PROTEIN EVOLUTION ON MEAT JUICE EXTRACTED FROM HEIFER MEAT OF HANWOO AS AFFECTED BY HEATING TEMPERATURES AND AGING DAYS

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Abstract –This study aimed to investigate the effect of heating temperatures and aging days on the protein degradation in meat juice extracted from heifer meat of Hanwoo. Each muscle sample was divided into four equal potions according to condition; (i) 3 day aging, heating at 60℃; (ii) 14 day aging, heating at 77℃; (iii) 3 day aging, heating at 77℃; (iv) 14 day aging, heating at 60℃. The resulting meat juice was used to analyze the protein evolution by SDS-PAGE and Western blot. SDS-PAGE revealed that higher molecular weight bands were found much more at rare temperature (60℃). It was also observed that the different degradation patterns of protein (less than 28 kDa products) by aging were only showed at well done temperature (77℃). Troponin-T known as indicator protein of tenderness was identified by immunological methods (western blot). Troponin-T fragments were much more generated by aging at well done temperature (77℃). These results indicate that protein evolutions in meat juice varied with cooking temperatures and aging days.

Key Words – Hanwoo, Heifer meat, Troponin-T

I. INTRODUCTION

Low molecular weight compounds such as free amino acids, peptides, nucleotides, sugars, and so on were important components to indicate taste of meat [1]. These components function as flavor compounds pool and flavor intermediates [2]. Of these, free amino acids and peptides produced by the proteolysis improved on the taste of meat [1]. Also, Davis and Anderson studied about chemical changes of proteins by cooking condition [3], also mechanism involved in the improvement of meat taste during postmortem aging have been studied by Nishimura [1]. In addition, a peptide produced by troponin-T known as indicator protein of tenderness contributed to improve beef taste [4]. Added to this there are many studies using SDS-PAGE and western methods for analyzing the polypeptide with 30 kDa which is derived from troponin T formed during aging [5, 6].

Therefore, the objective of this study was to investigate the protein degradation patterns indicating production possibility of low molecular weight compounds by interaction between heating temperatures and aging days.

II. MATERIALS AND METHODS

1) Treatment of animals and sample preparation

Hanwoo heifer (*M. longissimus dorsi* muscle, n=10) were used for the present investigation. Each muscle sample was divided into four equal potions per each treatment condition; (i) 3 day aging, heating at 60℃; (ii) 14 day aging, heating at 77℃; (iii) 3 day aging, heating at 77℃; (iv) 14 day aging, heating at 60℃. The samples were then vacuum packaged and age for the designed periods at 4℃.

2) Meat juice extraction

After aging, the samples were heated until core temperature of each sample reach 60℃ or 77℃ in water bath respectively. After heating, each sample was cooling in flowing water for 30 minutes and then chopped 5mm or less in size.

Meat samples chopped were centrifuged at 14,000 xg and 4℃ for 20 min, and the resulting supernatants were filtered through whatman paper (No.41) and were used to analyze the protein degradations.

3) SDS-PAGE and Western blot

After collecting the meat juice, its pH was adjusted to 2 mg/ml using SDS-PAGE sample buffer (pH 6.8). The samples were heated at 95 ℃ for 5 min and then were used for SDS-PAGE analysis. Each sample (5 ug) was loaded on 4% Stacking Gel, 12.5% Separating Gel with protein broad range marker (BIO-RAD). 20 cm gel was run at 150 V until dye reached to bottom. The gel was stained with R-250 (BIO-RAD).

After SDS-PAGE complete, the gel was used for western blotting. The proteins were transferred to 0.2 ㎛ PVDF membrane, Primer antibodies were diluted to 1:2,500 (Troponin T, JLT-12, SIGMA). Secondary antibody was also diluted to 1:2,500 ratios with TTBS. The bound antibodies were visualized by incubating membranes with HRP conjugate Substrate according to the manufacturer's guide. Images were taken by Versa Doc (3000, BIO-RAD, USA).

