Meat Tenderness of Thai Native Cattle from Different Area of Thailand

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Abstract— This study was conducted to determine the effects of cattle groups and ageing periods on meat quality. Four groups of Thai native bulls were 1) Tak (n = 10, TNT), 2) Ubonratchathani (n = 10, TNU), 3) Kanchanaburi (n = 8, TNK) and 4) Khao Lamphun (n = 8, KLP). The Thai native cattle were naturally raised under free range grazing except KLP cattle were stall-fed with pangola grass. *Longissimus* muscle samples were excised from carcasses at 6th-12th interface into 4 sections and aged at 2 - 4 °C for 2, 7, 14 and 21 d post-mortem. Warner Bratzler Shear force (WBSF), calpastatin protein (*CAST*) and soluble collagen (SC) were evaluated at each ageing period. The results showed that TNT cattle was the most tender and had the lowest *CAST* among TNU, KLP and TNK, respectively, contrasted to the highest amount of SC. Western blot analysis of Troponin-T (Tn-T) indicated that TNK cattle had the highest intensities bands of Tn-T products (28-30 kDa) than others. The study also showed that while post-mortem ageing time increased, the WBSF value and *CAST* decreased in all cattle groups. However, the SC and Tn-T products were increased during storage caused by proteolytic enzyme.

Keywords— Tenderness, Thai Native Cattle, Calpastatin, Troponin-T, Collagen

I. INTRODUCTION

Most of beef consumed in Thailand are come from Thai native cattle naturally raised under free range grazing or retired cattle from farm work. These cattle are fed for short period of time with concentrate before sale. Their meat is slightly tough, so it is suitable for Thai way of cooking like boiling or simmers. In contrast to western style that prefers tender meat for grill or barbecued. However, tenderness is the most important factor related to consumers' acceptability of meat, and it is known to improve during post-mortem storage. This improvement has been found to be influenced principally by the connective tissue, proteolysis of myofibrillar proteins, muscle study and the role of protease enzyme with the length of the ageing period [1]. Since, there had been a few reports on Thai meat**,** the aim of this present work was to determine meat quality of Thai Native cattle from different area of Thailand.

MATERIALS AND METHODS

A. Animals and muscle sampling

Four groups of Thai native bulls were 1) Tak (n = 10, TNT), 2) Ubonratchathani (n = 10, TNU), 3) Kanchanaburi (n = 8, TNK) and 4) Khao Lamphun (n = 8, KLP). The Thai native cattle were naturally raised under free range grazing except KLP cattle were stall-fed with pangola grass. *Longissimus dorsi* muscle samples (LD) were excised from carcasses at 6th-12th interface into 4 sections and aged at 2 - 4 °C for 2, 7, 14 and 21 d post-mortem. At the end of each ageing period, two steaks were cut, one for pH, colour (L* a* and b*), Warner Bratzler shear force (WBSF) and another one for study of calpastatin protein (*CAST*), soluble collagen (SC) and proteolysis of Troponin-T (Tn-T).

B. The pH and colour (L, a*, b*) measurement*

The pH value was measured at 2, 7, 14 and 21 d postmortem by pH Meter (SevenGo™ SG2). Then, LD with 1-cm thick anterior surface of each ageing period was removed. After exposing freshly cut surface to room temperature (25°C) for 45 min, instrumental colour was performed. CIE (L*, a* and b*) was obtained using Chromameter CR300 Colourimeter.

C. Warner Bratzler Shear Force (WBSF)

Each ageing period, LD steak was trimmed into a 2.5-cm thick steak before placing into a polypropylene bag and vacuum sealed. These bags were heated in a hot water bath and cooled down in running tap water for 20 min. Later 10 pieces of 1x3x1 cm cooked muscle cubes were cut, across and along the muscle fibers, from each LD for WBSF measurement [2].

D. Determination of Calpastatin (CAST)

The determination of *CAST* was performed according to Geesink [3]. Supernatant containing heat-stable proteins, including *CAST*, was used for determination of *CAST* by Indirect Enzyme Linked Immunosorbent Assay. Recombinant bovine *CAST* corresponding to domain I was expressed and purified as previously described by Tavitchasri [4].

E. Determination of soluble collagen

The soluble collagen contents were determined according to Hill [5].

