# Freezing, thawing and aging improves the tenderness of chuck muscles from young bulls of different phenotypes

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## Abstract

We evaluated eleven chuck muscles from phenotypically selected Brahman (BR, n =10) and Charolais (CH, n =10) young bull carcasses for the effect of freezing, thawing, and aging for 2, 9, 16, and 23 days on Warner-Bratzler shear force (WBSF). *Splenius*, *infraspinatus*, *teres major*, *rhomboideus*, and *subscapularis* from both phenotypes, and *complexus* from CH had tender ($\leq 40$ N) 9 d WBSF values. No further improvement in WBSF was observed beyond 9 days of aging. Overall, we observed freezing, thawing and aging of several chuck muscles from young bulls significantly improves beef tenderness. Further studies would help optimizing this tenderization strategy and assess its potential impact on other meat quality attributes.

*Keywords:* Beef quality; Aging; Forequarter muscles; Warner-Bratzler shear force; Young bulls.

## Introduction

Aging is one of the most commonly used postmortem techniques for improving beef tenderness, which is one of the most important factors determining beef quality. Numerous researchers (Belew *et al.*, 2003; Molina *et al.*, 2005; Rhee *et al.*, 2004; Von Seggern *et al.*, 2005; Yadata *et al.*, 2009) have studied the tenderization process of several beef muscles at different postmortem aging periods. However, most of these studies consider beef from steers and heifers while data from young bulls, which are the main source of beef in some countries (Mendez *et al.*, 2009) are very scarce.

The limited number of studies on young bulls (Chávez *et al.*, 2012; Hildrum *et al.*, 2009; Rubio *et al.*, 2007; Simões *et al.*, 2005; Vieira *et al.*, 2009) have shown meat produced by these animals is tough. The high WBSF values reported in most of these studies indicate this type of beef is resilient to aging. In that sense, it is well documented that freezing of meat prior to aging reduces calpastatin activity (Crouse & Koohmaraie, 1990; Koohmaraie, 1990). In fact, recent research has shown USDA Select beef previously frozen and thawed age faster than that which is never frozen (Grayson *et al.,* 2014). Therefore, we believe a freezing-thawing-aging strategy may be an effective means of tenderizing and adding value to muscles from young bulls

as well. The latter could be a feasible alternative in some specific markets. Hence, the objective of this study is to estimate the effect of freezing, thawing and aging on the tenderness of chuck muscles from young bulls that are exploited in Mexico.

## Materials and methods

### Muscle samples

We studied muscles from young bulls 18-24 months of age obtained from two commercial beef slaughter operations in Mexico. Since both cattle genotypes (*Bos taurus* and *Bos indicus*) are exploited in Mexico, we wanted to include in the study at least one of the most common breeds of each genotype. Therefore, trained personnel phenotypically selected *B. taurus* (Charolais, n = 10) and *B. indicus* (Brahman, n = 10) bulls in the lairage. Animals that did not fit into one of the phenotypes by visual appraisal were not selected for the study.

Due to the difficulty of finding a slaughterhouse where both phenotypes were available, the study was conducted as two independent trials. Cattle from each phenotype originated from two different feedlots and were slaughtered at two different slaughterhouses. However, we selected slaughterhouses and feedlot cattle suppliers with similar working conditions.

All animals had been concentrate-fed (sorghum, corn and soybean based diets plus vitamin and mineral premixes) for an average of 100 days. Moreover, animals were treated with the same commercial preparation of zilpaterol (Zilmax, Intervet México, S. A. de C. V.), added at 0.006 g/kg in the diet for the last 40 days before slaughter (no withdrawal period). Likewise, the distance from each of the feedlots to each of the slaughterhouses is < 100 km, and both slaughterhouses operate under Federal Inspection.

