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ARTICLE

Tenderization of Bovine *Longissimus Dorsi* Muscle using Aqueous Extract from *Sarcodon aspratus*

Ho-Kyoung Kim[1,†], Sang-Hoon Lee[2,†], and Youn-Chul Ryu*

Division of Biotechnology, College of Applied Life Sciences, Jeju National University, Jeju 690-756, Korea
[1]Department of Culinary & Food Service Management, Sejong University, Seoul 143-747, Korea
[2]Department of Nutritional Science and Food Management, College of Health Science, Ewha Womans University, Seoul 120-750, Korea

Abstract

The aim of this study was to investigate the effects of aqueous extract from *Sarcodon aspratus* on tenderization of the bovine *longissimus dorsi* muscles in comparison with commercial proteolytic enzymes. Furthermore, meat quality and muscle protein degradation were examined. We marinated meat with 2% *Sarcodon aspratus* extract, 2% kiwi extract, and 0.2% papain. Beef chunks (3×3×3 cm^3) were marinated with distilled water (control), *Sarcodon aspratus* extract (T1), kiwi extract (T2) or papain (T3) for 48 h at 4°C. There were no significant differences in muscle pH and lightness between control and treated samples. T1 had the lowest redness ($p<0.01$), and higher cooking loss and water holding capacity than control and T2 ($p<0.05$). T1 and T3 exhibited lower shear force values than control ($p<0.05$). Total protein solubility did not differ significantly between T1 and control, but T1 had less myofibrillar protein solubility than control and T2 ($p<0.001$). The degradation of myosin heavy chain in T1 and T3 was observed. This degradation of myofibrillar protein suggests that *Sarcodon aspratus* extract could influence tenderization. These results show that aqueous extract of *Sarcodon aspratus* extract actively affect the tenderness of the bovine *longissimus dorsi* muscle.

Keywords: *Sarcodon aspratus*, proteolytic enzymes, beef, tenderization

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Introduction

As of the recent improvement in living standard of contemporary man the consumer preference has been changed to health-conscious menu, considered both taste and quality, from existing quantitative aspect, so the consumption demand of high quality meat has shown the tendency of increase. Tenderness is the most importantly considered item in quality evaluation of meat quality by domestic as well as overseas consumers. To improve tenderness there are number of ways such as temperature treatment of carcass, electrical stimulation, suspension method and adding Tenderizer (Ahnstrom *et al.*, 2006; Hwang *et al.*, 2003; Koohmaraie *et al.*, 2002). For adding tenderizer the vegetable proteolytic enzymes, extracted from natural vegetables are widely used as of its safety and handiness (Gerelt *et al.*, 2000).

To apply proteolytic enzyme as a tenderizer mainly tropical fruits, such as papaya (papain), pineapple (bromelain), kiwi (actinidin) and fig (ficin) have been used (Douglas *et al.*, 1973; Kang and Rice, 1970; Kim and Taub, 1991). Also edible mushrooms have recently been reported as a healthy food and as a source of proteolytic enzymes. Those edible mushrooms used as a meat tenderizer are *Letinus edodes, Agaricus bisporus, Sarcodon aspratus* and so on (Ezmart *et al.*, 1979; Kim, 2013; Yamasaki and Suzuki, 1978). *Sarcodon aspratus* is an edible mushroom with pileus diameter of 15-20 cm, reseeded in the mountain side of humus soil rich broad-leaved forest in Korea and Japan.

In Korea traditionally it has been used as a single-medicine prescription for stomach-upset caused by meat. Also its various bioactive functions have been come to right by scientific research results (Kang *et al.*, 2000; Mizuno *et al.*, 2000; Song *et al.*, 2003). It is a healthy functional food in accordance with healthy food menu, recently the most keen-interest of consumers. As *Sarcodon aspratus* is known by its superior proteolytic effect, in this study, the

†These authors contributed equally to this work.
*Corresponding author: Youn-Chul Ryu, Department of Biotechnology, Jeju National University, Jeju 690-756, Korea. Tel: +82-64-754-3332, Fax: +82-64-725-2403, E-mail: ycryu@jejunu.ac.kr

possibility of its utilization and development as a tenderizer for beef has been appraised.