F. SDS-PAGE and immunoblotting

Samples (25 μl) containing myofibrillar protein was resolved by SDS-PAGE [6] with 4% stacking gel and 12.5% separating gel in Tris glycine buffer. Proteins were electrophoretically transferred to polyvinylidene difluoride membrane (Amersham bio-science,UK) for 1 hr at 100 (V) and 350 (mA) in buffer containing 25mM Tris, 192 mM Glycine, and 20% methanol. To prevent nonspecific antibody binding in immunoblotting, membranes were incubated with blocking buffer (0.05 M Tris, 3% non-fat milk) for 1 hr. Primary antibodies; mouse anti-troponin-T clone JLT-12 (Sigma,USA) diluted at 1:15,000 were used to soak membrane overnight at 4ºC, followed by an horseradish peroxidase conjugated anti-mouse IgG diluted 1:750 (Roche, Germany) for 1 hr. Membranes were washed 3 times with blocking buffer after each incubation. Troponin bands were visualized by using 3,3′,5,5′ tetramethyl-benzidine.

G. Statistical analysis

Analysis variance data of pH, color, WBSF, O.D.650 nm of *CAST*, SC, and intensity of Tn-T were performed using PROC GLM of the Statistical Analysis System. When significance was determined ($p < 0.01$), least squares group means were compared using the PDIFF test of SAS (2000).

RESULTS AND DISCUSSION

A. Effect of cattle groups

The influence of different cattle locations on meat quality showed that pH value of TNK steak was lowest ($p<0.05$) with highest L* among all groups while there were non-significant different values of other locations (Table 1). In agreement with Page [7] reported that L* values of beef had negative correlation to pH. At the low pH, proteins ability to bind with water are weak result in less water holding capacity, allowing more free water on meat surface to reflect light [8]. Whereas, a* and b* values of KLP were highest as their oldest average age presenting high myoglobin. Since, older animals had higher myoglobulin in muscle than the young one [9, 10]. Moreover, this group was stall-fed with high energetic diet explained higher a* and b* values than cattle raised under free range grazing. Close [11] also reported that feeding system had direct influence on carcass composition and rate of muscle growth which was ratio of protein and fat content. Therefore, fattening animal with high concentrate resulted in higher protein and intramuscular fat retention with high value of consumption.

The study also found that TNT cattle was the most tender and had the lowest *CAST* among TNU, KLP and TNK, respectively, contrasted to the highest amount of SC content (Table 1). Sethakul [12] revealed SC content in LD muscle of Thai native cattle with naturally free range grazing was lower than crossbred cattle with Thai meat production system, due to more muscle exercised and younger at slaughter age. This is similar to Johnson [13] presented that higher SC was found in young cattle than the old ones. Lawrie [8] reported that muscle with high *CAST* was tougher because calpain activity was inactivated.

Table 1 Influence (LSM±SE) of cattle groups and ageing periods on meat quality.

Parameters	Cattle groups (CG)				Ageing periods (A; day)				P-value		
	TNT	TNU	TNK	KLP	2	7	14	21	CG	A	G*A
pH	5.50 ± 0.03^{a}	5.46 ± 0.02^{a}	5.41 ± 0.02^{b}	5.48 ± 0.02^{a}	5.50 ± 0.02	5.44 ± 0.02	5.48 ± 0.02	5.43 ± 0.02	0.0467	0.0648	0.6163
L*	*	40.28 ± 0.46^{b}	42.63 ± 0.51^{a}	38.77 ± 0.51^{c}	38.52 ± 0.84^{z}	40.29 ± 0.54^{yz}	*	41.36 ± 0.54^{y}	<.0001	0.0158	0.9883
a*	*	15.80 ± 0.34^{c}	17.40 ± 0.38^{b}	21.63 ± 0.38^{a}	16.50 ± 0.62^{z}	18.52 ± 0.40^{y}	*	18.48 ± 0.40^{y}	<.0001	0.0087	0.9193
b*	*	4.21 ± 0.24^{b}	3.06 ± 0.27^{c}	5.19 ± 0.27^{a}	3.00 ± 0.44^{z}	4.84 ± 0.28^{y}	*	5.67 ± 0.28^{x}	<.0001	<.0001	0.9316
WBSF	6.21 ± 0.22^{b}	7.92 ± 0.22^{a}	8.37 ± 0.24^{a}	8.11 ± 0.24^{a}	9.67 ± 0.23^{x}	8.14 ± 0.23^{y}	6.68 ± 0.23^{z}	6.12 ± 0.23^{z}	<.0001	<.0001	0.4214
CAST[1]	0.16 ± 0.01^{c}	0.17 ± 0.01^{b}	0.20 ± 0.01^{a}	0.18 ± 0.01^{ab}	0.23 ± 0.01^{w}	0.19 ± 0.01^{x}	0.16 ± 0.01^{y}	0.13 ± 0.01^{z}	<.0001	<.0001	0.6850
SC[2]	0.25 ± 0.01^{a}	0.16 ± 0.01^{b}	0.15 ± 0.01^{b}	0.16 ± 0.01^{b}	0.12 ± 0.01^{z}	0.15 ± 0.01^{z}	0.19 ± 0.01^{y}	0.26 ± 0.01^{x}	<.0001	<.0001	0.4328
Tn-T 37-39	771.64±70.59	762.38±70.59	761.80±70.59	573.54±70.59	815.129±70.59	749.39±70.59	697.59±70.59	607.25±70.59	0.1583	0.2219	1.0000
Tn-T 28-30	421.68 ± 48.95^{b}	440.43 ± 48.95^{b}	661.92 ± 48.95^{a}	454.51 ± 48.95^{b}	394.67 ± 48.95^{y}	451.34 ± 48.95^{y}	523.66 ± 48.95^{yz}	608.86 ± 48.95^{z}	0.0046	0.0243	0.9887