Carcasses were electrically stimulated 1 to 3 min after bleeding, following the current commercial practices in Mexican slaughterhouses (680 V, 0.3-0.5 A for 10-15 s), and then refrigerated at 0 °C for 24 hours. Afterwards, trained personnel separated a total of 11 different muscles (*biceps brachii, brachialis, complexus, splenius, infraspinatus, teres major, rhomboideus, subscapularis, supraspinatus, triceps brachii* [long head], and *triceps brachii* [lateral head]) from the forequarters. Four steaks of 2.5 cm width and 6 cm diameter were hand-cut from each muscle within a carcass. The steaks were progressively removed, beginning at the anterior end, to make sure they came from the same location for each animal. Afterwards, the samples were labeled and vacuum packaged before they were taken to the Meat Science Laboratory at the Faculty of Veterinary Medicine of Universidad Nacional Autónoma de México, Mexico City. The packages were transported by road or air in insulated containers with coolant gels and arrived at the laboratory within 8-10 hours. Hence, the steaks have had a total postmortem aging time of 32-34 h before being frozen (-30 °C) in still air and held at this temperature for seven days. After this period, the samples were thawed for 24-30 hours at 2-4 °C and randomly assigned to one of the following additional aging periods: 0, 7, 14 or 21 days. Steaks assigned to 0-day additional aging were immediately measured to determine WBSF. The remaining three steaks were stored at 2-4 °C for the designated additional aging period. For practicality, the aging times were adjusted by

adding 2 days in order to compensate for the previous postmortem aging. Therefore, the aging times will be further referred to as 2, 9, 16 or 23 days.

### WBSF determination

WBSF was determined according to AMSA Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat (AMSA, 1995). We placed iron-constant thermocouples (Omega Engineering Inc., Stamford, USA) into the geometric centers of each muscle portion. All portions were cooked in groups of two on electric grills (model DEG-22 1500 W, Daewoo Electronics México, Mexico) to an internal temperature of 70 °C. The temperature was monitored using a portable thermometer.

After cooking, the steaks were allowed to equilibrate to room temperature (20-25 °C) before we removed 6-8 cores 1.27 cm in diameter from each steak, parallel to the muscle fiber, with an automated coring device. On the same day, the cores were sheared perpendicularly to the muscle fiber, with a Warner-Bratzler shear machine using a slice shear force blade from G-R Manufacturing (Manhattan, KS, USA). Peak shear force measurements were recorded and averaged to obtain a single WBSF value for each steak.

### Statistical analysis

We analyzed data with the general linear model procedure of Statgraphics Centurion XV (StatPoint, Inc., 1982-2007) and a model including the fixed effects of muscle, postmortem aging and the 2-way interaction. The data were sorted and analyzed by phenotype. When significant F-values were observed, means within each factor were discriminated using the Tukey's range procedure. A t-test was used to compare means from significant interactions.

## Results and discussion

Muscle and aging time significantly interacted to affect WBSF of beef from Charolais ($P = 0.0008$) and Brahman ($P < 0.0001$) bulls. Thus, analyzing WBSF of individual muscles across aging times provides more accurate information. The combined effects of freezing, thawing and aging significantly decreased ($P < 0.05$) WBSF of muscles from both phenotypes except values of *biceps brachii*, *brachialis*, and *supraspinatus* (Table 1). These results are in line with previous reports documenting the limited effect of aging on the WBSF of *brachialis* (Belew *et al.*, 2003; Von Seggern *et al.*, 2005) and *supraspinatus* (Hildrum *et al.*, 2009; Rhee *et al.*, 2004). Conversely, present findings failed to support previous studies, in which the *biceps brachii* from USDA Choice and Select carcasses was classified as tender (Belew *et al.*, 2003; Von Seggern *et al.*, 2005). It should be noted, however, that some bull muscles often have a poorer response to aging than those of steers and heifers (Claus *et al.*, 2010; Mach *et al.*, 2009; Rubio *et al.*, 2007). In aged *triceps brachii* (lateral head), WBSF remained high, which is comparable to results observed in previous studies with steers and heifers (Gruber *et al.*, 2006; Stelzleni *et al.*, 2007; Von Seggern *et al.*, 2005), and with bulls (Simões *et al.*, 2005).

