RELATIONSHIPS BETWEEN INTRAMUSCULAR FAT, COLLAGEN HEAT SOLUBILITY AND EHRLICH CHROMOGEN CONCENTRATION IN BOVINE *LONGISSIMUS THORACIS*

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Abstract- The effects of intramuscular fat on meat quality and collagen characteristics were investigated using 48 longissimus thoracis (LT, rib eye) muscles from four quality grades (Canada Prime, AAA, AA and A). Muscles were obtained within 72 hours postmortem. Mean shear force (SF) value decreased as marbling increased with Canada Prime and AAA having lower SF than Canada A and AA (P = 0.001). Canada Prime had the lowest moisture content but the highest intramuscular fat content (P < 0.0001). Collagen heat solubility was not affected by quality grade (P = 0.49); however, collagen heat solubility increased as total collagen content decreased (r = -0.47, P = 0.0007). Canada Prime LT showed a higher collagen content (2.49 mg/g raw muscle) than LT from AA (2.05 mg/g raw muscle), AAA (1.99 mg/g raw muscle) and A (1.92 mg/g raw muscle) (P = 0.0057). Canada Prime LT produced a greater proportion of perimysium (12.41%) than other quality grades with no difference in the Ehrlich chromogen (EC) concentration (mol/mol collagen) across grade. The results indicated that increased tenderness associated with increased intramuscular fat is not related to altered muscle EC concentration, suggesting that other factors such as myofibrillar proteolysis or changes in other mature collagen crosslinks may be operative.

Key Words– Intramuscular fat, Collagen content, Intramuscular connective tissue, Tenderness

I. INTRODUCTION

The Canadian beef grading system differentiates carcasses according to the amount of intramuscular fat present within the *longissimus thoracis* (LT, rib eye) muscle. The quality grades are Canada Prime (slightly abundant marbling), Canada AAA (small marbling), AA (slight marbling) and A (trace marbling) and are equivalent to United States Department of Agriculture (USDA) Prime, Choice, Select and Standard, respectively. Increased levels of marbling are associated with increased meat tenderness [1]. Beef tenderness is affected by many factors and is recognized as the main quality characteristic of beef affecting consumer satisfaction [2]. Studies have indicated that both intramuscular connective tissue (IMCT) and myofibrils contribute to cooked meat toughness [3]. Total collagen content shows a positive correlation with meat toughness, with 90% of intramuscular collagen found in the perimysium [4]. Studies have shown that not only the amount of collagen, but the combination of collagen cross-linking and perimysial collagen fiber diameter is responsible for beef toughness across different muscles [5].

The morphology, composition and amount of IMCT differ between various muscles, species, breed and animal age [6]. With the increase in physiological maturity of animals, the structure of collagen changes and its solubility decreases [7] as a result of the conversion of thermally labile intermediate (divalent) crosslinks into thermally stable mature (trivalent) crosslinks (EC and pyridinoline) [2, 8]. Increased total collagen content and mature collagen cross-linking appear to promote cooked beef toughness, while increased collagen solubility has been associated with increased marbling score possibly through modification of the collagen crosslinks during intramuscular fat deposition to those that are easily destroyed during cooking [8, 9]. The objective of this study was to compare the collagen heat solubility and EC concentration from bovine LT with different levels of marbling and test the hypothesis that increased intramuscular fat is associated with increased collagen content, decreased EC concentration, increased collagen

heat solubility and decreased cooked beef toughness.

II. MATERIALS AND METHODS

Twelve *longissimus thoracis* (LT, rib eye) muscles from each of the Canada quality grades (Prime, AAA, AA and A equivalent to USDA Prime, Choice, Select and Standard, respectively) were obtained from an abattoir within 72 hours postmortem. After 14 days aging, each muscle had three steaks removed from posterior: the first steak was for pH, color and proximate analysis, the second was for cooking loss and Warner-Bratzler shear force, and the third was for drip loss. The remainder of the muscle was frozen at -20 °C until analysis. Just prior to analysis, the remaining muscle was thawed and cut into 1 cm^2 cubes, mixed to homogeneity by hand, and then divided into two fractions. Fraction A was assessed as whole muscle, while Fraction B was used for isolation of connective tissue with a salt buffer extraction method. Fraction A was lyophilized, ground to a powder with dry ice pellets and then analyzed for proximate composition, total collagen content [10] and collagen heat solubility [7]. IMCT was isolated from Fraction B using de-ionized water and salt buffers [12, 13]. The isolated wet IMCT was then weighed and stored frozen at -80 °C until lyophilization. After lyophilization, the IMCT was defatted using chloroform/methanol (2:1 v/v), weighed and then stored at -20 °C protected from light. Total collagen content [10] and EC concentration in the lyophilized IMCT was estimated by tryptic digestion according to the method of Horgan *et al.* [14] and results were presented as EC nmol/g raw muscle and EC mol/mol collagen.

For total collagen analysis, 100 mg of lyophilized sample were placed in a glass tube and hydrolyzed with 6 M HCl at 110 °C for 20 hours. The hydrolysate was filtered through Whatman No. 4 filter paper, evaporated to dryness, reconstituted with 5.0 mL de-ionized water and then neutralized with 0.1 M NaOH. After neutralization, the hydrolysate was again evaporated to dryness, reconstituted to a final 5 mL volume with de-ionized water and stored at -20°C until total collagen content determination. The total collagen content was estimated by the determination of the hydroxyproline (HYP) concentration [10]. Collagen content was estimated by multiplying HYP concentration of the IMCT by 7.14 [11]. All measurements were performed in duplicate. Collagen heat solubility was performed on whole muscle (Fraction A) samples using the method of Hill [7].