Therefore, we examined the effects of aqueous extract from *Sarcodon aspratus* on tenderization of the bovine *longissimus dorsi* muscles in comparison with commercial proteolytic enzymes. Furthermore, meat quality and muscle protein degradation were examined.

Materials and Methods

Preparation of samples

The bovine *longissimus dorsi* muscles were obtained from a selected retail shop. Dried *Sarcodon aspratus* was collected from a retail mushroom market. Kiwi (New Zealand) were obtained retail market, and papain enzyme powder obtained from a standard supplier (76220, Sigma-Aldrich Co., USA). We marinated meat with 2% *Sarcodon aspratus* extract, 2% kiwi extract, and 0.2% papain. Beef chunks (3×3×3 cm^3) were marinated with distilled water (control), *Sarcodon aspratus* extract (T1), kiwi extract (T2) or papain (T3) for 48 h at 4°C *longissimus dorsi* muscles. After 48 h chilling, samples were taken to evaluate the meat quality, and for use subsequent analyses.

Preparation of enzyme solution

To extract crude enzyme from *Sarcodon aspratus*, 3 g of *Sarcodon aspratus* sample was taken, then homogenized with 30 ml of distilled water (6,000 rpm, 12 s; Ace Homogenizer AM-8, Nissei Co., Japan), while fruit flesh was homogenized for kiwi extract, thereafter those have been shaken for 2 h at 25°C. Thereafter those were centrifuged (10,000 *g*, 4°C, 20 min), then separated supernatants were filtrate by Whatman #2 (diameter 110 mm) (Naveena *et al.*, 2004). To use as the final aqueous extract, *Sarcodon aspratus* sample was 2 times, and kiwi extract sample was 1 time filtrated. Also papain sample was dissolved in 2 times distilled water for use.

Enzyme activity measure

The enzyme activity of 2% of *Sarcodon aspratus* aqueous extract, 2% of kiwi aqueous extract and 0.2% papain solution were measured by Hiizu *et al.* (1995) process. To measure enzyme activity 1mg of extract was mixed with 1 mL of 2 mM $CaCl_2$-0.2 M Tris-HCl (pH 7.5), then incubated 60 min at 35°C and adding 10 mg of 5% (w/v) trichloroacetic acid to stop the reaction. Thereafter it was centrifuged, and the absorbance at 280 nm was measured for the supernatant by spectrophotometer (DU 650, Beckman, USA). After the first absorbance measuring the sample was incubated 60 min at 35°C, and again its absorbance was measured at 280 nm. One unit of caseinolytic activity was defined as the amount of enzyme that caused of 0.1 absorbance units at 280 nm after 60 min incubation at 35°C.

Meat quality traits

For measuring pH of the sample, after taking the sample out of liquid nitrogen and reducing it to powder using a waring blender (HGB150, Christison Ltd., England), 5 g of it were mixed with 20 mL of distilled water and it was homogenized (6,000 rpm, 40 s; Ace Homogenizer AM-8, Nissei Co., Japan). After leaving it alone for five minutes, pH was measured using a portable pH meter (Model 290A, Orion Research Inc., USA).

Meat color was measured using a Minolta chromameter (CR-300, Minolta Camera Co., Japan).

For measuring WHC, the experimental method of Hamm and Deatherage (1960) was transformed to use. After grinding 10 g of the sample, which completed precipitation, using a Daunce homogenizer (7727-40, Pyrex, USA) 0.6 M NaCl 15 mL was added and it was homogenized for one minute. Then, it was homogenized again and centrifugation (3,000 *g*, 4°C, 25 min) was applied after leaving it alone for fifteen minutes at a temperature of 4°C. After centrifugation, supernatant's volume was measured. To measure WHC, the value to subtract supernatant's volume from NaCl 15 mL was multiplied by 10.