$^{a-c}$ Means within a row without a common superscripts letter differ significantly ($p<0.05$)
$^{w-z}$ Means within a row without a common superscripts letter differ significantly ($p<0.05$)
* = Missing data
L* = lightness, a*= redness, b* = yellowness
[1]Calpastatin = O.D. 650 nm
[2]SC = soluble collagen (mg/g)
Tn-T = Troponin-T (µg BSA-equivalent)

Study of Tn-T proteolysis by determination of band intensity demonstrated that TNK cattle had the highest intensity band of Tn-T products at 28-30 kDa where as TNT cattle had the lowest intensity band. There were non-significant different in Tn-T precursors at 37-39 kDa (Table 1). Thai native cattle studied in this experiment were *Bos indicus*. There was more red fiber ratio than white fiber in *Bos indicus* [14]. Further to this, red fiber had closely amount of calpain to *CAST* resulted in tougher meat than *Bos taurus*.

B. Effect of ageing periods

The study revealed that ageing had the influence on meat quality and *CAST* quantification for all cattle groups (Table 1). The pH values were non-significant different ($p<0.05$) to longer ageing period. Even on 21 days postmortem, pH value continuously declined, this could cause by an increase of lactic acid along glycolysis process and acid produced by anaerobic bacteria after postmortem [15]. On the contrary, L*, a* and b* value increased ($p<0.05$) along ageing period. The value of a* increased because of the reduction of enzyme activity that caused myoglobin in ageing meat attached to oxygen better [16]. While, changing of chemical properties in fat content increased b* value. This was supported by Page [7] and Berruga [17] reported that b* value was higher according to ageing period ($p<0.05$). It was also found that, pH value had negative correlation to L* value.

WBSF study showed direct relation to *CAST* protein in all cattle groups (Table 1). The lower WBSF correlated to reduction of *CAST* at each ageing period ($p<0.05$) from 2 to 21 day. These results indicated that meat tenderness increased over ageing period. As ageing period was longer, *CAST* activity declined enhanced calpain activity to degrade Tn-T [18]. According to Huff-Lonergan and Lonergan [19], *CAST* quantification in beef and lamb was associated with the process of postmortem mechanisms of meat tenderization. However, there were non-significant different in the degree of tenderization at 14 and 21 days postmortem. Hence, the suitable ageing time for all cattle was 14 day postmortem. Similar to Tavitchasri [4] reported that there was non-significant different in the decreasing of both *CAST* quantification and meat toughness in Kamphaeng Saen breed aged at 14, 21 and 28 d postmortem.

Ageing periods also had an effect on SC content, as prolonged meat ageing period increased SC content. Pearson and Young [20] stated that cathepsin activity was maximized at low pH, causing connective tissue degradation and Z-disks destroy into small fragmentations. This released isometric tension in muscle and decreased shear force values. Related to this, an increase of ageing time resulted in more degradation of Tn-T precursors and high density of Tn-T product bands mimicking the pattern observed by Ho [21]. This indicated the outcome of calpain activity in muscle during ageing.

II. CONCLUSION

The influence study of Thai native groups (TNT, TNU, TNK and KLP) from different area in Thailand and ageing period on meat quality revealed non-significant different on meat tenderness. However, TNT meat was the best quality in term of low WBSF and *CAST* by contrast to high SC content and Tn-T products. Prolong ageing period, increased meat tenderness, muscle colour SC content and Tn-T products. Nevertheless, further study on meat quality of Thai native cattle need to be investigated more in order to benefit value added products by improve processing method suitable for Thai native meat.

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