Thermal Stability of Connective Tissue from Beef from the Four Canadian Quality Grades

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Introduction

- The quality grading of Canadian beef is based on marbling score and is categorized in four quality grades, namely Prime, AAA, AA, and A from highest to lowest. Marbling is the distribution of visible intramuscular fat throughout a cut of beef and the higher the marbling score the higher the quality grade the cut of beef will be awarded.
- Tenderness is the most important quality trait to consumers and is affected by the main structural components of meat myofibers, connective tissue (collagen), and intramuscular fat. Increased intramuscular fat increases cooked beef tenderness in addition to post-mortem aging during which the meat myofibers breakdown and further tenderize the meat. Connective tissue, however, affects the background toughness of meat and collagen content or thermally stable collagen cross links are thought to be responsible for the toughness.
- Therefore, the aim of this study was to compare the meat quality traits and intramuscular connective tissue characteristics (collagen content, collagen cross-links and thermal stability) of rib steaks (*m. longissimus thoracis*) from the four Canadian quality grades.

Methodology

Nutritional Composition

- The nutritional composition of each quality grade of beef was determined through the analysis of the ash, crude protein, moisture, and fat content in freeze dried beef (n=12) from each grade by following the method of Association of Official Analytical Chemists (AOAC, 2000).
- Fat content was determined by placing 1 g freeze dried meat samples into a Soxhlet extractor. About 70 mL of petroleum ether was passed through in hot extraction mode at 135 °C for 1 h and 45 min. The extracted meat was then dried in an oven until a constant weight. Fat percentage was calculated by the following formula: Fat % = [(fat weight (g)/ freeze dried meat weight (g)) x 100 % x dry matter in freeze dried meat].

IMCT Isolation

- Intramuscular connective tissue (IMCT) was isolated by chopping muscle, blending it with water, and then passing the mixture through a 1mm^2 sieve, which retained the IMCT.

Shear Force

- To determine shear force 2.5 cm thick steaks were cut, their epimysium removed, and cooked in a 73 °C water bath until their internal temperature was 71 °C. Steaks were then cooled at 4 °C overnight and the next day 1.27 cm diameter cores were punched out parallel to the muscle fibers direction. The maximum force required to cut them perpendicular to the muscle fibers direction was recorded using a materials test machine (Lloyd Instruments).

Total Collagen Content

Freeze dried meat (0.1 g) hydrolyzed with 6M HCl at 110 °C for 20 h. → Filtered, evaporated, neutralized, and reconstituted with distilled water. → 1 mL hydrolysate solution is put in a test tube for hydroxyproline determination. → 2 mL isopropanol and 1 mL oxidant solution are added to the sample solution and it is left for 4 min.

13 mL Ehrlich reagent is added and the solution is mixed by vortexing and is heated at 60 °C for 25 min. → It is cooled down in ice water and diluted to 50 mL with isopropanol. → The absorbance is measured against a blank at 558 nm by a spectrophotometer. → Hydroxyproline content is calculated and converted to total collagen content (conversion factor of 7.14).

Methodology Cont'd

Soluble Collagen Content

12 mL of ¼ -strength Ringer's solution is added to 2 g of freeze dried meat. → It is heated for 63 min at 77 °C before being centrifuged for 30 min at 3500 g. → The supernatant solution is decanted and the pellet is suspended in more Ringer's solution before being recentrifuged.

The supernatant solutions are combined and hydrolyzed by HCl for 20 h at 110 °C. → The hydroxyproline content was determined using the method used to determine total collagen content. → The hydroxyproline content was converted to collagen content with a conversion factor of 7.14.

Pyridinoline Quantitation

- To determine the amount of mature cross links within the different grades we first had to hydrolyze the isolated IMCT. Then we dried and reconstituted it before using size exclusion column chromatography to separate the pyridinoline cross links. Cation exchange column chromatography was then used to purify the cross-links further and high performance liquid chromatography was used to determine the amount of pyridinoline cross links present.

Ehrlich Chromogen (EC) Quantitation

- To quantify the EC cross-links we first suspended the IMCT in a Tris/HCl buffer solution for 24 h at 4 °C. Then it is heated at 65 °C for 1 h and is equilibrated at 37 °C. Trypsin is then added and it is kept at 37 °C for 4 h. Then it is heated at 65 °C for 20 min and is then cooled to 25 °C. It was then centrifuged for 30 min at 28000 g and p-dimethylaminobenzaldehyde and perchloric acid were added to the supernatant before being centrifuged for 2 min at 14000 g.