For measuring cooking loss, after cutting the sample in a certain shape and weight (50±5 g) and putting it into an airproof polypropylene bag, it was put into a water bath with a temperature of 80°C and it was heated until the core temperature 75°C. After taking the sample out of it, it was cooled in ice slurry and left alone until the temperature 4°C. The surface of the sample, which was taken out of the Polypropylene bag, was dehydrated and its weight was measured. The value was expressed as a percentage (%).

The sample required for measuring shear force was cut in parallel with the muscle fibers (Diameter: 10 to 50 mm). Using an Instron (Series IX, Instron Corp., USA), the shearing cutting test was conducted for measurement. The chart speed was 120 mm/min, the maximum load was 10 kg, the measurement speed was 20 mm, the sample height was 20 mm, and it was measured using an adapter No. 4.

Protein solubility

Protein solubility was utilized as an indicator of protein denaturation (Joo *et al.*, 1999). Warner *et al.* (1997) process was taken to measure protein solubility. The sample,

kept in a deep freezer (-80°C) was powdered by using waring blender (HGB150, Christison Ltd., England). For total protein solubility 1 g of sample was mixed with 20 mL of 1.1 M KI/0.1 M potassium phosphate (pH 7.2), and for sarcoplasmic protein solubility 1 g of sample was mixed with 10 mL of 0.025 M potassium phosphate buffer (pH 7.2), then both were homogenized (6,000 rpm, 12 s; Ace Homogenizer AM-8, Nissei Co., Japan). Then those were centrifuged (1,500 *g*, 4°C, 20 min) after 24 h kept at 4°C. Thereafter, at 540 nm of spectrophotometer (DU 650, Beckman, USA), the absorbance of separated supernatant were measured by biuret method (Gornalle *et al.*, 1949). The myofibrillar protein solubility was calculated by the difference between total protein solubility and sarcoplasmic protein solubility

Myofibrillar protein and sarcoplasmic protein extract from muscle

To extract and analyze myofibrillar protein and sarcoplasmic protein the sample of quick-freeze and stored in deep freezer was used. The protein was extracted by Talmage and Roy (1993) process and sodium dodecyl sulfate- polyacylamide gel electorphoresis (SDS-PAGE) was carried out to analyze it by using modified Laemmli (1970) process. The sample taken out from deep freezer was powdered by using waring blender (HGB150, Christison Ltd., England), and 400 μL homogenization buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA, 20 mM Tris, pH 6.8) was injected to the sample 100 mg, then homogenized to make solution in the buffer and centrifuged (10,000 *g*, 4°C, 10 min). This supernatant was used for protein analysis. This process was carried out two more times repeatedly. To the settlement generated by the above process, 400 μL of wash buffer (175 mM KCl, 2 mM EDTA, 0.54% Triton X-100, 20 mM Tris, pH 6.8) was injected, then homogenized to make solution in the buffer and centrifuged (10,000 *g*, 4°C, 10 min). Again to the settlement after removing supernatant 400 μL resuspension buffer (150 mM KCl, 20 mM Tris, pH 7.0) was injected, then homogenized to make solution and stored at low temperature condition. As above all the process to extract myofibrillar protein were carried out under low temperature condition (4°C) to keep the sample stability.

To each myofibrillar protein and sarcoplasmic protein sample, each 300 μL of sample buffer (10% β-mercaptoethanol, 4% sodium dodecyl sulfate, 16% 1.0 M pH 6.8 Tris) was injected, the final protein concentration of sample was maintained around 2.00-2.50 μg/μL by biuret method (Gornall *et al.*, 1949). Samples were homogenized by vortex mixer, then heat treatment, 3 min in the boiling water, was taken.

