Korean J. Food Sci. Ani. Resour.
Vol. 29, No. 3, pp. 317~324(2009)

Application of a Kiwifruit (*Actinidia chinensis*) to Improve the Textural Quality on Beef *Bulgogi* Treated with Hydrostatic Pressure

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Abstract

In order to reduce the increased hardness of beef *bulgogi* due to hydrostatic pressure (HP), kiwifruit (*Actinidia chinensis*) was applied. To understand the changes of shear force in beef *bulgogi* with kiwifruit induced by HP, changes in chemical properties of myofibril (Mf) with 10% kiwifruit induced by HP were investigated. From the SDS-PAGE patterns of Mf with 10% kiwifruit, there was an observed increase in the degradation of myosin heavy chain (MHC) by HP (300-500 MPa) to that by 0.1 MPa. This result indicates that HP may enhance enzyme action from a kiwifruit for the degradation of MHC, and the similar phenomenon occurred in the beef *bulgogi* with kiwifruit induced by HP. The shear force of beef *bulgogi* without a kiwifruit induced by 400 and 500 MPa significantly increased compared to that by 0.1 MPa ($p<0.05$). However, in the beef *bulgogi* with 10% or 20% kiwifruit, the shear force induced by 400 or 500 MPa was similar or slightly lower than that by 0.1 MPa. Consequently, adding kiwifruit to *bulgogi* could reduce the hardness of HP-induced beef *bulgogi* due to the enzyme action in the kiwifruit accelerated by HP.

Key words : hydrostatic pressure, kiwifruit (*Actinidia chinensis*), bovine myofibril, beef *bulgogi*

Introduction

A beef *bulgogi* is a Korean traditional meat product, marinated beef with *bulgogi* sauce. Commercial beef *bulgogi* products are marketed as a chilled ready-to-cook product and its shelf-life is usually within 3-4 d. And hydrostatic pressure treatment (HP) represents a non-thermal technology for the preservation of meat product as well as affects the textural properties of meat product (Hong *et al.*, 2008; Kim *et al.*, 2007; Yamamoto *et al.*, 1990). In a meat product, above 400 MPa treatment was required to effectively inactivate the pathogen (Shigehisa *et al.*, 1991). The shear force of meat treated above 400 MPa was increased (Kim *et al.*, 2007; Margey *et al.*, 1997) although increase of activity of lysosomal enzyme, related with meat tenderness, was observed (Jung *et al.*, 2000). Therefore, in order to apply HP in meat product for safety, it needs to improve the meat tenderness.

The proteolytic enzyme plays a variable role in meat processing for quality improvements depending on its kinds. And HP treatment could be applied to increase the enzyme action. Trespalacios and Pla (2007) reported that under specific pressure condition, higher amount and heterogeneity of cross-links was produced in meat and egg proteins with microbial transglutaminase (MTGase). And simultaneous application of MTGase and HP in low-fat and low-salt chicken meat gels involved with the improvement of binding strength and textural properties (Pla and Trespalacios, 2007). Wada *et al.* (2002) reported that the unheated but kiwifruit protease-treated connective tissue with HP was obtained a soluble α-chain collagen. HP treatment after adding enzyme could affect the enzyme action. However, there are a few studies on the changes in meat induced by proteolytic enzyme and HP. Kiwifruit contains cysteine protease, called actinidin, which causes hydrolysis of proteins. Actinidin is stable and active over a wide range of conditions and applied as a meat tenderizer (Kamphuis *et al.*, 1985; Kim *et al.*, 2003). And Lewis and Luh (1988) reported that actinidin

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did not over-tenderize the beef steak surface. Therefore, kiwifruit could be applied in *bulgogi* sauce as an ingredient for improving textural properties.

In this study, to understand the changes in beef *bulgogi* with kiwifruit induced by HP, the effects of HP in Mf with kiwifruit except the interference compounds were investigated as evaluating the protein solubility and SDS-PAGE patterns, and sulfhydryl (-SH) contents.

