles and various quantities of intra muscular fat which may result in consumer dissatisfaction.

Descriptors of beef sensory attributes related to visual appeal and palatability are regarded as key attributes to predict consumers' initial and continued interest in the consumption of beef. McPeake (2001) stated that meat palatability is generally referred to as tenderness, juiciness and flavor of the product. Therefore consumers' satisfaction with desired attributes of that part of the muscle will determine if the purchase is repeated (Morgan *et al*., 1991).

Consumers relate the color of lean meat to freshness (Rentfrow, Linville, Stahl, Olson, & Berg, 2004; Faustman & Cassens, 1991) and quality is equated to a bright red color (Behrends, 2004) and surface discoloration is the ultimate criteria that the consumers use to reject meat (Renerre & Labas, 1987; O'Keefe & Hood, 1982). Hence the surface color at point of purchase strongly influences consumers' selection of whole and retail cuts (Jeremiah, 1982; Hood, 1980). McKenna, Mies, Baird, Pfeiffer, Ellebracht, & Savell (2005) and Behrends (2004) stated that beef muscles exhibit a range of shelf color stability over time.

Nevertheless, to date, there is no report on Ethiopian consumers' preference for the retail characteristics of Horro beef muscles. Hence, to lay the groundwork for sensory quality and retail life of individual Horro beef muscles, this study aimed to characterize six Horro beef muscles.

3.2. Materials and Methods

Two experiments were conducted. The first experiment (Exp. I) was assessment of sensory quality by a trained consumer panel while the second (Exp. II) evaluated the shelf stability of selected Horro beef muscles. The Horro breed is distributed in the Oromia region of Ethiopia in an area ranging from 6^0 to 10^0N and 35^0 to 38^0 E (Galal, 1983). The breed has been classified, though not extensively, as Sanga-Zebu type (Albero and Haile-Mariam, 1982). Often the breed is chestnut in color, uniform in body conformation, medium in size with small to medium hump.

3.2.1. Panel description

Twenty five individuals were prescreened as potential members of trained descriptive panel and twenty male were selected for training. The panel members were trained to do descriptive analysis in accordance with the guidelines of American Meat Science Association (AMSA, 1995). Training consisted of presenting four times replicated blind samples for each of three muscles in three preliminary sessions to familiarize them with the characteristics to be evaluated. The samples were collected from butchers and the cuts were of chuck, loin and round muscles. Hence, using these blind samples the panel was trained to improve the individual's ability to recognize and identify sensory attributes. They were further trained in the use of the rating anchors by pre-testing samples expected to differ in the intensity of various attributes, calibrated against endpoint-measures.

The ratings from each session were analyzed using one way ANOVA (SAS, 2003) to check the consistency of the individual panelists and of the whole panel. Based on consistency of perceived differences within and between panelists, nine were finally selected as the more consistent candidates. The skills of the

nine panelists were maintained by testing their reproducibility with blind samples and by reviewing their results regularly.

3.2.2. Sensory parameters and test procedures

The parameters studied include a nine-member trained panel description of palatability, tenderness, juiciness, amount of connective tissue (ACT), lean color and beef surface discoloration. Most of the parameters were evaluated by means of eight-point structured scales (8 = extremely palatable, extremely tender, extremely juicy, none, extremely bright cherry red and 1=extremely unpalatable, tough, extremely dry, abundant, extremely dark red respectively with palatability, tenderness, juiciness, ACT and lean color). Beef surface discoloration was evaluated on 7- point scales (7=total discoloration, 1=no discoloration). With the exception of discoloration, evaluation of each parameter started about 8 hrs after exsanguination. Beef surface discoloration was evaluated on the 6th day.

The test procedure involved evaluation of steaks from nine carcasses for five key attributes (except surface discoloration). Two sets of six muscles (3 carcasses x 6 muscles = 18 treatments) were randomly presented to each assessor in two sessions for the quantitative descriptive analysis. Each panelist evaluated two muscles per day for each attribute with 15 minutes between the sessions. The 54 treatments (9 carcasses x 6 muscles) were evaluated on three days. This was to achieve variables of interest (muscle effects on sensory attribute) within a block (USDA quality grade). For the surface discoloration, six muscles from fifteen carcasses (Exp. I+II) were used. The nine judges were asked to evaluate discoloration on day six relative to the color of the muscle on day 1.

3.2.3. Experimental animals and carcass grading

Fifteen steers were purchased from local livestock markets with anticipated uniformity in USDA beef quality grade and humanely slaughtered. Steaks from

carcasses of nine steers were assigned to the study of sensory quality (Exp. I) while steaks from all 15 carcasses were used to study retail life (Exp. II). The weights of hot carcasses were recorded and USDA quality and yield grades were evaluated as described by Hale, Goodson, & Savell, (2004).

USDA (1997) quality grade was evaluated based on marbling score and overall maturity of carcasses (ZoBell, Whittier, & Holmgren, (2005). USDA preliminary yield grade evaluation was based on external fat thickness on the cut surface of the ribeye, hot carcass weight, ribeye area and internal fat and then yield grade was calculated (ZoBell *et al.,* 2005). Samples of intact muscles from both sides of all carcasses were taken to the laboratory of BARC for evaluation.

3.2.4. Muscles and sample preparations

Once in the laboratory, muscles were dissected and total dissectible fat was eliminated manually. Muscles evaluated were *M. infraspinatus* (IS), *M. semimembranosus* (SM), *M. biceps femoris* (BF) and *M. rectus femoris* (RF) from both sides of the carcass, and *M. rhomboideus* (RM) and *M. longissimus lumborum* (LL) from medial plane of the carcass. Each muscle from the left side of nine carcasses (Exp.I) was divided into two (proximal and distal) sub-samples while medial plane muscles were split into three (cranial, middle part and caudal) sub-samples. Sub-sample one (n = 9) - proximal of the nine left carcass sides and cranial part of the medial plane muscles- were used. Sub sample two (n = 15)-distal part of the left carcass side muscles and middle part of medial plane muscles from the nine carcasses of Exp. I as well as distal part of left carcass sides and middle part of medial plane muscles from the other six carcasses were additionally used for the shelf life evaluation. Sub-sample three (caudal part of medial plane muscles and muscles from the right sides) from all carcasses were frozen in liquid nitrogen, packed in PVC film and stored at -80 ^{0}C for analysis of other parameters.

The fresh sub-samples assigned to the panel were sliced into two replicates for each parameter. Each muscle sample was cut across the fibers and slices were served to the panel on dark colored plastic plates. The average for each judge for each parameter was recorded.

3.2.5. Warner-Bratzler shear force determination

The Warner-Bratzler shear force (WBSF) of each uncooked muscle was evaluated using an Instron Universal Testing Machine model 4301 (Instron, Canton, MA). Three 1.27 cm-diameter cores were removed parallel to the longitudinal orientation of the muscle fibers from each of the six steaks. Each core was sheared once at its center, perpendicular to the muscle fiber orientation with a Warner-Bratzler shear attachment using a 50 kg load cell and 50 mm/min crosshead speed. Average WBSF values (kg/cm2) of the three cuts per muscle were recorded.

3.2.6. CIE 1976 L a* b* color values*

Six fresh muscle samples from fifteen carcasses were evaluated for CIE 1976 L*a*b* values over six days as described by AMSA (1995). Saturation index [Saturation = $(a^2+b^2)^{1/2}$] was calculated for each muscle. Each sample from each experimental muscle were sliced into 15 cm^2 steaks and displayed in air on black plastic trays under retail conditions. The retail conditions included incandescent light (60 watt lamp) one meter above the steaks, and mean temperature of 20 ^{0}C and 80% relative humidity (BARC, 2007). Thus, the samples were displayed for 6 days and on each day reflectance (every 24 hrs) measurements were made with a portable Minolta Chroma meter CR-100 colorimeter (Minolta Camera Co., Osaka, Japan) on three non-overlapping meat surfaces per sample. Therefore, each measurement was the mean of three readings. The measurements were taken with illuminant A, 10° standard observer settings and a 3.18 cm aperture.

Calibration of the instrument was performed using a light trap and the white tile supplied with the instrument.

3.2.7. Data analysis

Preliminary analysis was conducted using Proc NESTED of SAS (2003). Muscles were nested under USDA quality grade to examine its effect on each parameter. Carcass was considered as an experimental unit. When variance component analysis showed no significance for the USDA quality grade or interaction of the factors, USDA quality grade was removed from the analysis. When significant variation was explained by the factor, it was included in the model. Animal ages at slaughter were included as covariate in the model for increased precision in determining the effect of muscle types on all measurements. By inclusion of the covariate the model assumes that the slopes examining the chronological age records are parallel for all muscles. To test whether this assumption was valid chronological age x muscle interaction was entered as main effect. The analysis of chronological age x muscle interaction as main effect did not yield a significant difference.

Therefore, in the statistical treatment of the sensory data only the muscle effect was considered whilst for the analysis of shelf stability muscle and USDA quality grade were entered as main effects. Accordingly, data sets were analyzed either as one way ANOVA or RCB design using Proc GLM of SAS (2003). When *F* values were significant ($p < 0.05$), the mean separation technique of Tukey was used. Proc CORR of SAS (2003) was conducted to determine relationships among parameters.

3.3. Results

3.3.1. General carcass characteristics

The average weight of muscles varied from 1.14 kg for RF to 3.85 kg for LL (Table 1). The two muscles contributed 0.9 % and 3.0 % of the hot carcass weight, respectively.

3.3.2. Sensory attributes and WBSF values of beef muscles

Rated sensory attributes and measured Warner-Bratzler Shear Force (WBSF) gave significant differences ($p < 0.05$) among the six muscles (Table 2). Panelists rated palatability and tenderness in a similar order to acceptance. Generally speaking, steaks from IS gave the highest palatability and tenderness scores while steaks from SM and LL the worst. For both attributes the average scores of steaks from IS significantly differed from all other muscles except RF. The palatability and tenderness of steaks from RF were significantly higher compared to those of LL and SM. The muscles appeared to have interlinked significance of means for juiciness (Table 2). Notable significance was that juiciness of steaks from BF had a higher mean than steaks from LL and SM.

The amount of connective tissue (ACT) was significantly higher for steaks from LL and SM compared to steaks from the other muscles except RF (Table 2). The steaks from RF gave significantly higher ACT than BF. The steaks from LL gave significantly higher values of lean color than BF, SM, IS and RM. The lean color of steaks from RF was significantly different from steaks of IS and RM. Steaks from RF and RM showed significantly higher discoloration than steaks of all muscles except BF (Table 2). The color of the LL changed least in comparison to all muscles other than IS and SM. As indicated in Table 2, the steaks from SM

Table 3.1

Descriptive statistics of fifteen carcass traits.