SDS-PAGE analysis of myofibrillar protein

The analysis of myofibrillar protein was done by using SDS-PAGE (SE 260, Hoefer Pharmacia Biotech Inc., USA). Gel was consisted by Stacking gel 4%, separating gel 10%. The sample of myofibrillar protein was loaded 10 μg per lane, for running buffer, upper running buffer (0.1 M tris, 0.15 M glycine, 0.15% SDS) and lower running buffer (0.025 M tris, 0.2 M glycine, 0.1% SDS) were separately manufactured by modifying Laemmli (1970) process and used.

The operating condition of electrophoresis running was fixed by 4°C and 40 mA, and 2 h operated (Rivero *et al.*, 1997). To dye gel 0.05% Coomassie blue R-250 (w/v), 40% methanol and 7% acetic acid we used for 2 h at room temperature, so that to achieve sufficient dying, and 2 times bleaching were made by 40% methanol and 7% acetic acid.

To take gel image photo of myofibrillar protein Kodak DC290 (Eastman Kodak Company, USA) was used, and to analyze gel image Kodak 1D Image Analysis Software (Eastman Kodak Company, USA) was used (Huff-Lonergan *et al.*, 2002; Ryu *et al.*, 2005).

SDS-PAGE analysis of sarcoplasmic protein

To analyze sarcoplasmic protein SDS-PAGE was used (SE 260, Hoefer Pharmacia Biotech Inc., USA). The construction of Gel was 8% stacking gel and 15% separating gel. 10 μg of sarcoplasmic protein sample was injected into each lane. The running condition of electrophoresis and dying gel were identical to those of myofibrillar protein. To analyze sarcoplasmic protein the proportion of 16 bands in one lane from the gel image observed by electrophoresis (Ryu *et al.*, 2005).

Statistical analysis

For variance analysis General Linear Model (GLM) procedure of SAS (SAS Institute, USA) was used, and to verify significance F-test was used.

Results and Discussion

Enzyme activity

To analyze enzyme activity level of *Sarcodon aspratus* extract, kiwi extract and papain, available from market, were used for comparison (Table 1). The enzyme activity level of 2% *Sarcodon aspratus* extract was measured as

Table 1. Enzyme activity of *Sarcodon aspratus* extract, kiwi extract, and papain

	2% *Sarcodon aspratus* extract	2% kiwi extract	0.2% papain	Level of significance
Unit/mL	$683.2^{a}\pm96.49$[1]	$0.68^{c}\pm0.09$	$146.3^{b}\pm18.10$	***

[1]Result presented Means±SD.
Level of significance: ***$p<0.001$.
[a-c]Means with different superscripts within a row are significantly different ($p<0.05$).

Table 2. Meat quality traits of the bovine *longissimus dorsi* muscle treated with 2% *Sarcodon aspratus* extract (T1), 2% kiwi extract (T2), and 0.2% papain (T3)

	Control	T1	T2	T3	Level of significance
Meat pH	5.52 (0.03)[1]	5.51 (0.03)	5.53 (0.03)	5.45 (0.02)	NS
Lightness (L*)	39.13^{ab} (0.75)	40.22^{a} (0.75)	37.36^{b} (0.75)	40.12^{a} (0.67)	*
Redness (a*)	22.49^{a} (0.81)	18.29^{b} (0.81)	21.09^{a} (0.81)	22.23^{a} (0.72)	**
Yellowness (b*)	12.55^{ab} (0.34)	11.63^{bc} (0.34)	11.33^{c} (0.34)	12.82^{a} (0.31)	**
Cooking loss (%)	37.21^{c} (0.99)	41.13^{b} (0.99)	37.30^{c} (0.99)	50.52^{a} (0.99)	***
WHC (%)	22.33^{a} (10.48)	70.00^{b} (10.48)	26.33^{a} (10.48)	62.67^{b} (10.48)	*

[1]Standard error of least-square means.
Level of significance: NS=not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$.
[a-c]Means with different superscripts within a row are significantly different ($p<0.05$).
Abbreviation: WHC, water holding capacity.