Materials and Methods

Samples preparation

Bovine *semitendinosus* muscles were obtained from the carcasses (Holstein) after 24 h of slaughter. Bovine *semitendinosus* muscles were cut parallel to the myofibril axis in 1×1×3 cm. Soy sauce, garlic, onion, sugar, and kiwifruit (*Actinidia chinensis*) were purchased from local store and *bulgogi* sauce was prepared according to Table 1. Garlic, onion, and kiwifruit were juiced. The soy sauce (Sampyo Co., Icheon, Korea) had a salt content of about 15%. And beef *bulgogi* was made by mixing bovine *semitendinosus* muscles and *bulgogi* sauce (100 g : 50 g), and then marinated for 3 h.

Purification of myofibril

Minced muscle was homogenized with 25 volumes of extraction buffer, 20 mM tris-HCl including 0.1 M NaCl and 1 mM NaN_3, (0.1 M NaCl, pH 7.0). After centrifugation at 10,000×*g* for 30 min at 4°C, the pellets were resuspended in the extraction buffer and the same operation was carried out five times. After the last centrifugation, the pellets were again resuspended in extraction buffer (0.1 M NaCl, pH 7.0). In order to remove the connective tissue, the homogenate was filtered through a 20-mesh high density polyethylene (HDPE) net. And then, the 9 mL of myofibril (Mf) suspensions (10 mg/mL) with the 1 mL of 10% kiwifruit (Table 1) were sealed within polyethylene bags.

Pressure treatment

The samples were placed inside a high pressure vessel submerged in a hydrostatic fluid medium. The samples were pressured at 300, 400, or 500 MPa for 5 min, the initial temperature of the pressure vessel was 15±3°C with the isostatic pressure unit (Quintus food processor, QFP 6, ABB Autoclave systems Inc., Columbus, Ohio, U.S.A.). The control samples were maintained in 0.1 MPa (atmospheric pressure) at 4°C while the samples were being treated. After the treatment, all the samples were stored at 4°C until used for test. The rate of pressurization was 5 MPa/s. The pressure in the chamber was released within 10 s.

Protein solubility

The buffer solution including 1.1 M NaCl (pH 7.0) and treated aliquots of myofibril in 0.1 M NaCl (pH 7.0) were immediately mixed by 1:1 ratio and then centrifuged at 10,000×*g* for 30 min at 4°C after 1 h from the high pressure treatment to measure the protein solubility in 0.6 M NaCl (pH 7.0). Minced muscle was homogenized with 10 volumes of extraction buffer (20 mM tris-HCl including 0.6 M NaCl and 1 mM NaN_3, pH 7.0) to measure the protein solubility in 0.6 M NaCl (pH 7.0). Aliquots of meat suspension were centrifuged at 10,000×*g* for 30 min at 4°C after being stirred in 0.6 M NaCl buffer at 4°C during 1 d. Protein solubility was determined by the procedure established by Li-Chan (1983). And the protein concentrations were determined in accordance with the Biuret method originally described by Gornall *et al.* (1949). Also, protein concentration of the supernatant was determined by the Biuret method. The protein solubility was calculated as a percentage of the protein concentration in the meat suspensions. Protein solubility was calculated by the following equation:

$$\text{Protein solubility (\%)} = (\text{protein con.}_{\text{supernatant}}/\text{protein con.}_{\text{uncentrifuged sample}})\times 100$$

Table 1. The formula of *bulgogi* sauce and kiwifruit (Unit: g)

Sauces \ Seasoning	Soy bean sauce	Sugar	Onion	Garlic	Kiwifruit	Water
10% kiwifruit [1)]	0	0	0	0	10	90
Bulgogi without kiwifruit	40	28	6.8	3.2	0	22
Bulgogi with 10% kiwifruit	40	28	6.8	3.2	10	12
Bulgogi with 20% kiwifruit	40	28	6.8	3.2	20	2

[1)] 10% kiwifruit: Ten fold diluted kiwifruit juice without *bulgogi* sauce.
Bulgogi without kiwifruit: *Bulgogi* sauce without kiwifruit juice.
Bulgogi with 10% kiwifruit: *Bulgogi* sauce with 10 g of kiwifruit juice in 100 g of total seasoning.
Bulgogi with 20% kiwifruit: *Bulgogi* sauce with 20 g of kiwifruit juice in 100 g of total seasoning.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on gels of 10% polyacrylamide containing 1% SDS (Lawmmli, 1970). The 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 brillant blue was followed the method described by Neuhoff *et al.* (1988).

