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Heat-induced Toughening in the *Semitendinosus* Muscle of Beef Carcasses Held at Pre-rigor Room Temperature in Ethiopia

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Abstract

Carcasses from some Ethiopian public abattoirs are stored at room temperature for six to twelve hours before being sent to butcher shops where they are sold. The purpose of this study was to examine the effects of age, breed, and pre-rigor room temperature on the instrumental properties of the semitendinosus muscle. The blood samples, pH, and meat samples (48) were collected from 24 carcasses obtained from four cattle breeds under two age categories. The samples were held at pre-rigor room (24) and chill (24) temperatures for 24 hours and aged for 7 days to evaluate tenderness using Warner Bratzler-Shear Force, water holding capacity, cooking loss, thawing loss, and color (L, a*, b*). The results of the study revealed that the post-slaughter 6-hour mean temperature and pH of samples held in the pre-rigor room and chill temperatures were 29.5* ± 0.79 *°C, 6.09 ± 0.05, and* 25.35 ± 0.97*°C, 6.02 ± 0.04, respectively. The proportion of heat-induced toughening for semitendinosus muscle held at pre-rigor room and chill temperature were 12.5% and 4.2%, respectively. Heat induced toughening differed between breeds and age categories. Tenderness of muscle samples held at pre-rigor chill temperature was tender (36.05±1.22 N) while samples held at pre-rigor room temperature were intermediate (40.38±1.42 N). The water holding capacity of the muscle samples held at pre-rigor room temperature ranged from 62.83 to 73.2% while for samples held at pre-rigor chill temperature water holding capacity increased from 69.2 to 73.33%. Higher thawing and cooking losses were observed for samples held at pre-rigor room temperature compared to chilled one. In conclusion, keeping carcasses at pre-rigor room temperature has contributed to lowering the quality of beef produced at public abattoirs. It was recommended that a good practice of keeping carcasses at pre-rigor chill temperature until rigor mortis resolute need to be introduced in public abattoirs before being dispatched to butcher shops.*

Keywords: Pre-rigor temperature, Warner Bratzler-Shear Force, water holding capacity, color

Introduction

Africa's largest cattle population is found in Ethiopia. It was estimated that there were roughly 68 million cattle in the nation (CSA, 2022). More than 70% of the red meat produced in the nation was made up of beef (Issack et al., 2017). One of the main obstacles to Ethiopian beef exports is quality (Mummed, 2023).

A number of factors affect the quality of beef namely, pre-slaughter animal handling (nutrition, health status, age, sex, breed, level of stress), slaughter method, and post-slaughter carcass handling (Rodríguez-Vázquez et al., 2020). Among carcass handling, the temperature in which carcasses are managed and the rates of decline in pH affect quality parameters such as tenderness, color, and water-holding capacity of beef (Kim et al., 2014). The combination of rapid glycolysis (rapid pH fall) and slow cooling leads to high rigor temperatures there by heat toughening (CSIRO, 2002).

The core body temperature of grazing and feedlot cattle animals is about 38–39 °C (Cafe *et al*., 2011), but the interfacial seam between the semimembranosus (SM) and semitendinosus (ST) muscles can reach 42–44 °C post-mortem due to metabolic heat production (Jacob *et al*., 2014). Exposing pre-rigor muscle to high temperatures (> 35 °C), accompanied by a rapid pH decline, affects the quality of meat (Devine et al., 1999). Locker and Hagyard (1963) reported the presence of minimal shortening for pre-rigor muscles exposed to temperatures between 15 and 20 °C and substantial shortening for muscles exposed to pre-rigor temperatures above 20 °C. Similarly, Devine et al. (1999) reported the production of tender beef for muscle held at 15 °C during rigor development and resolution and the toughening of meat as the temperature increased beyond 20 °C. These researchers suggested the two main reasons for the toughening of meat: muscle shortening and inactivation of the calpain enzyme, both of which are dictated by pre-rigor temperature. Higher shear force values were reported for beef loins held at a high pre-rigor temperature of 38 °C (Kim et al., 2014) due to the effect of heat toughening. The same researchers reported the negative influence of heat toughening on the tenderness of aged and unaged meat. The influence is greater in the deep muscle compared to the intermediate and outer muscles. Pre-rigor temperature and pH were reported as one of the major factors responsible for meat toughness in the musculus longissimus thoracicum et lumborum, musculus semimembranosus, and musculus semitendinosus muscles (Devine et al., 1999). Differences in the quality of beef from bulls of Arsi, Bale, Borana, and Harar cattle breeds were reported in some studies conducted before (Gadisa et al., 2019; Birhanu et al., 2019; Dagne et al., 2021). The effects of production system, breed, and age were implicated as some of the reasons for the difference in the quality of beef between these breeds (Mummed, 2023). However, the effect of the breed of cattle on the quality of beef was minimized by finishing them under similar feeding conditions (Mummed, 2023). The management practices in public abattoirs were reported to contribute partly to the lower quality of beef produced in Ethiopia (Mummed and Webb, 2015). The researchers reported the rare practice of chilling carcasses in public abattoirs.

Abattoirs fabricate carcasses to be quartered on the slaughter floor and transported to butcher's shops. It took 4–10 hours from bleeding animals to transporting carcasses to the shops. Most butchers keep carcasses at room temperature until they finish selling the whole carcass, which usually takes 1-3 days. The time period between slaughter of cattle and transport of carcass to butcher's shops might not be enough for rigor to resolute, as rigor resolution needs 6–12 hours for the pH to fall from 5.4 to 5.8 and attain a carcass temperature of about 15 °C. This carcass temperature and pH are attained by keeping the carcass at 4°C in the chilling room for 24 hours.