Trait	Mean	SD	Minimum	Maximum
Age (months)	59.67	19.54	35	92
Hot carcass weight(kg)	127	5.95	117	135
USDA Yield Grade	1.76	0.14	1.58	1.98
Fat thickness (cm)	0.64	0.13	0.51	0.76
Ribeye area ($cm^{2)}$	54.75	2.26	50.97	57.42
Kidney, pelvic and heart fat (%)	1.40	0.39	1	2
Marbling score[a]	457	151	300	680
M. rhomboideus (kg)	2.58	0.26	2.28	3.13
M. infraspinatus (kg)	1.24	0.12	1.04	1.44
M. longissimus lumborum (kg)	3.85	0.13	3.67	4.08
M. semimembranosus (kg)	2.53	0.15	2.24	2.74
M. biceps femoris (kg)	2.81	0.15	2.51	3.05
M. rectus femoris (kg)	1.14	0.17	0.82	1.54

[a] $Traces^{00}$ = 300, $Slight^{00}$ = 400, $Small^{00}$ = 500, $Modest^{00}$ = 600

gave significantly higher LSMEAN of WBSF than all other muscles except LM. Steaks from both SM and LL showed significantly higher shear values than those from RF.

3.3.3. L-color values*

For all muscles, the steaks gave an over all significant difference ($p < 0.05$) of LSMEAN L*-color across the days (Table 3). The three USDA beef quality grades also generally showed a significant difference in the mean L*-color values of steaks across the days.

The L*-color value of RM significantly decreased on day six (Table 3). The effect of muscle and USDA standard quality grades significantly decreased the L* value of IS on days five and six compared to days 1-3 (Table 3).

The LSMEAN of L* value of LL was significantly affected ($p < 0.05$) by the main effects across days (Table 3). The muscle effect significantly decreased the L* value on day two followed by a further decrease on days five and six relative to

the first two retail days. The standard quality grade significantly decreased the L* value of LL on day six whereas the commercial quality grade gave a significantly higher L* value of LL on day one. The L* value of utility grade LL significantly decreased on day two accompanied by no change thereafter until day six where the value showed further decrease relative to the first three days. The L* values of steaks from SM were unaffected by the quality grades (Table 3). Only when the data were pooled over the quality grades did the steaks gave a significant LSMEAN decrease on day six compared to day 1.

The value of L*-color from steaks of BF were significantly affected by the muscle across days (Table 3). The steaks showed a significant decrease in the L* value on days five and six compared to the first two days. The L* value of steaks from RF were significantly affected at different levels of the main effects across days (Table 3). The effect of muscle gave a significantly higher L* value on day one and two as compared to the values on days five and six. Also a significant increase of L* values on day three over day six was noted. USDA standard quality grade significantly decreased the LSMEAN of L*-color on day four which remained unchanged thereafter. Likewise, a significant decrease of L* value of the steaks for USDA commercial grade were noted after retail day two compared with day one, on day four compared with days one and two, and on day six compared with all days other than day 5.

3.3.4. a-color values*

The muscles and USDA quality grades significantly affected ($p < 0.05$) a* values across days (Table 4). Muscle effects on the* values were highest for LL and smallest for IS across all days. In all cases, the highest mean of a* values for the quality grades were recorded on day one steaks for all muscles and showed a general significant decrease thereafter. Within muscles, the utility quality grades generally gave higher means of a* values followed by commercial quality grade for all muscles across the days.

Table 3.2

LSMEAN of sensory attributes and WBSF (kg/cm^2) for six selected muscles.

Muscles	Palatability[1]	Tenderness[1]	Juiciness[1]	ACT[a]	Lean color[1]	Discoloration[2]	WBSF
M. rhomboideus	5.22^{bc}	6.22^{bc}	4.67^{ab}	6.78^{ab}	4.78^{c}	3.89^{a}	50.68^{bc}
M. infraspinatus	6.11^{a}	7.11^{a}	5.00^{ab}	6.56^{ab}	4.89^{c}	3.00^{bc}	49.17^{bc}
M. longissimus lumborum	5.00^{c}	6.11^{c}	4.56^{b}	5.67^{c}	6.33^{a}	2.89^{c}	52.14^{ab}
M. semimembranosus	4.89^{c}	6.00^{c}	4.44^{b}	5.78^{c}	5.22^{bc}	3.00^{bc}	60.44^{a}
M. biceps femoris	5.22^{bc}	6.33^{bc}	5.22^{a}	7.22^{a}	5.44^{bc}	3.67^{ab}	45.34^{bc}
M. rectus femoris	5.89^{ab}	6.89^{ab}	4.67^{ab}	6.33^{bc}	5.78^{ab}	3.78^{a}	41.42^{c}
SE[*]	0.08	0.29	0.07	0.09	0.07	0.13	1.06

Within column LSMEAN having different superscripts are significantly different at $p < 0.05$.

ACT = Amount of connective tissue, WBSF = Warner Bratzler Shear Force

[*]SE is pooled standard error of mean.

[1] Traits were rated on eight-point structured scales (8 = extremely palatable, extremely tender, extremely juicy, none, extremely bright cherry red and 1=extremely unpalatable, tough, extremely dry, abundant, extremely dark red respectively with palatability, tenderness, juiciness, ACT and lean color).

[2] Surface discoloration was rated on 7- point scales (7=total discoloration, 1=no discoloration).

At all levels of the main effects, the average a* values for steaks from RM were significantly higher on day one than day six (Table 4). The LSMEAN of a* value was significantly decreased by the muscle on day four and thereafter compared to days one and two. For the utility quality grade, a significant difference of days four and five a* values from the day one value were also noted.

The muscle effect on steaks from IS gave significantly lower a* values on day five compared to days one and two, and on day six versus all other retail days (Table 4). The effect of standard and commercial quality grades showed a significant decrease in amounts of a* value for day four over day one. For utility quality grade, however, a non significant decreasing trend of average a* values was seen.

Table 3.3

LSMEAN of L*-values of steaks from six muscles over six retail case days.

Muscle USDA quality	N	Show days						SE[1]
		1	2	3	4	5	6	
M. rhomboideus	15	47.19^{a}	48.11^{a}	44.78^{ab}	43.08^{ab}	42.92^{ab}	41.85^{b}	3.54
Standard grade	5	54.04^{a}	54.21^{a}	51.34^{a}	50.47^{a}	50.08^{a}	49.10^{a}	
Commercial grade	5	44.95^{a}	45.85^{a}	42.45^{a}	40.94^{a}	40.87^{a}	40.59^{a}	
Utility grade	5	42.58^{a}	44.27^{a}	40.56^{a}	37.83^{a}	37.82^{a}	35.85^{a}	
M. infraspinatus	15	43.49^{a}	43.88^{a}	44.13^{a}	42.10^{ab}	40.78^{b}	40.23^{b}	0.86
Standard grade	5	45.04^{a}	45.33^{a}	45.35^{a}	42.24^{ab}	41.37^{ab}	40.73^{b}	
Commercial grade	5	43.91^{ab}	44.02^{ab}	44.61^{a}	43.69^{ab}	41.99^{ab}	41.40^{b}	
Utility grade	5	41.51^{a}	42.28^{a}	42.41^{a}	40.36^{a}	38.97^{a}	38.55^{a}	
M. longissimus lumborum	15	40.71^{a}	39.54^{b}	39.28^{bc}	38.96^{bc}	38.71^{cd}	38.25^{d}	
Standard grade	5	41.89^{a}	41.82^{a}	41.38^{a}	41.08^{a}	40.97^{a}	36.23^{b}	1.13
Commercial grade	5	40.71^{a}	39.21^{b}	39.04^{b}	38.74^{b}	38.50^{b}	37.83^{b}	
Utility grade	5	39.54^{a}	37.91^{b}	37.58^{b}	37.41^{bc}	37.07^{bc}	36.66^{c}	
M. semimembranosus	15	37.20^{a}	36.54^{ab}	36.46^{ab}	36.00^{ab}	34.17^{ab}	33.52^{b}	
Standard grade	5	41.42^{a}	40.71^{a}	40.65^{a}	39.80^{a}	38.69^{a}	38.14^{a}	2.13
Commercial grade	5	35.57^{a}	34.60^{a}	34.08^{a}	34.55^{a}	32.59^{a}	31.78^{a}	
Utility grade	5	34.59^{a}	34.30^{a}	34.65^{a}	33.65^{a}	31.22^{a}	30.64^{a}	
M. biceps femoris	15	39.19^{a}	39.72^{a}	38.16^{ab}	38.04^{ab}	36.94^{b}	36.69^{b}	1.57
Standard grade	5	42.24^{a}	42.20^{a}	41.13^{a}	41.28^{a}	40.27^{a}	39.80^{a}	
Commercial grade	5	38.30^{a}	38.99^{a}	37.81^{a}	36.95^{a}	36.05^{a}	35.77^{a}	
Utility grade	5	37.04^{a}	37.96^{a}	35.55^{a}	35.89^{a}	34.51^{a}	34.49^{a}	
M. rectus femoris	15	45.63^{a}	45.45^{a}	45.31^{ab}	45.08^{abc}	44.83^{bc}	44.73^{c}	0.78
Standard grade	5	46.76^{a}	46.70^{a}	46.61^{a}	46.18^{b}	46.10^{b}	46.02^{b}	
Commercial grade	5	46.16^{a}	45.90^{ab}	45.47^{bc}	45.29^{c}	44.95^{dc}	44.66^{d}	
Utility grade	5	43.96^{a}	43.84^{a}	43.76^{a}	43.75^{a}	43.51^{a}	43.43^{a}	

Means with different superscripts within each row are significantly different at $p < 0.05$.

Standard, commercial and utility grades are USDA (1997) based quality grade levels.

[1]SE is pooled standard error of mean.

The effects of type of muscle, standard and utility quality grades significantly lowered a* values on day six (Table 6). The effect of commercial quality grade could be noted at three levels. On days one and two means of a* value were significantly higher than days three, four and five which in turn exceeded the value of day 6.

For a* values of steaks from SM, significant differences were pronounced due to muscle effect (Table 4). The LSMEAN of a* values were significantly higher for

days one and two compared with days five and six. Further means of a* value on days four and five were significantly higher than on day six. The effects of both standard and commercial USDA quality grade gave significantly lower a* values on day six as compared to all other days.

Table 3.4

LSMEAN for a*-values of steaks from six muscles over six retail show days.