Western blotting

After SDS-PAGE, the proteins were transferred at 0.4 A to a 0.45 mm nitrocellulose membrane. A transfer buffer containing 20 mL/100 mL methanol, 25 mM tris, 192 mM glycine, and 0.01 g/100 mL SDS was used. The membranes were stained in Ponceau S staining solution (Sigma, Louis, MO, USA). Immunobloting for MHC was used as the primary antibody (Mouse monoclonal anti-myosin heavy chain, Novus Biologicals, Inc, Littleton, CO, USA) and the secondary antibody (anti-mouse Ig-G-HRP, Sigma). Protein bands were visualized using the substrate buffer (0.1 M Tris, pH 9.5; 0.1 M NaCl; 50 mM $MgCl_2$; 1 mM $ZnCl_2$).

Surface sulfhydryl (-SH) content

The surface -SH content was determined by using Ellman's reagent [5',5'-dithio-bis(2-nitrobenzoic acid), DTNB, Sigma] according to the method of Ellman (1959), as modified by Sompongse *et al.* (1996). The color developed was measured at 412 nm using spectrophotometer. The -SH content was calculated as mM.

Texture measurements

The samples were placed in polyethylene bags and cooked in a water bath to an internal temperature of 75°C, assessed using a thermocouple probe inserted into the meat. Cooking drips were measured by mass difference, and it was expressed as the percentage of loss related to the initial weight. Warner-Bratzler (WB) shear force was measured in 10-12 sub-samples to the direction of the blade attached to a texture analyzer (TA-XT2, Stable Micro System Ltd., Godalming, Surrey, UK).

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 (SAS Institute, Inc., Cary, N.C., USA, 1996).

Results and Discussion

Myofibril with/without kiwifruit induced by hydrostatic pressure

Protein solubility in 0.6 M NaCl (pH 7.0)

Fig. 1 shows the changes of protein solubility in 0.6 M NaCl (pH 7.0) of Mf with/without kiwifruit induced by HP (300, 400, and 500 MPa for 5 min). Mf in 0.6 M NaCl exists as a soluble monomeric protein but may be rendered insoluble by protein denaturation as well as

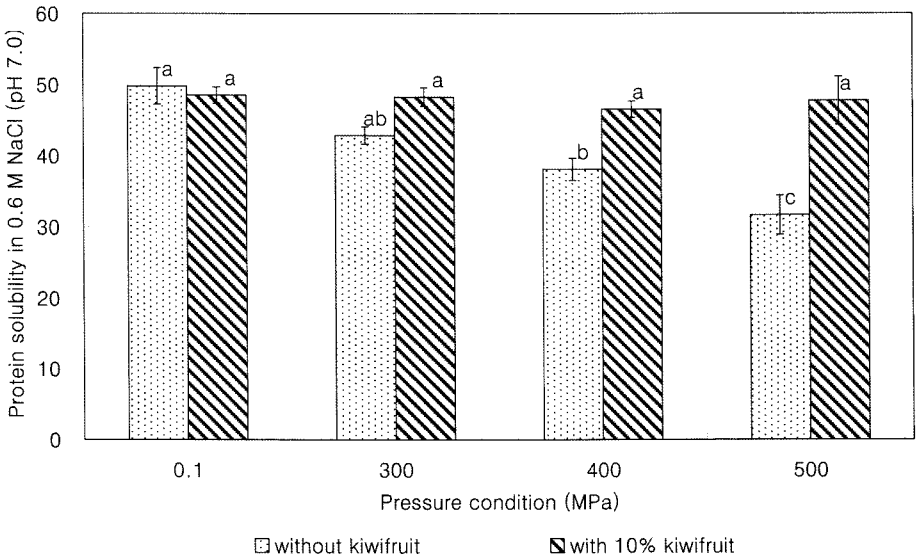

Fig. 1. Protein solubilities in 0.6 M NaCl (pH 7.0) of myofibril with/without kiwifruit induced by hydrostatic pressure ($p<0.05$). The 10% kiwifruit and myofibril (10 mg/mL) were mixed by a 1:9 ratio for 1 h in a refrigerator (4°C) and then hydrostatic pressure treated. Error bars represent standard deviations (n=3).

