



Research article

Assessment of soluble protein and collagen characteristics of dry-aged beef from Angus × Thai native and Charolais × Thai native crossbred steers

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Abstract

Importance of the work: The rising demand for premium beef necessitates more comprehensive indicators, in addition to traditional traits, to evaluate meat quality.

Objectives: To assess the meat quality of dry-aged steaks from crossbred cattle, focusing on soluble protein and collagen as supplementary indicators.

Materials and Methods: Dry-aged steaks from two crossbred cattle breeds were analyzed for soluble protein and collagen. Rib eye (*longissimus thoracis*) and strip loin (*longissimus lumborum*) cuts were compared from the cattle raised under normal and overfeeding conditions. The study examined the relationship between these components and conventional meat quality traits such as pH, cooking loss and shear force.

Results: Dry-aged beef steaks were examined from two cattle crossbreeds (Angus and Charolais with Thai native) raised under normal and overfeeding conditions. The soluble protein components were affected by both breed type and feeding condition. Additionally, there were significant differences in the sarcoplasmic protein and collagen contents between the two studied beef cuts that the tested conventional meat quality traits (pH, cooking loss and Warner-Bratzler shear force) could not differentiate.

Main finding: The soluble protein and collagen contents could serve as supplementary indicators for evaluating meat quality and offer a more nuanced assessment than using conventional methods. Further research is necessary to clarify the correlation between these chemical components, complex muscle characteristics and sensory quality.

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Introduction

The beef cattle population in Thailand was almost 9.4 million in 2022 with Thai native cattle (*Bos indicus*) accounting for 52% and crossbred cattle for approximately 43% of the population (Department of Livestock Development, 2023). The practice of cattle fattening involves overfeeding or supplementing with high-nutrient feed and reducing animal movements, or both, making up approximately 3% of Thailand's beef cattle production (Department of Livestock Development, 2023). It has been shown that consumers, especially in urban areas, are willing to pay a higher price for premium, tender beef with a high marbling (intramuscular fat) content (Shackelford et al., 2001; Bunmee et al., 2018). Thus, such consumer demand for premium meat cuts has created economic opportunities for improving cattle production and developing processes to improve the quality of meats.

Over decades, several beef breeds have been imported into Thailand, such as Charolais, Angus, Shorthorn, Santa Gertrudis and Brahman, and have been often crossed with Thai native cattle to improve their performance (Department of Livestock Development, 2023). The crossbreds of Angus (AT), Brahman (BT) and Charolais (CT) with Thai native cattle were reported to have good growth performance during the fattening period and to produce good carcass quality (Waritthitham et al., 2010a, b; Bunmee et al., 2017). Specifically, Bunmee et al. (2017) reported that CT produced the highest carcass weight, followed by AT and BT, respectively. In addition, BT had lower intramuscular fat and marbling scores than the others. The meat quality was superior in CT and AT compared to BT, although there was no significant difference in marbling scores and sensory evaluation between CT and AT (Bunmee et al., 2017).

Dry aging is a conventional method to improve meat quality (Dashdorj et al., 2016; Nair et al., 2018; Bulgaru et al., 2022). The dry-aging process involves keeping the meat at low temperature (0–4°C) and 61–85% humidity for a certain period, generally 14–30 d. Premium meat cuts are sometimes dry-aged inside water-permeable plastic bags to prevent microorganism contamination and to reduce excessive drying of the outer layer of meat, which otherwise must be trimmed off. During dry aging, the proteins and connective tissue are broken down by natural enzymes in the muscle meat and the moisture content is reduced by 10–15% after 20–30 d of aging (Dashdorj et al., 2016). The dry age process results in a unique, more condensed, flavorful aroma and improves the tenderness of the dry-aged meat (Dashdorj et al., 2016; Nair et al., 2018;

Bulgaru et al., 2022). Although premium beef meats are usually dry-aged to improve and maintain their quality, information on dry-aged, crossbred meats, such as CT and AT, remain unclear.