**Table 1.** Least square means of WBSF (N) for Brahman and Charolais chuck muscles (n = 10) frozen for one week, thawed for 24-30 h at 2-4 °C, and aged for different periods

| Muscle | Aging time, days | | | | SE ± |
|---|---|---|---|---|---|
| | 2 | 9 | 16 | 23 | |
| **Experiment 1: Brahman** | | | | | |
| *Biceps brachii* | 52.3 | 45.2 | 46.0 | 41.4 | 1.9 |
| *Brachialis* | 56.0 | 60.2 | 52.2 | 56.2 | 1.6 |
| *Complexus* | 62.2[a] | 44.8[b] | 46.1[b] | 36.4[c] | 1.3*** |
| *Splenius* | 62.4[a] | 39.1[b] | 39.6[b] | 38.6[b] | 1.2*** |
| *Infraspinatus* | 41.2[a] | 30.1[b] | 33.4[b] | 29.2[b] | 0.9*** |
| *Teres major* | 48.9[a] | 37.1[b] | 41.1[b] | 41.3[b] | 1.0*** |
| *Rhomboideus* | 49.0[a] | 35.4[b] | 34.0[b] | 39.3[b] | 1.2*** |
| *Subscapularis* | 51.7[a] | 39.4[b] | 37.3[b] | 39.2[b] | 1.4*** |
| *Supraspinatus* | 53.3 | 51.2 | 47.2 | 47.8 | 2.9 |
| *Triceps brachii* (long head) | 53.0[a] | 37.8[b] | 39.6[b] | 40.5[b] | 1.1*** |
| *Triceps brachii* (lateral head) | 63.1[a] | 56.2[b] | 53.4[b] | 50.2[b] | 1.3*** |
| **Experiment 2: Charolais** | | | | | |
| *Biceps brachii* | 50.0 | 44.0 | 45.0 | 45.9 | 3.6 |
| *Brachialis* | 58.2 | 51.1 | 59.3 | 57.3 | 1.3 |
| *Complexus* | 46.3[a] | 32.8[b] | 34.6[b] | 36.9[b] | 1.0*** |
| *Splenius* | 45.3[a] | 34.8[b] | 32.9[b] | 33.5[b] | 0.8*** |
| *Infraspinatus* | 34.4[a] | 29.8[b] | 27.2[b] | 27.4[b] | 0.7*** |
| *Teres major* | 46.9[a] | 38.1[b] | 38.0[b] | 38.4[b] | 2.4*** |
| *Rhomboideus* | 57.7[a] | 31.9[b] | 33.7[b] | 36.5[b] | 1.3*** |
| *Subscapularis* | 42.0[a] | 32.6[b] | 29.7[b] | 29.4[b] | 1.0*** |
| *Supraspinatus* | 48.3 | 43.0 | 42.2 | 43.9 | 1.8 |
| *Triceps brachii* (long head) | 51.6[a] | 37.1[b] | 39.0[b] | 37.2[b] | 0.8*** |
| *Triceps brachii* (lateral head) | 57.1[a] | 58.5[a] | 51.0[ab] | 48.4[b] | 1.4* |

[a, b] Means with different superscripts in the same row are significantly different (P<0.05)

* P < 0.05; *** P < 0.001

Interestingly, tenderization of muscles (except *complexus* from Brahman) occurred up to nine days while no further decrease in WBSF was observed at 16 or 23 days. The latter is difficult to explain since there is no agreement on what could be an appropriate aging time for this type of beef. Recommendations from other studies are quite variable (5 to 21 days) and usually depend on carcass quality grade and muscle (Bratcher *et al.*, 2005; Gruber *et al.*, 2006; King *et al.*, 2009; Nelson *et al.*, 2004; Von Seggern *et al.*, 2005). However, most of these studies consider beef from steers and heifers while data from young bulls, which are usually excluded from quality grading schemes, are very scarce. Moreover, the common methodology used to study meat tenderization follows an aging-freezing-thawing strategy. Under these circumstances, beef from young bulls has consistently exhibited high WBSF values (Hildrum *et al.*, 2009; Li *et al.*, 2012; Vieira *et al.*, 2009) even after relatively long aging periods. On the other hand, freezing of

meat prior to aging has been shown to drastically reduce calpastatin activity (Kooh-maraie, 1990; Crouse and Koohmaraie, 1990). Consequently, the strategy used in this study, which comprises freezing and thawing of muscles before aging, may have accelerated the tenderization process. Hence, the studied muscles may have reached the lowest WBSF possible within nine days. This reasoning is supported by findings from a recent study (Grayson et al., 2014) showing beef frozen, thawed, and aged for 14 days was more tender than that aged for 28 days. Therefore, the freezing-thawing-aging strategy appears to be a viable alternative to counteract the resilient response to aging of chuck muscles from young bulls.