683.2 unit/mL, the highest, while that of 2% kiwi extract was 0.68 unit/mL and that of 0.2% papain was 146.33 unit/mL.

According to the study of Lee and Jang (2005) the enzyme activity level of *Sarcodon aspratus* extract was 66 times higher than that of kiwi extract. Also it was proved from the test for this study that enzyme activity level of *Sarcodon aspratus* extract is quite higher than that of kiwi extract as well, and 4.6 times higher than that of 0.2% papain, widely used for the test condition of enzyme activity level.

Meat quality traits

To analyze the influence of vegetable proteolytic enzyme of *Sarcodon aspratus* extract to the quality of meat, the comparison analysis of meat processed with each kiwi extract and papain was carried out (Table 2). The influence of vegetable proteolytic enzyme to pH, there was no distinctive difference between sample group processed by *Sarcodon aspratus* extract and other sample groups as well as control group. For lightness (L*) there was significant difference ($p<0.05$) based on the sample groups, but between *Sarcodon aspratus* group and control group there was no significant difference, and kiwi extract group showed the lowest figure. For redness (a*) the sample group of *Sarcodon aspratus* showed the lowest figure ($p<0.01$). For yellowness (b*) there was no significant difference between sample group of *Sarcodon aspratus* and control group ($p<0.01$). As the idiopathic color of *Sarcodon aspratus* has been reflected, it is understood that redness (a*) of sample group *Sarcodon aspratus* was the lowest.

The cooking loss (%) of sample group of *Sarcodon aspratus* was lower than that of control group and sample group of kiwi extract ($p<0.001$), and there was no significant difference with sample group of papain. The adding of *Sarcodon aspratus* powder or *Sarcodon aspratus* extracted proteolytic enzyme gave influence to Water holding capacity (WHC) of meat, the WHC of sample group of *Sarcodon aspratus* was higher than that of control group, but similar to that of sample group of papain (Cho *et al.*, 2004). WHC of sample group of *Sarcodon aspratus* showed significant difference with control group as well as sample group of kiwi extract (70.00 vs. 22.33 vs. 26.33%, $p<0.05$), but showed similar result with sample group of papain (70.00 vs. 62.67%, $p>0.05$). This result is similar to the study result of Cho *et al.* (2004). For the Shear force (WBN), a mechanical figure representing tenderness (Fig. 1), sample group of *Sarcodon aspratus* was lower than that of sample group of kiwi extract (36.64 vs. 46.20 N, $p<0.05$), but no significant difference with sample group of papain (36.64 vs. 43.78 N, $p>0.05$), a marketable enzyme, so it was understood that the strong activeness of proteolytic enzyme of *Sarcodon aspratus* gave influence to the tenderness. As the above result of meat quality trait analysis, it is proved that the adding of *Sarcodon aspratus* extract is effective to improve the WHC and tenderness of meat.

Protein solubility

To analyze the level of proteolysis of meat, total pro-

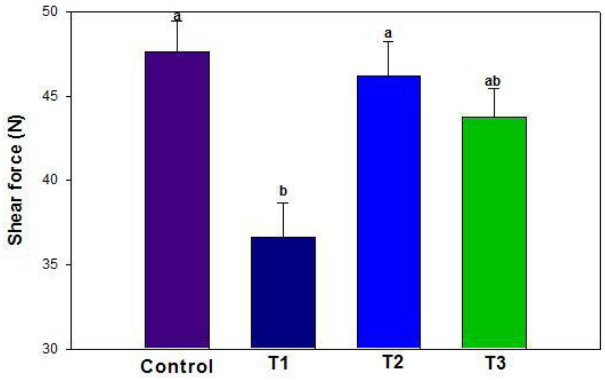

Fig. 1. Shear force of the bovine *longissimus dorsi* muscle treated with 2% *Sarcodon aspratus* extract (T1), 2% kiwi extract (T2), and 0.2% papain (T3). Results are expressed in mean values±SE. Significance (p<0.05) was indicated by differing letters.