Therefore, the current study aimed to assess the meat quality of dry-aged prime cuts (rib eye and strip loin) from two crossbreeds (AT and CT). Specifically, the contents were measured of soluble proteins and collagen, which influence meat texture based on the hypothesis that these two parameters in dry-aged beef would be affected by breed type, feeding conditions and the meat cut. The Tropical Animal Genetic Special Research Unit (TAGU) at the Faculty of Agriculture, Kasetsart University, Bangkok, Thailand provided meat samples from fattened beef cattle. The practical knowledge of cattle and meat production technologies is transferred to producers via a Beef Producer Network to enhance beef products. The insights gained from this study will support the production of high-quality beef cattle by such producers.

Materials and Methods

Meat samples

The study applied a completely randomized design to examine the impact of different feeding regimens on the meat quality of crossbreed steers and was conducted on a commercial farm in Buriram province (14°58'20.6"N 103°11'59.8"E), Thailand. In total, 16 crossbreed steers, comprising two breed groups, Aberdeen Angus × Thai native (AT; $n = 8$) and Charolais × Thai native (CT; $n = 8$), were divided equally into two feeding conditions: normal feeding (adequately fed twice daily with a diet of 12% crude protein) and overfeeding (receiving the same diet in unlimited amounts for an additional 60 d). The finished fattening age was 24–30 mth based on weight (>550 kg) and body condition. Slaughtering the fattened cattle involved several sequential steps to ensure both humane treatment and the production of high-quality meat products. Beginning with pre-slaughter handling and transportation from the feedlot to the slaughterhouse, the process included a period of rest and food withdrawal to prepare the animal for slaughter. According to the standard system, at the start of the slaughtering process, each beast was stunned to render it unconscious, followed by bleeding to drain the carcass of blood. Then, skinning and evisceration occurred to removing the hide and internal organs, respectively. Washing and inspection ensured cleanliness and adherence to quality and safety standards. Subsequently, the carcass was chilled to

reduce its temperature (between 0°C and 4°C) and inhibit bacterial growth, before further processing into prime or retail cuts, with careful attention being paid to animal welfare, hygiene and meat quality throughout each step. Samples of the rib eye (*longissimus thoracis*) and strip loin (*longissimus lumborum*) meat cuts were collected and dry-aged at 4°C for 30 d. Subsequently, the meat samples were stored at -20°C and transported to the Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand for chemical analysis.

In total, 57 pieces of dry-aged meat were analyzed (27 rib eye and 30 strip loin samples), with 25 pieces from AT (14 normal-fed and 11 overfed) and 32 pieces from CT (16 normal-fed and 16 overfed). Some samples were placed in water-permeable bags to prevent contamination and minimize weight loss. However, statistical analysis excluded the bagging method since no significant differences were found in protein content, collagen content or meat quality attributes (pH, cooking loss and Warner-Bratzler shear force) between bagged and unbagged samples, supporting other work (Ahnström et al., 2006; Dashdorj et al., 2016). Visual examination revealed marbling scores ranging from 0 to 3, grouping the samples into the same level of intramuscular fat. This experimental design facilitated a robust comparison of the effects of normal feeding versus overfeeding on meat quality across two crossbreed steers, with the randomized design controlling potential confounding variables, thereby ensuring that the observed effects were attributable to the feeding regimens and breed differences.

Warner-Bratzler shear force test

Warner-Bratzler shear force (WBSF) testing was performed on all cooked beef samples. Shear force was determined using a texture analyzer (TA.XT plus; Texture Tech. Corp.; Scarsdale, NY, USA) with a 2 kg load cell using a shearing blade. The cooked beef was cut into 1.5 cm long and 1.27 cm thick samples. The crosshead speed was set at 5 mm/s and the test was triggered by a 0.05 N contact force. The pre- and post-test speeds were set to 1 mm/s and 5 mm/s, respectively. Shear force was calculated as the area under the force deformation curve produced using the data from the texture analyzer.