The rare practice of keeping carcasses in the chilling room post-sale for 24-hours and the limited time for rigor to resolute suggested the possible negative contribution of heat-induced toughening to the lower qualities of beef (particularly on the interior portion of muscle such as semitendinosus) produced in the public abattoirs in Ethiopia.

However, there is no documented information on the degree of heat-induced toughening of beef in Ethiopia. This study was therefore conducted with the aim to determine the contribution of pre-rigor room temperature in heat induced toughening in *semitendinosus muscle* of beef in Ethiopia.

Materials and Methods

Animal management, feed composition and analysis

The study was conducted based on cattle fattened for 90 days at the Beef Farm of Haramaya University. A total of 24 intact bulls, which represented four breeds of cattle (Arsi, Borena, Harar, Holstein Frisian crossbreds six from each breed) under two age categories (2–3 and 4–5 years, 12 from each age category), were finished under similar feeding conditions composed of 60% roughage (grass hay and wheat straw) and 40% concentrate (34.78% wheat bran, 33.14% maize grain, 27.8% Guzotia Abyssinica cake, 1.7% limestone, 1.7% salt, and 0.88% ruminant premix). The chemical composition of the feed ingredients is presented in table 1.

Table 1. Chemical composition of experimental feed ingredients

	% of items on DM					
Feed type	**DM**	**Ash**	**CP**	**NDF**	**ADF**	**ADL**
Maize grain	87.35	1.80	10.06	42.68	6.64	3.79
Nuge cake	90.46	8.48	45.74	42.91	27.94	10.07
Wheat brane	88.81	5.24	17.19	54.44	9.92	4.22
Total mixed ration	89.81	9.99	21.69	51.28	14.54	6.93
Grass hay	89.29	8.59	5.83	77.05	44.14	8.41
Wheat straw	94.49	5.94	3.14	80.64	45.32	6.14

DM= dry matter, CP= Crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin

For chemical analysis 100 grams of samples of feeds were dried at 65 °C for 48 h. Then dried samples were then ground (1 mm screen) and stored for subsequent analyses of dry matter (DM), crude protein (CP), ash, neutral detergent fire (NDF) and acid detergent fire (ADF). DM, N and total ash were determined according to the official methods of (AOAC, 1990) and NDF and ADF according to (Soest *etal*., 1991). Dry matter content of the feed was determined by drying the samples in an oven at 105 °C overnight while ash content was determined by burning the samples at 550 °C for 5 h in a muff furnace. Nitrogen (N) was determined by Kjeldahl method (CP = N × 6.25).

Three percent of their body weight per day for total mixed ration was given in two equal meals at 8:00 AM in the morning and 3:00 PM in afternoon of the day and the amount were adjusted based on body weight once per every week. Clean water was available all the time. The amount of concentrate and roughage offered and refused were recorded daily to derive feed intake.

The study animals were slaughtered at Elfora, Bishoftu export abattoir, Bishoftu, Ethiopia, following the standard procedure of the abattoir. Carcasses were suspended at Achilles tendon and were not electrically stimulated.

Data collection

Temperature and pH

A wall thermometer in the abattoir was used to record room temperature. Room temperature on the day of slaughter, from bleeding time to post-sale 24-hours, was on average 25 °C (min 22.5 °C and max 27.5°C). Moreover, the temperature and pH of the meat samples (*Semitendinosus* muscle) kept at chill and room temperature were measured using a portable pH/ORP/Temp meter at 45 minutes, 3, 6, 12, and 24 hours post-sale. To calibrate the pH meter, a probe was inserted into distilled water and a buffer solution (pH 4, pH 7, and pH 10) after each reading. The pH value was read about 30 seconds after inserting the probe into the incised semitendinosus muscle (ESVLDM, 2005).

Blood and carcass sampling

Blood samples were collected in heparinized tubes at exsanguination from the 24 bulls and immediately placed in an icebox for plasma and serum separation.

The carcass samples were collected from *semitendinosus* (part of round) muscle from 24 carcasses. Duplicate samples, each sample weighting 200 gm was collected and kept at chill (48 samples) and room temperature (48 samples) for 24 hours. Then after the collected samples were packed into the plastic bag, sealed, stored in the icebox and then transported to meat processing technology laboratory at Oda Bultum University located at West Hararghe, Oromia Regional State. The

samples were kept at 4 ℃ for 7 days to determine WBSF, color, WHC, TL and CL.

Evaluation of carcass quality parameters

Heat induced toughening

The percentage of heat-induced toughening was calculated by dividing heat-toughened meat by the total number of meat samples and multiplying by 100. Heat-induced toughening of meat samples was evaluated based on two models. The first model was the Meat Standard Australia model, which considered heat-induced toughening for those meat samples that had a pH less than 6 and a temperature above 35 °C at 6 hours post-sale (Meat Technology Update, 2011a). The second model was one used by Devine et al. (1999), which considered heat-induced toughening for those meat samples that had a pH less than 6 and a temperature of 20–25 °C at 6 hours post-sale.

Glucose and insulin

The plasma was separated by centrifugation at 2000 rpm for 10 minutes at room temperature. The serum was decanted into Eppendorf tubes and frozen at -20 °C until analysis for insulin and glucose at the Public Health Institute, Addis Ababa. The insulin resistance score (HOMA-IR) was computed with the formula: fasting plasma glucose (mmol/L) times fasting serum insulin (μu/mL) divided by the constant number 22.5. HOMA-IR = {[glucose (mmol/L) × insulin (μu/mL)] / 22.5} (Muniyappa *et al*., 2008).

