HYDROSTATIC PRESSURE EFFECT ON THE CHANGES OF TEXTURE AND PROTEIN SOLUBILITY IN BEEF *BULGOGI* USING SOY SAUCE

Eun-Jung Lee[1], Se-Wook Oh[2], Nam-Hyouck Lee[3], Young-Ho Kim[3], Yun-Ji Kim[1*]

[1]Food Safety Research Group, Korea Food Research Institute, Seongnam-si, Gyeonggi-do, 463-746, South Korea

[2]Department of Food and Nutrition, Kookmin University, Seongbuk-Gu, Seoul, 136-702, South Korea

[3]Neo Food Resources Research Group, Korea Food Research Institute, Seongnam-si, Gyeonggi-do, 463-746, South Korea

[*]Corresponding author (phone: +82-31-780-9160; fax: +82-31-780-9185; e-mail: yunji@kfri.re.kr)

***Abstract*—The changes of shear force and protein solubility in beef *bulgogi* were evaluated, to understand the properties on hydrostatic pressure (HP)-treated beef *bulgogi*. Shear forces of 0.1 and 300 MPa treated beef *bulgogi* showed no significant differences. However, those of 200, 350 and 400 MPa treated beef *bulgogi* showed significantly higher than that of 0.1 MPa sample. As increasing pressure size, protein solubility of beef *bulgogi* was decreased due to the decrease of soluble myosin heavy chain (MHC) and actin. For evaluating the influence of soy sauce as a ingredient for *bulgogi* seasoning, the changes of protein solubility in 300 MPa treated Mf suspended with soy sauce (4, 12 and 20%) were compared with those with NaCl (0.1, 0.2, 0.4 and 0.6 M). Protein solubility of Mf induced by increasing NaCl concentration was increased, and soluble Mf in 0.4 and 0.6 M NaCl was not precipitated by 300 MPa treatment. However, protein solubilities of 0.1 and 300 MPa treated Mf were not increased as increasing soy sauce concentrations. In the SDS-PAGE patterns of soluble protein of Mf with increasing soy sauce concentrations, increase of MHC band was not observed in 0.1 and 300 MPa samples. And dissociation of MHC induced by soy sauce was observed.**

***Index Terms*—hydrostatic pressure, beef *bulgogi*, soy sauce, shear force, protein solubility**

I. INTRODUCTION

The application of *bulgogi* as an item of ready-to-cook meat product is confronted by the difficulties of the preservation during distribution, the output for standard product and the quality control for preference of consumer. Therefore, it is necessary to use non-thermal techniques to overcome these difficult problems. Hydrostatic pressure (HP), one of the non-thermal processing, is continually being investigated by the industry in the producing better quality foods when it is compared with heating processing. Consequently, the characteristics of HP-treatment are adapted for keeping and controlling the product in the ideal standard state for the commercial application.

There are two types of beef *bulgogi* seasoned with soy sauce-based sauce and salt-based sauce in Korea. Soy sauce has been used as a usual ingredient for seasoned meat products in Korea, and it contains salt, amino acid, peptide and hydrolyzed compounds (Luh, 1995). Kim, Jeong, Choi, Seo and Lee (2003) were reported that quality characteristic of cured pork meat with soy sauce was improved by tumbling. Soy sauce treated pork showed the decrease of shear force during storage compare with other Korean traditional sauce such as Kimchi-based sauce, pickled shrimp-based sauce and onion-based sauce (Moon, Jun, Kim, Park and Hah, 2006). Myofibril (Mf), salt-soluble protein in meat, is the major factor affecting meat tenderness. A direct relationship has been reported to exist a relationship between the tenerness of meat and the weakening of Mf (Koohmaraie, Seideman, Schollmeyer, Dutson & Babiker, 1988). However, there are few studies about the effect of soy sauce in Mf which could be related with meat tenderness. Therefore, the changes of shear force and protein solubility in HP treated beef *bulgogi* based soy sauce were investigated in this study. And the effect of HP and soy sauce in Mf was evaluated by protein solubility.