Muscle / USDA quality	N	Show days 1	2	3	4	5	6	SE[1]
M. rhomboideus	15	22.39^{a}	21.95^{a}	21.60^{ab}	20.98^{b}	20.80^{bc}	20.15^{c}	0.63
Standard grade	5	21.47^{a}	21.03^{a}	20.68^{ab}	20.06^{ab}	19.86^{ab}	19.23^{b}	
Commercial grade	5	22.11^{a}	21.66^{ab}	20.71^{ab}	20.36^{ab}	20.14^{ab}	19.46^{b}	
Utility grade	5	23.60^{a}	23.16^{a}	22.81^{ab}	22.19^{b}	22.01^{bc}	21.36^{c}	
M. infraspinatus	15	19.76^{a}	19.23^{ab}	18.50^{abc}	17.93^{bc}	17.13^{c}	14.95^{d}	1.23
Standard grade	5	17.24^{a}	16.68^{ab}	15.85^{ab}	15.35^{abc}	14.59^{bc}	13.53^{c}	
Commercial grade	5	20.85^{a}	20.29^{ab}	19.46^{ab}	18.96^{ab}	18.20^{b}	13.59^{c}	
Utility grade	5	21.18^{a}	20.72^{a}	20.19^{a}	19.49^{a}	18.61^{a}	17.72^{a}	
M. longissimus lumborum	15	23.84^{a}	23.82^{a}	23.56^{a}	23.46^{a}	23.37^{a}	21.63^{b}	0.44
Standard grade	5	23.24^{a}	22.85^{a}	22.92^{a}	22.83^{a}	22.78^{a}	21.36^{b}	
Commercial grade	5	24.19^{a}	23.77^{a}	23.01^{b}	22.91^{b}	22.82^{b}	21.63^{c}	
Utility grade	5	24.11^{a}	24.84^{a}	24.74^{a}	24.63^{a}	24.52^{a}	21.89^{b}	
M. semimembranosus	15	19.96^{a}	19.83^{a}	19.30^{ab}	18.95^{ab}	18.37^{b}	16.26^{c}	1.07
Standard grade	5	17.77^{a}	17.65^{a}	17.30^{a}	16.61^{a}	16.47^{a}	14.05^{b}	
Commercial grade	5	20.79^{a}	20.76^{a}	20.60^{a}	20.55^{a}	19.75^{a}	17.23^{b}	
Utility grade	5	21.31^{a}	21.08^{a}	20.01^{a}	19.68^{a}	18.89^{a}	17.49^{a}	
M. biceps femoris	15	19.80^{a}	19.47^{a}	18.92^{ab}	18.49^{b}	18.16^{bc}	17.49^{c}	0.83
Standard grade	5	17.63^{a}	17.17^{ab}	16.62^{ab}	16.19^{abc}	15.78^{bc}	14.97^{c}	
Commercial grade	5	20.74^{a}	20.47^{a}	19.92^{ab}	19.49^{bc}	19.16^{c}	18.71^{c}	
Utility grade	5	21.04^{a}	20.78^{a}	20.23^{a}	19.80^{a}	19.53^{a}	18.78^{a}	
M. rectus femoris	15	22.53^{a}	22.59^{a}	21.32^{b}	20.49^{c}	19.28^{d}	18.14^{e}	0.85
Standard grade	5	21.13^{a}	21.32^{a}	20.08^{b}	19.43^{c}	18.04^{d}	16.91^{e}	
Commercial grade	5	22.30^{a}	22.23^{a}	20.85^{b}	19.90^{c}	18.99^{d}	17.89^{e}	
Utility grade	5	24.18^{a}	24.23^{a}	23.05^{b}	22.14^{c}	20.83^{d}	19.62^{e}	

Means with different superscripts within each row are significantly different at $p < 0.05$.

Standard, commercial and utility grades are USDA (1997) based quality grade levels.

[1]SE is pooled standard error of mean.

In case of BF as well, main effects decreased a* values over days (Table 4). Due to muscle effect, the LSMEAN of a* value showed a significant decrease on

day four and thereafter compared to the values on days one and two. Moreover, a significant decrease of a* value on day six compared with values on days one to four was noted. The steaks from RF showed a consistent significant change of a* values at all levels of main effects over days (Table 4). The LSMEAN of a* values significantly decreased after day two and each day thereafter.

3.3.5. b-color values and saturation indices*

In terms of b*-values, a change exhibited over days was limited. Preliminary analysis of the b* value for interaction of main effects gave non significant means. Therefore, data were pooled across days and analyzed for effects of muscle and USDA quality grades on b* value. Saturation indices were calculated and entered as main effect to further characterize muscles.

Table 3.5

LSMEAN of b*-values and saturation index of six muscles.

Main effect	N	b* value		Saturation	
		LSMEAN	SE[a]	LSMEAN	SE[1]
USDA quality					
Standard grade	5	4.30^{a}	0.31	19.19^{c}	0.74
Commercial grade	5	3.36^{b}	0.31	20.66^{b}	0.74
Utility grade	5	3.37^{b}	0.31	21.74^{a}	0.74
Muscles					
M. rhomboideus	15	3.14^{c}	0.31	21.51^{b}	0.74
M. infraspinatus	15	3.42^{bc}	0.31	18.33^{e}	0.74
M. longissimus lumborum	15	3.45^{bc}	0.31	23.45^{a}	0.74
M. semimembranosus	15	2.02^{d}	0.31	18.95^{d}	0.74
M. biceps femoris	15	6.40^{a}	0.31	19.87^{c}	0.74
M. rectus femoris	15	3.63^{b}	0.31	21.07^{b}	0.74

Means with different superscripts within column of each main effect are significantly different at $p < 0.05$.

Standard, commercial and utility grades are USDA (1997) based quality grade levels.

[1]SE is pooled standard error of mean for the respective parameter.

USDA quality grades and muscles significantly affected the LSMEAN of both b*-color and saturation index (Table 5). The LSMEAN of standard quality grade b* value was significantly higher than the values of both commercial and utility grades. In terms of saturation, however, utility quality grade gave the most significant LSMEAN followed by commercial and then standard quality grades.

In terms of muscle effects, steaks gave the highest significant LSMEAN of b* value for BF (Table 5). The b* values showed a significant decrease of means for steaks of RF, RM, and SM in that order. The saturation of steaks from LL showed the highest while those from IS gave the least significantly different LSMEAN. The saturation index mean of steaks from RF and RM showed significantly higher means over BF, followed by SM.

3.3.6. Relationships among sensory attributes and objective parameters

Generally, the rated sensory parameters and objective measurements showed significant relationships ($p < 0.05$ or $p < 0.01$) for key parameters (Table 6). The

Table 3.6

Correlation coefficients of sensory & shelf stability parameters of six muscles.

Parameters	Disco-loration	Palat-ability	Tend-ness	Juic-iness	ACT	Lean color	WBSF	L*	a*	b*
Discoloration	1									
Palatability	0.01	1								
Tenderness	0.03	0.07	1							
Juiciness	0.0332	0.15	0.07	1						
ACT	0.2433	-0.31[a]	-0.25	-0.21	1					
Lean color	-0.06	0.06	-0.15	0.05	0.04	1				
WBSF	0.06	-0.24	-0.29[a]	-0.43[aa]	0.15	0.08	1			
L*	0.08	0.02	0.18	-0.05	0.13	0.06	0.32[a]	1		
a*	0.19	-0.15	-0.03	-0.15	0.4[aa]	0.12	0.10	-0.08	1	
b*	0.31[a]	0.24	0.31[a]	0.09	-0.16	-0.02	-0.26	0.0	0.11	1
Saturation	0.23	-0.11	0.02	-0.12	0.36	0.11	0.07	-0.1	0.9[aa]	0.3

[a] Correlation coefficients with single letter superscript show significant ($p < 0.05$) association of the parameters.

[aa] Correlation coefficients with double letters superscript show significant ($p < 0.001$) association of the parameters.

ACT= amount of connective tissue, WBSF= Warner Bratzler Shear Force

ACT was correlated negatively and significantly ($p < 0.05$) with palatability. The association of WBSF with tenderness and juiciness gave negative and significant coefficient at $p < 0.05$ and $p < 0.01$ respectively.

The L* value gave a positive and significant ($p < 0.05$) correlation with WBSF. The a* value was related positively and significantly ($p < 0.01$) to ACT. The association between b* value & surface discoloration and the b* value with tenderness gave a positive and significant value ($p < 0.05$). The relation between color saturation and a* value gave a positive and significant coefficient ($p < 0.01$).

3.4. Discussion

The age at which the steers were slaughtered depended on economic factors related to the producer. Hence the average slaughter age reported in this study was a common feature of steers from the breed. The weights of hot carcass, RM and LL were in the ranges of values of carcass characteristics reported by Tesfaye, Tesfa, Amsalu, & Tekle (2007) for Borana bulls from Ethiopia. However, in our study the weight of hot carcass was slightly higher while percentage of abdominal fat was lower than the values reported by Ashenafi, Abule, Tadesse, Adane, & Belete (2007) for similar indigenous breeds.

3.4.1. Sensory and color attributes of beef muscles

The higher rate for IS and lowest rates for both SM and LL in palatability and tenderness attributes reported in the present findings were in a general in

agreement with the results of Rhee, Wheeler, Shackelford, & Koohmaraie, (2004) and Carmack, Kastner, Dikeman, Schwenke, & Garcia Zepeda, (1995).

However, relative to the SM and RF, rates of tenderness and WBSF observed for LL did not confirm the findings of Dransfield and Jones (1981). The authors reported significantly higher tenderness of LL than SM and no difference in WBSF between LL and RF. Also reports of Whiting & Strange (1991) indicated higher tenderness value for LL relative to RM. The lower palatability and tenderness rates of LL differed from the findings of Rhee *et al.* (2004), Carmack *et al.* (1995) and Whiting & Strange (1991). The reason could be explained by the preslaughter management of the steers in this study. The farmers in the study area use sticks to hit the steers on areas of LL at the time of ploughing and hence this may have increased secondary fiber content that resulted in higher background toughness of the muscle. The present rates for juiciness of muscles agree with findings of several authors (Kukowski *et al.,* 2004; Rhee *et al*., 2004; Carmack *et al*., 1995). However, our study was not in agreement with Browning, Huffman, Egbret, & Jungst (1990) who found difference of juiciness between IF and SM as well as LL.

The results for both ACT and WBSF were in accordance with the reports of Rhee *et al.* (2004) except that RM was not considered in their report and that the rate for ACT of BF was different. Johnson, Chen, Muller, Costello, Romans, & Jones (1988) found that RM had lower ACT than LL and IS, and lower WBSF of RM than LL, results that generally support our findings. Unlike our results, Rhee *et al.* (2004) indicated significantly lower ACT for BF relative to muscles considered in both studies. Also not consistent with our reports, Browning *et al*. (1990) found significant WBSF difference between IF and LL and no difference between SM and BF. Our result was comparable with the discoloration of round muscles over three retail days reported by Hall, Savell, & Smith (1980).

L* and a* values of the six muscles varied based on quality grades and significantly decreased across days. McKenna *et al.* (2005) also observed a significant change of L* values of beef muscles over retail days. The seven days cold storage (4 ^{0}C) of three beef muscles (SM, LL and *M. psoas major*) studied by Jeong *et al.* (2008), however, did not yield a significant difference in L* values.

The a* value of muscles agree with McKenna *et al.* (2005) and Isdell, Allen, Doherty, & Butler, (1999). The significant effect of quality grade on b* value observed in our study was not in agreement with findings of Kim and Lee (2003). McKenna *et al*. (2005) asserted that b* value had no clear measure of muscles color stability. To our knowledge, there have been no reports on effect of quality grade on saturation index of beef and hence the significant effect of quality grade on the saturation index reported here remains a subject of future research.