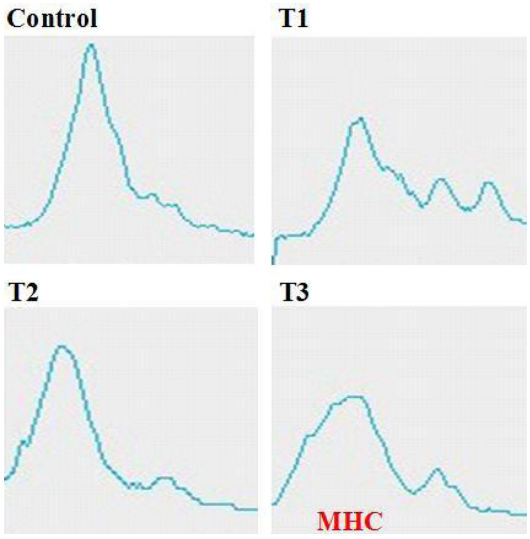

Fig. 2. Change of myosin heavy chain (MHC) in SDS-PAGE profile of the myofibrils prepared from crude enzyme-treated bovine *longissimus dorsi* muscle. Abbreviations: T1, 2% *Sarcodon aspratus* extract treatment; T2, 2% kiwi extract treatment; T3, 0.2% papain treatment.

tein, solubility of sarcoplasmic protein and myofibrillar protein have been measured from the meat processed by proteolytic enzyme and stored 48 h at 4ºC (Table 3). Total protein content of control group showed no significant difference with both sample groups of *Sarcodon aspratus* and kiwi extract, but showed significant difference with sample group of papain (p<0.05). The sarcoplasmic protein solubility of sample groups *Sarcodon aspratus* was not significantly different from that of sample group of papain (56.92 vs. 58.40 mg/mL, p>0.05), but showed higher figure against control group (56.92 vs. 42.77 mg/mL, p<0.001). The myofibrillar protein solubility of sample group *Sarcodon aspratus* showed lower figure than that of control group (102.39 vs. 123.15 mg/mL, p<0.001) and sample group of kiwi extract (117.31 mg/mL). The lower total protein solubility of sample group papain was more related to myofibrillar proteolysis, instead of sarcoplasmic proteolysis, and the sample group of *Sarcodon aspratus* showed similar tendency.

In this study myofibrillar protein solubility of sample group *Sarcodon aspratus* showed lower figure than that of control group and sample group of kiwi extract, so it was analyzed that the level of proteolysis was higher. When comparing this test result with Table 2, it is understood that the proteolytic capability of *Sarcodon aspratus* proteolytic enzyme affected to the myofibrillar proteolysis, and this proteolysis affected the tenderness of meat, so that the figure of shear force was low.

SDS-PAGE analysis of myofibrillar protein

To closely observe the change of muscle protein, when meat processed by proteolytic enzyme of *Sarcodon aspratus* extract, the SDS-PAGE gel pattern of myofibrillar protein was analyzed (Fig. 2). According to the study of Gil *et al.* (2006) calpain and cathepsin, it is reported that the proteolytic enzyme in the muscle, gave influence to myofibrillar protein fragmentation, it has co-relation with tenderness, but little influence to the decomposition of

Table 3. Protein solubility of the bovine *longissimus dorsi* muscle treated with 2% *Sarcodon aspratus* extract (T1), 2% kiwi extract (T2), and 0.2% papain (T3)