II. MATERIALS AND METHODS

A. Samples preparation

Bovine skeletal muscles (*semitendinosus*) were obtained from the carcasses (Holstein) after 24 h of slaughter. *Semitendinosus* muscles were cut parallel to the muscle fiber axis in 1×1×3 cm. Soy sauce, sugar, garlic, onion and water were purchased from local market. And for preparing *bulgogi* sauce, soy sauce, sugar, onion, garlic and water were mixted by a 200:140:34:16:11 ratio. Fermented soy sauce (Sampyo Co., Icheon, Korea) had a salt content of about 15~18% (coressponded to 2.57~3.08 M NaCl). The *bulgogi* sauce and beef were mixed by a 1:2 ratio for 3 hr in a refrigerator (4°C) before HP treatment. For preparing Mf, minced muscle was homogenized with 25 volumes of extraction buffer, 20 mM tris-HCl including 0.1 M NaCl (pH 7.0). After centrifugation at 10,000×g for 30 min at 4°C, the pellet resuspended in the extraction buffer and the same operation was arried out 3 times. After the last

centrifugation, the pellets were again resuspended in 20 mM tris HCl (pH 7.0) including 0.1, 0.2, 0.4 and 0.6 M NaCl or adding 4, 12 and 20% soy sauce (corresponded salt concentration about 0.2, 0.4 and 0.6 M NaCl, respectively).

B. Pressure treatment

Smples were placed in a high pressure vessel submerged in a hydrostatic fluid medium. The samples were pressurized at 300 MPa for 5 min at 25±3°C with an isostatic pressure unit (Quintus food processor, QFP 6, Ohio, USA). Control samples were maintained in atmospheric pressure (0.1 MPa) at 4°C while the samples were being treated. Soon after the treatment, all the samples were stored at 4°C until required.

C. Texture measurements

The muscle sample was placed in a polyethylene bag, then heated in a water bath at 75°C. Warner-Bratzler (WB) shear force was measured in 10-12 samples to the direction of the blade attached to a texture analyzer (Stable Micro System Ltd., UK).

D. Protein solubility

Protein concentration in an aliquot of the liquid was determined using the Biuret method (Gornall, Bardawill, & David, 1949). This method was also used to estimate total protein present in the supernatants of myofibrils. Amount of soluble protein was calculated as a percentage of the protein present in the suspension of myofibrils.

E. SDS-PAGE

SDS-PAGE was performed on gels of 12.5% polyacrylamide containing 1% SDS. Samples for electrophoresis were dissolved in Tris-HCl buffer (pH 7.5) containing 8 M urea, 2% SDS and 2% 2-mercaptoethanol and heated at 100°C for 2 min. Fixation and staining by Coomassie brilliant blue was followed the method described by Neuhoff *et al*. (Neuhoff, Arold, Taube, & Ehrhardt, 1988).

F. Statistical analysis

The data were analyzed by ANOVA using the SAS statistical program, and significant differences among various treatments were compared using Duncan`s multiple range tests.

III. RESULTS AND DISCUSSION

Shear forces of 0.1 and 300 MPa-treated beef *bulgogi* showed a similar degree (fig. 1). Those of 200, 350 and 400 MPa-treated beef *bulgogi* showed significantly higher than that of 0.1 MPa beef *bulgogi*. However, the shear force of HP-treated beef *bulgogi* was lower than that of 0.1 MPa beef without seasoning. The protein solubility in 0.6 M NaCl of 0.1 MPa beef *bulgogi* was significantly lower than 0.1 MPa beef without seasoning and it was decreased as increasing pressure size (fig. 2-(a)). The decrease of protein solubility was due to the decrease of soluble MHC, α-actinin and actin in SDS-PAGE (fig. 2-(b)). From this result, the increase of shear force induced by HP except 300 MPa was observed, but the shear force of HP-treated beef *bulgogi* was lower than that of 0.1 MPa beef without seasoning. It was need to understand the effect on individual ingredients of the seasoning on beef *bulgogi*.