aggregation and coagulation. In the Mf without kiwifruit, protein solubility in 0.6 M NaCl (pH 7.0) was significantly decreased as increasing pressure condition ($p<$ 0.05). This result agreed with our previous data which the decrease of protein solubility in 0.6 M NaCl (pH 7.0) was due to the protein denaturation of Mf induced by HP (Lee *et al.*, 2004; Lee *et al.*, 2007). However, there was no significant difference among 0.1 MPa sample and HP treatments in Mf with kiwifruit. Therefore, kiwifruit might affect the protein denaturation of Mf induced by HP.

SDS-PAGE patterns of soluble protein in 0.6 M NaCl (pH 7.0)

SDS-PAGE patterns for total and soluble protein contents were observed, and western blot for the follow-up of MHC was performed (Fig. 2) in order to elucidate the protein contents in Mf with 10% kiwifruit induced by HP. SDS-PAGE patterns of total and soluble protein in 0.6 M NaCl (pH 7.0) of Mf without kiwifruit were similar to Mf with 10% kiwifruit induced by 0.1 MPa (Fig. 2-(a) and (b)). However, from the western blot, some of MHC band degraded into lower molecular weight products with size ranging from 116 to 205 kDa in 0.1 MPa with 10% kiwifruit (Fig. 2-(c) and (d)). Although the similar band density between SDS-PAGE and western blot could not be observed due to the use of the monoclonal primary antibody in this study, but the result of western blot could suggest that the band between 116 and 205 kDa was came from MHC. A notable degradation of MHC into lower molecular weight products was shown in Mf with 10% kiwifruit induced by 300, 400, or 500 MPa compared with that by 0.1 MPa from the SDS-PAGE and western blot. The enzyme action from kiwifruit in Mf might be accelerated by HP treatment. The band of MHC was not observed by SDS-PAGE patterns of soluble protein in 0.6 M NaCl (pH 7.0) for Mf with kiwifruit induced by 400 or 500 MPa (Fig. 2-(b)). However, in SDS-PAGE patterns of the total protein for Mf with 10% kiwifruit induced by 400 or 500 MPa, the band corresponding to 205 kDa was observed and that band might be the MHC from the result of western blot (Fig. 2-(a), (c)). Therefore, remained MHC which did not degrade into low molecular products induced by 400 or 500 MPa might be precipitated as the band of 205 kDa was observed in total protein, while it was not observed in soluble protein in 0.6 M NaCl (pH 7.0). Consequently, it could be simultaneously occurred the MHC degradation as well as MHC aggregation in Mf with kiwifruit induced by HP.