3.4.2. Relationships among studied parameters

Significant associations of key quality parameters were observed. The significant negative correlation of ACT with palatability was reasonable because ACT was known to decrease palatability (Jeremiah, Dugan, Aalhus, & Gibson, 2003). The inverse relationship between tenderness and WBSF was also documented by Whiting & Strange (1991). Similarly, beef moisture content was identified to negatively affect WBSF (Cross, Stanfield, & Franks, 1978).

The positive correlation of L* color value with WBSF can be explained in terms of a muscles fiber type composition. Muscles with increased *α*-white fibers possess connective tissues and were responsible for increase of WBSF (Calkins, Dutson, Smith, Carpenter, & Davis, 1981). As five of the six muscles studied were classified as white muscles (Kirchofer, Calkins, & Gwartney, 2002) they may have possessed numerous fibers that affected the L* value and WBSF in same direction. The ß-fiber type responsible to give red color of the muscles (Kirchofer

et al., 2002) was attributed to the positive correlation of a* value (redness) with ACT.

The significant positive correlation of b* value with surface discoloration was not well documented and may need further assessment. Moreover, the positive and significant correlation of b* value with tenderness had no obvious explanation nor does the positive correlation between a* value and saturation index.

3.5. Implications

The carcass fabrication and quality evaluation suggested differential Horro beef muscle qualities and shelf stabilities. Hence butchers of Horro beef could better satisfy their customers and obtain premium values when retailing individual muscle cuts rather than sales of mixed parts. Retailing based on muscle's color stability basis could also help butchers to extend shelf life and hence curtail consumer complaints.

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Chapter 4:

The effect of *postmortem* storage on μ-calpain activity, proteins Titin & Troponin-T, and derivatives of myoglobin in six Horro beef muscles in Ethiopia[1]

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The effect of *postmortem* storage on μ-calpain activity, proteins Titin & Troponin-T, and derivatives of myoglobin in six Horro beef muscles in Ethiopia

Abstract

*Reports on quality parameters of Horro beef muscles are scanty. To document baseline data, we characterized six muscles, namely, Longissimus lumborum (LL), Rhomboideus, (RM), Semimembranosus (SM), infraspinatus (IS), Biceps femoris (BF) and Rectus femoris (RF). Samples were taken from these muscles at death (0.04 day) days 1, 7 and 13 postmortem (p. m.) to assess temporal changes of μ-calpain activity, myofibrillar protein degradation (titin and troponin-T, Tn-T), Warner-Bratzler shear force (WBSF), percentages of oxymyoglobin (OMb) and metmyoglobin (MMb), metmyoglobin reductase activity (MRA), and CIE 1978 L*a*b* values. The p. m. ageing and muscle effect had significant ($p<0.01$ and $p<0.05$, respectively) effect on the relative rate of tenderization activities of μ-calpain. The degradation product (intact and isoforms) of titin and Tn-T were immunodetected and the extents of degradation of both proteins were affected by p. m. temporal changes. Ageing significantly ($p<0.05$) affected a* values of muscles, % OMb and MMb. Also muscle effect on % OMb and MMb was significant ($p<0.05$).*

Key words: calpain, titin, troponin, myoglobin, metmyoglobin reductase, Horro beef muscles, Ethiopia

4.1. Introduction

The quality of beef is the major factor that influences the purchase decision and use by consumers. Although beef quality issues include many parameters, tenderness and lean color are ultimate criteria used by consumers to judge beef quality. Whilst the degree to which muscle tenderizes after slaughter is the most

important factor contributing to beef quality (Savell et al., 1989; Warkup, Marie & Harrington, 1995), colour is also important factor that affects consumers' perception of the freshness of the muscles and influences their buying decision. Both *postmortem* tenderization and color of beef are effects of a multifaceted biochemical processes.

The key biochemical *postmortem* process for tenderization is unanimously known as an enzymatic process of proteolytic systems, where ubiquitous calpains are the single most proteases responsible for the tenderization (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002). The roles of the calpains in *postmortem* muscles have linked to degradation products of myofibrillar proteins, for example titin and Troponin-T. It is generally accepted that μ-calpain is largely responsible for *postmortem* proteolysis (Geesink, Kuchay, Chishti, & Koohmaraie, 2006; Koohmaraie, Schollmeyer, & Dutson, 1986) because of the *postmortem* muscle calcium concentration is sufficient to activate μ-calpain but not m-calpain activity (Koohmaraie, 1992).

The most noticeable change occurring in the myofibrillar proteins during the aging process is the degradation of troponin-T (Tn-T) and titin (Ilian et al. 2001). As ageing progress, decrease in calpain activity was linked with decrease in degradation of protein (Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst, 2001). The degradation of myofibril proteins directly impacts the structural integrity of the muscle and is associated with indices of meat tenderness (Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson, & Robson, 1996; Taylor et al., 1995). It is apparent, therefore, that rate and extent of the proteolysis of key proteins within muscle fibers is significant determinant of ultimate tenderness (Kemp, Sensky, Bardsley, Buttery, & Parr, 2009).

Among the biochemical factors that contribute to lean color of beef, the myoglobin reductase activity and forms of expressed myoglobin species are the major concerns. The chemical properties of myoglobin change when muscle is

converted to beef and the change is what primarily affects the color of beef (Ledward, 1992). Deoxymyoglobin and oxymyoglobin are easily oxidized to metmyoglobin due to their high affinity for oxygen resulting in discoloration of the beef surface (Giddings, 1977). Maintenance of oxymyoglobin and thus a desirable appearance in fresh beef at the retail level could result in added value (Liu, Lanari, & Schaefer, 1995).

Subject to the proteolytic enzymes, the rate and extent of *postmortem* tenderization of beef can be variable and these result in the inconsistency of beef tenderness which is found at the consumer level (Koohmaraie and Geesink, 2006). The extent of p. m. proteolysis varies among different muscles (Kołczak, Pospiech, & Palka, 2003). And the deterioration in color stability due to accumulation of met myoglobin at retail properties can lead to consumer dissatisfaction. Proteolyses of myofibrillar proteins of individual muscles *per se* with respect to the calpain activities and the rate at which chemical state of myoglobin influence muscle color stability in Horro beef have not been reported. Therefore, the objective of this study was to characterize selected Horro beef muscles in terms of tenderizing activity of calpain and rate of myoglobin autoxidation on muscle color properties.

4.2. Materials and Methods

4.2.1. Management of the animals and the skeletal muscles

Six skeletal muscles from twenty Horro steers were used in these experiments. Additionally, caudal parts of *Longissimus lumborum* and the right carcass sides of *Rhomboideus* and *Semimembranosus* muscles from previous experiments (Yadata et al., 2009) were used for shear force measurements. Management and slaughter of the experimental animals, and carcass grading was as previously described by Yadata et al. (2009). The six muscles identified include *M. rhomboideus* (RM), *M. infraspinatus* (IS), *M. longissimus lumborum* (LL), *M.*

semimembranosus (SM), *M. biceps femoris* (BF) and *M. rectus femoris* (RF). Each muscle sample were subdivided in to three cuts and randomly allocated for three aging days *postmortem* (day 1, day 7 and day 13 p. m.). From each muscle steak (12 gm/muscle), an at-death samples (0.04 day p. m.) were collected and designated day 0 p. m. sample. All the muscle samples were processed at room temperature for about 5 hours and then chill stored at about 4 ^{0}C for the respective aging days. At the end of each aging day, samples were snap frozen in liquid nitrogen, packed in PVC film and stored cold (at about -80 ^{0}C) in the Anatomy Laboratory of Faculty of Veterinary Medicine (FVM). Finally, these samples were shipped on dry ice to Meat Science Laboratory of University of Goettingen for analysis.

4.2.2. Calpain zymogram

The calpain zymography procedure described by Arthur and Mykles (2000) were followed with slight modifications for the calpain activity determination. Stock buffers and aliquots used for this experiment were stored at 4 ^{0}C except aliquots of ammonium persulfate (APS) which were kept at -20 ^{0}C. All solutions were prepared with deionized and distilled water.

4.2.2.1. Native casein gels

Native resolving gels were prepared from 10% polyacrylamide slab (acrylamide: bisacrylamide ratio of 35.5:1, w/v), casein (10 mg/ml) dissolved in 0.75 M (pH 8.8), 1.5 M Tris-HCl (pH 8.8), APS (0.05%, w/v) and N,N,N',N'-Tetramethylethylenediamine (TEMED) (0.05, v/v). Stacking gels were prepared from 4.8 % polyacrylamide, 500 m*M* Tris-HCl (pH 6.8), APS (0.25%, w/v), and TEMED (0.1%, v/v). The stacking gels contained no casein.

4.2.2.2. Sample homogenization

Four gram of samples were homogenized in 10 volume of lysis buffer, pH 7.6 (50 mM HEPES, 150 mM NaCl, 10% [v/v] glycerol, 0.1 % [v/v] Triton X-100, 5 mM EDTA, and 10 mM β-ME) on ice using Rotters tissue homogenizer (B. Braun Biotech International, Germany). The homogenates were centrifuged at 12000 x g at 4 ^{0}C for 10 min and the supernatant filtered with filter paper. The protein concentrations of filtered homogenates were determined according to Bradford (1976) procedure using premixed reagents (Sigma-Aldrich, Germany). Samples were mixed with equal volumes of gel sample buffer, pH 6.8 (100 m*M* Tris-HCl, 20%, [v/v] glycerol, 10 mM EDTA, 0.02% [w/v] bromophenol blue and 10 mM β-ME), vortexed briefly and were made ready for mounting onto the gels for electrophoresis.

4.2.2.3. Electrophoresis

The non-denaturing 10% PAGE casein gels were pre-run at 125 V for 15 min in running buffer (pH 8.8). The running buffer used in the upper and lower reservoirs prepared as 5x stock solution contained 125 m*M* Tris-HCl, 625 m*M* glycine, 5 m*M* EDTA and 1 mM DTT. The stock solution was diluted (1x) every time before electrophoresis. Muscle protein samples (15-μg*)* were loaded immediately onto the gels per lane. All experiments were standardized at 15-μg loads to allow comparisons among the different treatments used. For identification of protein bands, crystallized porcine Calpain-2, 109 kDa (Calbiochem®, Germany) (4 μg/lane) was randomly run along side as standard. Also, independent day 0 p. m. samples were run at random position of lanes as a reference standard at times when Calpain-2 standard was not included. Gels (6 cm wide x 9 cm tall x 0.75 mm thick) were run in Bio-Rad Power Pac Basic (Bio-Rad, USA) electrophoresis units at a constant voltage (125 V) for approximately 150 min on ice.

