



## The Effects of Oxidation on the Physicochemical Properties of Semitendinosus and Vastus Lateralis Muscles from Thai Native Cattle (*Bos indicus*)

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Article History: 23-142

Received: 08-Feb-23

Revised: 28-Mar-23

Accepted: 14-Apr-23

### ABSTRACT

The objective of this study was to examine the effects of oxidation on the Semi-tendinosus (ST) and Vastus lateralis (VL) muscles of Thai native cattle (*Kamphaeng Saen* beef cattle). The muscle samples ST and VL (n = 6) were collected from three cows and sliced into 3 cm steaks. These steaks were stored at 50°C for seven days and analyzed for proximate and myoglobin content on day 0. The pH, expressible drip, cooking loss, surface color L\*(lightness), a\* (redness/greenness), and b\*(yellowness/blueness), hardness and values of Thiobarbituric Acid Reactive Substances (TBARS) were monitored from day 0 to day 7 of storage. The results indicated that the vastus lateralis muscle was more susceptible to lipid oxidation in terms of TBARS compared to the ST muscle, which was linked to its higher percentage of myoglobin on days 5 and 7 of storage. Additionally, a decline in the redness/greenness of surface color (a\* value) of the vastus lateralis muscle was observed on days 5 and 7, concomitantly with an increase in myoglobin and TBARS content, indicating discoloration. These changes were attributed to the differences in anatomical position and physical activity of these muscles. These findings suggest that muscle source can influence the rate of deterioration and discoloration during chilled storage.

**Key words:** Lipid Oxidation, Semitendinosus, Vastus lateralis, Discoloration, Thai Native Cattle.

### INTRODUCTION

In general, meat at meat shops or retailers is supplied in packaged form and kept at a refrigerated temperature. The cold chain system is employed to prolong the shelf life, maintain the freshness, and decrease the risk of spoilage due to microbes and lipid oxidation during storage. However, lipid oxidation and myoglobin redox can take place in the meat during its storage under refrigerated environment, leading to discoloration and a potential reduction in the quality of the stored meat (Mitacek et al. 2019; Terevinto et al. 2019; Wibowo et al. 2022).

Color is widely recognized as an important indicator of the freshness of meat or meat products from the perspective of consumers and can influence their purchasing decisions (Mohan et al. 2010). However, maintaining a visually appealing red color of meat during post-mortem storage and display is quite problematic

(Bjelanovic et al. 2016). According to several studies (Mohan et al. 2010; Bjelanovic et al. 2016; Canto et al. 2016), discoloration of meat is caused by the oxidation of myoglobin. The fresh color of meat is primarily regulated by its myoglobin (Mb) content, which is influenced by various intrinsic and extrinsic factors (Canto et al. 2016). The type of muscle, its anatomical position in the body, temperature and duration of storage can all influence the redox state of myoglobin on the meat surface (Berian et al. 2009; Canto et al. 2016).

In the past, the research on meat color has primarily been focused on ante-mortem and post-mortem management (King 2011). It has been shown that intrinsic factors such as genetic variation, anatomical location, type of muscle, breed and sex of the animal play a significant role in meat color stability and oxidation (Xiong et al. 2007; King 2011; Canto et al. 2016). According to Canto et al. (2016), muscle source can significantly affect the color attributes and oxidative stability of meat, with

**Cite This Article as:** Wibowo A, Fajrih N, Panpipat W, Riebroy S, Chaijan M and Suhardi, 2023. The effects of oxidation on the physicochemical properties of semitendinosus and vastus lateralis muscles from Thai native cattle (*Bos indicus*). International Journal of Veterinary Science x(x): xxxx. <https://doi.org/10.47278/journal.ijvs/2023.044>

Longissimus lumborum steaks exhibiting a greater color compared to Psoas major from *Bos indicus* (Nellore) bulls. Similarly, Behrends et al. (2003) have reported that muscle type can affect meat color due to differences in metmyoglobin content during cold storage. These workers also found that Semimembranosus muscle had the highest percentage of metmyoglobin content compared to Biceps femoris and Semitendinosus muscles.

The scientific community of meat researchers has primarily focused their attention on the color and oxidative stability of beef from *Bos taurus* cattle, but information regarding oxidative stability and the physiochemical changes of different muscle sources from *Bos indicus* during chilled storage is limited. The purpose of this study was to examine the changes in oxidative stability and physiochemical properties of Vastus lateralis and Semitendinosus muscles from Thai Native Cattle during a 7-day period of chilled storage at 5°C.