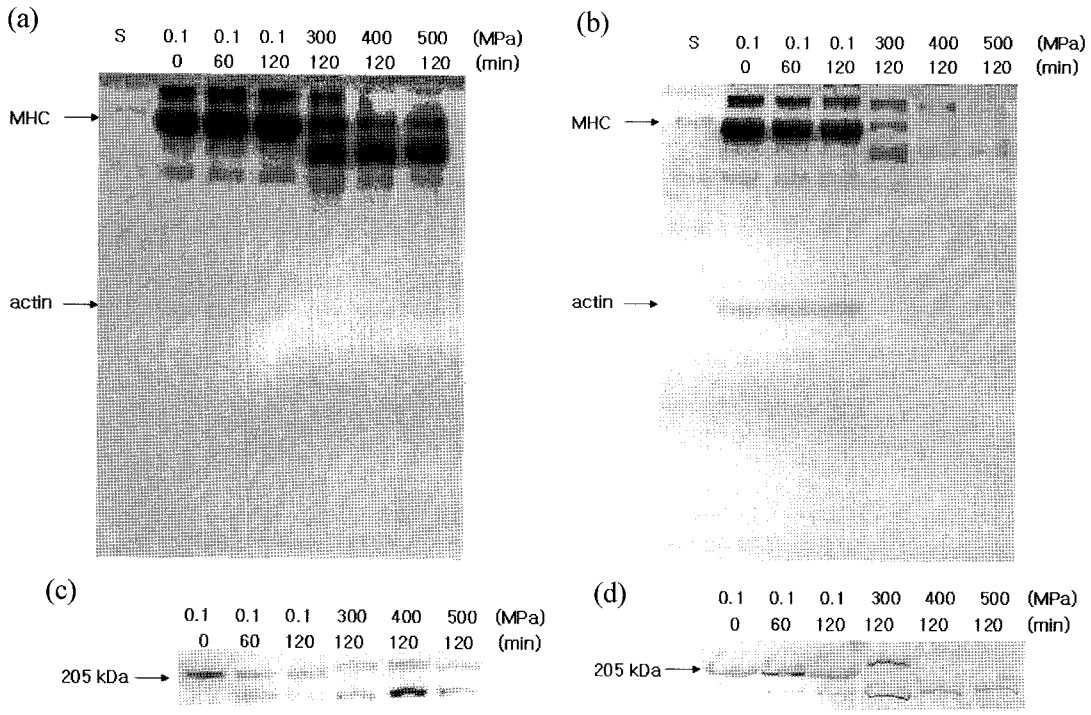

Fig. 2. SDS-PAGE patterns and western blot of total protein and soluble protein in 0.6 M NaCl (pH 7.0) of myofibril with kiwifruit induced by hydrostatic pressure. The 10% kiwifruit and myofibril (10 mg/mL) were mixed by a 1:9 ratio for 1 h in a refrigerator (4°C) and then hydrostatic pressure treated. Lane S means protein standard with molecular weights indicated on the left margin (a) SDS-PAGE patterns of total protein; (b) SDS-PAGE patterns of soluble protein in 0.6 M NaCl (pH 7.0); (c) western blot of MHC on total protein; (d) western blot of MHC on soluble protein in 0.6 M NaCl (pH 7.0)

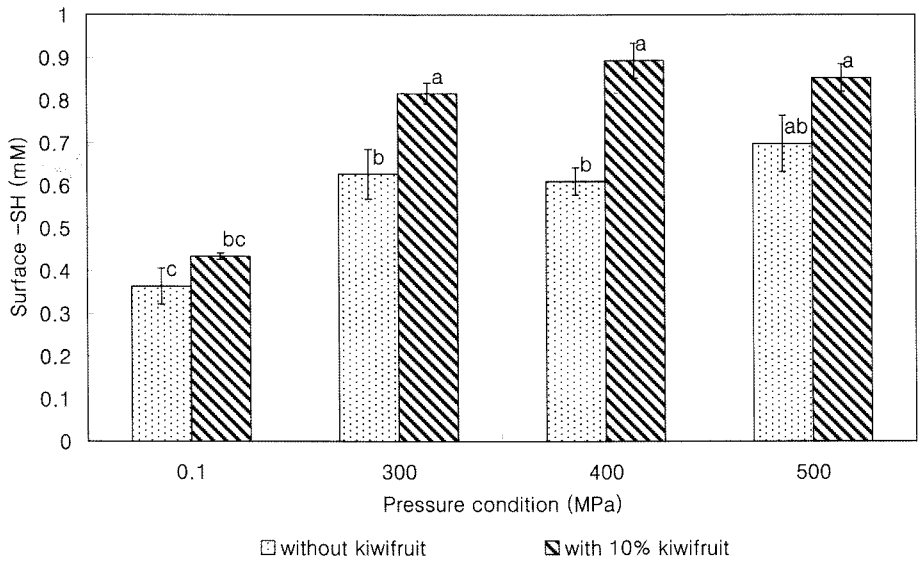

Fig. 3. Surface sulfhydryl group of myofibril with/without kiwifruit induced by hydrostatic pressure ($p<0.05$). The 10% kiwifruit and myofibril (10 mg/mL) were mixed by a 1:9 ratio for 1 h in a refrigerator (4°C) and then hydrostatic pressure treated. Error bars represent standard deviations (n=3).