Warner Bratzler-Shear Force, thawing and cooking losses

Instrumental tenderness was determined using the WBSF device. The device is a G-P*P* shear machine model (No. GR-151; serial number 1612021) produced by G-P-Electrical Manufacturing Company LLC. The beef samples aged for 7 days at 4 °C were exposed to room temperature for 12 hours before determining WBSF. The steak preparation procedure of AMSA (2015) was followed. The cooking pan was heated for about 205 °C before placing the steak on the pan. The steak, which was cooked at 70 °C, was allowed to cool down to room temperature for about an hour to evaluate instrumental tenderness using WBSF. After cooling, heavy connective tissue was cut across the long axis of the steak to determine the fiber direction by using a knife. The steak was cut to 1 inch (2.5 cm) in thickness perpendicular to the long axis of the semitendinosus (ST) muscle, and six cores parallel with the muscle fibers were removed from the steak. Each core was cut across the middle (center) and expressed by Newton (N). The values for each core were averaged for the determination of a single value for each steak (AMSA, 2015). Thawing and cooking losses were determined based on the steak used to

determine WBSF. The difference in the weight of beef samples before and after thawing, divided by the weight before thawing and multiplied by 100, was used to determine thawing loss. The difference between the weight of the steak before and after cooking, divided by the weight before cooking multiplied by 100, was used to determine cooking loss.

Color

The color of the meat samples was determined using a Mini Scan EZ machine (model number MSEZ-4500L, Serial No. MSEZ1547, 45°) with a 20 mm diameter measurement area, illumination/viewing system, D65 light source, and 10˚ standard observer angle. The machine was calibrated before taking measurements using the black and white standardized tile samples provided for this purpose.

A 3 cm-thick meat sample was taken from round muscle, particularly *semitendinosus* muscle, that was removed from the sirloin area of the carcass in the free fat area. Measurements were made after 30-minute exposure to air (bloom time) at the different locations of the surface of the muscle. Three readings were taken on each sample by rotating the Color Guide 90˚ between measurements so as to obtain the average value for the color. The meat color was expressed using the CIELAB color space (L* = lightness, a* = redness, and b* = yellowness) according to the CIE system (Chulayo and Muchenje, 2016).

Water holding capacity

The water holding capacity of the samples was measured in triplicate using the method suggested by Whiting and Jenkins (1981) after removing the samples from the refrigerator overnight. Two Whatman number-1 filter papers were weighed (A), and 0.5 gram of meat sample (C) was placed between two filter papers, which in turn were placed between two glass sheets. Over it, an object weighing 2.015 kg was placed, while the glass sheet weighed 0.8278 kg, giving a total compression weight of 2.8428 kg for 5 minutes. Then the weight was removed, the meat was separated from the filter papers, and it was weighed (D). At the end, the filter paper was dried, and the weight was recorded (B). After that, the amount of protein attached to the filter paper and the actual weight of the meat after pressure treatment was determined.

Amount of protein attached to the filter paper (E) = B - A
Actual weight of meat after pressure treatment (F) = E + D
% Water holding capacity of the meat (WHC) = (C-F)/2*100

Statistical Analysis

The qualities of beef from four breeds, namely Harar, Borena, Arsi, and Holstein Friesian cross, under the two age categories (2–3, 4–5 years), which contained meat samples from chilled and not chilled, were analyzed using the General

Leaner Model (GLM) procedure of SAS 9.1 software. Where a significant difference between effects was observed, mean separation was done by Tukey Test at $P < 0.05$. Besides the correlation between carcass temperature, pH and WBSF were evaluated using Pearson correlation.

Different models were used to see the effect of breed, age and pre-rigor temperature on instrumental qualities of *semitendinosus* muscle.

Model 1. $Y_{ij\gamma t} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha*\beta*\gamma)_l + e_{ijkl}$ used for analysis of effects of breed, age and pre-rigor temperature on *semitendinosus* muscle temperature, pH, WBSF, WHC, CL, TL and color, where; $Y_{ij\gamma}$ = the response variable

μ = Overall mean
α_i = Effect of breeds
β_j = Effect of age
γ_k = Effects of pre-rigor temperature
$(\alpha*\beta*\gamma)_{ijk}$ = Interaction effect
e_{ijkl} = Random error

Model 2. $Y_{ij\gamma} = \mu + \alpha_i + \beta_j + (\alpha*\beta)_{k\gamma} + e_{ij\gamma}$ used for analysis of effects of breed and age on glucose, insulin, insulin resistance, p8 fat thickness where, $Y_{ij\gamma}$ = the response variables,

μ = Over all mean
α_i = Effects of breeds
β_j = Effects of age
$(\alpha*\beta)_{k\gamma}$ = Interaction effect
$e_{ij\gamma}$ = Random error

Results and Discussion

Temperature and pH of *Semitendinosus* Muscle Held at Pre-Rigor Room and Chilled Temperature

Mean temperature of *semitendinosus* muscle held at pre-rigor room and chilled temperature for breeds of cattle under two age categories is shown in Table 2. The overall mean temperature post slaughter 45 minute for all samples (pre-rigor room and chilled temperatures) was 33.91 ℃. The overall mean *semitendinosus* muscle temperature post slaughter 24-hours for samples held under pre-rigor chill temperature was 15.85 ℃ while for those samples held under pre-rigor room temperature was 22.36 ℃. Post slaughter 24-hour temperatures of *semitendinosus* muscle in the range from 10 to 15 ℃ were reported to be associated with highest degree of tenderness, while above this range, heat induced toughening may occur that can increase toughness of the meat (Devine et al., 1999). Post slaughter 6-

