

EFFECT OF COLLAGEN TYPE ON BEEF MEAT TOUGHNESS

M.N.Uddin, B.V.Reddy, J.S.Lee and I.H.Hwang

Department of Animal Science, Chonbuk National University, 567, Jeonju city,
Republic of Korea

Abstract – The aim of the present study was to investigate the effects of extracted collagen (type I and type III) and its ratio from five different muscles (TL, LO, ST, KN and FR) of Korean native cattle Hanwoo, which is closely related to meat tenderness. The total collagen content, and types of collagen were significantly different among muscles. From our current results we suggested that the ratio of collagen type I and Collagen type III and the amount of solubility of collagen are greatly related to meat tenderness.

Key Words – Hanwoo beef, collagen, tenderness, collagen type

I. INTRODUCTION

Meat tenderness is one of the most important quality attributes in the consumption for human. It is greatly influenced by several factors such as growth rate of live animals, age, thermal condition (post slaughter storage), pH and connective tissue content [1]. Connective tissue protein constitutes from 2 to 6 % of the total protein of meat [2]. Collagen, the major connective tissue protein, is an integral constituent of muscle, and mainly contributing in meat tenderness. The collagen content in muscle leads to tough. The high ratio of type III collagen may result in a decrease in meat tenderness but increase in toughness. Type III collagen is smaller in diameter than Type I collagen fibrils, suggesting they should possess less resistance to shear force [3]. Collagen is found in several locations within the muscle. Collagen is present in 19 different forms, each having a different role in biological systems [3]. Connective tissue is a very important structural component relative to muscle function. The degree to which connective tissues affect tenderness is determined by the type and amount of collagen. Type I collagen was largely associated with meat tenderness and it is found in skin, muscle, tendons, organs, and bones. Type III collagen, often found alongside type I,

is the main component of reticular fibers. Type I and type III, collagens given emphasis for meat tenderness. The amount of collagen present in muscle tissue is important in understanding the effect on meat quality parameters; however, the type of collagen is a more direct measure of the tenderness and acceptability. The aim of the present research was to determine the extraction process of collagen isolation and its amount of collagen (total collagen and heat soluble)/ratio of type I & type III collagen from five different muscles of same carcass related to toughness of Hanwoo beef.

II. MATERIALS AND METHODS

Muscle sampling The muscle samples was obtained from Hanwoo steer carcass to extract collagen, determine total collagen and heat collagen content. Five muscles were used in this experiment. The muscles were *Semitendinosus* (ST, eye of round), *Psoas major* (PM, TL, tenderloin), *Gluteus medius* (GM, Top-sirloin butt, KN), *Longissimus thoracis* (LT, LO, Loin) and *short plate* (FR, Diaphragm).

Measurement of total collagen and heat soluble collagen contents Total collagen content was measured by Kurt Kolar, (1990) [4] colorimetric determination method. The heat soluble collagen content was determined by the method of Hill, (1966) [5] with slight modifications. Total collagen and heat soluble collagen contents were expressed in gm/100gm of muscle sample.

Extraction of collagen from muscles of Hanwoo beef In the following extraction process of collagen from muscles the operational temperature was regulated at 4°C in chilling room. Thirty gram (30gm) of fresh deboned, fatless meat was cut into pieces of 2 mm to 5 mm in size, three times washed the chopped pieces of meat sample with sodium chloride at a ratio of :6 (w/v) in 1000 ml glass beaker every

times 10 minutes then three times washed with cold distilled water after that the meat sample was treated with sodium hydroxide at a ratio of 1:10 (w/v) for continuous magnetic stirrer for three days and every day NaOH solution was changed and finally washed with cold distilled water. Following washing with cold distilled water the meat sample were homogenized two times with tris maleate and KCl. For gradual dissolution added 0.5 M acetic acid with 5 mM EDTA at a ratio of 1:15 to homogenized meat sample and stirred by magnetic stirrer for 48 h, then pepsin was added and the homogenate was centrifuged. The supernatant was then salt out with 4 M sodium chloride and centrifuged at 13000g for 15 min to collect precipitate. The precipitate collected from salt out contain pure collagen that was dissolved in minimum volume of 1 M acetic acid. Finally the precipitate solutions were dialyzed against 0.02M Na₂HPO₄ for 48 h with daily changes of solution.

SDS-PAGE After extraction of collagen from different muscles, the protein concentration was determined using Bio-Rad protein assay kit (Bio-Rad). SDS-PAGE was performed by the method of Laemmli, (1970) [6] with slight modification, using 6% separating gel and 4% stacking gel. According to protein concentration the extracted collagen was dissolved in 1 ml of sample buffer (Tris-HCl, pH 6.8 containing 2-mercaptoethanol, bromophenol blue, 10% SDS) and heated at 50°C for 10 minutes. After that 25 µl of sample protein were loaded in each well of the gel. High molecular weight protein markers (5µl, Precision Plus protein TM dual

color Standards, Bio-Rad laboratories, USA) were used to estimate the molecular weight of proteins. Rat tail tendon type I collagen and collagen type III from human placenta (Sigma-Aldrich Co. LLC. USA) were used as standard collagen. Electrophoresis was done at constant 100 V for 2 h 10 min. After electrophoresis the gel were stained with Commassie Brilliant Blue R-250 in 5% (v/v) methanol and 7.5% (v/v) acetic acid for overnight then de-stained using 7.5% (v/v) of acetic acid and 25% (v/v) methanol for about 5 h with a change of solution every hour to clearly visualize the desire protein band in gel.