## MATERIALS AND METHODS

### Meat Samples and Experimental Design

A quasi-experiment was carried out to examine the quality of Semitendinosus and Vastus lateralis muscles in Thai native beef cattle (*Bos indicus*- *Kamphaeng Saen*). The muscle samples (ST and VL) were collected from three cows (36 months old with initial body weight of 350±5.0kg) slaughtered at a local commercial slaughterhouse in Thasala, Nakhon Si Thammarat, Southern Thailand. All the samples (n=6) were trimmed the fat surface, connective tissue and cut into 3cm cube steaks, packaged using polyethylene film and stored in a refrigerator at 5°C for 7 days. Proximate and myoglobin content were analyzed on day 0, while cooking loss, expressible drip, hardness, myoglobin redox, pH, Thiobarbituric Acid Reactive Substances (TBARS), and color changes were monitored throughout the 7-day storage period.

### Proximate Composition

Moisture, protein, fat and ash contents of six beef cuts were determined according to the methods of Official Methods of Analysis of Association of Official Analytical (AOAC 1995).

### Zinc and Iron Determination

The Fe content in six beef cuts was assessed using flame atomic absorption spectrophotometry (Analyst 300, Perkin Elmer, USA), as previously described by Ramos et al. (2012) and the Official Methods of Analysis of Association of Official Analytical (AOAC 1990).

### Measurement of pH

Each sample was homogenized using an IKA Labortechnik homogenizer (Selangor, Malaysia) with 10 volumes of deionized water (w/v). The pH was measured using a calibrated pH meter (Cyberscan 500, Singapore), following the method described by Chaijan et al. (2004).

### Expressible Drip

Expressible drip was measured according to the method of Ng (Ng 1987). Briefly, the sample with a thickness of 0.5cm was weighed and placed between two

pieces of Whatman filter paper No. 1 at the top and three pieces of the same type of filter paper at the bottom. The standard weight (5kg) was placed on the top of the sample and maintained for 2min. The sample was then removed and weighed again. Expressible drip was calculated and expressed as percentage of sample weight.

### Color Measurement

The surface color of Semitendinosus and Vastus lateralis muscle samples was measured using a Hunterlab Miniscan/EX instrument (Hunter Assoc. Laboratory; VA, USA) with 100 standard observer, Illuminant D65, and a 2.54cm aperture size. The tristimulus values L\* (lightness), a\* (redness/greenness) and b\* (yellowness/blueness) were obtained by averaging 2 scans per sample (Holman et al. 2017). The redness index (a\*/b\*) was calculated as described by Holman et al. (2017).

### Texture Analysis

Texture analysis of meat was performed using a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, and Surrey, UK), as described earlier (Onega et al. 2005). Samples were prepared as described for cooking loss determination. Hardness was measured using the texture analyzer equipped with a spherical plunger (diameter 5mm; depression speed 60mm/min).

### Cooking Loss Analysis

The analysis of cooking loss was carried out according to the method described by Purslow et al. (2016). Meat samples were cut along the muscle fiber direction, and their raw weight was recorded. They were then placed in plastic bags and heated in a temperature-controlled water bath (Mettler, Schwabach, Germany) set at 75°C for 60min. After cooling in an ice bath for 15 min, they were dried, weighed, and their cooking loss was calculated.

### Total Myoglobin Content

The total myoglobin (as deoxymyoglobin or DMb) content was determined in accordance with the procedure outlined by the American Meat Science Association (AMSA 2012). The extraction of myoglobin in all its forms (DMb, oxymyoglobin or OMb, and metmyoglobin or MMb) was carried out using a cold 0.04M phosphate buffer at pH 6.8. The conversion of the extracted myoglobin to its deoxy form was achieved by the addition of a reducing agent (sodium dithionite). The concentration of DMb was then determined by measuring the absorbance of the Soret peak at 433nm. The total myoglobin content was calculated based on the DMb concentration and expressed as mg/g.

### Myoglobin Redox

Measurement of myoglobin redox was conducted by the method described by Faustman and Philips (2001). Briefly, the meat sample (25g) was homogenized for 30-45 sec with 75mL of 40mM sodium phosphate buffer (pH 6.8). The homogenate was filtered through double-layer cheesecloth and the absorbance was recorded at 500 to 600nm using buffer as blank. The proportions of the two myoglobin forms (oxymyoglobin and metmyoglobin)

were calculated using a modified Krzywicki's equation (Tang et al. 2004) as follows:

$$[\text{Oxymyoglobin}] = 0.722R1 - 1.432R2 - 1.659R3 + 2.599$$

$$[\text{Metmyoglobin}] = -0.159R1 - 0.085R2 + 1.262R3 - 0.520$$

Where R1 = A582/A525, R2 = A557/A525 and R3 = A503/A525.

### Thiobarbituric Acid Reactive Substances (TBARS) Analysis

The TBARS assay was conducted according to Al-Hijazeen et al. (2016). For this purpose, 0.5g ground sample was homogenized in a 2.5mL solution containing thiobarbituric acid (0.375% w/v), trichloroacetic acid (15% w/v), and 0.25N HCl. The mixture was heated in a boiling water bath for 10 minutes until a pink color appeared, then cooled and centrifuged at 3600g at 25°C for 20 minutes. The absorbance of the supernatant was measured at 532nm. A standard curve was created using 1, 1, 3, 3-tetramethoxypropane at concentrations from 0 to 10ppm. The TBARS results were expressed as mg malonaldehyde (MDA) per kg of meat sample.