III. RESULTS AND DISCUSSION

Figure 1 show that protein degradation patterns of the meat juices extracted from Hanwoo heifer beef as affected by different cooking temperatures and aging periods. In general, bands at low temperature were intense and bands at high temperature were very weak. This was assumed that low molecular weight peptides back out of the acrylamide consist of gel were many at high cooking temperature. Also, at 77℃ the different degradation patterns by aging, the less than 28 kDa products were observed.

Western blot results are showed in Figure 2, at 77℃, more troponin-T fragments at different aging periods were found. These results corresponded with Cheng's results in increase of 30 kDa components and low molecular weight components by troponin-T degradation with an increase in heating temperature [6].

Consequently, troponin-T fragments by aging were much more generated at well done temperature than rare temperature, this result showed possibility to difference taste by interacting between heating temperatures and aging days.

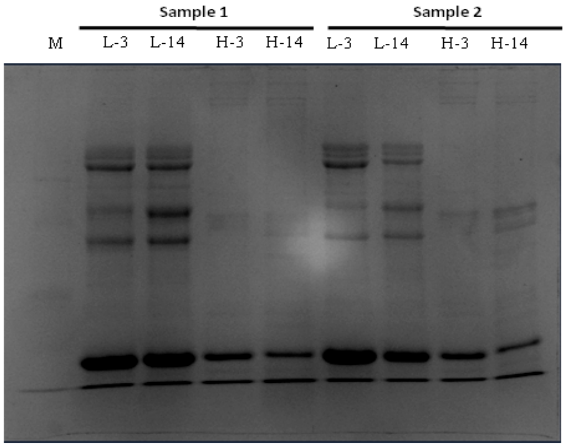

Figure 1.SDS-PAGE patterns on meat juices as affected by cooking temperatures and aging days (M; Standard markers, L; Low temperature-60℃, H; High temperature-77℃).

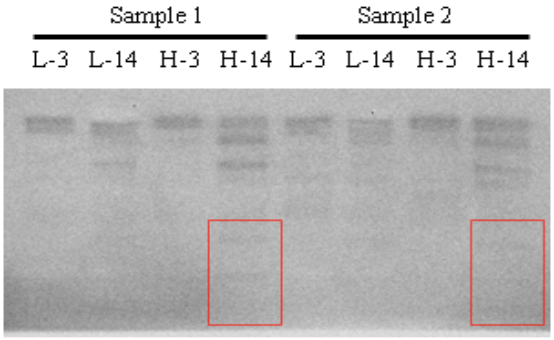

Figure 2.Troponin-T degradation patterns on meat juices as affected by cooking temperatures and aging days using Western blot methods (L; Low temperature-60℃, H; High temperature-77℃).

IV. CONCLUSION

The results obtained from SDS-PAGE and Western blot analysis using meat juices extracted from heifer meat of Hanwoo treated with two different heating temperatures and aging days. Notable differences were found in the formation of troponin-T fragments from meat juices aged 14 days at 77℃. These results imply that protein contents of meat juices to feel the taste moving inside the mouth by mastication differ in condition (e.g., heating temperature and aging).

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REFERENCES

1. Nishimura, T. (1998). Mechanism involved in the improvement of meat taste during postmortem aging. Food science and technology international 4: 241-249
2. Spanier, A. M., McMillin, K. W. & Miller, J. A. (1990). Enzyme Activity Levels in Beef: Effect of Postmortem Aging and End-point Cooking Temperature. Journal of food science 55(2): 318-322
3. Davis, C.E. & Anderson, J. B. (1984). Size exclusion/HPLC of heated water soluble bovine and porcine muscle proteins. Journal of food science 49: 598
4. Nishimura, T. (2001). Palatability on texture, aroma, and taste of meat. Japanese journal of taste and smell research, 8, 161-168.
5. Penny, I.F. & Ferguson-Pryce, R. (1979). Measurement of autolysis in beef muscle homogenate. Meat science 3: 121-141
6. Negishi, H., Yamamoto, E. & Kuwata, T. (1996). The origin of the 30 kDa components appearing during post-mortem aging of bovine muscle. Meat science 42: 289-303
7. Cheng, C. S. & Parrish, F. C. (1979). Heat-induced changes in myofibrillar proteins of bovine, longissimus muscle. Journal of food science 44: 22