Soy sauce is one of main ingredient of beef *bulgogi*. Notable differences in soluble protein compositions of HP-treated beef *bulgogi* were in Mf. Therefore, Mf suspended in 20 mM tris-HCl buffer (pH 7.0) including NaCl (0.1, 0.2, 0.4 and 0.6 M) or soy sauce (4, 12 and 20%) was treated with 300 MPa for evaluating the influence in Mf structure induced by soy sauce. The protein concentration of the supernatant of HP-treated Mf in 20 mM tris-HCl buffer (pH 7.0) including NaCl (0.1, 0.2, 0.4 and 0.6 M) or soy sauce (4, 12 and 20%) were performed after centrifugation for protein solubility. And the protein composition of the supernatant and total protein of suspension was analyzed by SDS-PAGE (fig. 3). The protein solubilities were increased as increasing NaCl concentrations. The protein solubility of Mf with 0.4 M NaCl (pH 7.0) was over 70% in 0.1 and 300 MPa samples (data not shown). Mf existed as soluble monomeric proteins in 0.4 and 0.6 M NaCl (pH 7.0) was not aggregated by 300 MPa. In Mf with 0.1 and 0.2 M NaCl (pH 7.0), protein solubilities of 300 MPa treatments was higher than those of 0.1 MPa samples. It was due to the increase of low molecular weight protein of Mf induced by 300 MPa. Protein solubilities of Mf with soy sauce were not dramatically increased as increasing the addition of soy sauce regardless of HP treatment (data not shown). According to the results of SDS-PAGE in fig. 3-(a), soluble MHC was not observed in Mf with soy sauce induced by both 0.1 and 300 MPa. Soluble protein compositions in Mf induced by soy sauce were different from those by NaCl. It might reflect that the properties of salt-based seasoned beef were different from the properties of soy sauce-based seasoned beef. It needs to

perform further study. Soy sauce-induced decrease of MHC band in the SDS-PAGE pattern of total protein was observed (fig. 3-(b)). It might be induced by the action of proteolytic compounds in soy sauce.

IV. CONCLUSION

The shear force of beef *bulgogi* by 300 MPa was similar with 0.1 MPa sample. The protein solubility of beef *bulgogi* was decreased by seasoning and HP. Soy sauce, as an ingredient of *bulgogi* sauce, influenced in MHC dissociation. However, the protein solubility in Mf induced by soy sauce was not increased.

ACKNOWLEDGEMENT

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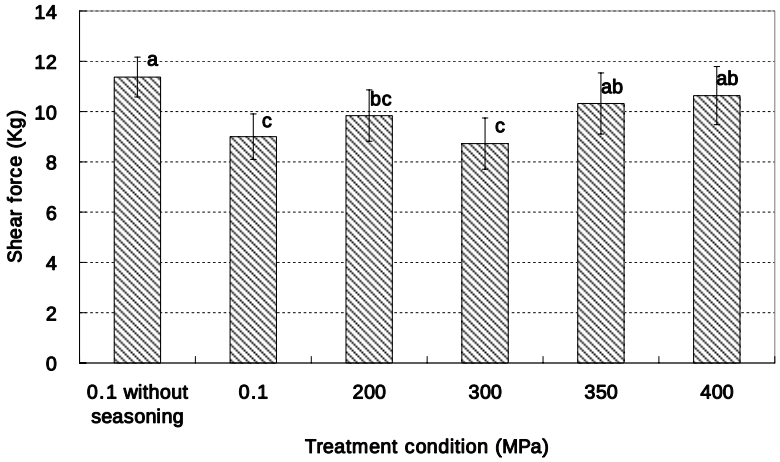

Fig. 1. The changes of shear force in hydrostatic pressure-treated beef *bulgogi*.
Each bar represents the mean±SD (n=10). Different alphabet represents the significant differences among groups at p<0.05 by Duncan`s multiple range test.