### Statistical Analysis

The data were analyzed through ANOVA and means were compared using Duncan's multiple range test to identify significant differences ( $P < 0.05$ ), as described earlier (Steel and Torrie 1980). The analysis was done using SPSS v.23 to evaluate the parameter changes and the interrelationships between the parameters.

## RESULTS AND DISCUSSION

### Proximate Composition

The proximate composition of the Semitendinosus (ST) and Vastus lateralis (VL) muscles of Thai Native cattle is presented in Table 1. The results indicated a non-significant difference in protein content between the two muscles. However, significant differences were observed in the fat and ash contents, with the ST having a higher percentage of fat and ash compared to the VL ( $P < 0.05$ ), indicating that the muscle source can significantly affect the chemical composition of beef cuts. Samootkwam et al. (2015) also found non-significant difference in protein content between the Longissimus dorsi (LD) and Semitendinosus (ST) cuts of Thai Native cattle (21.6% protein). A comparative study of specific muscle sources was also conducted by Canto et al. (2016), who found significant differences in moisture, protein, and lipid contents between the Longissimus lumborum (LL) and Psoas major (PM) muscles from Brazil native cattle, with higher levels of protein and lipid in the PM compared to the LL.

On the other hand, ash and fat contents in ST and VL muscles from Thai Native cattle were found to be significantly different. This suggests that muscle source has a distinct chemical composition. However, it is important to note that other factors such as breed and genetic traits can also influence the chemical composition, oxidative stability, and color of different beef muscles. This is further supported by the findings of Mohan et al. (2010), who reported that the muscle source (Semitendinosus and Biceps femoris) from *Bos taurus* cattle had a higher fat content compared to *Bos indicus*,

demonstrating the important role that breed and genetic traits play in the chemical composition of beef.

### Zinc and Iron Content

The zinc and iron contents in ST and VL muscles from Thai Native cattle are also presented in Table 1. The zinc content was found to be significantly different ( $P < 0.05$ ) between the ST and VL cuts, with 19.54mg/kg in the Semitendinosus and 30.99mg/kg in the Vastus lateralis. Differences in zinc content can be attributed to factors like breed, age, and muscle source. Ramos et al. (2012) also reported that muscle source had a significant effect ( $P < 0.05$ ) on zinc content in the Pectoralis major, Gluteus medius, and Longissimus dorsi muscles, while the effect of age and breed on zinc content in beef was less pronounced. Other studies have also supported these findings. For example, Lombardi-Boccia et al. (2005) found that zinc content in meat samples collected from five different muscle sources in Italy ranged from 34 to 100mg/kg. The USDA has also documented that zinc content in meat ranges from 34 to 100mg/kg (Cabrera et al. 2010). Therefore, it is believed that muscle source is a significant factor in determining the variability of zinc content in beef.

The iron content in Semitendinosus and Vastus lateralis muscles demonstrated slightly different values, with 12.12mg/kg of raw meat in Semitendinosus and 12.94mg/kg of raw meat for Vastus lateralis, the difference was non-significant. The difference of Fe content in meat has strongly been correlated with breed, sex, physical activity, nutrition plan, and anatomical location or source of muscle. Ramos et al. (2012) emphasized that breed, source of muscle and feed were the main factors which influenced the Fe content in meat postmortem. According to Ramos et al. (2012), the Fe content in Hereford breed meat was 34-45mg/kg, while in Bradford breed meat it was 30-38mg/kg. Purchas et al. (2005) reported that the iron contents in meat were associated with the animal's nutrition plan. In their study, pasture-finished animals from New Zealand showed significantly higher levels of iron in their meat (22.3mg/kg wet tissue) compared to feedlot-finished animals from the USA, which had a value of 16.5mg/kg wet tissue. This indicates that the feeding regime of animals can influence the iron content of meat. However, it can be postulated that combination between feeding system and anatomical location or different muscle sources can affect Fe contents in meat.

### Myoglobin Content

The total myoglobin (Mb) contents in two beef muscles (Semitendinosus and Vastus lateralis) from Thai Native cattle are depicted in Table 1. Concentration of Mb was higher ( $P < 0.05$ ) in Vastus lateralis (2.67mg/g) compared to Semitendinosus (1.41mg/g) counterparts. The variability in myoglobin content among different muscle sources is shown to be closely related to several factors, including anatomical position, physical activity, and the type of muscle fibers. King et al. (2011) found that the myoglobin content in five different beef muscles Longissimus lumborum (LL), Semimembranosus (SM), Biceps femoris (BF), Gluteus medius (GM), and Triceps Brachii (TB) collected from *Bos indicus* varied between

**Table 1:** Proximate composition and myoglobin content in Semitendinosus and Vastus lateralis steaks (n=6) from Thai Native cattle

Chemical Composition	Type of Muscles	
	Semitendinosus	Vastus lateralis
Moisture (%)	76.56±1.01a	77.51±0.89b
Protein (%)	21.52±0.44a	22.03±0.54a
Fat (%)	0.65±0.47b	0.51±0.30a
Ash (%)	1.28±0.17b	1.19±0.15a
Zinc (mg/kg)	19.54±0.23a	30.99±1.05b
Iron (mg/kg)	12.12±0.82a	12.94±0.05a
Myoglobin (mg/g)	1.41±0.55a	2.67±0.28c

Values are given as mean±SD from triplicate determinations. Different letters in the same row indicate significant differences (P<0.05).