hour average temperature of *semitendinosus* muscle samples held under pre-rigor room temperature was about 29.5 ± 0.79 ℃ while those held under pre-rigor chill temperature was about 25.35 ± 0.97 ℃ in the present study. High temperature accelerated glycolysis (pH decline; Kim et al. 2012). Substantial shortening of muscle fibers was observed in muscles when exposed to higher than 20 ℃ pre-rigor temperatures (Locker and Hagyard, 1963). The post slaughter 6-hour temperature of *semitendinosus* muscle varied ($p < 0.01$) across breeds and ages in the present study. Meat sample from Arsi and HF-Cross breeds that was held at pre-rigor room temperature had attained relatively higher temperature (31.98, 30.55 ℃) compared to meat samples of the same breeds held at pre-rigor chill temperature (26.55, 28.16). The temperature in which the muscle managed might implicate to the difference in the ultimate temperature. The slow cooling of muscle was reported to lead to high rigor temperatures (CSIRO, 2002). Meat samples from bulls slaughtered at 4-5 years of age had exhibited higher carcass temperature for both categories of meat samples held under pre-rigor room (31.55 ℃) and chill temperature (27.3 ℃), compared to meat samples from bulls slaughtered at 2-3 years of age. The amount fat and weight of carcass might be implicated for difference in the temperature between the two age groups. The heavier the carcass and fat stored, the higher the temperature of carcasses was reported by Warner et al. (2014). Six-hours post slaughter pH and temperature are important to determine the degree of heat toughening in meat. According to Meat Standard Australia, heat induced toughening occur at temperature above 35℃ and pH less than 6 at 6 hours post slaughter (Meat technology update, 2011a). Some other studies suggest the possibilities of heat induced toughening at lower temperature. For instance, Locker and Hagyard (1963) and Devine et al. (1999) reported the incidence of heat toughening at temperature 20-25℃ and pH less than 6 at 6-hours post slaughter. The justification according to these researchers were the breakdown of actino-myosin muscle bondage at the specified temperature by calpain enzyme contributing positively to tenderization of the meat. This is because elevated pre-rigor temperature inactivated calpain enzyme activities there by toughness of the meat. According to the report by Wahlgren et al. (1997), carcasses kept at constant pre-rigor temperature of 35 ℃ for 5 h after slaughter lost about 80% of the m-calpain activity while only about 20% of the activity lost when exposed to a constant rigor temperature of 15 ℃ for 27 h post slaughter. Devine et al. (1999) reported that muscles held at 15 ℃ showed the least shortening while for those held at 30-35℃ maximum shortening of 25% occurred. The average 6 and 24-hour post slaughter temperature (29.5 ± 0.79; 22.36 ± 0.46) in the present study suggest the possibilities of loss of some of the activities of m-calpain which may trigger heat toughening incidence.

Table 2. Effects of breed, age and pre-rigor temperature on *semitendinosus* muscle temperature

	Temp 45 minute Mean ± SE	Temp 3 hour Mean ± SE	Temp 6 hour Mean ± SE	Temp12 hour Mean ± SE	Temp 24 hour Mean ± SE
Overall Temp	33.91 ± 0.56	30.48 ± 0.77	27.43 ± 0.68	23.65 ± 0.70	19.11 ± 0.54
Breed					
Arsi	37.4^{a} ± 0.81	32^{a} ± 1.13	29.27^{a} ± 1.67	25.483^{a} ± 1.91	19.81 ± 1.3
Borena	31.9^{b} ± 0.70	28.15^{bc} ± 0.69	25.86^{b} ± 0.79	22.592^{ab} ± 0.95	18.63 ± 0.87
HF-Cross	34.35^{b} ± 1.49	30.76^{ab} ± 1.56	29.36^{a} ± 1.60	24.675^{ab} ± 1.46	19.69 ± 1.24
Harar	31.95^{b} ± 0.43	26.76^{c} ± 0.79	25.24^{b} ± 0.90	21.879^{b} ± 1.04	18.31 ± 0.91
P - Value	***	***	**	**	NS
Age (year)					
2-3	32.32^{b} ± 0.64	28.46^{b} ± 0.60	25.43^{b} ± 0.62	22.17^{b} ± 0.75	18.58^{b} ± 0.65
4-5	35.49^{a} ± 0.81	32.5^{a} ± 1.3	29.43^{a} ± 1.09	25.15^{a} ± 1.13	19.64^{a} ± 0.87
P - Value	***	***	***	**	*
Pre-rigor temperature					
Room	33.90 ± 0.80	31.55^{a} ± 0.86	29.51^{a} ± 0.79	26.83^{a} ± 0.84	22.36^{a} ± 0.46
Chill	33.90 ± 0.80	29.42^{b} ± 0.88	25.36^{b} ± 0.97	20.49^{b} ± 0.68	15.86^{b} ± 0.28
P - Value	NS	**	***	***	***
Breed*Age	***	***	***	*	*
Age * Pre-rigor temp	NS	NS	NS	NS	NS
Breed *Pre-rigor temp	*	*	*	*	*
Breed*Age * Pre-rigor temp	*	*	*	*	*

Mean values under the same category that bear different superscript letters are significantly different, ***= P<0.001, **= P<0.01, *= P<0.05, SE= standard error of mean, HF-cross= Holstein Frisian cross breed, Temp = temperature, NS= not significant

Mean pH of meat samples held at pre-rigor room and chilled temperature for breeds of cattle under two age categories is presented in Table 3. The overall mean post slaughter 45 minutes pH of meat samples held under pre-rigor room and chilled temperature was 6.73 ± 0.06 ℃. The overall mean post slaughter 24-hour pH of meat samples held under pre- rigor room and chilled temperature was 5.6 ± 0.02 ℃. The average post slaughter 6-hour pH of meat samples held under pre-rigor room and chilled temperature were almost similar (6.09 ± 0.05 and 6.02 ± 0.04, respectively). Meat samples which attained pH less than 6 at 6-hours post slaughter are expected to exhibit heat toughening (Locker and Hagyard (1963); Devine *et al*., 1999). With respect to this criterion, meat samples from Arsi cattle breed (held under pre-rigor room temperature), meat samples from Boran and HF-Cross (managed under pre-rigor chilled temperature) and meat samples from bulls slaughtered at 4-5 years of age (held under both pre-rigor room and chilled temperatures) exhibited heat toughening. Devine *et al*. (1999) reported heat induced toughening for carcass which had post slaughter 6-hour pH less than 6 and temperature above 20 ℃ while MSA anticipate the presence of heat toughening for carcass above 35 ℃ (Meat technology update, 2011a).