Surface sulfhydryl (-SH) group

Fig. 3 shows the changes in surface -SH contents of Mf with/without kiwifruit induced by HP. Accessibility for the relatively large reagent (DTNB), restricts the method to detect only the -SH groups that are located on the surface or in accessible cavities (Visschers and Harmen de Jongha, 2005). Surface -SH contents in Mf without kiwifruit induced by 0.1, 300, 400, and 500 MPa were 0.36, 0.63, 0.61, and 0.70 mM, respectively. This result agreed with the report by Hsu *et al.* (2007) that HP treatment induced the increase of surface -SH content in Mf. Surface -SH contents in Mf with 10% kiwifruit induced by 0.1, 300, 400, and 500 MPa were 0.43, 0.82, 0.89, and 0.85 mM, respectively. Actinidin from kiwifruit contains a free -SH group, essential for activity (McDowall, 1970). The presence of -SH groups indicated that the enzyme was a cysteine protease (Mohamed *et al.*, 2005). HP treatment could affect the exposure of -SH amino groups buried in the interior of the native muscle proteins (Ishioroshi *et al.* 1980; Kato and Nakai, 1980). Adding kiwifruit might affect the increase of surface -SH content under same pressure condition due to contain -SH groups.

Beef *bulgogi* with/without kiwifruit induced by hydrostatic pressure

Protein solubility in 0.6 M NaCl (pH 7.0)

Fig. 4 shows the protein solubilities in 0.6 M NaCl (pH 7.0) of beef *bulgogi* with/without 10% or 20% kiwifruit induced by HP. In the group of beef *bulgogi* without kiwifruit, protein solubilities in 0.6 M NaCl (pH 7.0) induced by 0.1, 300, 400, and 500 MPa were 39.1, 31.9, 29.9, and 26.0%, respectively. In the group of beef *bulgogi* with 10% kiwifruit, protein solubilities in 0.6 M NaCl (pH 7.0) induced by 0.1, 300, 400, and 500 MPa were 42.4, 39.1, 33.0, and 29.6%, respectively. In the group of beef *bulgogi* with 20% kiwifruit, protein solubilities in 0.6 M NaCl (pH 7.0) induced by 0.1, 300, 400, and 500 MPa were 47.0, 41.4, 37.9, and 36.1%, respectively. Protein solubilities in 0.6 M NaCl (pH 7.0) under same kiwifruit concentration were significantly decreased as increasing pressure size, and those under same HP condition were significantly increased as increasing kiwifruit concentration in *bulgogi* sauce ($p<0.05$).

SDS-PAGE patterns of soluble protein in 0.6 M NaCl (pH 7.0)

SDS-PAGE patterns of soluble protein in 0.6 M NaCl (pH 7.0) of beef *bulgogi* with/without kiwifruit induced by HP are showed in Fig. 5. The band of 205 kDa corresponding to MHC was decreased by HP treatment. HP-induced phenomena in myosin such as aggregation and gelation were observed (Yamamoto *et al.*, 1990; Yamamoto *et al.*, 1993) and the head is the most pressure-sensitive portion in myosin molecule (Iwasaki and Yamamoto, 2002; Iwasaki and Yamamoto, 2003; Yamamoto *et al.*, 1994). Protein solubility in beef *bulgogi* without kiwifruit might be showed the complex effect by the mixture of *bulgogi* sauce, and it might be possible to have a difference between beef and beef *bulgogi*. Addition kiwifruit