4.2.2.4. Calpain activity assay

Upon completion of electrophoresis, the casein gels were removed, rinsed with deionized H_2O, and then incubated with shaking for 30 min at room temperature (~22 to 24°C) in 50 ml activity assay buffer, pH 7.5 (50 mM Tris-HCl and 1 mM DTT) containing 5 mM $CaCl_2$ ($2H_2O$) to reactivated the embedded calpain. Casein gels were transferred to fresh activity assay mixture and made to slightly shake for further 20 minutes. Following equilibration, the casein gels were incubated in fresh activity assay overnight (11 hours) with gentle swirling. Control assay (where 5 mM $CaCl_2$ ($2H_2O$) was replaced by 5 mM EDTA) were randomly included. The following day, the reaction was stopped by adding 20 ml of de-staining solution (10% [v/v] acetic acid and 40% [v/v] methanol). The zymograms were fixed in the de-staining for 20 minutes and stained in staining solution (10 % [v/v] acetic acid and 40 % [v/v] methanol and 0.1 [w/v] Coomassie brilliant blue) under slow shaking for 30 min.

Finally, the zymograms were de-stained with three times washes by destaining solution for 45 min. Calpain activities were indicated by clear zones in the zymograms. For better clarity of the activity bands, zymograms were immersed in boiling water for 8 min. The scan image of the zymograms were taken using CanoScan 4400F scanner (Canon Instruments, China) to determine band intensity using the Quantity One Basic tools provided in the program Quantity One® software (Bio-Rad, USA). The activities of *μ*-calpain at different times of p. m. storage were recorded as band density units/gm tissue. In addition to the three p. m. ageing days' data record, the density of bands from at-death reference samples within the gel was also collected.

4.2.3. Western blotting

The Western blotting and blot membrane scanning were conducted with slight modification of Melody, Lonergan, Rowe, Huiatt, Mayes, & Huff-Lonergan (2004).

4.2.3.1. SDS- PAGE analysis of titin and troponin-T

Discontinuous SDS-PAGE resolving gels were prepared from 10 % polyacrylamide gel (acrylamide: bisacrylamide ratio = 35.5:1), 0.1% [w/v] SDS, 0.5% [v/v] TEMED, 0.1% w/v] APS, and 1.5M Tris-HCl (pH 8.8) to determine degradation of titin. Also, SDS-PAGE separating gels were made from 12.5 % polyacrylamide gel (acrylamide: bisacrylamide ratio = 35.5:1), 0.1% [w/v] SDS, 0.05% [v/v] TEMED, 0.1% [w/v] APS, and 1.5M Tris-HCl (pH 8.8) to determine troponin-T (Tn-T) degradation. In both cases, 4 % polyacrylamide gel (acrylamide: bisacrylamide ratio = 35.5:1), 0. 1% [w/v] SDS, 0.1% [v/v] TEMED, 0.5% [w/v] APS, and 0.5 M Tris-HCl (pH 6.8) stacking gel was used.

4.2.3.2. Sample homogenization

Muscle sample (1 gm) from each treatment group was homogenized in 10 ml buffer (10 mM EDTA and 50 mM Tris-HCl, pH 8.3) for 20 min. using Rotters tissue homogenizer. Homogenate (0.5 ml) was diluted (1:1) in protein denaturing buffer (PDB) (125 mM Tris-HCl, 4% [w/v] SDS, 20% [v/v] Glycerol) and incubated at 50 ^{0}C for 20 min in water bath. The incubated homogenate was vortexed for 1 min and re-incubated for 5 min. The re-incubated homogenate was centrifuged for 20 min at 16,000 x g. Protein concentration of the homogenate was determined according to Bradford (1976) and the sample was adjusted to 3 μg/μl protein in PDB. Directly after protein adjustment of protein concentration, 1 mL of protein solution was mixed with 10% [v/v] β-ME and 0.008% [w/v] bromophenol blue.

4.2.3.3. Electrophoresis and membrane transfer condition

Gels (8 cm wide x 6 cm tall x 0.75 mm thick) for analysis of titin and Tn-T degradation were electrophoresed in Bio-Rad Power Pac Basic (Bio-Rad, USA) electrophoresis units. Western blot electrophoresis running buffer was identical to

electrophoresis buffer in the calpain experiment (2.2.3). Gels were loaded with 60 μg per lane of total protein and run at a constant voltage of 200 V for 45 min.

Immediately before transfer, the polyvinylidene difluoride (PVDF) membranes (Roti®-PVDF, Carl Roth GmbH+Co., Germany) were equilibrated for 5 min in 20 % methanol. Further, the membranes along with filter papers and pads were equilibrated for 15 min in a transfer buffer (192 mM glycine, 15% [v/v] methanol and 25 mM Tris-HCL, pH 8.3). After electrophoresis, gels were electroblotted on PVDF membranes at a constant ampere setting of 100 mA for 1.5 h using a Mini Trans-Blot Cell (Bio-Rad, USA) equipped with a bio-ice cooling apparatus. For both titin and Tn-T blots, the temperature of the transfer buffer was maintained at sub zero on ice. To verify transferred proteins, a broad range Dalton pre-stained molecular weight marker (Sigma-Aldrich Co., Germany) was used. Gels after transfer were stained with Coomassie blue to examine the efficiency of transfer.

4.2.3.4. Immunoblotting assay of titin and troponin-T

Post-transfer membranes were blocked for 1 hour at 25 ^{0}C with gentle shaking in Tris-buffered saline Tween (TBST) (Tween 20 [0.5%, v/v], Tris-buffered saline, pH 7.4) and 0% skim milk produced by Tetra Pack® purchased from retail supermarket. Blots were incubated with primary antibodies overnight at 4 ^{0}C in solutions containing dilutions of primary antibodies in TBST. Primary antibodies used in the Western blotting procedure included monoclonal anti-titin antibody produced in mouse, Clone T11 (clone T11, Sigma Chemical Co., Germany) diluted 1: 100 (Sigma) and monoclonal anti-Tn-T antibody produced in mouse, clone JLT-12 (T6277, Sigma-Aldrich® Chemical Co., Germany) diluted 1:200.

Incubated blots were washed three times, 10 min per wash, in TBST. Bound primary antibodies were labeled with monoclonal anti-mouse IgG-alkaline phosphatase antibody produced in rabbit (clone GT-34, Sigma-Aldrich® Chemical Co., Germany), diluted 1:140000 in TBST at approx. 22 ^{0}C for 60 min. Labeled

blots were rinse washed in TBST three times, 10 min per wash. Bands were stained with 20 ml BCIP®/NBT premixed staining solution (B6404, Sigma-Aldrich® Chemical Co., Germany) for 30 min. Blots were scanned using Canon CanoScan 4400F scanner (Canon Instruments, China) and protein band densities were manipulated using Quantity One® band density analysis software (Bio-Rad)

4.2.4. Myoglobin species

4.2.4.1. Equine metmyoglobin preparations

Tang, Faustman, & Hoagland (2004) reported that equine metmyoglobin (MMb) can be used instead of bovine MMb. Therefore, equine myoglobin (M-0830, Sigma Chemical Co., Germany) was prepared as substrate for the metmyoglobin reductase activity (MRA) with slight modification of the method by Mikkelsen, Juncher, & Skibsted, 1999). The equine myoglobin was dissolved in 2.0 mM phosphate buffer (pH 7.0) and oxidized with slight excess of ferricyanide. The solution was centrifuged for 10 min at 15000 x g) in 5000 molecular weight cut-off membrane (VIVASPIN 20, Sartorius Stedim Biotech GmbH, Germany) and further centrifuged against 2.0 mM phosphate buffer (pH 7.0) at 4 ^{0}C with three 75 ml changes of the buffer. Scan data were collected over 400-710 nm using Spectronic® Genesis™ Spectrophotometers (Spectronic Instruments, Inc, Germany) to make sure that myoglobin were fully oxidized. MMb solution in 2.0 mM phosphate buffer (pH 7.0) was stored in multiple aliquots at -20 ^{0}C until used for MRA determination.

4.2.4.2. Extraction of the samples

A sample of 4 g (free from fat and connective tissue), drawn from six different muscles of 10 carcasses were used to measure the MRA and percentage of oxymyoglobin (OMb) and MMb. Muscle sample extracts for the assay were prepared with slight modification of the method described by Reddy and

Carpenter (1991). Briefly, the samples were homogenized in 25 ml of 2.0 mM phosphate buffer, pH 7.0, using Rotters tissue homogenizer for 45 sec. The homogenate was centrifuged in cold (4 ^{0}C) at 23,000 x g for 30 min and the supernatant filtered using filter paper to remove the fat layer. Hemoproteins (oxyhemoproteins) in the extract were oxidized with 1-2 crystals of potassium ferricyanide, poured into 5000 molecular weight cut-off membrane (VIVASPIN 20, Sartorius Stedim Biotech GmbH, Germany) and centrifuged at 15,000 x g for 20 min in cold (4 ^{0}C) with three 75 ml changes of 2.0 mM phosphate buffer, pH 7.0. The final muscle extract was made to 25 mL with 2.0 mM phosphate buffer, pH 7.0 and were used for determination of MRA and myoglobin (Mb) content.

4.2.4.3. Determination of MMb reductase activity

Metmyoglobin (MMb) reductase activity in the beef muscle extracts was measured spectrophotometrically (Spectronic Instruments, Inc, Germany) using the method of modified method of Reddy and Carpenter (1991). The enzyme assays were carried out at 30 ^{0}C in 1 cm path length cuvette with 1.0 mL final reaction volumes. The assay mixture had a pH of 6.4 and contained the following: 0.1 mL 5 mM disodium EDTA, 0.1 mL 50 mM citrate buffer, pH 5.60; 0.1 mL 3 mM $K_4Fe(CN)_6$; 0.3 mL 0.5 mM equine 0.5 MMb in 2 mM phosphate buffer, pH 7.0; 0.1 mL distilled-deionized water; 0.2 mL muscle extract; and 0.1 mL 1.0 mM NADH. Blanks contained all the additions except NADH which was replaced by water. The assays on each muscle extract were carried out in triplicate.

The cuvettes were placed in a 8 cell thermostat-controlled sample holder Spectronic® Genesis^{TM} Spectrophotometers for 10 min, to attain desired temperature. The reaction was initiated with addition of NADH to the sample cuvette. The spectrophotometer was programmed to record absorbance at 582 nm (the wavelength at which the difference in absorption between OMb and MMb is maximal) and to measure every 2 sec. Molar absorptive difference at 582 nm

in the linear phase of the reaction using Beer's law with a molar extinction coefficient of 14.4 x 10^3 M^{-1} cm^{-1} was used to calculate metmyoglobin reduced (equal to the amount of oxymyoglobin formed). MMb reductase activity was expressed as the mean of three replicates and one unit of MMb reductase activity was defined as that amount which would reduce 1 nmoles of MMb per minute per gram of muscle. These values were divided by the myoglobin content (mg Mb/g muscle) of the respective muscles to obtain MetMb reductase activity in terms of nmol MetMb reduced/min/mg Mb in the original extract.

4.2.4.4. Myoglobin species concentration

The content of myoglobin species was determined using the muscle extract from the MRA assay. A 2 ml of the muscle extract was poured into cuvette and the absorbance was read at 582, 557, 525, and 503 nm using Spectronic® Genesis™ Spectrophotometers. The relative proportions of oxymyoglobin (OMb), deoxymyoglobin (DMb) and MMb were calculated according to Tang et al. (2004) using a molar extinction coefficient ($\varepsilon_{\lambda, /mM/cm}$) of 14.4, 12.3, 9.8 and 7.6.