Table 3. Effects of breed, age and pre-rigor temperature on *semitendinosus* muscle temperature

	Temp 45 minute Mean ± SE	Temp 3 hour Mean ± SE	Temp 6 hour Mean ± SE	Temp12 hour Mean ± SE	Temp 24 hour Mean ± SE
Overall Temp	6.73 ± 0.04	6.41 ± 0.04	6.05 ± 0.03	5.8 ± 0.02	5.6 ± 0.01
Breed					
Arsi	6.80^{ab} ± 0.03	6.44^{a} ± 0.04	6.02^{ab} ± 0.06	5.78^{a} ± 0.04	5.59 ± 0.03
Borena	6.52^{b} ± 0.13	6.29^{a} ± 0.12	5.96^{b} ± 0.08	5.76^{ab} ± 0.05	5.58 ± 0.02
HF-Cross	6.73^{ab} ± 0.05	6.33^{a} ± 0.08	6.01^{ab} ± 0.07	5.75^{ab} ± 0.07	5.59 ± 0.05
Harar	6.88^{a} ± 0.03	6.60^{a} ± 0.03	6.22^{a} ± 0.03	5.89^{b} ± 0.03	5.66 ± 0.01
P - Value	*	NS	*	NS	NS
Age (year)					
2-3	6.76^{a} ± 0.05	6.46^{a} ± 0.05	6.14^{a} ± 0.05	5.86^{a} ± 0.03	5.64^{a} ± 0.02
4-5	6.71^{a} ± 0.05	6.37^{a} ± 0.06	5.97^{b} ± 0.04	5.74^{b} ± 0.03	5.57^{b} ± 0.02
P - Value	NS	NS	*	*	*
Pre-rigor temperature					
Room	6.73 ± 0.06	6.45 ± 0.05	6.09 ± 0.05	5.8 ± 0.04	5.60 ± 0.02
Chill	6.73 ± 0.06	6.38 ± 0.06	6.02 ± 0.04	5.79 ± 0.03	5.61 ± 0.02
P - Value	NS	NS	NS	NS	NS
Breed*Age	NS	NS	NS	NS	NS
Age * Pre-rigor temp	NS	NS	NS	NS	*
Breed *Pre-rigor temp	NS	NS	NS	NS	NS
Breed*Age * Pre-rigor temp	NS	NS	NS	NS	*

Mean values under the same category that bear different superscript letters are significantly different, ***= P<0.001, **= P<0.01, *= P<0.05, SE= standard error of mean, HF-cross= Holstein Frisian cross breed, Temp =Temperature, NS= not significant

Percentage of heat induced toughening for meat samples held under pre-rigor room and chilled temperature are presented in Table 4. The table shows that

percentage of heat induced toughening for meat samples held at pre-rigor room and chilled temperature were 12.5% and 4.2%, respectively, based on the criteria for Meat Standard Australia (Meat technology update, 2011a). Based on this model, meat samples from Arsi breed carcass held at pre-rigor room scored relatively higher percentage (8.3%), followed by HF Crossbred (4.2%) while no heat induced toughening was anticipated for samples from Borena and Harar cattle carcasses. Based on the same criteria, no heat toughening was occurred for all meat samples (held at pre-rigor room and chilled temperature) from bulls slaughtered at 2 - 3 years of age while 12.5% heat toughening observed in beef samples from bulls slaughtered at 4 - 5 years of age. Based on the second model (Locker and Hagyard 1963; Devine *et al*. 1999) carcasses managed under pre-rigor chilled temperature exhibited a total 29.2% heat induced toughening with carcasses from Arsi breed (4.2%), Borena and HF cross 12.5% each and Harar 0%. Relatively lower percentage of heat toughening for age group 2 - 3 years (8.3%) compared to age group 4 - 5 years of age (12.5%). The difference in percentage of heat toughening between breeds and age categories in the present study might be associated with difference in proteolytic potential, level and rate of glycolysis, weight and fat deposition on the carcasses (CSIRO, 2002; Warner et al. 2014).

Table 4. Percentage of heat induced toughening for *semitendinosus* muscle managed under pre-rigor room and chilled temperature

Heat Toughening Models	Semitendinosus muscle	Breed					Age group (years)		
		Arsi	Borana	HF cross	Harar	Total	2 -3	4 - 5	Total
MSA	Pre-rigor room	8.3	0	4.2	0	12.5	0	12.5	12.5
	Pre-rigor chilled	0	0	4.2	0	4.2	0	4.2	4.2
Devine et al. (1999)	Pre-rigor room	12.5	8.3	8.3	0	29.2	8.3	20.83	29.2
	Pre-rigor chilled	4.2	12.5	12.5	0	29.2	8.3	12.5	29.2

The occurrence of high rigor temperature across beef processing plants in Australia ranges from 56 to 94% (Warner *et al*., 2014). The lower incidence of heat toughening in the present study compared to the report by the former researchers might be associated with the difference in the weights of the carcasses. The average hot carcass weight of bulls under the present study was about 86.8 kg (Musa et al., 2021). Carcasses evaluated by Warner et al. (2014) in the Australia were heavy (243- 432 kg). The same researchers reported strong correlation between heavy carcass weight and higher rigor temperature. The likely occurrence of heat toughening in modern processing plants was reported by Meat technology update, (2011a). Chilling carcass had minimized the rate of heat toughening in the present study. However, the incidence of heat toughening in chilled *semitendinosus* muscle in the present study suggest the need to adjust the slaughter and other management practice at abattoirs so that incidence of heat toughening

would be minimized. Minimizing number of hour carcass stay in the slaughter floor, providing high thermal conductivity path using 'heat tubes,' and vascular infusion of cold solution to beef carcass can be considered as some of the alternatives strategies to minimize the temperature in the deep muscle of beef carcasses (Meat technology update, 2011b).