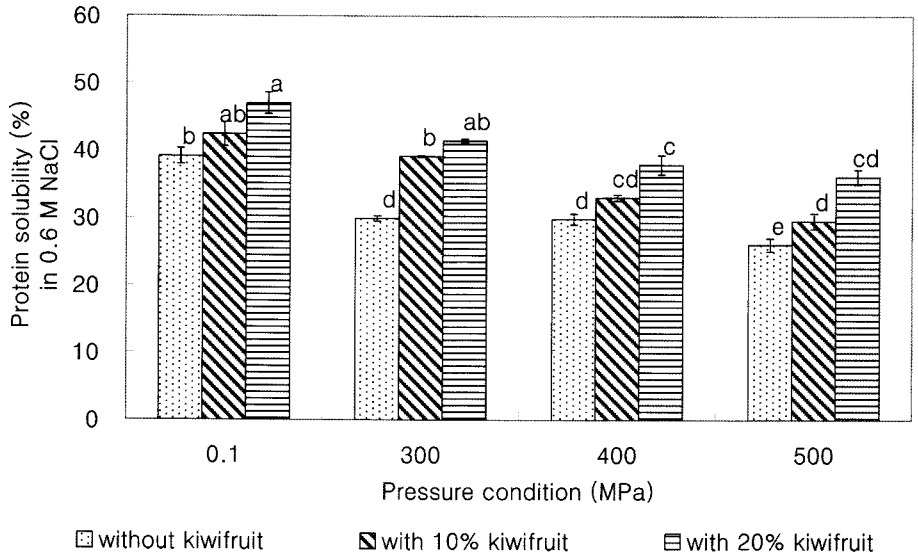

Fig. 4. Protein solubility in 0.6 M NaCl buffer (pH 7.0) of beef *bulgogi* with/without kiwifruit induced by hydrostatic pressure ($p<0.05$). The *bulgogi* without kiwifruit, *bulgogi* with 10% kiwifruit, and *bulgogi* with 20% kiwifruit sauce and beef were mixed by a 1:2 ratio for 3 h in a refrigerator (4°C) and then hydrostatic pressure treated. Error bars represent standard deviations (n=3).

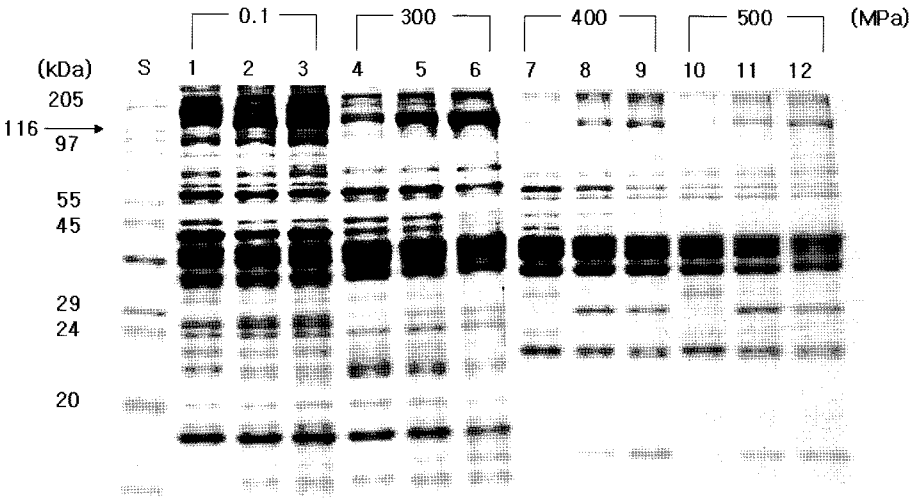

Fig. 5. SDS-PAGE pattern of soluble protein in 0.6 M NaCl buffer (pH 7.0) of beef *bulgogi* with/without kiwifruit induced by hydrostatic pressure. The sauce and beef were mixed by a 1:2 ratio for 3 h in a refrigerator (4°C) and then hydrostatic pressure treated. Lane S: protein standard with molecular weights indicated on the left margin; lane 1, 4, 7, and 10: beef *bulgogi* without kiwifruit induced by 0.1, 300, 400, and 500 MPa, respectively; lane 2, 5, 8, and 11: beef *bulgogi* with 10% kiwifruit induced by 0.1, 300, 400, and 500 MPa, respectively; lane 3, 6, 9, and 12: beef *bulgogi* with 20% kiwifruit induced by 0.1, 300, 400, and 500 MPa, respectively