3.67 to 5.21mg/mL. These workers also reported that the myoglobin content in Psoas major (PM) (5.54mg/g) was greater than that in LL muscle (4.96mg/g).

King et al. (2011) also recorded variations in the distribution of muscle fiber types in livestock depending on the muscle's function and position. A higher concentration of red fibers, which is linked to the large number of mitochondria, can lead to decreased color stability due to an increased oxygen consumption rate. The anatomical location of the muscle can also influence the concentration of myoglobin. Thus, the concentration of myoglobin in muscle or beef cuts can be influenced by the anatomical position of the muscle within the animal, as noted by Lawrie and Ledward (2006).

### pH Values

The pH plays a crucial role in determining the eating quality, physical and chemical properties, and other changes that occur in meat during cold storage. Therefore, monitoring the ultimate pH of meat has become a crucial aspect of the beef industry. Meat scientists have extensively studied the relationship between pH and meat quality (Jankowiak et al. 2021), as noted by Rios-mera et al. (2017). The pH values of the Semitendinosus and Vastus lateralis muscles from Thai Native cattle during chilled storage are presented in Table 2. On day 0 of storage, there was non-significant difference between the pH values of Semitendinosus (6.8±0.57) and Vastus lateralis (6.9±0.20), and the same was true on Day 2 of storage. This finding is slightly different from a previous study on the Semitendinosus (ST) and Longissimus dorsi (LD) muscles of Thai Native cattle, which showed pH values of ST (6.68) and LD (6.84) on day 0 post-slaughter (Jaturasitha et al. 2009).

In the present study, difference in pH values was seen between the Semitendinosus and Vastus lateralis beef cuts of Thai Native cattle during storage. From days 1 to 7, the pH value in the Vastus lateralis was higher compared to Semitendinosus. This variation in ultimate pH (pHu) and pH values could be attributed to the muscle source, with differences in anatomical position, physical activity, and metabolic systems playing an important role.

### Expressible Drip

Table 2 also shows the results of expressible drip percentage in Semitendinosus and Vastus lateralis muscles. There was no difference between the two muscles in terms of expressible drip percentage during seven days of storage at 5°C. However, the

Semitendinosus muscle tended to have a slightly higher expressible drip percentage compared to the Vastus lateralis muscle. The ability of meat to retain water during storage is closely related to its pH value. During the first two days of storage, there was no difference between the two muscles in terms of expressible drip percentage and pH value. However, from day 2 until the end of the storage period on day 7, there was a noticeable but non-significant difference in expressible drip percentage between the two muscles. This was concurrent with the significant difference (P<0.05) in pH values during the same period. This can be explained by the fact that the pH of meat gradually declines during cold storage and approaches the isoelectric point (pH=5.4) during the rigor stage (Hopkins 2016). The pH also affects the activity of proteolytic enzymes during storage.

Previous studies have shown a clear relationship between the pH of meat and the degradation of myofibrillar proteins (Li et al. 2014). Moreover, meat or carcasses that have an intermediate pHu value (pH 5.8-6.2) are able to reduce the degradation of desmin and troponin-T, which is associated with lower drip loss. This indicates that pH plays an important role in the degradation of myofibrillar proteins and, consequently, the quality of meat. This study showed that the pH value of the Vastus lateralis was closer to the intermediate pH from day 2 to 7 of storage compared to the Semitendinosus. This indicates that the Vastus lateralis has a better ability to retain water during chilled storage compared to Semitendinosus. This difference in expressible drip can be attributed to differences in anatomical location, type of muscle fiber, physical activity, or source of Semitendinosus and Vastus lateralis muscles.

### Cooking Loss

Cooking loss plays a crucial role in evaluating the quality of raw beef and its products, as it is influenced by the storage duration (Dominguez-Hernandez et al. 2018; Jezek et al. 2019; Vavoska et al. 2020). Higher cooking temperatures result in lower water content due to structural changes in the muscle fibers caused by temperature and cooking time. These changes affect physical properties of meat, as well as its juice, flavor, and micronutrient contents (García-Segovia et al. 2007; Oillic et al. 2011; Schönfeldt and Strydom 2011).