Fat thickness, glucose and Insulin resistance among cattle breeds under study

Least square means of glucose, insulin, insulin resistance and p8 fat thickness of cattle breeds under the study are presented in Table 5. The value of glucose, insulin and insulin resistance (IR) of the studied cattle breeds were significantly ($p < 0.05$) different between breeds under the study. Age groups had affected the level of glucose. Bulls from Arsi and Boran cattle breed had higher glucose in their blood at slaughter compared to HF-cross and Harar cattle breeds under the present study. Bulls slaughtered at older age contained more glucose level in their blood compared to their young counterpart in the present study. Meat technology update, (2011a) reported lower incidence of heat toughening (46%) for cattle feed 60-70-day pre-slaughter, with the increase in the incidence as the number of feeding periods increased to 340-350 days (94%). The higher incidence of heat toughening in the latter case might be associated with heavier weight, and faster fall of pH of carcass compared to lighter cattle (Meat technology update, 2011a). Arsi and Harar cattle had relatively higher insulin and insulin resistance over Borena and HF crossbreds in the present study. Higher plasma insulin levels at slaughter were associated with a higher temperature at pH 6 (Warner et al., 2014). The same researchers further suggest the compromise of high insulin resistance to thermoregulation ability which can exacerbate stress. The higher concentration of insulin resistance in Arsi breed might contribute to higher level of heat toughening of the muscle in this breed. Fat thickness didn't significantly differ between breed and age categories in the present study. The average fat thickness of carcasses in the present study was 4.59 ± 0.5mm. P8 fat thickness is a good indicator for overall carcass fat content (Taylor *et al*., 1996). Grass-fed lean cattle P8 fat thickness of 5 mm had a relatively low incidence of heat toughening (54%) compared to cattle P8 fat thickness of 30 mm with higher (87%) incidence of heat toughening. The higher concentration of plasma glucose, insulin and insulin resistance in bulls from Arsi cattle breed might be contributed to relatively higher incidence of heat toughening in the *semitendinosus* muscle from the breed.

Table **5**. Least square means of glucose, insulin, insulin resistance and p8 fat thickness

Variables	Glucose (mg/dL)	Insulin (µu/ml)	IR (mg/dL/µu/ml)	P8 fat thickness (mm)
Breeds	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Arsi	72.92^{a} ± 1.16	0.92^{a} ± 0.08	2.97^{a} ± 0.32	5.55 ± 1.2
Boran	74.71^{a} ± 3.14	0.45^{b} ± 0.08	1.48^{b} ± 0.26	4.04 ± 1.3
HF-Cross	60.59^{b} ± 6.53	0.62^{b} ± 0.1	1.77^{b} ± 0.46	3.91 ± 0.84
Harar	67.8^{ab} ± 2.72	0.73^{ab} ± 0.12	2.15^{ab} ± 0.31	4.85 ± 0.65
P - Value	*	*	*	NS
Age (year)				
2-3	64.64^{b} ± 3.3	0.67^{a} ± 0.06	1.97^{a} ± 0.22	3.62 ± 0.45
4-5	73.37^{a} ± 2.23	0.69^{a} ± 0.1	2.22^{a} ± 0.33	5.56 ± 0.83
P - Value	*	NS	NS	NS
Overall	69.008 ± 2.15	0.68 ± 0.05	2.098 ± 0.20	4.59 ± 0.5
A * B	NS	NS	*	NS
CV	10.67	33.15	33.31	53.23

R=insulin resistance; Mean values under the same category that bear different superscript letters are significantly different, A* B = age and breed interaction, ***= P < 0.001, **= P < 0.01, *= P < 0.05, SE= standard error of mean, CV= coefficient of variation, HF-cross= Holstein Frisian cross breed, NS= not significant

Effect of Breed, Age and Pre-Rigor Temperature on WBSF value of *Semitendinosus* muscle

The effect of breed, age and pre-rigor temperature of carcass on WBSF value of *Semitendinosus* muscle aged for 7 days is presented in Table 6. Breed, age and pre-rigor temperature significantly affected the WBSF tenderness. *Semitendinosus* muscle from Borena and Harar bulls were more tender than the muscle from Arsi and crossbred of HF cross. In the previous part of the manuscript, beef from Arsi and HF cross exhibited heat toughening (MSA model) while Boran and Harar didn't. Similarly, the relatively higher value of WBSF for cattle slaughtered at 4-5 years of age compared to those slaughtered at 2-3 years might be associated with the heat toughening condition exhibited by cattle slaughtered in the former age categories compared to the later. The finding further indicated the effect of the pre-rigor temperature on the WBSF value, with pre-rigor chilled muscle yielded tender beef while meat samples held at pre-rigor room temperature yielded intermediate tender. The adverse impacts heat induced toughening in beef was reported by a number of studies (Devine et al. 1999; Rosenvold et al. 2008; Thomson et al. 2008). Devine et al. (1999) reported lower values of WBSF (10N) for beef at held at 15 ºC which increased to WBSF above 40N as the rigor temperature increased from 20 to 30 ºC. Citing a number of studies, Jian et al. (2023) reported the effect of high pre-rigor temperature coupled with a fast pH decline that led to toughened meat. The possible cause for the toughening might be associated with heat-induced sarcomere shortening or reduced postmortem proteolysis of calpain activities (Kim et al., 2014).