*4.2.5. CIE 1976 L*a*b* color values*

Steaks from the frozen storage were transferred to refrigerator (+ 4^0C) for 24 hours for equilibration of CIE 1976 L*a*b* values measurement as described by AMSA (1995). The steaks were unpacked from the PVC film and oxygenated for 15 minutes. Reflectance measurements of each 15 cm^2 steaks of day 7 age sample from each muscle was made with Minolta Chroma Meter CR-410 (Minolta Camera Co., Osaka, Japan) on three non-overlapping meat surfaces per sample. Therefore, each measurement was the mean of three readings. The chroma meter was operated using illuminant A, 10^0 standard observer settings and a 3.18 cm aperture. Calibration of the instrument was performed using a light trap and the white tile standard (L^* = 98.14, a^* = 0.23, and b^* = 1.89) supplied with the instrument.

4.2.6. Cooked meat WBSF evaluation

4.2.6.1. Cooking procedure

Steaks from the frozen storage were transferred to refrigerator (+ 4^0C) for 24 hours for equilibration to pre cooking temperature. At the time of cookery, the weight of each day 7 aging steak was measured and the steak wrapped in a plastic bag (Siegelrandbeutel PA/PE 90, 150 x 200 mm, Germany). To monitor the temperatures of the cooking samples, an insertion type thermometer /Physitemp Model BAT-12 digital thermometer/ (Physitemp Instruments Inc, Clifton) was inserted to the approximate geographic center of the steak. The steaks were cooked to an internal endpoint temperature of 70 ±0.5 ^{0}C in a hot water bath (IKA® -Labortechnik, GMBH & Co. KG, Germany) at boiling water temperature of approx. 80 ^{0}C. Once the steaks reached the desired internal temperature endpoint, they were cooled to room temperature for approximately 30 minutes and post-cooking weight measured. Cooking loss was expressed as a percentage of weight difference due to cookery to the raw steak weight. The cooked steaks were used to measure shear force of the probes.

4.2.6.2. Shear force evaluation

Tenderness of all muscle (n = 20) steaks for day 7 treatments was determined objectively from the cooked steaks used for cooking loss determination. Additionally, steaks of fifteen carcasses (n = 15; 5/treatment) from three muscles (RF, SM & LL) were taken for coked shear force analysis. Three round cores (1.27 cm diameter/muscle/treatment) were removed parallel to the longitudinal orientation of the muscle fibers. Each core was sheared once at the center, perpendicular to the fiber orientation, using Warner-Bratzler shear attachment (50 kg load cell and 50 mm/min crosshead speed) attached to Instron Universal Testing Machine model 4301 (Instron, Canton, MA). Average WBSF values (kg/cm^2) of the three cuts per muscle/treatment were recorded. The tenderness

(i.e., the WBSF from n =20) results will be reported in tabular form while the values of tenderness from the 15 carcasses will be given with every representative zymogram shown in the results section.

4.2.7. pH measurements

The pH of each muscle was determined according to Wojtysiak, Połtowicz, & Karasinski (2008) with a portable Hanna HI 991001 pH meter (Hanna Instruments, Japan) by direct insertion of the combined glass electrode in the muscle. For each muscle of all ageing days, acidity at 14 hours p. m. was taken as ultimate value (Morton, Bickerstaffe, Kent, Dransfield, Keeley, 1999).

4.2.8. Statistical analysis

The analyses of data were initially done with the Proc NESTED of SAS (2003). The data were arranged as a balanced split-plot design with three experimental plots: each carcass as main plot A, individual muscles as subplot B and p. m. aging days as sub-sub plot C. Thus sub plots were assumed fixed effects nested under whole plots which were randomly assigned to three USDA quality grade used as blocks and arranged in a randomized complete-block design. When nesting resulted to non significant variability, the factors were removed and data were analyzed using p. m. aging dates as fixed effect.

Therefore, Proc GLM SAS (2003) was run. For analysis data from calpain zymogram, ultimate pH and day 0 measurements were entered as covariate. Covariates were removed from the model when their interaction was non significant. Mean separation of significant model ($p < 0.05$) was conducted as of Duncan multiple range test. The Western blot analysis data were reported qualitatively.

4.3. Results and Discussion

4.3.1. The activity of calpains

In our experimental condition set for day 1, 7 and 13 p. m. storage we observed μ-calpain activity bands only on days 1 and 7. Representative zymogram obtained for μ-calpain at days 1 and 7 p. m. storage of six beef muscles was shown in Fig. 1. With the zymogram assays it was possible to estimate μ-calpain activity in crude muscle extracts without having to remove calpastatin. Similar procedure had been used by Camou, Marchello, Thompson, Mares, & Goll (2007).

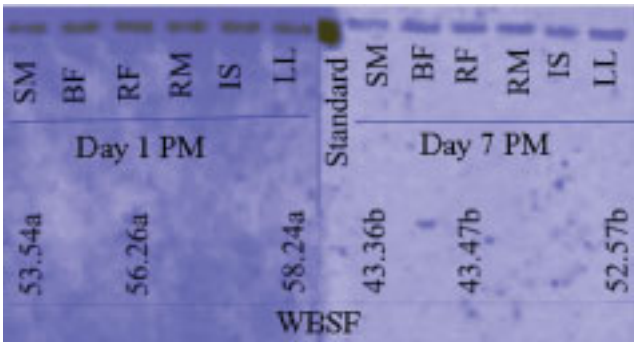

Figure 4.1. Representative zymogram of temporal changes in μ-calpain activity bands for six Horro beef muscles: *Biceps femoris* (BF), *Infraspinatus* (IF), *Longissimus lumborum* (LL), *Rectus femoris* (BF) *Rhomboideus* (RM) and *Semimembranosus* (SM). The μ-calpain activity bands were from whole muscle extract taken from the six muscles steaks aged for 1 and 7 days *postmortem*. Along with the zymograms, mean value of shear force (RWBSF) data from uncooked steaks corresponding to three muscles (SM, RF & LL) for each ageing days were given. The standard lane contained crystallized porcine Calpain-2, 109 kDa (Calbiochem®, Germany).

The zymogram illustrates substantial difference of band densities between the ageing days than when compared within ageing day. For all muscles, day 1

compared to day 7 gave significantly higher LSMEAN band density. Among the muscles, intra-aging day's band density showed seemingly no qualitative band intensity difference. Likewise, uncooked steak shear force (RWBSF) for the three muscles (Fig. 1) gave significant difference ($p < 0.05$) between the ageing days while comparison among the three muscles for same ageing day showed non significant difference ($p>0.05$).

In the quantitative expression of the zymogram data, similar to the qualitative data, the LSMEAN of μ-calpain activity showed significant decrease ($p< 0.01$) over the p. m. ageing days for all muscles (Table 1). In all muscles day 1 μ-calpain activity was higher than day 7 activity. Within ageing day comparison sorted muscles into three and two significantly different ($p<0.05$) groups respectively for ageing days 1 and 7. On day 1 ageing p. m., LSMEAN band intensity of μ-calpain activity for muscles BF, LL, RF, and RM was significantly higher than SM followed by IS. On day 7 p. m., BF, LL and RF gave significantly higher band intensity compared to SM and IS. The standard error around all muscle means were many folds below the respective means of the muscles indicating replications of the experiment had minimum difference.

In terms of the CWBSF, muscles BF and RF gave lowest shear force (most tender) mean compared to IS and RM while the other muscles (LL and SM) tended in between. IS gave the largest percentage CL followed by RM while SM showed the lowest percentage CL.

It was well documented that μ-calpain involve in p. m. beef tenderization. Our results for day 1 and 7 p. m. tenderization activity of μ-calpain as measured across the two ageing days agree with reports of Koohmaraie, Seideman, Schollmeyer, Dutson, and Crouse (1987) who indicated that activity of μ-calpain was responsible for meat tenderization and that it gradually declined during the 7

Table 4.1

Mean band density of μ-calpain activity (image density units/gm tissue), CWBSF (kg/cm^2) and cooking loss (%) for six Horro beef muscles aged for day 1 and 7 *postmortem*

Muscle	N*	Day 1		Day 7		CWBSF	CL
		LSMEAN	SE	LSMEAN	SE		
Biceps femoris	18	1062.93^{a1}	0.55	486.24^{a2}	0.92	47.32^{c}	31.36
Infraspinatus	18	989.83^{c1}	0.55	481.32^{b2}	0.92	55.29^{a}	32.64
Longissimus lumborum	18	1064.47^{a1}	0.55	486.05^{a2}	0.92	49.65^{bc}	30.31
Rectus femoris	18	1060.48^{a1}	0.55	485.59^{a2}	0.92	47.63^{c}	30.36
Rhomboideus	18	1059.13^{a1}	0.55	483.32^{ab2}	0.92	54.12^{ab}	31.78
Semimembranosus	18	1015.26^{b1}	0.55	480.47^{b2}	0.92	50.00^{bc}	29.42

*N is number of muscles used for experiment

CWBSF, cooked Warner Bratzler Shear Force; CL, Cooking Loss

LSMEANS with different number superscript within rows are different at $p < 0.001$ while LSMEANS with different letter superscripts within column are different at $p < 0.05$.

SE is pooled standard error of the mean

days *postmortem* aging of the meat. Similarly, Boehm et al. (1998) concluded that μ-calpain activity at day 7 p. m. decreased to less than 4% of its at-death value with the consequent change of the 80-kDa subunit to the 76-kDa autolyzed form. Our results were consistent with p. m. temporal changes of μ-calpain activity observed Boehm et al., (1998) in SM of beef and Nagaraj, and Santhanam (2006). The present finding was in agreement with results of Nagaraj, and Santhanam (2006) in *Longissimus dorsi*, BF, SM muscles of goat. Our findings were not consistent with reports of Veiseth, Shackelford, Wheeler, & Koohmaraie, M. (2004) who sated an eventual decrease of μ-calpain activity led to undetectable bands within 72 hours p. m.

The zymograms across all muscle steaks aged for day 13 p. m. didn't show detectable activity bands. The absence of activity band in the zymograms could be attributed to calpain autolysis. Several reports indicated that μ-calpain activity enabling environment is inherently available in p. m. aging beef muscles and one of the mechanisms for activation of the activity is autolysis (Molinari, Anagli, &

Carafoli, 1994; Saido, Suzuki, Yamazaki, Tanoue, & Suzuki, 1993; Suzuki, Tsuji, Ishiura, Kimura, Kubota, and Imahori, 1981). The p. m. autolysis of calpains begins by the autolysis of the small (30kDa) subunit on binding Ca^{2+}. Following, dissociation of the smaller subunit, the larger (80 kDa) subunit μ-calpain maintains its activity over long period of time. Thus, the onset of autolysis results in an increase in Ca^{2+} sensitivity of the enzyme (Suzuki, Tsuji, Kubota, Kimura, & Imahori, 1981) and ultimately in the loss of enzyme activity (Crawford, Willis, & Gagnon, 1987) because the dissociated large subunits (76- or 78-kDa) aggregate to form dimers and trimers, which are proteolytically inactive (Li, Thompson, & Goll, 2004).