The results of the cooking loss measurements for the Semitendinosus and Vastus lateralis muscles from Thai Native cattle are shown in Table 2. There was no difference in the percentage of cooking loss between the two muscle samples on days 0, 1 and 7 of chilled storage. However, there was a significant difference (P<0.05) in cooking loss between days 2 and 5, with Semitendinosus showed higher cooking loss compared to Vastus lateralis. However, these results are not consistent with those of a previous study, where the Semitendinosus, Longissimus dorsi (LD) and Infraspinus (IS) cuts showed cooking losses greater than 30% at 24 hours post-slaughter (Jaturasitha et al. 2009). These differences may be related to the pH values, as Semitendinosus showed a pH of 5.8 on day 1 of storage in our study, while Jaturasitha et al. (2009) reported a pH of 5.5 at the same storage time. Nevertheless, both cuts showed a gradual decrease in pH over the seven days of storage.

Heat transfer and protein denaturation also have an important influence on cooking loss in beef (Purslow et al. 2016). Thus, the denaturation or degradation of protein play an important role in percentage of water release during aging and cooking of beef, in which it can be associated with pH value (Li et al. 2014). Li et al. (2014) also showed interrelationship between pH and degradation of myofibrils protein, with an intermediate  $pH_u$  (pH 5.8-6.2) can reduce degradation desmin and toponin-T, which contributes to retain purge of water. This is consistent with our data where Semitendinosus and Vastus lateralis on day 1 of storage had a pH value close to an intermediate  $pH_u$  (5.8-6.4), thus both of these beef cuts had lower cooking loss percentage on day 1 compared to day 2, 3, 5, and 7 of chilled storage. In addition to cooking loss, differences between two beef cuts are associated with anatomical location, physical activity, type and size of muscle fiber, and the response of muscle fibre to heat treatment during cooking loss test.

### Hardness Index

Biological factors such as age, type of muscle, species, and sex also have an important role in tenderness and hardness of beef (Jeleníková et al. 2008). The level of hardness in Semitendinosus and Vastus lateralis during seven days of chilled storage is presented in Table 2. The results showed a significant difference ( $P<0.05$ ) in the hardness index between Semitendinosus (5.03) and Vastus lateralis (7.14) on day 5 of storage. However, there was no difference in hardness index between two beef muscles on 0, 1, 2, 3 and 7 days of storage.

The decrease in hardness values observed in both beef muscles during storage can be attributed to the role played by proteolytic enzymes in reducing the toughness of muscle. Both muscles showed a declining trend in hardness values from day 2 to 7 of storage. Even though there was non-significant difference in hardness values between Semitendinosus and Vastus lateralis, the latter had slightly higher hardness value at day 7 of storage. This difference is likely related to the anatomical position, physical activity, and post-mortem pH of each muscle.

Previous studies (Li et al. 2014; Wu et al. 2014) have shown that  $pH_u$  is strongly associated with beef tenderness and plays a crucial role in tenderness changes during post-mortem storage. The pH of beef can be categorized into three levels: low  $pH_u$  (<5.8), intermediate  $pH_u$  (5.8-6.2), and high  $pH_u$  (>6.2). The differing  $pH_u$  values are associated with the acceleration of the proteolysis and the activity of proteolytic enzymes, which degrade beef during post-mortem and storage.

Our results indicated that the hardness values of the two beef cuts, which had high  $pH_u$  values at day 0 of storage, declined during storage. This decrease was particularly noticeable in Semitendinosus and Vastus lateralis and can be explained by the relationship between pH and tenderization of beef during storage.

### Instrumental Color

Color of meat can influence consumer acceptance and food choice toward meat and meat products (Shuang et al. 2020). Hence, it has become the most critical trait that

influences the purchase decision of consumers (Wu et al. 2015).

Colors ( $L^*$ ,  $a^*$  and  $b^*$ ) of two beef muscles (Semitendinosus and Vastus lateralis) from Thai Native cattle during chilled storage are presented in Table 3. Significant differences ( $P<0.05$ ) were observed in the lightness index of the two muscles from day 0 to day 7 of storage. These differences can be attributed to the higher concentration of myoglobin (Mb) in Vastus lateralis compared to Semitendinosus, as it is known that myoglobin content is positively correlated with the  $L^*$  value.

Canto et al. (2016) also observed that the Psoas major muscle had lower  $L^*$  values compared to the Longissimus lumborum muscle on days 3 and 9 of storage, which was attributed to a higher concentration of Mb in this muscle. However, the phenomenon on  $L^*$  values in all of these beef muscles tended to decrease gradually during chilled storage for seven days. Another study by King (2011) indicated that Semitendinosus and Vastus lateralis muscles underwent decline in  $L^*$  values during retail display in cold temperature for nine days, while (Mckenna, 2003) found that  $L^*$  values in Semitendinosus and Vastus lateralis tended to decrease during five days storage under chilled temperature.

