MUSCLE PROTEIN DEGRADATION AND TENDERIZATION OF THE BOVINE LONGISSIMUS MUSCLE USING VARIOUS MUSHROOM EXTRACTS

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Abstract - The aim of this study was to investigate the effects of mushroom extract on tenderization of the bovine longissimus muscle. Muscle protein degradation were also examined. Beef cuts were marinated with distilled water (control), 5% Sarcodon aspratus extract (SA), 5% Agaricus bisporus (AB) or 5% Letinus edodes (LE). SA and AB showed lower shear force values than control (p<0.001). Particularly, SA has shown the degradation capability of mvofibrillar and sarcoplasmic proteins that was not appeared in the AB and LE. This degradation of muscle protein suggests that SA extract could influence tenderization. These results show that aqueous extract of Sarcodon aspratus extract actively affect the tenderness of the bovine longissimus muscle.

Key Words – mushroom, tenderization, myofibrillar and sarcoplasmic protein degradation

I. INTRODUCTION

Mushroom usually means one that does not have toxicity and is edible. It has been known that there are about 20,000 types, and it contains various bioactive materials as well as a plenty of nutrients such as various types of carbohydrates, protein, minerals, vitamins, and nucleic acid [1, 2]. In addition to the nutritional value of mushroom. there are various studies currently being performed about various enzymes contained in edible mushroom and it has been known that Sarcodon aspratus, Agaricus bisporus, and Letinus edodes contain proteolytic enzymes [3, 4, 5]. Particularly, Sarcodon aspratus has been used as a single treatment for indigestion after eating meats in traditional medicine for a long time, and Lee [6]'s study has scientifically shown that Sarcodon aspratus contains a plenty of proteolytic enzymes. According to the study of shin et al. [7], it has been reported that addition of *Sarcodon asparatus* increases water holding capacity and myosin heavy chain degradation, and thus increases the tenderness of meats. Therefore, this study analyzed the effect of increasing tenderness of meat by using edible mushrooms containing proteolytic enzymes.

II. MATERIALS AND METHODS

Sample preparation

Mushroom: Sarcodon aspratus, Agaricus bisporus, and Letinus endodes were sliced as 0.3mm, freeze dried (Model FD-5518, Ilshin Lab Co., Korea), and dehydrated. Dry samples were ground (Food mixer, Hanil Co., Korea), sieved through 30 mesh, made into powder, and stored in a freezer at -21° C before used as a sample.

Measurement of shear force

The sample for shear force measurement was sliced in thickness of 10mm and diameter of 50mm in parallel to muscle fibers, and the force was measured when the sample was perpendicular to the muscle fibers after Warner-Bratzler shear was installed using Instron (Series IX, Instron Corp., USA). For analytical conditions, chart speed was 120 mm/min, and maximum load was 10 kg. Measured speed is 20 mm, and the sample height is 20 mm, adapter No.4

Measurement of hardness

In order to investigate the texture of meats mixed with edible mushroom, the sample immersed for 48 hours (beef, loin muscle) was put in a vacuum polypropylene bag, and heated in 85°C water bath until the core temperature was 75°C. Then, the sample was taken from water bath, cooled down at room temperature, and measured using texture analyzer (TA-plus, Lloyd Instruments Ltd. England). The measured data were analyzed using NEXYGEN Plus Material Test and Data Analysis Software (Lloyd Instruments Co Ltd., UK).

Sensory quality evaluation

Sensory quality evaluation was performed in order to investigate the difference of tenderness of beef mixed with edible mushroom. 12 panel were recruited as sensory quality evaluators and trained according to the method of American Meat Science Association (AMSA, 1995) for 2 weeks. Tenderness (1: very tough, 5: neither tough nor tender, 9: very tender) based on a 9-point scale.

Protein degradation analysis

For myofibril protein analysis, SDS-PAGE was performed (SE 260, Hoefer Pharmacia Biotech Inc., USA). The component of gel was 4% stacking gel and 10% separating gel. 10 μ g sample of myofibril protein was distributed per lane, and running buffer was made by the revised method of Laemmli [8], which separated upper running buffer (0.1 M tris, 0.15 M glycine, 0.15% SDS) from lower running buffer (0.025 M tris, 0.2 M glycine, 0.1% SDS). For sarcoplasmic protein analysis, the component of gel was 8% stacking gel and 15% separating gel. 10 μ g sample of sarcoplasmic protein was distributed per lane. The condition of electrophoresis included 4°C and 40 mA current for 2 hours. Gel was stained using 0.05% Coomassie blue R-250(w/v), 40% methanol, and 7% acetic acid at room temperature for 2 hours, and decoloration was repeated twice using 40% methanol and 7% acetic acid. Gel imaging of myofibril protein was performed using Kodak DC290 (Eastman Kodak Company., USA).