The image of gel was taken using Versadoc Imaging system model 3000 Bio-Rad with quantity software. The presence of collagen type I and Collagen type III in gel were determined by SDS-PAGE according to standard collagen type I & III. The quantification of collagen protein bands from SDS-PAGE gel using Image J software.

Data were analyzed using SAS (Version 9.1 ED.), SAS institute, Cary, NC, USA. The level of significance was preset at p<0.05.

III. RESULTS AND DISCUSSION

The amount of total collagen, heat soluble collagen, collagen type I and collagen type III and its ratio were shown in Table 1. Total collagen content was highest in FR muscle and lowest in LO whereas heat soluble collagen highest in ST muscle. The type I and type III collagen were identified by SDS-PAGE and quantified using Image J software.

Table 1: Estimation of Total collagen, Heat soluble collagen, collagen type I collagen type III and ratio of collagen type I and collagen type III.

Muscle name	Total collagen (gm/100gm)	Heat soluble collagen (gm/100gm)	Collagen type I	Collagen type III	Ratio (Type I/Type III)
TL	0.4180 ^{dc}	0.3140 ^a	5122.6 ^{ba}	2647.7 ^{dc}	1.9374 ^a
LO	0.3574 ^{dc}	0.1696 ^b	5144.5 ^{ba}	2522.0 ^d	2.0392 ^a
ST	0.7577 ^{ba}	0.5850 ^a	5417.5 ^a	3605.9 ^a	1.5024 ^b
KN	0.4578 ^{bdc}	0.2510 ^{ba}	4564.0 ^{bc}	3192.6 ^{ba}	1.4299 ^b
FR	0.8323 ^a	0.5550 ^a	4929.4 ^{bac}	3622.0 ^a	1.3611 ^b

^{a-d} means with in a column with different superscript differ significantly. (p<0.05)

TL-Tenderloin, LO- Loin, ST-Eye Round, KN-Top sirloin butt, FR-Short plate muscle

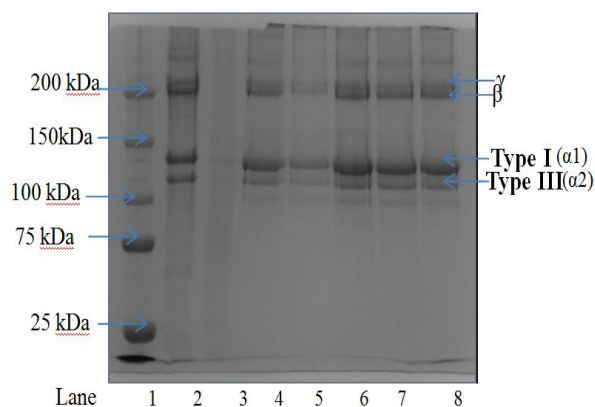
Figure 1 showed the SDS-PAGE patterns of collagen extracted from different muscle. The β dimmers and γ trimmers are inter and intra muscular components of collagen. The $\alpha 1$ and $\alpha 2$ indicates the Type I and Type III collagen compared with standard collagen.

We found that ST muscle contain highest amount of type I collagen whereas collagen type III were highest in FR muscle (Fig.1). Type I collagen is mostly associated with meat tenderness it is true fact but when muscle contain higher amount of type I collagen then the meat become tough.

Type III collagen is especially characteristics of embryonal and young tissues with the advances in animal age collagen type III started to replace by collagen type I [7]. Higher collagen solubility may be associated with increased tenderness in muscles.

Current result indicated that ST muscle contains more heat soluble collagen than the other muscles. So ST muscle is more tender followed by FR, TL, KN and LO.

Figure1. SDS-PAGE patterns on 6% gel of collagens from the muscle of Hanwoo beef



Lane 1 Molecular weight marker, Lane 2: Pure Coll type I, Lane-3: Pure coll Type 3, Lane 4: ST, Lane 5: KN, Lane 6: FR, Lane 7: TL, Lane 8: LO.

IV. CONCLUSION

Our current results suggested that the meat tenderness or toughness can be evaluated by collagen solubility and types (I & III) and its ratio.

ACKNOWLEDGEMENTS

We are pleased to inform that the current study was supported by Research Program for Agriculture Science & Technology Development (Project No PJ010170) and grant from the Next-Generation BioGreen 21 Program (Project No PJ011101), Rural Development Administration, Republic of Korea.

REFERENCES

1. Van, E.C., Clinquadt, A., Uyterhaegenn, L., Hornick, J. L., Demeyer, D., Istasse, L., Bakdwin, P. & Clayes, E. (1994). Post-mortem proteases activity in relation to muscle protein turnover in Belgian blue bulls with different growth rates. *Sciences des Aliments* 14: 475–483.
2. Pospiech, E., Grzes, B., Lyczynski, A., Borzuta, K., Szalata, M., Mikolajczak, B. & Iwanska, E. (2003). Muscle proteins, their transformations and meat tenderness. *Mieso I Wedliny* 1: 26-33
3. Bailey, A. J. (1998). Connective tissue and meat quality. In *Proceedings 34th International Congress of Meat Science and Technology* (pp. 152-160), 29 August- 2 September 1988, Brisbane, Australia.
4. Kurt, K. (1990). Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. *Association Official Analytical Chemistry* 73 (1): 54-57.
5. Hill, F. (1960). The solubility of intramuscular collagen in meat animals of various ages. *Food Science* 31(2): 161-166.
6. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227 (5259): 680-685.
7. Light, N., Champion, A.E., Voyle, C & Bailey, A. J. (1985). The role of epimusial, perimusial and endomysial collagen in determining texture in 6 bovine muscles.