involved the increase of the band between 116 and 205 kDa under same HP condition. This protein might come from the degradation of MHC as the result of SDS-PAGE patterns of Mf with kiwifruit induced by HP (Fig. 2). Therefore, the similar phenomenon was occurred in beef *bulgogi* with kiwifruit induced by HP, which the degradation of MHC into low molecular weight protein was observed in Mf with kiwifruit induced by HP.

Shear force

Fig. 6 shows the changes of shear force value in beef *bulgogi* with/without kiwifruit induced by HP. In the group of beef *bulgogi* without kiwifruit, shear force value induced by 300 MPa was 8.9 kg, and it was elucidated lower than 11.8 kg of that by 0.1 MPa. While shear force values in beef *bulgogi* without kiwifruit induced by 400 and 500 MPa were 13.0 and 15.5 kg, respectively, and both of them were significantly higher than that by 0.1

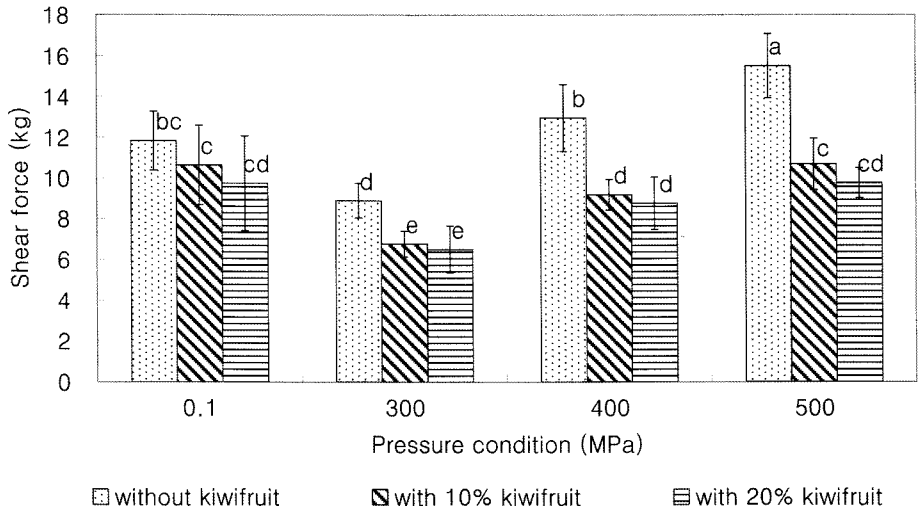

Fig. 6. Shear force of beef *bulgogi* with/without kiwifruit induced by hydrostatic pressure ($p<0.05$). The sauce and beef were mixed by a 1:2 ratio for 3 h in a refrigerator (4°C) and then hydrostatic pressure treated. Error bars represent standard deviations (n=10).

MPa ($p<0.05$). In the group of beef *bulgogi* with 10% kiwifruit, shear force values induced by 400 and 500 MPa were 9.2 and 10.6 kg, respectively. And in the group of beef *bulgogi* with 20% kiwifruit, shear force values induced by 400 and 500 MPa were 8.8 and 9.8 kg, respectively. The shear force values in beef *bulgogi* with 10 or 20% kiwifruit induced by 400 or 500 MPa were significantly lower than those in beef *bulgogi* without kiwifruit induced by 0.1 MPa ($p<0.05$) and similar or slightly lower than in beef *bulgogi* with 10% kiwifruit induced by 0.1 MPa. Therefore, adding kiwifruit could reduce the increased hardness in beef *bulgogi* induced by over 400 MPa.

Acknowledgements

This work was conducted with the support by the Korea Food Research Institute project E 084002.

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(Received 2009.4.29/Accepted 2009.6.16)