Table **6**. Effects of breed, age and pre-rigor temperature on WBSF (Mean±SE) of *semitendinosus* muscle aged for 7 days

Category	WBSF (N)
Breed	
Arsi	41.29±1.38[a]
Borana	32.76±1.22[b]
HF cross	42.63±2.25[a]
Harar	36.19±1.50[b]
p-value	***
Age	
2 - 3	34.71 ±1.02[b]
4 - 5	41.73±1.35[a]
p-value	***
Breed*Age	NS
Pre-rigor temperature	
Chill	36.05±1.22[b]
Room	40.38±1.42[a]
p-value	***
Breed* Pre-rigor temperature	NS
Age* Pre-rigor temperature	NS
Breed*Age*Pre-rigor temperature	NS

Mean values under the same category that bear different superscript letters are significantly different, ***= P<0.001, **= P<0.01, *= P<0.05, SE= standard error of mean, HF-cross= Holstein Frisian cross breed, WBSF= Warner - Bratzler Shear Force,, NS= not significant

Influence of Pre-Rigor Temperature on Color of Semitendinosus Muscle

Color is the primary attribute by which meats are judged by the consumer before purchase. The desirable color of meat is usually reddish-pink (or bright cherry red), which make the purchaser assume that the product is wholesome and edible (Kim et al., 2014). Influence of pre-rigor temperature on color of *semitendinosus* muscle is presented in Table 7. Lightness (L*) of meat of color was not affected by breed, age and pre-rigor temperature in the present study. Depite the absence of significant difference in L* value, sample held under chill pre-rigor temperature was relatively lighter (35.01 ± 0.82) compared to samples held under pre-rigor room temperature (32.8 ± 1.27). Farouk and Swan (1998) reported higher L* value for semitendinosus muscle held 35 ºC. The rapid pH decline at high muscle temperature condition is a well-known fact that affects both meat color and stability at grading. Pre-rigor higher temperature yields paler color and reduce color stability of meat which can be primary attributed to protein denaturation (particularly myoglobin and/or myofibrillar), and possibly to altered oxygen consumption by endogenous enzymes and/or metmyoglobin reducing ability (Kim et al., 2014). The paler color due to higher muscle temperature can be attributed to light scattering. PSE-like qualities in semitendinosus muscle was reported by (Hunt and Hedrick 1977). The redness (a*) was influenced by breed and pre-rigor temperature. and yellowness (b*) of semitendinosus muscle. The *semitendinosus* muscle from Boran was relatively redder (P<0.05) compared to the muscle from Harar cattle breed. The redness (a*) and yellowness (b*) of semitendinosus muscle kept at room and chilled pre-rigor temperature were significantly different.

The values of a* and b* were lower for muscle kept at pre-rigor chill temperature. Similar to the present study, Farouk and Swan (1998) reported higher value at higher temperature. Pre-rigor chilling temperatures of carcasses improve meat color and color stability. However, stepwise chilling temperature needs to be optimum to benefit the industry further in the future (Kim et al., 2014).

Table 7. Effects of breed, age and pre-rigor temperature on color of semitendinosus muscle aged for 7 days

Category	L* Mean ± SE	a* Mean ± SE	b* Mean ± SE
Overall	33.90 ± 1.27	10.87 ± 1.49	12.91 ± 0.56
Breed			
Arsi	33.54 ± 1.32	10.56 ± 0.43^{ab}	12.05 ± 0.46
Borena	35.13 ± 1.40	12.10 ± 0.59^{a}	13.76 ± 0.60
HF-Cross	33.11 ± 1.02	10.77 ± 0.37^{ab}	12.75 ± 0.98
Harar	33.83 ± 2.19	10.03 ± 0.38^{b}	13.07 ± 0.70
P - Value	**NS**	*	**NS**
Age			
2-3	32.91 ± 1.28	10.81 ± 0.39	12.62 ± 0.54
4-5	34.89 ± 0.81	10.92 ± 0.30	13.19 ± 0.46
P - Value	NS	NS	NS
Pre-rigor temperature			
Room	32.80 ± 1.20	11.45 ± 0.38^{a}	14.21^{a} ± 0.48
Chill	35.01 ± 0.82	10.28 ± 0.25^{b}	11.60^{b} ± 0.37
P - Value	NS	*	***
Breed*Age	NS	NS	NS
Age * Pre-rigor temp	NS	NS	*
Breed *Pre-rigor temp	NS	*	*
Breed*Age * Pre-rigor temp	NS	*	*

Mean values under the same category that bear different superscript letters are significantly different, ***= P<0.001, **= P<0.01, *= P<0.05, SE= standard error of mean, HF-cross= Holstein Frisian cross breed, NS = not significant, L*- lightness, a*- redness, b* yellowness

Influence of Pre-Rigor Temperature on Water Holding Capacity, Cooking and Thawing Loss of *Semitendinosus* Muscle