Determination of soluble protein

The protein contents of the beef samples were determined based on soluble nitrogen using the modified micro-Biuret

method described by (Joo et al., 1999). Each ground, homogenized beef sample (0.25 g) was immersed in 20 mL of 1.1M of KI in 0.1M phosphate buffer (pH = 7.2) for total soluble protein extraction, while the sample was immersed in 10 mL of 0.025M phosphate buffer (pH = 7.2) for sarcoplasmic protein extraction. The mixture was centrifuged at 1500×g for 20 min; then, the supernatant in each sample was collected as the soluble protein concentration determined by the micro-Biuret method using an ultraviolet (UV)-visible spectrometer (Spark™ 10M microplate reader; TECAN; AG, Switzerland). The myofibrillar protein was calculated as the difference between the total soluble protein and sarcoplasmic protein (Equation 1):

$$\text{Myofibrillar protein} = \text{Total soluble protein} - \text{Sarcoplasmic protein} \quad (1)$$

Determination of collagen

The collagen content was analyzed from the isolated hydroxyproline in the homogenized beef samples, according to the method of Kolar (1990). This method involved the hydrolysis of 4 g of each meat sample with 30 mL of 7 N sulfuric acid and heating at 105°C for 16 hr. Then, 1 mL of the extraction solution was mixed with chloramine-T as an oxidizing agent and mixed with 1 mL of 4-dimethylaminobenzaldehyde solution. The hydroxyproline concentration was quantitatively determined by measuring absorbance at 560 nm using a UV-Visible light spectrometer (V750; JASCO; Tokyo, Japan). The total collagen concentration was calculated assuming a collagen weight of 7.25 times that of hydroxyproline. The conversion factor used to convert the hydroxyproline content to estimate the total collagen content was 7.25, as collagen has been reported to have a high protein content (Starkey et al., 2015).

Statistical analysis

The data were statistically analyzed using the general linear model procedure in the SAS software (SAS® Studio Release 3.81; SAS Institute; Cary, NC, USA). A three (2 × 2 × 2)-factorial analysis of variance was applied to test the effects of breed group (BG), feed condition (FC), and cutting part (CP) and their first- and second-order interaction terms on the soluble protein and collagen contents (Equation 2):

$$y_{ijkl} = \mu + BG_i + CP_j + FC_k + BG_i|CP_j|FC_k + e_{ijkl} \quad (2)$$

where, y_{ijkl} represents the dependent variables (total soluble protein, sarcoplasmic protein, myofibrillar protein and collagen content, respectively); μ is the overall mean; BG_i , CP_j , and FC_k , are main effects; $BG_i|CP_j|FC_k$ represents the interaction terms; and e_{ijkl} is the random error.

Levene tests were performed to verify the homogeneity of variance. The soluble protein contents passed the Levene tests; however, the null hypothesis of equal variance was rejected for the collagen content. Therefore, logarithmic transformation was applied to the collagen contents to address variance inequality. Multiple comparisons were performed with Bonferroni adjustment at a significance level of $p < 0.05$. Additionally, Pearson’s correlations (r) were calculated to examine the relationships between the dependent variables.

Results

This study examined the physical and chemical properties of dry-aged meat samples, focusing on the effects of breed types, cutting parts and feeding conditions (normal versus overfeeding).

Physical properties

The conventional meat quality traits (pH, cooking loss and Warner-Bratzler shear force) measured in the dry-aged beef samples are summarized in Table 1. Notably, the AT samples had a highly significantly ($p < 0.001$) greater pH compared to

the CT samples, likely due to genetic differences (Chambaz et al., 2003; Waritthitham et al., 2010b). The pH values did not differ significantly among the different cutting parts and feeding conditions. Furthermore, no significant differences were observed in the cooking-loss and shear force values.

Soluble protein and collagen contents

The total soluble protein, consisting of sarcoplasmic (water-soluble) and myofibrillar (salt-soluble) proteins, as well as collagen, were extracted and analyzed. The total soluble protein content was significantly dependent on breed group, feed condition and cut of meat (Table 2). The sarcoplasmic protein content was significantly affected by breed group and cut of meat but not by feed condition. The myofibrillar protein content was significantly influenced by both breed group and feed condition. However, the collagen content was dependent only on the cut of meat. The only significant interaction was between feed condition and cut of meat.

Variations in these biochemical contents due to breed type, cutoff meat and feeding condition are shown in Table 3. The CT samples had higher total soluble protein, myofibrillar protein and sarcoplasmic protein contents than the AT samples. However, the collagen content did not differ between breeds.