The high lightness index observed in the two beef muscles on day 0 of storage in this study indicates that they were in the deoxygenation form, or deoxymyoglobin, due to high activity of mitochondria consuming oxygen, which was reflected by the high pH on day 0 of storage. Mckeith et al. (2016) have suggested the significant role of mitochondria in contributing to dark cutting beef, due to high oxygen consumption because of high pH values. Theoretically, decreased oxygenation of myoglobin occurs when mitochondrial respiration outcompetes myoglobin for oxygen, reducing the initial development and intensity of the red color. However, our findings showed that the Semitendinosus had a greater lightness index from the start to the end of the storage period of 7 days, suggesting that differences in muscle source or location can influence the lightness index during cold storage.

On the flip side for  $a^*$  values, there were non-significant differences between the two beef muscles on Days 1, 2, 3, and 5 of chilled storage (Table 2). However, on days 0 and 7 of storage, the two beef muscles were significantly different ( $P<0.05$ ) for  $a^*$  values. On day 0, Semitendinosus had a greater  $a^*$  value ( $P<0.05$ ) compared to Vastus lateralis, while reverse was true on Day 7. Nevertheless, during storage the Semitendinosus had greater  $a^*$  values on days 2 and 3, while on day 5 Vastus lateralis was higher in  $a^*$  numerically. Marked elevations of  $a^*$  values were seen on days 1 and 2 in both beef muscles and it could be associated with the oxygenated myoglobin on the surface of meat during storage, which from deoxymyoglobin form becomes oxymyoglobin. In partial agreement with our findings, King et al. (2011) and Mckenna (2003) documented that Semitendinosus and Vastus lateralis showed gradual decrease in  $a^*$  values during cold storage at retail display, except on day 7 of storage. Our findings indicate that  $a^*$  values in Vastus lateralis tended to increase on the seventh day of chilled storage.

**Table 2:** Meat pH, expressible drip, cooking loss, and hardness of Semitendinosus (ST) and Vastus lateralis (VAL) (n=6) from Thai Native cattle during 7-days of chilled storage at 4°C.

Attributes	Muscle	Days of Storage					
		0	1	2	3	5	7
Meat pH	ST	6.8±0.57b	5.8±0.57a	5.7±0.10a	5.6±0.37a	5.5±0.49a	5.5±0.37a
	VAL	6.9±0.20b	6.4±0.20b	5.9±0.15a	5.7±0.10b	5.7±0.15b	5.6±0.40b
Expressible Drip (%)	ST	8.03±1.91a	16.57±4.35a	18.94±1.24a	19.11±3.08a	19.99±3.48a	20.97±3.34a
	VAL	7.45±3.51a	15.50±1.75a	15.65±1.68a	17.66±2.53a	18.63±2.29a	19.04±4.96a
Cooking loss (%)	ST	11.50±5.91a	16.64±1.15a	24.14±3.42a	26.52±0.70a	24.93±1.12a	26.37±0.63a
	VAL	8.68±1.62a	15.89±0.67a	16.70±1.94b	19.78±2.42b	19.89±1.00b	22.78±4.48a
Hardness (N)	ST	10.24±4.09b	9.45±2.95b	7.86±1.31b	7.31±1.61b	5.03±1.11b	4.41±1.62a
	VAL	10.45±3.98b	9.50±2.59b	7.02±2.07b	6.74±1.03b	7.14±2.13a	5.11±2.54a

Values are given as mean±SD from triplicate determinations. Different letters in the same row indicate significant differences (P<0.05).

During chilled storage, the beef muscle Semitendinosus demonstrated greater values of yellowness (b\*) compared to Vastus lateralis on days 0, 1, 2, and 3 (P<0.05), as shown in Table 3. However, on days 5 and 7, there was non-significant difference between the two muscles, although numerically Semitendinosus remained higher than Vastus lateralis. Similar results regarding b\* values in Semitendinosus and Vastus lateralis were also documented by King et al. (2011) and McKenna (2003), where Semitendinosus had greater b\* values compared to Vastus lateralis during retail display for 7 and 9 days of cold storage. The trend of changes in the b\* values of both beef cuts differed from previous finding (King et al. 2011), where a steady decline in b\* values was reported during storage from days 0 to 7. In contrast, our results showed an increase in b\* values on day 7 of chilled storage for both Semitendinosus and Vastus lateralis muscles.

### Percentages of OxyMb and MMB

Oxidation of Mb or Oxymyoglobin produces MMB, and this process is well known as autoxidation (Tofteskov et al. 2017). Oxidation of unsaturated fatty acids in phospholipids and triglycerols results in lipid oxidation which contributes to off-flavor in beef (Faustman et al. 2010). Subsequently, lipid oxidation and myoglobin oxidation in meat are coupled and both reactions apparently become reciprocal between each other to cause deterioration in the quality of meat and meat products (Chaijan, 2008). The percentages of OxyMb and MMB in two beef muscles from Thai Native cattle are depicted in Table 3. On day 0 of storage, *Vastus lateralis* had significantly higher percentage of OxyMb (P<0.05) compared to Semitendinosus. Nevertheless, on days 1 and 2 of chilled storage both beef muscles were non-significantly different for OxyMb percentage. Conversely, on days 3, 5, and 7 of storage, Semitendinosus became dominant over Vastus lateralis for percentage of OxyMb. It can be seen clearly that the percentage of OxyMb in Vastus lateralis tended to increase on day 1, followed by a gradual decline until the end of storage. On the flip side for Semitendinosus, the OxyMb forms exhibited steady decline from days 1 until day 7 of storage.