Most muscles from both phenotypes had 9 d WBSF means lower than those reported elsewhere. For instance, Vieira et al. (2009) reported high WBSF values (> 46 N) in muscle *longissimus thoracis* from young bulls aged for 10 days and then frozen for up to 90 days. Likewise, Li et al. (2012) reported WBSF > 60 N in *longissimus lumborum* from Chinese Yellow cattle subjected to different chilling regimens and aged for up to 21 days. Another study with Norwegian Red bulls (Hildrum et al., 2009) reported WBSF values above 50 N for *triceps brachii* [long head] aged for 9 days (never frozen). This is roughly 15 N higher than the average 9 d WBSF we observed for the same muscle. Likewise, the 9 d WBSF reported by Hildrum et al. (2009) for *infraspinatus* is about 10 N higher than that observed here. Interestingly, muscles *teres major*, *rhomboideus*, and *triceps brachii* [long head] had average 9 d WBSF equal to or even lower than those reported else-where in steers for beefsteaks aged 14 d (Bratcher et al., 2005; Rhee et al., 2004; Stelzleni et al., 2007).

Given these facts, it is worth mentioning that differences in methodology make direct comparisons of actual WBSF across studies very difficult. However, four muscles from Brahman bulls and seven from Charolais reached WBSF values < 38.22 N, which are typical of tender beef (Belew et al., 2003). Despite the an-imals were slaughter in different slaughter houses, these results support findings documenting the superior tenderness of beef from *B. taurus* breeds as compared to the *B. indicus* ones (Bidner et al., 2002; Bonilha et al., 2008; Shackelford et al., 1995). Moreover, current results confirm the widely documented superior ten-derness of *infraspinatus* (Bratcher et al., 2005; Gruber et al., 2006; Rhee et al., 2004; Searls et al., 2005; Stelzleni et al., 2007), as well as the potential for value addition of other beef forequarter muscles. Our results also support prior sugges-tions (Bratcher et al., 2005) that fabrication and merchandizing decisions should be made on an individual muscle basis.

In summary, these findings show some forequarter muscles from young bulls are able to produce tender beef if frozen and thawed before aging. The latter does not seem to be affected by zilpaterol supplementation, which was administered to all of the animals used in the experiment. Hence, freezing and thawing before aging could serve as an effective means of improving the tenderness consistency of meat from young bulls. We believe this is a striking outcome since young bulls conform nearly 80% of the Mexican beef slaughter population (Mendez et al., 2009) and zilpaterol is widely used in Mexican feedlots (Camponova, 2006).

Even though it is unlikely that this strategy would be massively used by the meat industry in the future, it may be a viable alternative in some specific markets. Particularly, when dealing with tough meat that needs long aging times to tender-ize. In the studied sample, several chuck muscles from young bulls reached WBSF

typical of tender beef when kept frozen for one week and aged for nine days. Given these facts, we believe further research is needed in order to assess the effects of freezing on the sensory properties and other quality attributes of economical importance in beef. The latter is vital in order to optimize the tenderization strategy studied here.

## Conclusions

Data from the present investigation show freezing, thawing and aging of several chuck muscles from young bulls significantly improves beef tenderness. Further research is needed to optimize the tenderization process and assess its potential impact on other meat quality attributes.

## Conflicts of interest

None of the authors has any conflict of interest in regard with this article.

## Author contributions

María Salud Rubio Lozano: Directed the research, reviewed the data, reviewed the manuscript critically, and directed revisions.

Esmeralda Vanessa Pérez Buitrón: Conducted the research, compiled data, analyzed results, wrote the thesis in Spanish, and reviewed the manuscript in its English version.

Rubén Danilo Méndez Medina: Helped design the research, analyzed results, and reviewed the manuscript.

Adrián Chávez Gómez: Participated in conducting the research, compiled data, and reviewed the manuscript.

Enrique Jesús Delgado Suárez: Contribuited to the research design, helped with the statistical analysis, and drafted the manuscript in English.

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