The cut of the meat also significantly affected the protein content (Table 3). Although rib eye and strip loin are similar in terms of muscle type and conventional traits, the strip loin samples had higher total soluble protein and sarcoplasmic protein contents (but a lower collagen content) than the rib eye.

Table 1 Effects of breed, cut of meat, and feed condition on pH, shear force, and cooking loss of meats from Angus × Thai native (AT) and Charolais × Thai native (CT) crossbred cattle

Parameter	Breed		Cut of meat		Feed condition	
	AT	CT	Rib eye	Strip loin	Normal	Overfed
pH (aged 30 d)	6.30 ± 0.03**	6.10 ± 0.03**	6.21 ± 0.03	6.19 ± 0.03	6.19 ± 0.03	6.20 ± 0.03
Shear force (N)	25.90 ± 1.76	28.13 ± 1.64	28.13 ± 1.70	25.90 ± 1.70	27.20 ± 1.42	25.92 ± 1.57
Cooking loss (%)	22.83 ± 0.74	22.86 ± 0.70	22.47 ± 0.72	23.22 ± 0.72	23.60 ± 0.91	22.51 ± 0.99

Data shown as least square mean ± SE, with number of observations, $n = 32$ for each case
** = highly significant difference ($p < 0.001$) between two asterisked values; no significant difference was observed among the remaining pairs.

Table 2 Statistical significance of effects of breed group (BG), feed condition (FC), cut of meat parts (CP) and their first- and second-order interactions on soluble protein and collagen contents

	BG	FC	CP	BG×FC	BG×CP	FC×CP	BG×FC×CP
Total soluble protein	*	*	*	ns	ns	ns	ns
Sarcoplasmic	*	ns	*	ns	ns	ns	ns
Myofibrillar	*	*	ns	ns	ns	*	ns
Collagen	ns	ns	*	ns	ns	ns	ns

* = significant ($p < 0.05$) ns = not significant ($p \geq 0.05$)

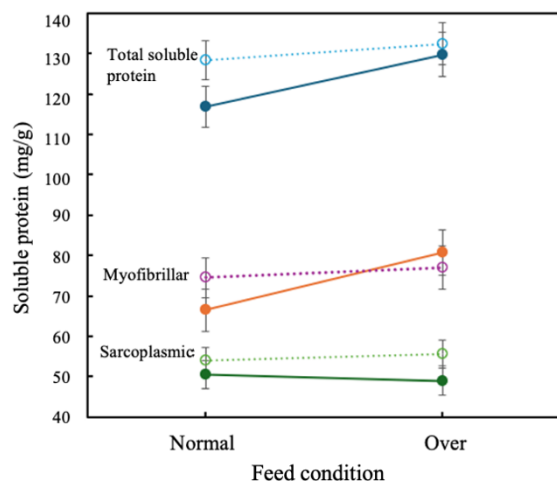
Table 3 Effects of breed, feeding condition and cut of meat on chemical composition of meats from Angus × Thai native (AT) and Charolais × Thai native (CT) crossbred cattle

	<i>n</i>	Total soluble protein (mg/g)	Sarcoplasmic protein (mg/g)	Myofibrillar protein (mg/g)	Collagen (mg/g)
Breed					
AT	25	121.0 ± 1.9	49.5 ± 1.3	71.4 ± 2.0	9.1 ± 0.6
CT	32	132.9 ± 1.7	55.0 ± 1.1	77.9 ± 1.7	9.6 ± 0.6
<i>p</i> value		< 0.001	0.003	0.02	0.53
Feed					
Normal	30	122.7 ± 1.8	52.2 ± 1.2	70.5 ± 1.8	9.0 ± 0.6
Overfed	27	131.1 ± 1.9	52.3 ± 1.2	78.9 ± 1.9	9.7 ± 0.6
<i>p</i> value		0.002	0.98	0.002	0.37
Cut of meat					
Rib eye	27	123.3 ± 1.9	49.7 ± 1.2	73.6 ± 1.9	10.8 ± 0.6
Strip loin	30	130.5 ± 1.8	54.8 ± 1.2	75.8 ± 1.8	7.9 ± 0.6
<i>p</i> value		0.007	0.005	0.41	0.001

Data shown as least square mean ± SE; *n* = number of observations.