Table 3 also shows MMB percentages on Semitendinosus and Vastus lateralis muscles from Thai Native cattle. On day 0, Semitendinosus had higher MMB percentage (P<0.05) compared to Vastus lateralis, while on days 1, 2, and 3 of storage, these two beef muscles were non-significantly different for MMB contents,

although on days 1 and 3, the percentages of MMB were numerically higher in Vastus lateralis compared to Semitendinosus. Subsequently, on days 5 and 7 of storage, Vastus lateralis showed significantly higher (P<0.05) percentages of MMB compared to Semitendinosus.

McKenna (2003) provided supportive evidence for our results by measuring the resistance to induced metmyoglobin formation (RIMF) in both Semitendinosus and Vastus lateralis muscles during a 5-day retail display. He reported that the percentage of MMB increased gradually in both muscle sources. Additionally, McKenna (2003) emphasized that beef cuts with lower color stability were more prone to metmyoglobin formation. That study also revealed that on the 5th day of retail display, Semitendinosus had a slightly induced metmyoglobin level of no more than 70%, while Vastus lateralis showed 86.6% induced metmyoglobin at the same time. These findings are consistent with our results, which showed that Vastus lateralis had higher percentage of induced metmyoglobin than Semitendinosus on days 5 and 7 of storage (Table 3).

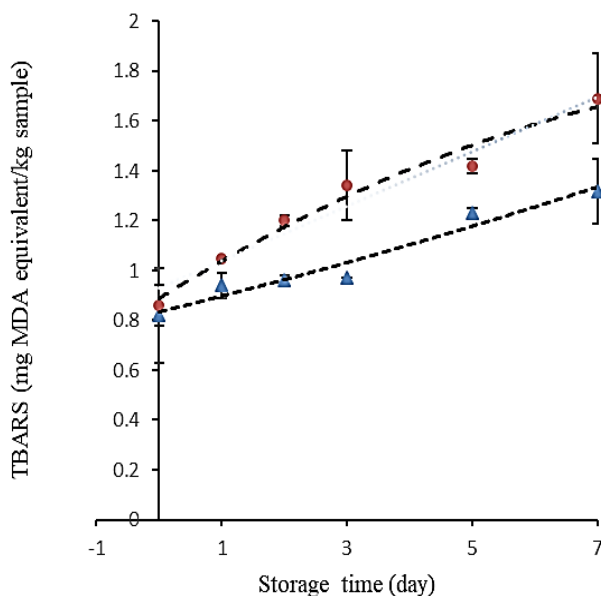
### Thiobarbituric Acid Reactive Substances (TBARS)

In this study, lipid oxidation products were detected in both beef muscles (Semitendinosus and Vastus lateralis) from day 0 of storage and tended to increase gradually with the duration of storage. The TBARS contents in muscles from both sources are presented in Fig. 1. It shows that Vastus lateralis had higher TBARS values than those of Semitendinosus during chilled storage. Although there were non-significant differences in TBARS values between Semitendinosus and Vastus lateralis on days 0 and 1 of the storage, the numerical values were higher in Vastus lateralis. This suggests that there may still be a trend towards higher levels of lipid oxidation in Vastus lateralis during the early stages of storage, despite the lack of statistical significance. Furthermore, during the later days (2, 3, 5, and 7) of the chilled storage period, Vastus lateralis displayed significantly higher TBARS content (P<0.05) compared to Semitendinosus, suggesting a greater degree of lipid oxidation occurring in Vastus lateralis compared to Semitendinosus during extended periods of chilled storage. Higher content of TBARS in Vastus lateralis could be attributed to higher myoglobin and iron contents in this muscle compared to Semitendinosus (Table 1). The myoglobin content in Vastus lateralis was found to be 2.67 mg/g, whereas in Semitendinosus it was only 1.41 mg/g. On the other hand, the iron content was 12.94

**Table 3:** Meat color ( $L^*$ ,  $a^*$ ,  $b^*$ ), and proportion of myoglobin forms of Semitendinosus (ST) and Vastus lateralis (VAL) (n=6) from Thai Native cattle during 7-days of chilled storage at 4°C.