	Control	T1	T2	T3	Level of significance
Protein solubility (mg/mL)					
TPS	165.92^{a} (3.66)[1]	159.30^{ab} (3.66)	156.88^{ab} (3.66)	149.78^{b} (3.66)	*
SPS	42.77^{b} (2.98)	56.92^{a} (2.98)	39.57^{b} (2.98)	58.40^{a} (2.98)	***
MPS	123.15^{a} (3.52)	102.39^{b} (3.52)	117.31^{a} (3.52)	91.37^{c} (3.52)	***

[1]Standard error of least-square means.
Level of significance: *p<0.05, ***p<0.001.
[a-c]Means with different superscripts within a row are significantly different (p<0.05).
Abbreviations: TPS, total protein solubility; SPS, sarcoplasmic protein solubility; MPS, myofibrillar protein solubility.

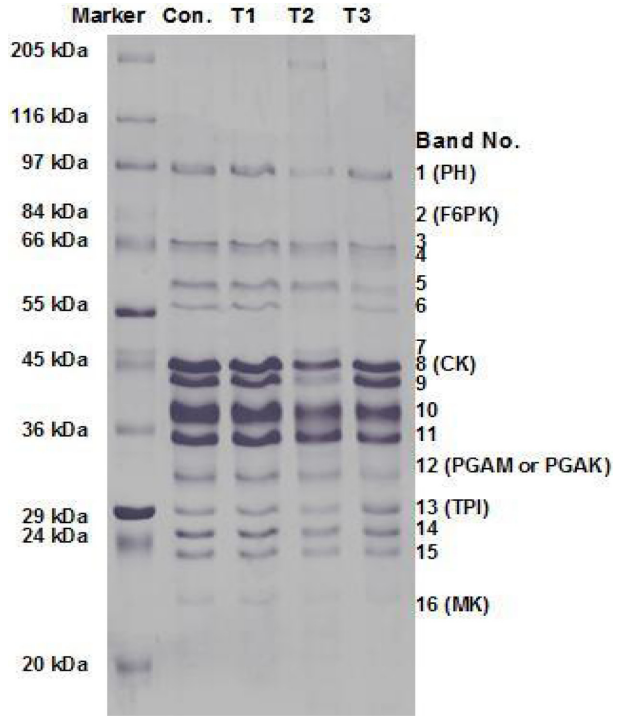

Fig. 3. SDS-PAGE patterns of sarcoplasmic proteins for crude enzyme-treated bovine *longissimus dorsi* muscle. Sixteen bands were used to calculate the relative percentage using image analysis system. Abbreviations: Con., control; T1, 2% *Sarcodon aspratus* extract treatment; T2, 2% kiwi extract treatment; T3, 0.2% papain treatment.