Data were analyzed using R software (Package "CRAN", version 3.2.1, Foundation for statistical computing, Vienna, Austria). The effects of grade on beef quality and collagen characteristics were analyzed by one way analysis of variance using lm() function and statistical model; $yij=\mu+\tau i+\varepsilon ij$, where *i* indexes the treatment levels (grade) and *j* the observation within the *i*th grade, μ is the overall population mean of the response variable, τi is the effect of the *i*th grade on μ, and εij is the error component that are independent and identically normally distributed, with mean zero and homogeneous variance, within each grade ; $\varepsilon_i \sim N(0,\sigma^2)$. Mean differences between sources of variation and all possible pair-wise comparisons were estimated by Honest Significant Difference (Tukey's) multiple range test with a default alpha 0.05 using lsmeans() function, while Pearson correlations estimating linear relationships between the measurements were performed with cor.test() function with significant difference at $P < 0.05$.

III. RESULTS AND DISCUSSION

Moisture content of LT from Canada Prime carcasses was significantly lower than that of LT from carcasses of other grades and moisture content declined with intramuscular fat content (Table 1). This result agrees with previous research showing that marbling score is negatively correlated with moisture content [1, 15]. Mean WBSF value was lower for LT from Canada Prime and AAA carcasses than that from Canada A and AA carcasses (Table 1), supporting a positive correlation ($r = 0.45$, $P = 0.001$) between intramuscular fat and meat tenderness [1, 9, 15]. Grade had no effect on pH, cook time and drip loss ($P > 0.05$). However, Canada Prime LT had a lower mean cooking loss than LT from other quality grades (data not shown), most likely due to its increased

intramuscular fat content, as there were strong correlations between intramuscular fat and moisture (r = -0.54), intramuscular fat and protein (r = -0.53), and cooking loss and moisture (r = 0.40) and cooking time (r = 0.36).

Table 1. Effect of quality grade on *longissimus thoracis* proximate, Warner-Bratzler shear force, collagen and EC concentration measurements

Measurements	Grades A (n=12)	AA (n=12)	AAA (n=12)	Prime (n=12)	SEM[1]
Whole muscle (Fraction A)					
Crude fat (%)	2.92[a]	3.11[a]	4.62[b]	11.57[c]	0.49
Moisture (%)	72.14[a]	71.99[a]	70.1[a]	65.70[b]	0.87
WBSF[2] (kg)	44.10[a]	52.65[b]	35.72[c]	39.6[a,c]	2.88
Total collagen (mg/g raw muscle)	1.92[a]	2.05[a]	1.99[a]	2.49[b]	0.09
Solubility (%)	24.75	22.73	20.88	20.16	2.27
Intramuscular connective tissue (Fraction C)					
Perimysium (% of raw muscle)	8.18[a]	8.94[a]	9.03[a]	12.41[b]	0.86
Moisture (%)	89.06	88.11	87.79	90.54	1.08
Total collagen (mg/g raw muscle)	2.09[a]	2.51[b]	2.39[b]	2.54[b]	0.11
Ehrlich chromagen (nmol/g raw muscle)	2.06	2.25	2.16	2.35	0.16
Ehrlich chromagen (mol/mol collagen)	0.29	0.31	0.30	0.28	0.02

[1]SEM: Standard error of means
[2]WBSF: Warner- Bratzler shear force
[a,b,c] Within a row, means without a common superscript letter differ (P < 0.05)

Muscle with the highest proportion of intramuscular fat appeared to have the greatest amount of IMCT. The amount of wet IMCT recovered as a percentage of wet tissue was significantly higher (P < 0.05) for LT from Canada Prime carcasses than that from carcasses of other grades, opposing results of Li *et al*. [16]. Total collagen content in whole muscle was highest in LT from Canada Prime carcasses, and so was associated with increased marbling score; however, calculation of collagen content from IMCT collagen concentration did not agree with this result although total perimysium IMCT isolated did (Table 1) [16]. These results agreed with those of Kim and Lee [1] who found no difference in the amount of collagen across grades.

A negative correlation between collagen heat solubility and total collagen content (r = -0.47) was observed. This was not accompanied by significant differences in mean collagen heat solubility across the grades, although there was a numerical decline in mean collagen heat solubility as grade intramuscular fat content increased, disagreeing with previous findings [6, 16] that showed collagen solubility increased with marbling score. The results did agree with others who found that collagen heat solubility decreased with increased marbling score [8].

Increased collagen content in highly marbled, tender LT suggested that the development of intramuscular fat may contribute to beef tenderization by changing the structure of IMCT, possibly by decreasing heat-stable collagen cross-links [8]. Analysis of variance showed no significant difference in EC concentration across quality grades. As well, there were no linear relationships observed between shear force, fat, moisture, collagen and IMCT contents and EC concentration or collagen solubility nor was there a linear relationship between the collagen heat solubility and EC concentration. Overall results confirmed that Canada Prime and AAA LT with inherent increased intramuscular fat content are more tender than Canada AA and A LT. Increased collagen content and decreased collagen heat solubility were not accompanied by changes in Ehrlich chromogen concentrations in muscle and collagen, indicating other factors were important in tenderization of high intramuscular fat beef

IV. CONCLUSION

Beef tenderness is influenced by many factors and our results implied that increased tenderness associated with increased intramuscular fat is not related to altered muscle EC concentration, suggesting that other factors such as myofibrillar proteolysis or changes to other collagen crosslinks may be responsible. These results will assist Canadian beef branding and marketing based upon muscle composition and their effects on eating quality particularly in the light of USDA guaranteed beef tenderness program. Determination of types of collagen involved and pyridinoline cross-link concentration in IMCT will further assist in understanding the role of

mature cross-links formation and their effect on cooked beef toughness.

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