Therefore, for Horro beef muscles aged under the aging condition we used, the μ-calpain seems to loose its activity in between day 7 and 13 due to autolysis. Hence, the activity lost μ-calpain was not detectable on 13 days p. m. in our run of the zymogram. Li, Thompson, & Goll (2004) reported that only unautolyzed μ-calpain was active while autolyzed μ-calpain was not. Further, Veiseth, Shackelford, Wheeler, and Koohmaraie (2001) reported the autolysis of calpains in lamb longissimus muscle and loss of 95 % of its activity after 3 days p. m. normal storage conditions. The authors detected no calpain activity after 15 days p. m. However, Camou et al. (2007) detected that autolyzed μ-calpain migrated slightly faster than unautolyzed μ-calpain in the casein gel as degraded faint bands. Also, Camou et al. (2007) indicated that the μ-calpain activity can be detected in zymogram assays of muscle extracts from 11- to 13-d *postmortem* muscle.

4.3.2. Western blot analysis of myofibrillar proteins titin and Troponin-T

Western blot assays were used to measure the kinetics of proteolysis of titin and Tn-T in IS, RM, LL, SM, BF and RF muscles during p. m. storage. The results revealed that multiple degradation products of titin and Tn-T were generated in all the six beef muscles at different rates during p. m. aging. Figures 2 and 3 were

representative temporal changes in Western blot analysis of the immunoreactive bands for titin and Tn-T, respectively. The pattern and trends exhibited were similar across muscles within the aging dates for each protein. P. m. ageing progressively accelerated breakdown of both proteins in all muscles. Although the muscles were different based on their day 7 p. m. WBSF measurements, the degradation of the proteins among muscles showed no clear qualitatively expressed difference. This observation is not consistent with results of Huff-Lonergan, et al. (1996) who found that Tn-T degradation products appeared at markedly faster rate in beef samples having lower shear force compared to those with higher shear force samples.

For titin (Fig. 2), in day 1 p. m. muscles, T1 (approximately 3000 kDa) polypeptide reacted with the anti-titin monoclonal antibody and only single band was visible with no further detectable degradation product of the protein. On day 7, two bands (T1, T2 ≈ 2400 kDa) and on day 13, three bands (T1, T2, and T3 ≈ 1200 kDa as well as additional small molecular weight bands) per lane were occurred. The clarity of bands decreased for T1 and T2 across ageing days and the proteins migrated as faint bands while T3 was migrated as very clear bad on day 13 p. m. The three molecular masses detected might represent intact titin and its isoforms.

Our findings for titin degradation was in agreement with Huff-Lonergan, Parrish, and Robson (1995) who reported that SDS-PAGE analysis of titin showed that it often migrated as three closely-spaced bands (T1, T1-2, T2) and that with advances in time p. m., intact titin (T1) decreased and degraded titin (T2) increased in all beef samples. Nagaraj and Santhanam (2006) also reported, for goat *Longissimus dorsi* muscle, clearly visible electrophoretic patterns of titin in

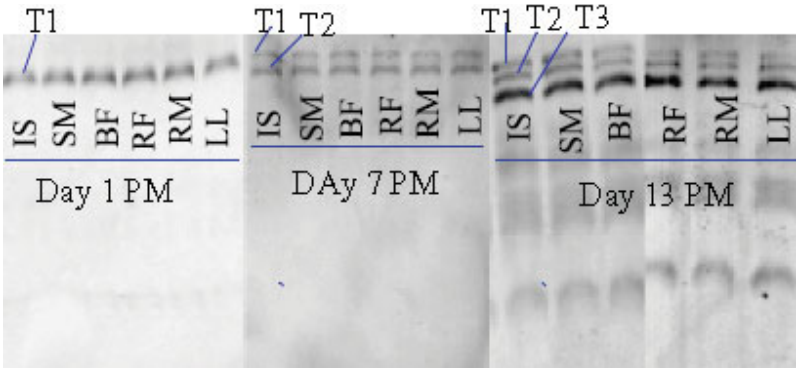

Figure 4.2. *Postmortem* titin degradation in six Horro beef muscles aged for days 1, 7, and 13 p. m. at 2 ^{0}C. The bands represent ten independent experiments (n = 10) for the rate of titin degradation, determined after SDS PAGE followed by immunoblotting with monoclonal anti-titin antibody.

that intact titin (they named it T1) appeared as a closely spaced protein doublet with T2 (which may correspond to our T1) in early p. m. but the intact titin band disappeared within 24 hours p. m. and their T2 remained less visible band as p. m. ageing of the muscle progressed. Our findings were not consistent with Huff-Lonergan, Mitsuhashi, Parrish, & Robson (1996) in temporal degradation titin for T3 (1200 kDa) band because we observed doublet bands as of day 7, the T3 band on day 13 p. m. but not as early as day 0 for doublet bands nor as of day 3 p. m. for T3 band as on their SDS-PAGE.

For Tn-T (Fig. 3), day one p. m. samples revealed only one immunoreactive band (Tn-T ≈ 40 kDa) per lane. In the day 7 and 13 p. m. samples two and more than three bands per lane, respectively, were evident. In both days' samples, the clarity of the first two bands (T1 ≈ 34 kDa, T2 ≈ 30 kDa) was similar while faint bands were additionally detected in the day 13 samples (T3 ≈ 23 kDa). The molecular masses detected might represent intact Tn-T and its isoforms. This finding agrees with reports of Huff-Lonergan et al. (1996) and Ho, Stromer, & Robson (1994) who observed the 30-kDa band recognized by the Tn-T monoclonal antibody on day 7 p. m. was a degradation product of Tn-T and that an entire family of lower molecular mass polypeptides originates from it.

Moreover, our results agree with at least four degradation fragments findings of Muroya, Nakajima, Oe, & Chikuni (2006) but inconsistent with their observation of increasing band intensity across the p. m. ageing dates. Our finding for Tn-T nearly coincides with results of Negishi, Yamamoto & Kuwata, (1996) who investigated the 40.4 kDa Tn-T and noted the disappearance of Tn-T within 24 hours. However, inconsistent to their recognition of the 23.3 kDa band as the major bands on SDS-PAGE in p. m. beef muscles stored for 3 days, because we identified the comparable polypeptide band only on day 13 p. m.

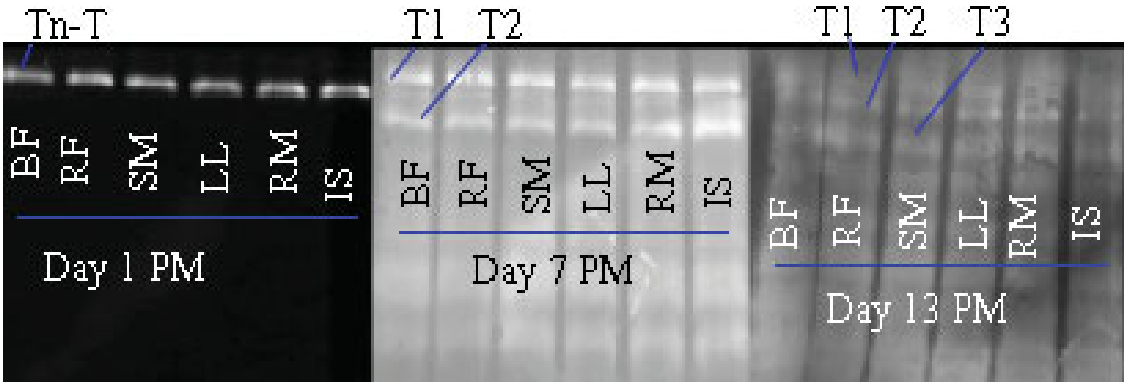

Figure 4.3. *Postmortem* troponin-T degradation in six Horro beef muscles aged for days 1, 7, and 13 p. m. at 2 ^{0}C. The bands represent ten independent experiments (n = 10) for the rate of troponin-T degradation, determined after SDS PAGE followed by immunoblotting with monoclonal anti-troponin-T antibody.

4.3.3. CIE lab values, Myoglobin species, and myoglobin reductase activity

Postmortem storage up to day 7 at 2 ^{0}C had non significant effect ($p>0.05$) on the L* value muscles (Table 2). For the a* value, however, ageing gave significantly higher ($p<0.05$) LSMEAN for BF, IS, RF, and SM on day 1 compared to day 7 p. m. In our previous study (Yadata et al., 2009), these six muscles gave significantly higher values of L* and a* on days 1 and 6 p. m. In the present study, however, that L* values showed non significant change over the storage days may be related to the difference in p. m. storage condition (mainly temperature and exposure to light) of the experiments.

Table 4.2

LSMEAN of L*a*b* values, MRA (activity/mg Mb), Oxymyoglobin (%) and Metmyoglobin (%) of six Horro beef muscles

Muscle	L*		a*		MRA		% OMb		% MMb	
	d1	d7	d1	d7	d1	d7	d1	d7	d1	d7
BF	39.9	37.6	22.5^{a}	20.1^{b}	118.3^{a1}	104.6^{b1,2}	62.3^{a}	42.5^{b1,2}	35.6^{b}	56.2^{a2}
IS	45.1	42.2	23.2^{a}	19.2^{b}	114.6^{a1,2}	102.2^{b1,2}	59.1^{a}	45.3^{b1,2}	38.1^{b}	55.2$^{a 2}$
LL	34.4	32.5	22.6	20.7	117.9^{a1}	108.5^{b1}	65.4^{a}	47.4^{b1}	36.8^{b}	49.4^{a3}
RF	38.4	37.6	24.9^{a}	20.9^{b}	120.3^{a1}	96.6^{b1,2}	68.6^{a}	30.7^{b3}	29.8^{b}	67^{a2}
RM	51.5	46.4	7.3	6.6	112.6^{a2}	92.7^{b2}	59.6^{a}	34.5^{b3}	37.8^{b}	66.3^{a1}
SM	41.7	37.9	23.4^{a}	19.6^{b}	115.4^{a1,2}	98.3^{b1,2}	58.4^{a}	40.2^{b2}	34.8^{b}	59.3^{a2}

MRA, myoglobin reductase activity; OMb, Oxymyoglobin; MMb, Metmyoglobin; RM, *Rhomboideus*; IS, *Infraspinatus*; LL, *Longissimus lumborum*; SM, *Semimembranosus*; BF, *Biceps femoris*; RF, *Rectus femoris*

For each parameter, LSMEANS with different letter superscript within row and for MRA, day 7 OMb and MMb different number superscript within column are significantly different as $p<0.05$.