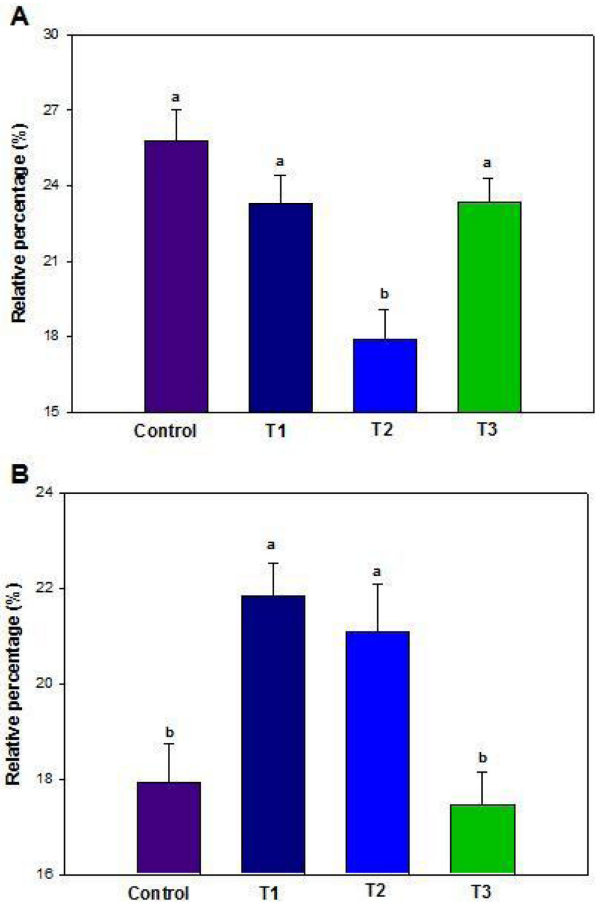

Fig. 4. Relative percentage of decreased (A) and increased (B) sarcoplasmic protein fractions in the bovine *longissimus dorsi* muscle treated with 2% *Sarcodon aspratus* extract (T1), 2% kiwi extract (T2), and 0.2% papain (T3). Results are expressed in mean values±SE. Significance (p<0.05) was indicated by differing letters.

myosin and actin. On the other hand, thiol group vegetable proteolytic enzyme, contained in kiwi, papaya, pineapple and etc., gave influence to the decomposition of sarcoplasmic protein, so it is known as effective to improve tenderness (Wada *et al.,* 2002). In this study, according to the observation result of MHC (myosin heavy chain) by using SDS-PAGE, it was proved that the decomposition level of sample group *Sarcodon aspratus* relatively higher than that of control group as well as that of sample group kiwi. Comparing with sample group papain, the decomposition level was similar. This result is identical to that of the study result of Kim (2013). Like this, the proteolytic enzyme extracted from *Sarcodon aspratus* gave influence to the decomposition of MHC, and this is related to the tenderness of sample group *Sarcodon aspratus*.

SDS-PAGE analysis of sarcoplasmic protein

Fig. 3 is the SDS-PAGE gel pattern of sarcoplasmic protein, when processing meat by proteolytic enzyme of *Sarcodon aspratus* aqueous extract. According to the analyzing result there were differences among gel pattern of sarcoplasmic protein depending on the processing groups, but there were similar tendency between sample group of *Sarcodon aspratus* and papain at bands 1, 2, 5, 6, 7, 9 and 12 and between sample group of *Sarcodon aspratus* and kiwi at bands 1, 2, and 7. Fig. 4 indicated the sum of protein content, more decreased than control group (1, 5, 6. 9) and protein content more increased than control group (2, 7, 8, 12) among band analyzed in this study. For the sum of more decreased bands, sample group of Kiwi was the lowest, but for the sum of more increased bands sample group of *Sarcodon aspratus* and kiwi were relatively higher than other processing groups, so it is understood that the proteolytic effect of *Sarcodon aspratus* and kiwi is high (p<0.05).

Sarcoplasmic protein is a water soluble protein, and has no direct relationship with tenderness, but other various proteins are used as an indicating factor of meat quality as

well as protein denaturation. Like this the intensity of various Sarcoplasmic protein bands can be used as an indicator of protein denaturation, in this study PH, CK and unknown protein (about 55 and 60 kDa) showed low intensity by the treatment, but F6PK and phosphoglycerate mutase or phosphoglycerate showed high intensity according to the treatment, so it is understood that those are proteins co-related with the level of proteolysis. Especially an unknown band near 45 kDa, the protein not found in previous Ryu *et al.* (2005) test, was found from *Sarcodon aspratus*, which has high treatment effect of proteolytic enzyme, so it is understood that it has relationship with the level of proteolysis.

Conclusion

The treatment by *Sarcodon aspratus* extraction liquid is effective in improvement of meat tenderness, and it is related to the proteolysis, especially decomposition of MHC, so it has high usefulness for meat tendering agent. However the inherent color of *Sarcodon aspratus* aqueous extract may affect to the redness of meat, so it is understood that the refined proteolytic enzyme of *Sarcodon aspratus* aqueous extract, instead of the extraction liquid as it is, is better to improve tenderness of meat without meat color transition.

Acknowledgements

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