III. RESULTS AND DISCUSSION

Tenderness

The results of texture of the beef treated with Sarcodon aspratus (SA), Agaricus bisporus (AB), and Letinus edodes (LE) are shown in Figure 1. For hardness, which has closest association with the effect of increasing tenderness of beef, the SAtreated group showed significantly lower than the control group (p<0.001). SA-treated group also showed lowest shear force value compared to ABtreated group and LE-treated group (p<0.001). AB-treated group had lower value than LE-treated group, but there was no significant difference. The control group showed highest shear force value compared to mushroom-treated groups (p<0.001). SA-treated group and AB-treated group had significantly higher score of sensory tenderness quality than LE-treated group and a control group (p<0.001).

Muscle protein degradation

The changes in myofibrillar and sarcoplasmic proteins were observed after the treatment of meat samples with the mushroom extracts (Figure 2). The changes of myosin heavy chain (MHC), actin and sarcoplasm proteins were observed in SA, AB, and LE treated samples. Mushroom marinated groups effectively degraded MHC among the various muscle proteins. The group marinated with SA has shown superior degradation capability of sarcoplasm proteins, in addition, than the groups marinated with AB and LE.

IV. CONCLUSION

Freeze drying powder of SA, AB, and LE used in this study had the effect of increasing tenderness of beef, and particularly the SA has a greater effect on the degradation of myofibrillar and sarcoplasmic proteins. It is supposed that this would highly increase the tenderness of beef with the treatment of SA compared to the treatment with AB and LE. Therefore, the use of *Sarcodon aspratus* extract represents the effective treatment for the improvement of proteolysis and tenderness of the bovine *longissimus* muscle. Figure 1. Tenderness and hardness of the bovine *longissimus dorsi* muscle with 5% *Sarcodon aspratus* (SA), 5% *Agaricus bisporus* (AB), *Letinus edodes* (LE)



Sensory evaluation score (tenderness) using 9 point intensity scale (1: very weak, 5: neither strong nor weak, 9: very strong); ^{a-c}Means with different superscripts within a row are significantly different (p<0.05).

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- C0: Control was the bovine *longissimus dorsi* muscle before treated
- C10: Control was the bovine *longissimus dorsi* muscle treated only distilled water
- SA: The bovine *longissimus dorsi* muscle treated by Freeze drying *Sarcodon aspratus* powder 5% (w/v)
- AB: The bovine *longissimus dorsi* muscle treated by Freeze drying *Agaricus bisporus* powder 5% (w/v)
- LE: The bovine *longissimus dorsi* muscle treated by Freeze drying *Letinus edodes* powder 5% (w/v)

REFERENCES

- 1. Bai, Y.H. & Rho, J. H. (2000). The properties of proteolytic enzymes in fruits (pear, kiwifruit, fig, pineapple and papaya). Korean Journal for Food Science of Animal Resources 16:363-366.
- Kim, B. K., Shin, G. G., Jeong, B. S. & Cha, J. Y. (2001) Cholesterol lowering effect of mushroom power in hyperlipidemic rats. Journal of the Korean society of food science and nutrition

30:510-515.

- Ezmart, M. E. & Ialaki, M. A. (1997). Edible mushrooms as producers of amylases. Food Chemisty 4:203-211.
- Eun, G. S., Yang, J. H., Cho, D. Y., Lee, T. K. & Park, I. H. (1989). Studies on higher fungi in Korea (Ⅱ) proteolytic enzyme of *Agaricus bisporus* (Lange) Sing. Journal of Korean Pharmaceutical Sciences 19:9-14.
- Eun, J. S., Yang, J. H., Cho, D. Y. & Lee, T. K. (1989). Studies on higher fungi in Korea (I) activity of proteolytic enzyme from *Sarcodon aspratus* (Berk) S. Ito. Journal of Pharmaceutical Investigation 18:125-131.
- Lee, T. K. (1986). Purification and some characteristics of the proteolytic enzyme in fruitbody of Neungee [*Sarcodon aspratus* (Berk.) S. Ito]. Journal of the Korean Society of Food Science and Nutrition 15:276-285.
- Shin, H.G., Choi, Y.M., Kim, H.K., Ryu, Y.C., Lee, S.H., Kim, B. C., (2008) Tenderization and fragmentation of myofibrillar proteins in bovine *longissimus dorsi* muscle using proteolytic extract from *sarcodon asparatus*. Journal of the Korean Society of Food Science and Nutrition 41:193-201
- 8. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 227:680-685.