Attributes	Type of muscles	Storage time (days)					
		0	1	2	3	5	7
$L^*$ Value	ST	27.60±0.73b	27.17±1.34b	26.22±0.67b	26.25±0.69b	25.07±2.12b	26.63±2.94b
	VAL	26.10±1.49c	25.51±1.16c	22.15±1.12c	23.13±2.56c	22.20±2.06c	21.16±5.10c
$a^*$ Value	ST	7.07±1.24b	9.38±0.91a	9.38±0.91a	8.57±0.89a	6.27±1.20a	6.14±1.04 a
	VAL	5.66±0.84a	9.90±1.70a	9.90±1.70a	7.54±2.71a	7.27±1.99a	9.77b±1.42b
$b^*$ Value	ST	6.63±0.73a	11.66±0.94a	10.60±0.64a	10.60±0.64a	8.56±1.03a	8.94±0.61a
	VAL	5.26±0.78b	9.10±1.18b	8.27±1.26b	8.27±1.26b	7.70±1.11a	8.90±0.92a
OxyMb (%)	ST	53.00±0.00a	68.50±0.70a	59.50±0.70a	60.00±0.00a	55.00±0.00a	54.50±0.70a
	VAL	58.5±0.70b	67.50±0.70a	62.50±0.70a	51.50±0.70b	49.00±0.00b	42.00±0.00b
MMb (%)	ST	29.00±0.00a	17.50±0.70a	26.50±0.70a	31.00±1.41a	31.00±0.00a	30.50±0.70a
	VAL	25.00±0.00b	19.00±0.00a	24.50±0.70a	31.50±0.70a	36.00±0.00b	41.00±0.00b

Values are given as mean±SD from triplicate determinations. Different letters in the same row indicate significant differences ( $P<0.05$ ).



**Fig. 1:** Changes of TBARS of Semitendinosus (▲) and Vastus lateralis (●) during storage time for 7-days of chilled storage.

mg/kg in Vastus lateralis and 12.12 mg/kg in Semitendinosus. Although there was statistically non-significant difference in the iron content between the two muscle groups, numerically Vastus lateralis showed higher value compared to Semitendinosus. In support to our results, some researchers pointed out that rates of lipid oxidation are associated with high iron and myoglobin contents.

Traditionally, the occurrence of lipid oxidation is interlinked with oxidation OxyMb and  $a^*$  value where pH can be considered as a triggering factor for lipid oxidation (Amaral et al. 2018; Dominguez et al. 2019; Barahona et al. 2021). This phenomenon was noted in both beef cuts during chilled storage for seven days. During early storage time of day 0, Semitendinosus and Vastus lateralis had higher pH (6.8 and 6.9, respectively) compared to the others day of storage (1, 2, 3, 5, and 7). Furthermore, Vastus lateralis still had high pH value of 6.4 till day 1 of storage. Under a high muscle pH, the mitochondria are outcompeting in oxygen consumption compared to myoglobin. Hence, abundance of mitochondria in some muscles would be associated with high oxygen consumption (Mckeith et al., 2016).

Based on our observations, it appears that the percentage of OxyMb tended to decrease and was followed by decline of  $a^*$  values. Conversely, the MMb tended to incline markedly in Vastus lateralis and Semitendinosus during chilled storage. These findings indicated that oxidation of OxyMb had occurred and it was changed to MMb forms. A previous study emphasized that changes in OxyMb and  $a^*$  values appeared to be driven by lipid oxidation and were correlated strongly with TBARS (Faustman et al., 2010). In consequence, the pH level, myoglobin concentration, iron content, and oxidation of OxyMb to MMb are interrelated factors which can synergistically influence one another during chilled storage of beef muscles. The interaction between these factors may ultimately lead to increased lipid oxidation, which is known to cause discoloration of meat and results in decreased  $a^*$  values.

## Conclusion

In conclusion, results of the present experiment showed that source of muscle from different anatomical locations in Thai Native cattle could affect eating quality, physicochemical properties, and rate of lipid oxidation during chilled storage for seven days. Semitendinosus tended to show low rate of lipid oxidation compared to Vastus lateralis, which could be associated to differences in myoglobin concentrations, iron contents, and pH values during early storage time.

## Acknowledgement

This work was supported by Kaltim Cemerlang Scholarship, Indonesia and Walailak University, Research Fund, Thailand.

## Author's Contribution

Ari Wibowo: Overall conceptualization of the study, acquisition, analysis and interpretation of data, formulation of proposed strategies, manuscript writing, as well as finalization of edits and revisions necessary. Nurul Fajrih : Provided support in the conceptualization of the research design and proof reading the manuscript. Suhardi: Provided substantial contribution to theory development and organization of data. Worawan Panpipat: Provided substantial contribution in organization of data and responsible for lipid oxidation, meat color (myoglobin redox) and hardness index in muscles analysis. Siriporn Riebroy Kim: Provided

material discussion on theory development and critical evaluation of research data and content. Manat Chaijan: Assisted in the conceptualization and streamlining of the research, contributed in the research design, performed data analysis and strategy development, critical evaluation of research data and content, fund and facility sourcing as well as checking for manuscript revisions necessary.

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