Overfeeding significantly increased the total soluble protein and myofibrillar protein contents (Table 3) but did not affect the collagen content. Notably, the interaction between the feeding condition and cut of meat was also significant (Fig. 1). In the rib eye, the total and myofibrillar protein contents were significantly higher in samples subjected to overfeeding, while in strip loin, the protein contents were not significantly changed by the feeding conditions.

Pearson's correlations were calculated to examine the relationships between the studied variables. No significant correlation existed between the collagen and soluble protein contents. As expected, strong correlations were observed among different soluble protein types due to their related quantities. The myofibrillar protein content was calculated by subtracting the salt-soluble protein from the water-soluble protein.

**Fig. 1** Different soluble protein contents in rib eye (solid lines) and strip loin (dotted lines) plotted as function of feed condition

Discussion

Conventional meat quality traits

The pH values of the meat dry-aged for 30 d were only significantly different between the two breed groups, likely due to genetic differences (Chambaz et al., 2003; Waritthitham et al., 2010b). The other factors had no significant impact on the pH value. The relatively high pH values aligned with observations in other Thai crossbred beef studies (Waritthitham et al., 2010b; Bunmee et al., 2017), suggesting pre-slaughter stress and Thailand's hot, humid climate may be contributing factors.

The shear force and cooking loss values of the dry-aged samples in the current study were comparable to those reported in the literature for dry-aged meat (Dashdorj et al., 2016; Nair et al., 2018; Bulgaru et al., 2022). Chambaz et al. (2003) found that Angus and Charolais had similar meat quality traits, with the Charolais having slightly higher WBSF values but a lower cooking loss than Angus meat. The current results also showed a similar trend, with slightly higher WBSF values in the CT samples than the AT samples, although the differences were not statistically significant.

Despite genotype differences, Angus and Charolais have been reported to have comparable growth performance and meat qualities, even though the purebred Charolais breed is classified as late-maturing, characterized by better muscle tone and less fat than the earlier-maturing Aberdeen Angus (Sakowski et al., 2022). Chambaz et al. (2003) reported that the meat qualities of *M. longissimus dorsi* samples dry-aged

for 14 d of Angus and Charolais steers, at the same intramuscular fat content, displayed similar quality traits. Their study reported that even trained panels of tasters gave similar scores for tenderness, flavor intensity and preference, though noticeably higher juiciness scores were given to Charolais. Bunmee et al. (2017) studied the fattening performance, carcass and meat quality of Charolais, Black Angus and Brahman crossbred with Thai native cattle. They reported that the Angus and Charolais crossbreeds had comparable fattening performance that was superior to that of the Brahman crossbred. The meat qualities of Angus and Charolais crossbreeds were also comparable in terms of both physical traits and sensory perceptions. (Bunmee et al., 2017) Other literature has suggested that Angus and Charolais share comparable overall meat quality, making their meat products indistinguishable based conventional quality traits (Bunmee et al., 2017; Chambaz et al., 2003).

Soluble protein and collagen content as differentiation factors

While conventional meat quality traits (pH, cooking loss, and shear force) could not be used to differentiate among meat samples of comparable quality in the current study, the soluble protein and collagen contents were significantly different, with the soluble protein contents being significantly dependent on breed, cut of meat and feeding condition. In contrast, the collagen contents differed significantly only between the different muscle types. Overfeeding significantly increased the total soluble protein and myofibrillar protein contents. Notably, the effect of overfeeding was not uniform across both cuts. There was a significant interaction between feeding condition and cut of meat (Fig. 1). In rib eye, overfeeding resulted in a substantial increase in both the total and myofibrillar protein contents. Conversely, overfeeding did not significantly affect the protein content in the strip loin. These findings suggested that the soluble protein and collagen contents could be considered sensitive indicators for differentiating meats from different breeds, meat cuts and feeding histories.