Influence of pre-rigor temperature on water holding capacity (WHC), cooking (CL) and thawing loss (TL) of *semitendinosus* muscle is presented in Table 8. The WHC, CL and TL of the *semitendinosus* muscle were significantly influenced by pre-rigor temperature. Keeping muscle under pre-rigor chill temperature significantly (P<0.001) reduce thawing loss. The influence of pre-rigor temperature similarly influenced the WHC and CL. The WHC of muscle held under pre-rigor room temperature was 67.38 ± 1.19 while for those samples held at chilling pre-rigor temperature was 72.98 ± 2.0. Similarly, the CL was remarkably reduced by keeping the meat sampled under chilling condition at pre-rigor period. This confirm the importance of keeping beef carcass under pre-rigor

chilling condition which was not widely practiced for fresh meat supplying abattoirs and butcher shops in Ethiopia. Similar to the present finding, Jian et al. (2023) reported the effect of high pre-rigor temperature in decreasing water holding capacity. The same authors reported the higher purge and cooking loss for meat sample kept at high temperature. Similar to the present finding, Warner et al. (2014) reported higher purge, surface exudate and cooking loss for *semitendinosus* muscle held at 37ºC. Decrease in proteolysis due to lower m-calpain activities because of higher temperature might induces an increased shrinkage of the muscle cell, creating channels for dripping moisture out of muscle bundles and thus results in greater drip loss (Huff-Lonergan and Lonergan 2007). Poor WHC results in high drip and purge loss, which can represent significant loss of weight from carcasses which may affect the yield and quality of processed meat. In addition, inferior WHC can negatively affect the appearance of meat, and this can influence consumer willingness to purchase the product.

Table 8. Effects of breed, age and pre-rigor temperature on TL, CL and WHC muscle aged for 7 days

Category		TL Mean ± SE	CL Mean ± SE	WHC Mean ± SE
Overall		11.24 ± 1.57	28.07 ± 1.37	70.23 ± 0.16
Breed				
	Arsi	14.09 ± 3.02	30.47 ± 2.92	68.56 ± 0.33
	Borena	13.99 ± 3.98	29.08 ± 3.09	72.70 ± 0.29
	HF-Cross	8.37 ± 2.13	25.66 ± 1.88	68.08 ± 0.31
	Harar	8.52 ± 3.11	25.08 ± 2.93	73.54 ± 0.34
	P - Value	**NS**	**NS**	**NS**
Age				
	2-3	11.25 ± 2.24	24.84 ± 2.20	70.27 ± 0.23
	4-5	11.23 ± 2.24	25.31 ± 1.71	69.18 ± 0.23
	P - Value	**NS**	**NS**	**NS**
Pre-rigor temperature				
	Room	18.17 ± 2.3[a]	31.0 ± 2.0	67.38 ± 1.19
	Chill	4.32 ± 0.47[b]	27.47 ± 1.7	72.98 ± 0.2
	P - Value	***	*	*
Interaction				
	Breed*Age	NS	NS	NS
	Age * Pre-rigor temp	NS	NS	NS
	Breed *Pre-rigor temp	NS	NS	NS
	Breed*Age * Pre-rigor temp	NS	NS	NS

Mean values under the same category that bear different superscript letters are significantly different, ***= P<0.001, **= P<0.01, *= P<0.05, SE= standard error of mean, CV= coefficient of variation, HF-cross= Holstein Frisian cross breed, NS = not significant, TL = thawing loss, CL = cooking loss, WHC = water holding capacity, yrs =years

Correlation between Pre-Rigor Temperature, pH and WBSF value of *Semitendinosus* Muscle

Correlation between meat sample parameters and WBSF is presented in Table 8. The correlation in the present study indicated that carcass temperature significantly and highly influenced the WBSF value of *semitendinosus* muscle. As the temperature increased, the WBSF value increased. As the values of WBSF increase, the values for tenderness decreased, leading to toughness of the meat. The increase in temperature with simultaneous increase in the value of WBSF clearly indicates the negative influence of higher pre-rigor temperature, which is influencing the tenderness of the muscle. In general, the consequences of keeping muscle at pre-rigor room temperature was heat induced toughening. Moreover, the concentration of glucose, insulin and insulin resistance in the blood influenced the WBSF at lower level, moderate and higher level, respectively. The correlation further confirms on the importance of keeping muscle at chill temperature during pre-rigor development and resolution to produce tender beef for the consumers.

Table 8. Correlation between meat sample parameters

	Carcass Wt	Fat p8(mm)	Temp carcass	pH carcass	WBSF (N)	Glucose (mmolL)	Insulin (µuml)	I R
Carcass Wt (kg)	1							
Fatp8(mm)	-0.005	1						
Carcass Temp (°C)	0.134	-0.157	1					
Carcass pH	-0.033	0.085	-0.302	1				
WBSF(N)	0.180*	0.057	0.642***	-0.123	1			
Glucose (mmolL)	0.429	0.154	0.232	-0.272*	0.186**	1		
Insulin (µuml)	-0.118	0.110	0.387	0.110	0.371**	0.106	1	
IR	-0.045	0.127	0.442	0.012	0.430**	0.326	0.969	1

*** = P<0.001, ** = P<0.01, * = P<0.05, WBSF = Warner - Bratzler Shear Force, Temp = temperature, Wt- weight

Conclusion and Recommendations

Semitendinosus muscle held at pre-rigor room temperature exhibited higher-induced heat toughening leading to reduction in quality of the meat. Pre-rigor chilling temperature improved instrumental tenderness and water holding capacity of the muscle by reducing thawing and cooking loss. The correlation between pre-rigor temperature, pH, and the WBSF value of semitendinosus muscle further confirms the importance of chilling muscle during pre-rigor development and resolution. It is therefore recommended that awareness should be created among people working in public abattoirs and butchers about the importance of holding carcasses in a chilling condition for 6–12 hours post-slaughter to produce quality beef for the market. The use of heat tubes and the vascular infusion of a cold

solution in the carcass to minimize heat load in the interior muscle need to be considered as an alternate means of reducing heat induced toughening in the future.

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Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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