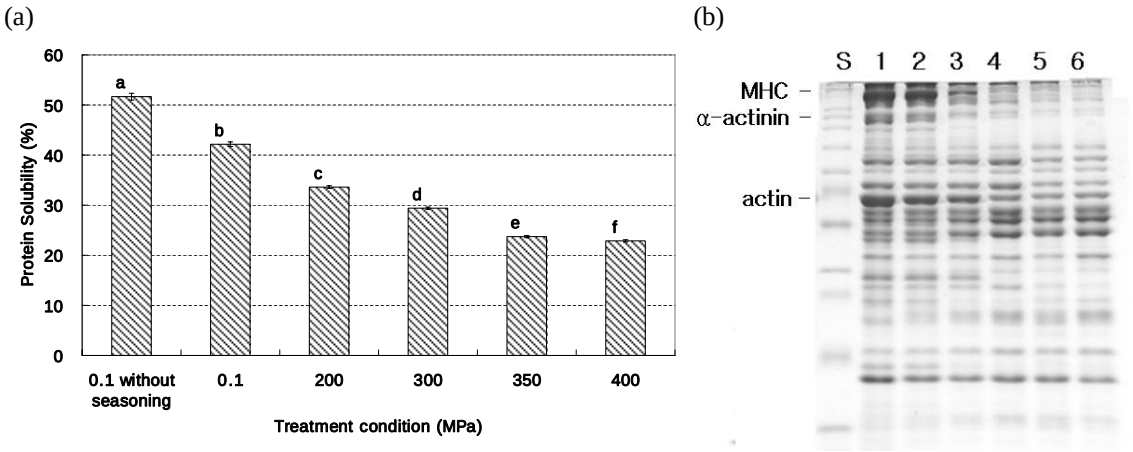

Fig. 2. Protein solubility (a) and SDS-PAGE pattern of soluble protein (b) in 0.6 M NaCl (20 mM tris-HCl buffer, pH 7.0) of hydrostatic pressure-treated beef *bulgogi*. (a) Each bar represents the mean±SD (n=3). Different alphabet represents the significant differences among groups at p<0.05 by Duncan`s multiple range test. (b) Lane S: protein standard with molecular weights indicated on the left margin; lane 1: 0.1 MPa sample without seasoning; lanes 2: 0.1 MPa beef *bulgogi*; lanes 3, 4, 5 and 6: high pressure treatment respectively (200 MPa), (300 MPa), (350 MPa), (400 MPa).

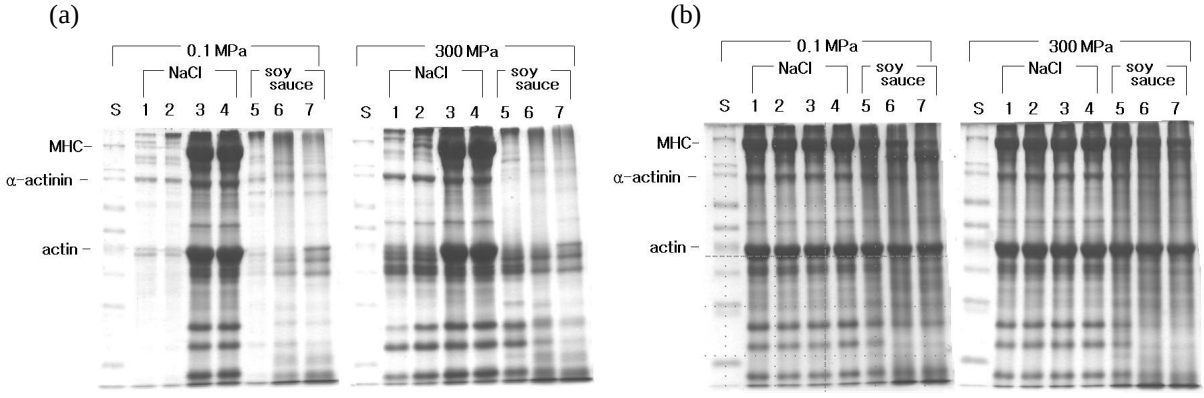

Fig. 3. SDS-PAGE patterns of soluble protein (a) and total protein (b) hydrostatic pressure-treated myofibril depending on NaCl and soy sauce concentrations.

Lane S: protein standard with molecular weights indicated on the left margin; lane1: Mf suspended with 0.1 M NaCl (pH 7.0); lane 2, 3 and 4: Mf suspended with 20 mM tris-HCl buffer including 0.2, 0.4 and 0.6 M NaCl (pH 7.0), respectively; lane 5, 6 and 7: Mf suspended with 20 mM tris-HCl buffer (pH 7.0) adding 4, 12 and 20% soy sauce, respectively.