While the soluble protein content is linked to meat quality, the specific relationship remains unclear. Some studies suggested correlations between protein types and traits, such as water-holding capacity (WHC), in pork (Joo et al., 1999), though the connection was not always consistent across meat types. In broiler breast meat, while the overall myofibrillar and sarcoplasmic protein solubility did not correlate directly with WHC, denaturation of sarcoplasmic proteins after slaughter appeared to impact WHC directly (Bowker and Zhuang, 2015). There are no known benchmark values for protein solubility

because protein solubility depends on several parameters, such as the method of extraction, muscle tissue structure, pH, and post-mortem muscle transformations, as well as the aging process (Joo et al., 1999). Reports have indicated that increased myofibrillar and sarcoplasmic protein contents are associated with beef tenderization, since during dry aging of beef, the soluble protein, sarcoplasmic protein, and myofibrillar protein contents changed with aging time, impacting the texture profile of the dry-aged beef (Nair et al., 2018; Bulgaru et al., 2022)). The use of other enzymes (meat marinating) can also lead to tenderizing effects due to protein degradation by enzyme activities (Kalahrodi et al., 2021). For example, when semitendinosus beef steaks were marinated with asparagus juice for 48 hr, the myofibrillar protein content increased from 24 ± 7 mg/g to 55 ± 7 mg/g, and the sarcoplasmic protein increased from 24 ± 3 mg/g to 38 ± 3 mg/g (Kalahrodi et al., 2021). The current reported values for both the myofibrillar and sarcoplasmic protein contents were consistent with these reported values.

Collagen is a primary protein found in connective tissue, providing support, protection and structural integrity to muscles (López-Bote and Calvo, 2023). Collagen is commonly regarded as an indicator of meat tenderness. For example, Hammond et al. (2022) that the more tender muscles (*longissimus thoracis* and *rectus abdominis*) had lower collagen contents than the *pectoralis profundus*, *rectus femoris*, *semitendinosus*, and *triceps brachii* muscles. Past and recent meta-analyses (Chriki et al., 2013; Li et al., 2022) based on extensive literature have revealed that the collagen content varies with muscle types and animal age. A positive correlation has been observed between collagen content and WBSF, with the strength of this correlation varying, depending on the muscle type, with greater correlations found in other beef muscles compared to the loin (*longissimus thoracis* or *lumborum*). In the current study, the average collagen content—with strip loin containing significantly less collagen (7.9 ± 0.6 mg/g) than ribeye (10.8 ± 0.6 mg/g)—aligned with the original JAOAC International report (Kolar, 1990), which found 6.6 mg/g in sirloin meat.

The current findings suggested that the collagen and sarcoplasmic protein contents could serve as valuable indicators to differentiate among breeds and similar muscle cuts of meat. Despite being neighboring primal cuts in the short loin with comparable conventional quality traits (pH, cooking loss and shear force) and having similar muscle-fiber types, the rib eye and strip loin meat cuts had distinct biochemical profiles, specifically their collagen and sarcoplasmic protein contents,

likely contributing to their differing sensory characteristics. According to recent research by Bulgaru et al. (2022), different chemical properties affect the quality of dry-aged beef, with correlations found between meat texture attributes and physicochemical properties, including the soluble protein and collagen contents. For example, the hardness of texture is largely influenced by the content of soluble proteins, fats, soluble collagen and WHC, while chewiness and gumminess are mainly influenced by fats, soluble proteins, and soluble collagen. (Bulgaru et al., 2022). Hammond et al. (2022) highlighted that multiple biochemical factors such as proteolysis, sarcomere length, collagen content, collagen crosslinks and lipid content, contribute to overall tenderness across different muscles to varying degrees. However, this area of research is still nascent, and further studies are necessary to translate biochemical and physicochemical profiles into meat quality and sensory perceptions.

In conclusion, the current study investigated the effects of breed, feeding regimen and meat cut location on the protein and collagen contents in dry-aged beef. Conventional quality traits could not differentiate the variations attributed to these factors. However, the soluble protein levels were influenced by both breed and meat cut. Notably, overfeeding increased the protein content only in the rib eye and not in strip loin cut, revealing a significant interaction between feeding condition and cut location. The collagen content was influenced solely by cut location, with strip loin having a lower collagen content than rib eye. These results underscored the complexity of factors that contribute to meat quality. Additionally, the results suggested the potential of using the soluble protein and collagen contents as indicators to differentiate meat characteristics. However, further research is needed to explore the relationship between these biochemical components and the sensory quality of beef, with the results contributing to deeper insights into how these factors impact meat quality and consumer preferences.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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