Differential Scanning Calorimetry (DSC)

- Isolated IMCT was thermally analyzed in triplicate using a Multi-Cell Differential Scanning Calorimeter. The freeze dried IMCT was weighed, soaked in deionized water overnight at 4 °C, and then blotted and put into stainless steel ampoules. They were heated from 20 °C to 100 °C at a rate of 1°C/min and after the scanning was completed the raw data was analyzed for the temperature at denaturation and the amount of energy required for denaturation.

Statistical Analysis

- Data were analyzed using Statistical Analysis Software (SAS institute Cary, North Carolina). The effects of grades on beef quality and collagen characteristics were analyzed by one way analysis of variance with grade as the sole source of variation with significance at $P < 0.05$.

Results

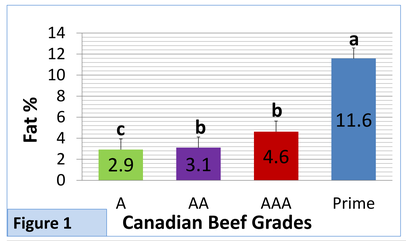

Figure 1

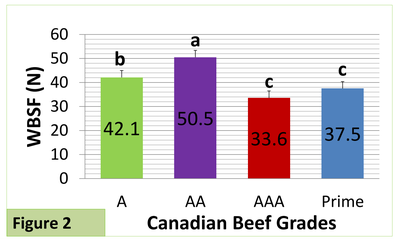

Figure 2

The different letters above the bars indicate a significant difference among the beef grades.

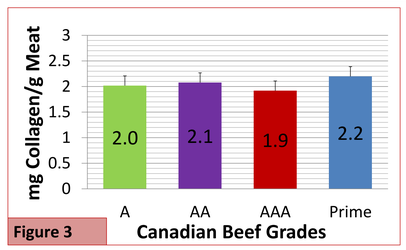

Figure 3

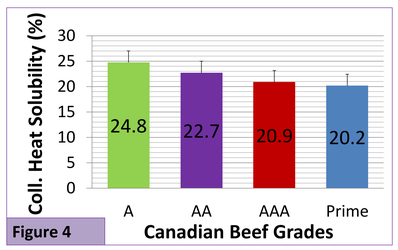

Figure 4

Results Cont'd

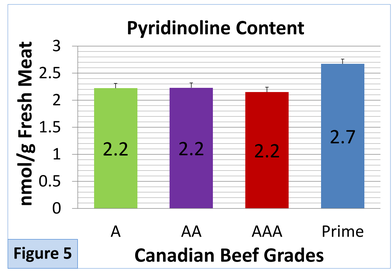

Figure 5

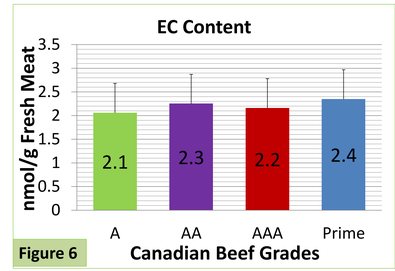

Figure 6

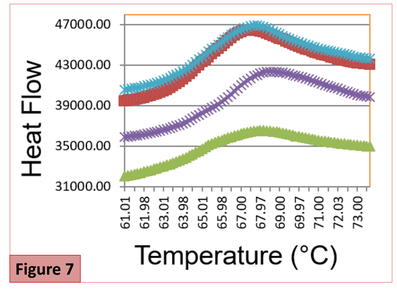

Figure 7

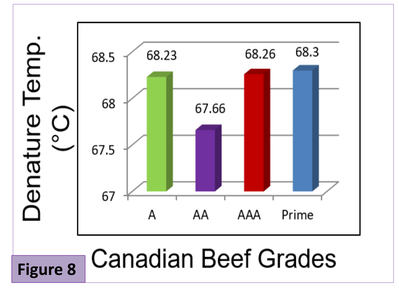

Figure 8

Conclusions

- Fat content (a meat quality trait) increases as beef quality grades increase and generally when fat content increases shear force decreases. However, when comparing Figure 1 and Figure 2 it is clear that increased fat content in lower grades does not have a lowering effect on shear force, where as in the two highest grades increased fat content does lower shear force. Thus fat content has an effect on tenderness in high fat content beef, but not in low fat content beef.
- Collagen characteristics, such as total collagen content, soluble collagen and collagen cross-links, have little to no effect on tenderness in the muscle used (*m. longissimus thoracis*), possibly because it is known to have a total collagen percentage that is significantly lower than other beef muscles.

Acknowledgements

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Literature Citations

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