Both p. m. ageing and muscles affected MRA at $p<0.05$ (Table 2). The highest significant difference MRA was observed on day 1 p. m. compared to day 7 for all muscles. Muscles BF, RF and LL gave significantly higher MRA compared to RM on day 1 while only the difference between LL and RM was significant on day 7 for all muscles. It is logical that previously defined as color stable muscles (Yadata et al., 2009) would have greater reductase activities (Reddy and carpenter, 1991). Our finding of the ageing effect on MRA was similar to reports of (McKenna, Mies, Baird, Pfeiffer, Ellebracht, & Savell, 2005; Sammel, Hunt, Kropf, Hachmeister, & Johnson. 2002; Madhavi and Carpenter, 1993) for RF, LL, IS, SM, and BF in beef. However, muscle effect in the studies of Renerre and Labas (1987) didn't explain the differences in MRA.

p. m. ageing affected the percentage of both pigments. All muscles had significantly higher % OMb on day 1 compared to day 7 p. m. Conversely, all muscles showed significantly higher % MMb on day 7 relative to day 1 p. m. The

six muscles had significantly different pigment contents ($p<0.05$). Muscle LL gave the highest OMb content while RM had the lowest on day 7 p. m. The MMb of RM gave the highest while LL showed the least content on day 7 p. m. Equating lean color to % OMb and discoloration to MMb, the present finding is in agreement with the trained panel assessment (Yadata et al. 2009) for lean color and discoloration parameters of both LL and RM muscles. Our findings were in agreement with Sammel, Hunt, Kropf, Hachmeister, & Johnson (2002) who reported significant decrease and increase respectively for % OMb and MMb on days 1 and 5 p. m. in beef SM. The authors also reported a higher activity of MRA on day 1 than day 14 a trend which agrees with our report.

4.4. Implications

Muscle profiling indicated that Horro beef cattle shares the quality parameters reported for intensively managed improved cattle breeds. Hence, with future improvement of level of genetic and environmental characters, the breed could be of industrial value.

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Chapter 5:
General Discussion

General Discussion

The issue of beef safety and quality may begin from beef cattle production at the farm level. In our diagnostic survey experiment, data indicated that Horro beef animals sold for slaughter were semi-intensively managed, used for draft purposes before sale for slaughter, their market age ranged from 35-92 months and no grading system (live animal or carcass) was officially adopted. Taking account of these data, one can conclude that the beef production and consumption in the case of most of the local cattle breeds of Ethiopia are alike. Consequently, today's beef industry in the country seems at a low level of development where slaughter stocks are purchased from non-specialized beef production systems and safety and quality aspects at all stages of the supply and processing chain are given minimal attention.

It is well established that improved production and management systems that are compatible with the consumers need are required for pre-slaughter animal handling. As well, carcass grading and fabrication systems need to address consumer preferences. However, observed butchery retail practices in Ethiopia make sub-primal cuts (muscle groups) available to consumers. Retailing carcasses without regard to muscle location and fiber orientation may compromise the quality and accentuate beef quality problems. Typical to traditional cutting systems, steaks consisting of a number of different muscles and various quantities of intra-muscular fat could lead to meat being merchandised in the form of low-priced roasts.

Nevertheless, an intuitive judgment indicates that the trend of beef carcass retailing is moving toward a concept of value-based marketing which prices slaughter stock and carcasses on individual muscle merit rather than the sub-primal (or whole) cuts. The evolving trend may also lead to translation of the individual muscle into consumer-oriented product development. As consumers' perception and state of the safety and quality of the muscles can ultimately affect

the acceptance of a beef product and purchasing habits, progress towards product development necessitates profiling of the muscles. Consequently the level of Horro beef safety was investigated and butchery of significant muscles characterized based on trained panel judgment and biophysical merits.

Slaughter stocks from Horro beef cattle are mostly destined to the Addis Ababa abattoir. Following the production to consumption chain, we assessed the safety level at all stages based on the principles of HACCP. As HACCP requires a good understanding of the relationship between cause and effect in order to be more pro-active, it is a key element in total safety management. Moreover the identified risk posing severe hazards like *E. coli O157:H7* and Salmonella species requires that consumers' health-endangering hazards need to be eliminated or reduced to an acceptable level. It is also important that pre-requisite programs such as good production practices and hygienic carcass handling practices must be working effectively within a supply and processing chain before HACCP is applied. If these pre-requisite programs are not functioning effectively then the introduction of HACCP will be complicated, resulting in a cumbersome and over-documented system.

On the subject of sensory attributes, our findings indicated that muscles showed differences in their sensory quality. Therefore it is important that butchers of Horro beef carcasses practice dissection and retail of individual muscles based on inherent quality features. For instance, in our study muscles like *Infraspinatus* and *Rectus femoris* were significantly better than other muscles in terms of their palatability and tenderness, implying the greater consumers' preference toward these muscles when dissected individually. In a similar study, Meade *et al*. (1992) reported that many muscles are very desirable in palatability and tenderness if they are removed intact and cut in the proper muscle fiber orientation. This means that the diverse palatability characteristics of muscles can not be further amplified when removed intact.

It was not uncommon to hear Horro beef consumers complain over shelf stability attributes of beef, despite their willingness to pay premium prices for tender and glossy cut parts (personal communication). The dissatisfaction and claim of consumers was especially intense when consumers perceived that the carcass stayed for a longer time on shelf. They commonly judged the shelf life against beef showing a bright cherry red color. Our study revealed that shelf stability was related to individual muscle merit. To this end, Abd El-Rhman *et al.* (1998) have reported that beef muscles showed a range of shelf stability days. Therefore, it is important that butchers could follow the inherent characteristics of the Horro beef muscles when longer shelf life with minimum spoilage (discoloration) is expected.

Lean color and tenderness are main aspects of *postmortem* beef muscle quality. Whilst lean color influences the purchase decision, the degree to which muscle tenderizes after slaughter determines re-purchase and use of the beef by consumers (Warkup, Marie and Harrington, 1995). Both lean color and tenderness are governed mainly by proteolytic calpain enzymes, and redox state of myoglobin species and myoglobin reductase, respectively. Thus, comparison of Horro beef on the basis of muscle inherent qualities and *postmortem* ageing main effects gave significant differences of parameters such as μ-calpain activity, degradation of myofibrilar proteins (titin and Troponin-T), the activity of metmyoglobin reductase, and relative distribution of myoglobin species.

The implication of the significant difference of the studied parameters based on the main effects could define a number of use values to the butchers and end users of Horro beef muscles. For instance, muscles of relatively higher oxymyoglobin concentration are likely to attract the consumers for purchase and those of higher metmyoglobin reductase activity are considered relatively longer shelf stable as the role of the enzyme is to reduce metmyoglobin to its reduced state. Therefore, both butchers and end users can benefit by appropriate choice of the muscles based on the reported inherent quality and metmyoglobin reductase activity of the muscle. Similarly, the fact that μ-calpain activity varied

among muscles is directly related to the tenderness of the muscles. Those muscles with apparently higher μ-calpain activity can be assumed of high tenderness and hence enables butchers to add value for that muscle category. Similarly consumers can also benefit form the inherent quality of the muscles by purchasing and aging of the muscles to the optimum ageing date of that specific muscle.

In general, the significance of the study can be thought of baseline work on the state of safety of Horro beef farmers to consumers chain, and profiling of muscle quality in terms of lean color and tenderness. The assessment made, therefore, was only one aspect of the many broad issues to be addressed regarding safety and quality of beef derived from the Horro cattle breed. Future studies should possibly consider various issues like adopting grading systems, introduction of voluntary or mandatory HACCP programs, detailed biophysical evaluation of fresh and aged muscle cuts, and estimation of candidate genes for tenderness.

Summary

Beef is the most favored food consumed either cooked and/or uncooked in Ethiopia. The sources of the beef consumed are animals slaughtered either formally in the city slaughter houses or informally otherwise. The slaughter beef stocks are either trekked or loaded to slaughter points from extensive or semi intensive management and production systems. Where formal slaughtering is practiced, an official veterinarian undertakes classical gross inspection. Approved carcasses are stamped and immediately loaded on a loading truck and sent away to butcheries for retailing. Obviously, such gross inspection is not strict way to assure safety of the carcass dispatched from the production line.

Nevertheless, it is clear that beef animals can be reservoir of pathogens and carcasses can be good medium for spoilage and pathogenic organisms which impose risk of public health. On the other hand, neither level of safety nor epidemiological data following this supply and slaughter chain were documented as no health care and surveillance system adequate to produce such data were operating. Under such unreliable inspection systems and scanty data, spoilage of the beef and public health issues endangered by beef-borne hazards came to fore and were one of the major concerns of the investigators. The need of the country to improve safety and quality of carcasses not only for domestic consumption but also to comply with international beef export standards were as well taken into consideration.

Therefore, with the interest to develop own baseline reference data on hazards or potential hazards for the supply and processing chain of Horro beef and to customize available generic HACCP plans to match site specific circumstances and processes, a thorough investigation was conducted. In the chain, production to consumption of Horro beef, effective baseline data on the types and levels of contamination at each stage of production and processing were reported. Notably, hazards like *Klebsiella pneumoniae, Morganella morganii,*

Pseudomonas aeruginosa, Kluyvera species, Salmonella species, and *E. coli O157:H7* were identified as most worrisome for the consumers' health. Following the chain, for the identified hazards, critical control points were suggested and critical limits were set forth.

On the subject of Horro beef quality and use, it is not uncommon to see that the butchers fabricate the carcass without regard to muscle fiber orientation and retail mixed parts of the muscle in an attempt to compromise quality, accentuating beef quality problems. Whereas literature based evidences show that cutting practices such as following appropriate fiber orientation or removal of intact muscles positively affect palatability particularly tenderness of beef. This means that the diverse palatability characteristics of muscles are further amplified by traditional way of fabricating the carcass. Thus, when not properly fabricated carcass is translated in to lesser quality end product for use by consumers and hence it may result to consumer dissatisfaction.

Muscle characterization based on appropriate muscle dissection could facilitate that butchers might be willing to follow appropriate muscle fabrication steps to reduce consumer claims. Consequently, butchery men may be able to add value based on quality with possibility to increase their over all marginal profit and yet recouping of the customer consumers. Muscle characterization may also lead to translation of the individual muscle in to consumer-oriented product development. Consequently, we selected six muscles of most butchery importance and characterized in terms of consumers' perception of the sensory attributes which can ultimately affect their purchasing habits toward beef and acceptance of the beef product. As well, progress towards product development necessitates muscles quality assessment based on knowledge of physio-biochemical properties and shelf stability of the individual muscles.

Descriptors of beef sensory attributes related to visual appeal and palatability were found to be muscle dependent and were reported as key attributes to

predict consumers' initial and continued interest in the consumption of Horro beef. The shelf characteristics of Horro beef muscles, reported here in, contribute to adding value of superior muscles and scale down consumers' complaint. Physio-biochemical properties of Horro beef muscles namely, proteolytic activities of μ-calpain and yield of degradation products of myofibrillar proteins along with autoxidation properties and reducing activities of metmyoglobin reductase were found to be muscle and *postmortem* ageing related. This could help to explore the potential technologies that can be developed based on this knowledge to reduce variation in tenderness, thus, improving consumer acceptance of beef.

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